CLINICAL ORTHOTOPIC AND HETEROTOPIC HEART TRANSPLANTATION: ASPECTS OF THE UNIVERSITY OF CAPE TOWN EXPERIENCE.

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A dissertation submitted for consideration for the degree of Doctor of Medicine of the University of London.

VOLUME 2

JUNE 1985
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Experimental Development of Cardiac Transplantation

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Heart transplantation has in recent months become a clinical fact. Its experimental development reaches back over 60 years, and has been augmented, particularly in the field of immunosuppression, by knowledge gained from studies of kidney transplantation. Research workers were faced with a number of questions. Would a denervated heart function effectively, if at all? How would it respond to various pharmacological agents? Would the pattern of "rejection" be similar to that in other organs? How could immunological rejection of the heart be assessed clinically? What would be the most effective form of immunosuppression? And, finally, would the findings gained from experimental work on dogs be applicable to man? Today most of these and other questions have been answered, at least in part, and this paper traces the many stages of progress in this field, from the pioneering work of Carrel and Guthrie in 1905 to the first attempts at cardiac transplantation in man by Hardy and his associates in 1964 and by Barnard and his colleagues in 1967.

Introduction

Surgeons concerned with the transplantation of vital organs have recently taken a major step forward with the attempts in South Africa (C. N. Barnard, 1967; Thomson, 1968) and elsewhere to transplant the human heart. These efforts have been made possible by the considerable amount of experimental work carried out throughout this century. Such studies, mainly in the U.S.A., have embraced the technical, physiological, and immunological aspects of cardiac transplantation.

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This paper endeavours to review the experimental surgical techniques which have been evolved and utilized by surgeons and the results obtained. Several workers have attempted transplantation of the heart together with both lungs. Such attempts will be mentioned briefly for the sake of completeness, but have been reviewed fully elsewhere (Cooper, 1968).

Experimental work on cardiac transplantation has evolved through several phases, though there has been considerable overlap between them. Firstly, animals were given a second, often parasitic, heart which enabled certain physiological, pharmacological, and pathological studies to be made. The locus chosen was usually the neck, though the abdomen and inguinal regions have also been used. Suggestion was then made that an additional heart might act as an auxiliary pump in certain circumstances, and this led to the evolution of techniques of inserting the donor heart into the chest in circuit with the recipient organ. With the advent of hypothermia and the pump-oxygenator, total excision and replacement of the heart became more feasible, and, finally, when technical and physiological problems had been studied and minimized, efforts were made to combat the immune response with immunosuppressive drugs. By 1964 the state of our knowledge regarding cardiac transplantation was sufficient for one group of surgeons to attempt transplantation in man.

Transplantation of an Accessory Heart

The first reported attempts at experimental heart transplantation were by Carrel and Guthrie in 1905 (Carrel and Guthrie, 1905; Carrel, 1907). They transplanted the heart in several different ways, but their most fully described technique is shown in Fig. 1. The heart of a small dog was transplanted into the neck of a larger one, the procedure taking about 75 minutes. Some 20 minutes after the re-establishment of the circulation the blood was actively circulating through the coronary system. Strong fibrillar contractions soon occurred. Contractions of the atria appeared, and about one hour after the operation effective contractions of the ventricles began. The transplanted heart beat at the rate of 88 per minute, while the rate of the normal heart was 100 per minute. Carrel (1907) reported that "owing to the fact that the operation was made without aseptic technic, coagulation occurred in the cavities of the heart after about two hours, and the experiment was interrupted."

Carrel and Guthrie also attempted transplantation of the heart and both lungs into the neck of a cat, "but the lungs
soon became oedematous, and distension of the right part of the heart occurred” (Carrel, 1907).

Carrel also studied replantation of the limbs, thyroid gland, ovary, loop of intestine, and one or both kidneys, all based on his techniques of blood-vessel anastomosis. He clearly visualized organ transplantation as we are just beginning to see it today. His contribution to surgical thought equals his contribution to surgical technique.

Coronary Perfusion

Mann et al. (1933) developed a technique of cervical transplantation by which the crucial factor of donor coronary perfusion (viviperfusion) was brought about more simply. The recipient common carotid artery was anastomosed to the donor aorta, thus directly perfusing the donor coronary system. Coronary sinus blood was returned via the right atrium, right
ventricle, and pulmonary artery to the recipient jugular vein.

Two variations were in fact used; in some cases the peripheral end of the divided common carotid artery was used for anastomosis to the aorta, whereas in others the central end was utilized. The main cause of failure with this technique was distension of the heart with blood before its beat was established. The valves of the heart functioned inadequately if the cardiac muscle tonus was abolished or low. Many different expedients were tried to overcome this difficulty, and some were found to be successful.

The heart usually began to contract immediately after the coronary circulation was established, the heart rate being generally 100-130 per minute. The rate was reported as being "surprisingly constant," but did increase if the animal exercised or struggled. E.C.G. tracings were "surprisingly normal." In several experiments the host was given intravenous injections of thyroxine. Within 18 hours the pulse rate of the transplanted organ was appreciably increased, whereas the rate of the host's heart was not yet affected. As had been indicated in previous papers (Lewis and McEachern, 1931; Priestley et al., 1931; Yater, 1931) this experiment was interpreted as demonstrating that the tachycardia after injections of thyroxine was independent of the central nervous system, and that the part played by the central nervous system was of an inhibitory nature, since the denervated heart was more sensitive to the accelerating influence of the drug.

After from one to eight days irregularities in the pulse rate became apparent and were soon followed by absence of pulsation in the transplanted organ, or by fibrillation. The longest period of survival was eight days and the average was four days. The authors do not state how many experiments they performed. When the heart was removed, just before it became quiescent, the left atrium was found to be filled with a clot, and the right atrium and ventricle were distended. The surface of the heart was covered with mottled areas of ecchymosis, and the heart was friable on section. Histologically, the heart was completely infiltrated with lymphocytes, large mononuclear cells, and polymorphs. In some instances a heart that was beating feebly at the time of removal showed few normal muscle fibres when examined microscopically.

The authors concluded that functioning cardiac muscle appeared to be no less resistant as a homotransplant than similarly transplanted kidney. Even when the heart of a small dog was transplanted into the neck of its mother the result was the same. Function could cease very suddenly, pulsation stopping completely after a period of vigorous, healthy con-
traction s. With regard to short experiments—that is, within 24 hours of transplantation—the authors had no hesitation in saying that the transplanted heart should be a valuable test object for the investigation of various physiological problems. They concluded that, although failure of the grafted organ to survive was occasionally due to intravascular thrombosis, in most cases it was not due to technical difficulties and failures but to “some biologic factor which is probably identical to that which prevents survival of other homotransplanted tissues and organs.”

Numerous investigators have subsequently used the Mann technique, or modifications of it, in studying the problems of heart transplantation. Download (1953) used the technique in an effort to elucidate the nature of the tachycardia found in heparinized dogs. He transplanted a series of 30 hearts, of which 23 survived. They beat on average for 129 hours, and one heart survived for 245 hours and another for 240 hours.

Wesolowski and Fennessey (1953) surrounded the donor heart with a plastic bag. The results of the experiment showed no differences from those performed without the bag, thus adding support to the view that rejection was mediated through blood-borne factors.

Sayegh and Creech (1957) used newborn pup and foetal hearts in the Mann preparation, but without improved results.

Recenttma et al. (1960), Bing et al. (1961, 1962), and Chiba et al. (1962) have utilized the Mann technique, or slight modifications of it, to study the metabolism of the transplanted hearts and immunological changes in the recipient (Chiba et al., 1963, 1966; Ramos et al., 1963). Barsamian et al. (1960) and Connaughton and Lewis (1961) have used it also in studying methods by which the heart might be preserved in an extracorporeal phase before its transplantation. Jin (1960) and Manax et al. (1965, 1967) have also used modifications of the technique as a basis for their studies, and DePasquale et al. (1965) have used it in a study of the E.C.G.s of both the transplanted and recipient hearts (DePasquale et al., 1965). Robicsek et al. (1967a, 1967b) utilized Lillehei's modifications of the Mann technique, as well as several other techniques, in his studies of the heart-lung preparation.

Marcus et al. (1951, 1953), in the U.S.A., modified the Mann technique very slightly, anastomosing both ends of the recipient's divided common carotid artery to the donor aorta to perfuse the donor coronary system (Marcus I technique). They modified it yet again to impose a work-load on the donor left ventricle, anastomosing the recipient proximal common carotid
artery to the donor left atrium (Marcus II technique). The
donor left ventricle therefore supplied its own coronary system,
the remaining blood in the donor aorta passing to the recipient's
brain through an anastomosis with the recipient distal carotid
artery.

Sinitsyn (1948), in the U.S.S.R. had described a technique
identical to this Marcus II technique.

The Marcus I technique, in which only the right ventricle
was functioning as a pump, was carried out successfully 15
times by Marcus and his group. On 13 of these occasions,
before being inserted into the recipient, the donor heart was
kept viable by being perfused with blood from a third animal—
a technique developed by these workers and named by them
"interim parabiotic perfusion." Maximal survival times were
45 and 48 hours.

Transplantation in group 2 fashion (all four chambers
functioning) was accomplished successfully on 22 occasions.
Though average survival time had increased, maximal survival
time was still 48 hours.

At the end of this period the transplanted heart and sur-
rounding tissues exhibited an angry-looking inflammatory
oedema, but histological examination was not reported.

Some Major Difficulties

Marcus and his associates listed disturbances of rhythm by
reflex or mechanical causes as among the major difficulties
to be avoided in heart transplantation. They found that reflex
causes could usually be prevented by means of bilateral cervical
vagotomy in the heart donor at the very beginning of the
experiment. Mechanical causes were due to overdistension of
the donor right heart, either from obstruction of venous return
to the recipient or from greatly increased coronary venous
return to the right atrium, as a result of hypoxia from any
cause. Maraist and Glenn (1952) showed that hypoxia could
increase the coronary sinus outflow sixfold.

Electrocardiographs and phonocardiograms were obtained
showing the presence of the two hearts with entirely separate
and independent rates. Pharmacological studies showed that
the two hearts reacted with different patterns to the drugs
adrenaline and digitalis (Luisada and Marcus, 1954).

These workers also developed a third surgical technique in
which donor heart and both lungs were transplanted into the
abdomen of the recipient, being anastomosed into the aorta
and inferior vena cava (Marcus III technique). Eight such
experiments were performed, with survival of the donor organs
up to nine hours. The possibility of using a heterologous heart-lung preparation as an extracorporeal pump during intracardiac procedures was suggested. In the addendum to their paper the authors reported one six-day and one six-and-a-half-day survival of transplanted hearts when using their second technique.

Webb and Howard (1957a) and Lee and Webb (1959) utilized the Marcus II or Simtysyn technique to study maintenance and metabolism of the excised heart.

The addition of a second heart into the neck of the recipient has therefore been studied fairly extensively, all of this work being based on Carrel and Guthrie's original experiments.

Abdominal Accessory Hearts

In more recent years other workers have described techniques for transplanting the donor heart into the abdomen of the recipient, anastomoses being made between the recipient abdominal aorta and the donor ascending aorta, allowing perfusion of the myocardium through the coronary arteries. Coronary venous return drains through the right heart and pulmonary artery, which is anastomosed to the recipient inferior vena cava. This technique has been used mainly for the study of the immune response and its modification by therapeutic agents, and has been described in dogs (Boake and Fols, 1967), and, using microsurgical techniques, in rats (Abbott et al., 1964a, 1964b, 1965; Oliver et al., 1965; Tomita, 1966; Bui-Mong-Hung and Vigano, 1966; Ono et al., 1967).

Stansel and Terino (1965) described an ingenious method of heterotopic cardiac homotransplantation requiring a single anastomosis. The donor heart is excised; the pulmonary artery stump is doubly ligated, the vena caval and pulmonary vein orifices are excised from the atria, as are the atrial septum and tricuspid valve leaflets. The remains of the atrial walls are then brought together and sutured, forming a single atrium. The aortic stump is anastomosed to the recipient's abdominal aorta. In recipient systole the donor coronaries are perfused, the donor aortic valve remaining competent (confirmed by cineangiography). Coronary venous return enters the single atrium and is expelled via the left ventricle through the aorta into the recipient. Obviously this last event can take place only if the donor heart can generate a pressure higher than the recipient's diastolic pressure. However, the authors performed this experiment on 16 animals and obtained survivals up to 36 days, using mercaptopurine as an immunosuppressive. In one animal cineangiography revealed aortic valve insufficiency on the fourteenth postoperative day, which was corrected by
the twenty-eighth day following a course of hydrocortisone, suggesting that a rejection phenomenon was temporarily reversed by the steroids.

Transplanted Heart as Auxiliary Intrathoracic Pump

In the 1940s Demikhov, in the U.S.S.R., was attempting to transplant a second heart into the thorax of the recipient animal. He believed that "because of its anatomical and physiological features the heart can only function actively when it is transplanted into the thorax. If it is transplanted to the vessels of the neck or into the inguinal region it cannot take an active part in the movement of the blood, and is a neutral organ, living on the recipient's blood" (Demikhov, 1962). In 1940 he himself had attempted to transplant the heart of a warm-blooded animal to the vessels of the inguinal region. Ognev (1947), also in the U.S.S.R., described transplantation of the heart into the inguinal region, after which the heart survived for a period of 50 minutes.

Demikhov pointed out that when the heart was outside the thorax it did not communicate with the lungs, and therefore was unable to carry out one of its principal functions. Moreover, the left side of the heart was either empty—for example, Mann or Marcus I technique—or excessively overfilled—for example, Sinitsyn, Marcus II, or Ognev technique. This overfilling was due to the fact that blood was pumped into the left atrium at arterial pressure. He also felt that scar formation around the peripherally placed transplanted heart would disturb its contractile activity.

In 1946 Demikhov began his extensive studies on transplantation of the heart into the thorax. These involved the addition of a second heart (with occasionally an attached lobe of a lung) as an auxiliary pump (Demikhov, 1950b, 1962), homograft replacement of the heart and both lungs (Demikhov, 1947, 1950a, 1951, 1952, 1955, 1962), and of the heart alone (Demikhov, 1962).

No such ambitious attempts had been made previously, though in 1945 Sinitsyn had published his work on transplantation of the heart in a frog, with complete replacement of the recipient organ (Sinitsyn, 1945, 1948, 1951). The frog, however, offers many favourable conditions when compared with warm-blooded animals—notably that the heart can normally contract for some days after removal from the body (if perfused with saline solution and protected against desiccation), and that a disturbance of pulmonary respiration is not of decisive importance to the frog, for it has a well-developed
The ambitious nature of Demikhov's attempts can be appreciated best when it is remembered that supportive techniques such as hypothermia and cardiopulmonary bypass were not available to him, as their development had not taken place at this time.

In all, Demikhov described 24 variants of his technique to place an additional heart within the thorax, utilizing most of the major vessels within that cavity. He performed 250 operations on dogs, using these techniques. Forty-three animals died on the operating table, 87 during the first two days, 97 between the 2nd and 12th day, and 13 between the 12th and 19th day. One dog survived for 32 days with two hearts functioning within its chest. In these experiments Demikhov anastomosed the vessels by several different techniques, ranging from ordinary suturing with silk to the insertion of various forms of tubing over which the vessel ends were ligated. The best results as regards functional activity of the transplanted heart and the survival and preservation of its structure were observed after operations using scheme 24 (Fig. 2). Apart from operative and immediate postoperative deaths and deaths from other technical failures and postoperative complications, the principal causes of death of the experimental animals were: (1) thrombosis at the sites of vascular suture with disturbance of the circulation; (2) pleurisy and pericarditis around the transplanted hearts; (3) infarcts of the transplanted hearts; and (4) infarcts of the kidneys and mesenteric arteries.

It is of interest to note that Demikhov was not convinced that tissue incompatibility played an important part in the deaths of these animals. He considered that the myocardial infarcts could be caused by emboli arising from thromboses at the sites of blood-vessel anastomosis. Frequently, however, inflammation of the transplanted heart was mentioned, with histological evidence of round-cell infiltration of the pericardium spreading into the depths of the myocardium.

From the physiological aspect the transplanted heart was distinguished by the comparative constancy of its rhythm and by its greater resistance to the action of toxic doses of various cardiac glycosides. After the injection of large doses of strophanthidin into the dog a marked bradycardia of the dog's own heart was observed, but no changes took place in the rhythm of the transplanted heart. When lethal doses of cardiac glycosides were given the animal's own heart stopped beating 10 to 15 minutes before the transplanted heart. It was also observed that when a dog died from acute blood loss or from peritonitis the co-ordinated activity of the animal's own heart ceased long before that of the transplanted heart.
Many years later Reemtsma (1964) described a method of inserting an additional intrathoracic heart as an auxiliary pump. The donor inferior vena cava was anastomosed to the recipient's right atrial appendage, followed by anastomosis of the two left atrial appendages, and end-to-side anastomoses of the two pulmonary arteries and aortae. Function as an auxiliary pump was maintained for a maximum period of 72 hours.

Sen et al. (1965) in India described a further technique in which the transplanted heart supported only the systemic circulation of the recipient. This auxiliary heart functioned in one animal for 48 hours, when it was surgically removed and the animal was supported solely by its own heart once again.

Barrié et al. (1966), in France, reported two techniques very similar to those of Reemtsma and Sen respectively.

Johansson et al. (1967), in Sweden, developed a similar technique in which the transplanted heart acts as a left heart bypass,

Fig. 2.—Demikhov's scheme 24 for the intrathoracic transplantation of a second heart (shaded), which functioned as accessory support of the host animal.
and reported some success in maintaining the circulation while the recipient's own heart was in ventricular fibrillation.

In our own laboratory (Longmore et al., 1968) we have experimented with a simple technique for the insertion of a cardiac homograft as an auxiliary intrathoracic pump, based on Demikhov's original method of cardiopulmonary transplantation. The donor heart is grafted along with one or both lungs, the two right atrial appendages being anastomosed, and the donor aorta being joined end-to-side to the recipient ascending aorta. The donor bronchus or trachea is then anastomosed to that of the recipient or brought out as a tracheostomy. The major disadvantage with this technique is that to accommodate the donor organs in the chest it is often necessary to remove at least one lobe of the recipient's own lungs.

Orthotopic Transplantation of Heart

Between 1946 and 1955, using an ingenious method, Demikhov carried out 67 attempts to transplant the heart and both lungs in dogs, with survival up to the sixth day. Deaths resulted mainly from thrombosis at the sites of blood-vessel anastomosis, or from bronchopneumonia in the lower lobes.

On 25 December 1951 Demikhov made his first attempt (and the first described in the literature) to replace the heart alone. Without the availability of hypothermia or pump-oxygenator support the technical problems are forbidding. His technique was complicated, but basically consisted of end-to-side anastomoses between the corresponding thoracic aortae, superior venae cavae, inferior venae cavae, and pulmonary arteries. The two inferior pulmonary veins of the donor were joined together and connected to the recipient's left atrial appendage. After these anastomoses the ascending parts of the recipient's thoracic aorta and pulmonary artery were ligated, and by means of a purse-string suture the recipient's left atrium was indrawn at its border with the ventricle. The entire part of the recipient's heart excluded from the circulation of blood was removed.

Demikhov performed this procedure on 22 occasions, and on two of these (in January 1955) was successful in obtaining good cardiac function for periods of 11½ and 15½ hours. In the former of these two cases death was due to thrombosis at the superior vena cava anastomosis, and in all other cases death resulted from technical problems. However, these were the first described experiments where dogs survived, even for a few hours, solely on the activity of the transplanted heart without participation of their own. From these experiments Demikhov concluded that, from the surgical and physiological aspects, replacement of the heart alone was possible, but much
further work was required in order to overcome the technical problems involved.

In reviewing the literature on this subject it is obvious that Demikhov's contribution to the field of experimental organ transplantation is considerable, and subsequent workers rarely appear to have paid sufficient attention or given adequate credit to his pioneering work, possibly as his original papers were published in Russian. His work has subsequently been translated into English (Demikhov, 1962) and reported in the German literature (Uebermuth, 1959).

Advent of Supportive Techniques

With the advent of means of supporting the recipient during the operative procedure workers in this field became more ambitious. Neptune et al. (1953) used hypothermia in three attempts at homotransplantation of the heart and both lungs, with survival for up to six hours. Webb and Howard (1957b) performed cardiopulmonary replacement, the animal being maintained on mechanical pump-oxygenator support, with survival up to 22 hours. They also studied homologous cardiac and unilateral pulmonary transplants, and autotransplants of the heart and lungs and of the heart alone. In these latter cases the pulmonary vessels were not divided at all, and thus the heart was not completely removed at any time. Couples were used to anastomose the cavae, though the sortae were sutured. Webb and Howard were mainly interested in the problem of respiratory function following transplantation of both lungs and did not report the length of survival of the control dogs, in which the heart alone was autografted. However, in a subsequent paper (Webb et al., 1959) they reported survivors from 23 minutes to seven and a half hours following homotransplantation of the heart alone. Their technique involved the anastomosis of the superior and inferior venae cavae and all four pulmonary veins with couples, followed by pulmonary artery and aortic anastomoses. The donor heart was preserved by refrigeration in cold Tyrode's solution containing 10% serum.

In the previous year homologous cardiac transplantation had been carried out, again using pump-oxygenator support (Golberg et al., 1958; Berman et al., 1958). Their technique differed from Webb's subsequent technique in that they preserved a left atrial cuff in the recipient as Demikhov had done, thus nullifying the need to anastomose the pulmonary veins. They reported three experiments, with survival for only 21, 117, and 86 minutes, and in two of these cases pacemakers had to be inserted to maintain an adequate heart rate.
In the same year Blanco et al. (1958) attempted total heart-lung homotransplantation in eight dogs, with survival up to four and a half hours.

First British Work

In 1959 there appeared the first report of experimental heart transplantation in the British literature. Cass and Brock (1959) reported six attempts at autotransplantation and homotransplantation. Basically they used two main methods, that described by Golberg et al. and an advance on this where both atria were left intact in the recipient, thus simplifying the procedure even further. Anastomoses of the atria, aorta, and pulmonary artery were now all that were required. This identical procedure was later described by Lower and Shumway (1960), whose results were more successful. Shumway and his colleagues have since carried out extensive work using this technique (which has subsequently become known as “Shumway’s technique”), and it is this technique which has formed the basis of most of the recent attempts at human cardiac transplantation. It is therefore of interest to note that it was first described in a British journal (Guy’s Hospital Reports). Cass and Brock (1959) used it in performing five autotransplants and one homotransplant, but in these six attempts the grafted heart was able to maintain an adequate blood pressure for up to one hour only. Using the Golberg technique to perform an autotransplant, they had a similar result.

It was therefore not until 1960 that the major advance was made, Lower and Shumway reporting a series of eight consecutive homotransplantations, with five of the recipient animals living for 6 to 21 days. During convalescence the dogs ate and exercised normally. The pulse rate was variable and increased moderately with exercise. They noted that E.C.G.s taken only a few hours before death were virtually normal, showing no evidence of arrhythmia or conduction defects. The terminal course was usually rapid, occurring over the span of about 24 hours, during which time the animal became lethargic and progressively stuporuous. Post-mortem examination of the heart showed it to be ecchymotic and oedematous, with a fibrinoid pericarditis and generalized dilatation. Microscopical examination of sections demonstrated severe myocarditis with massive round-cell infiltration, patchy necrosis, interstitial haemorrhage, and oedema. The regional lymph nodes were large, but microscopical examination showed a non-specific increase in plasma cells and histiocytes.
The authors concluded that "if the immunologic mechanisms of the host were prevented from destroying the graft, in all likelihood it would continue to function adequately for the normal life span of the animal."

In the following year Lower et al. (1961a) reported complete homograft replacement of the heart and both lungs with survival time of up to five days, with deaths from respiratory insufficiency, which was apparently due to infiltration of mononuclear cells into the lung parenchyma.

These authors and their colleagues have subsequently confined their studies to autotransplantation (Harley et al., 1962; Dong et al., 1964a) and homotransplantation (Lower et al., 1961b, 1962, 1965) of the heart alone, and have achieved long-term survivors. They have contributed massively to our knowledge of this subject (Shumway et al., 1963; Dong et al., 1964b; Shumway and Lower, 1964; Hurley et al., 1965; Lower, 1966; Lower et al., 1966; Angell et al., 1967; Cleveland and Lower, 1967). They have emphasized the value of their technique in preserving the volume receptor nerve supply to the posterior walls of the recipient atria; they have studied the function of the transplanted heart and found that it has the capacity to adequately increase cardiac output (mainly by increasing stroke volume) under a variety of physiological stresses, including hypoxia and exercise; they have demonstrated normal cardiac output one year after homotransplantation and five and a half years after autotransplantation; and they have shown clear evidence of autonomic reinnervation of the heart after autotransplantation. From time to time they have reviewed their progress in this field (Shumway, 1963a, 1963b; Dong et al., 1965; Shumway et al., 1966a, 1966b, 1967a, 1967b; Lower and Cleveland, 1967).

Technique of Autografting

Willman et al. (1962a) produced the first of several papers from their group on the subjects of myocardial structure and function following autotransplantation of the heart. Their technique differed from those of previous workers in being a piecemeal division and resuture in turn of each of the vessels connecting the heart to the rest of the body. Polyvinyl catheters were introduced into the superior vena cava and inferior vena cava and joined to establish continuity between the cavae. Two snare of umbilical tape were tied down 1 cm. apart over the superior vena cava catheter; the superior vena cava was divided between the umbilical tape snares and immediately resutured. The snare were released and the inferior vena cava was similarly divided and sutured.
The vena caval catheters were then joined to the inflow line of an extracorporeal circuit and the animal was perfused with the perfusate at 15°C. A polyvinyl catheter was introduced through the apex of the left ventricle and this chamber was perfused with heparinized Ringer lactate solution at 2-4°C. The aorta and pulmonary artery were then clamped across, the left atrium was divided and immediately sutured, followed in turn by division and suture of the aorta and pulmonary artery. The animal was rewarmed with the perfusate at 40°C, defibrillated, and weaned off extracorporeal circulatory support. The perfusion period averaged 70 minutes and the cardiac arrest time was generally 50 minutes.

Twenty-seven out of 40 animals undergoing the operation died within two days, mainly from technical causes, particularly those associated with haemorrhage from the aortic suture line. Thirteen animals survived longer than two days, all survivors appearing critically ill during the first week. They generally attempted to eat and drink by the second day but became anorexic and apathetic by the fourth day. Some degree of oedema became evident on the fourth to the eighth day. During this period parenteral fluids, blood, and diuretics were administered as indicated. The oedema disappeared during the second week from those which survived and they began to eat and became more active. At the time of publication of the authors' first paper one animal was alive and active eight months after autotransplantation.

Six animals died within four weeks with clinical evidence of congestive heart failure, and in four of these histological study of the heart, lungs, spleen, and liver confirmed this diagnosis. All survivors had disturbances of cardiac rhythm demonstrated by radiotelemetry in the unanaesthetized state. Although their cardiac rate was not fixed they had lost the normal sinus arrhythmia and the rate failed to alter in response to activity.

In four animals subjected to perfusion, hypothermia, and cardiac arrest without transplantation there were three survivors, none of whom developed oedema in the postoperative period and all of whom remained active four months after the sham operation.

The authors concluded that the difference in the course of the two groups indicated a specific adverse effect of severing the heart from the body. They suggested that this deleterious effect might reside in severance of the extrinsic innervation, or the lymphatic drainage, or both, and quoted work by Cooper et al. (1961) and by Miller et al. (1960, 1961) supporting these possibilities. The specific mechanism of adaptation by which long-term survival was achieved remained unclear. Shumway's group, in Stanford, later disputed these pessimistic findings on the basis of autotransplants in dogs which survived for over
16 months (Hurley et al., 1962). The disparity between the findings of these two groups has never been fully explained.

Willman, Cooper, Hanlon, and their associates have subsequently used their technique to carry out extensive studies of the physiology (Willman et al., 1962b, 1964a, 1966, 1967; Cooper et al., 1966; Daggett et al., 1967), histology (Napolitano et al., 1964; Cooper et al., 1964a), metabolism (Willman et al., 1964b; Cooper et al., 1965; Potter et al., 1965), and pharmacology (Cooper et al., 1964b) of the autografted heart, and have studied cardiac autotransplantation in the primate (Willman et al., 1965).

Use of Profound Hypothermia

Kondo et al. (1965a, 1965b, 1965c, 1967) have used Shumway's technique in the transplantation of homologous puppy hearts, but performed the procedure under profound hypothermia rather than with the pump-oxygenator. Total body-cooling by iced-water immersion of both recipient (to 16-17°C rectally) and donor (to 27-29°C rectally) was carried out, allowing complete circulatory arrest of the recipient for the 45±5 minutes required as actual operative time. Heart massage was begun immediately the anastomoses were completed, and the animal was rewarmed by body immersion and flushing of the chest cavity with warm saline. When warm enough (26-28°C rectal temperature) the heart was electrically defibrillated, and re-warming continued until the temperature returned to normal.

In their first reported series of 40 experiments this group obtained 24 survivors of more than one day, 13 of more than seven days, and one which was alive and well 112 days postoperatively. None were given immunosuppressive therapy, and yet histological and other signs of rejection occurred much later than in previously reported series. The surprisingly long survival of some of their animals was attributed to accidental histocompatibility, though the fact that 3-month-old puppies were used for these experiments was thought to be a significant factor in the relative mildness of the immune response observed.

In France Dubost's group have experience of both the Shumway technique (using pump-oxygenator support) and Kondo's technique (using profound hypothermia) (Cachera et al., 1966a). Although they obtained survival of a few days with both methods, they found Kondo's technique was the simpler of the two, and in their subsequent work they have used hypothermia exclusively. They have reported survivors of two and three months following autographs, and of 10 and
20 days following homografts (Cachera et al., 1966b). Leandri (1967) and Leandri et al. (1967) reviewed the histological findings in these series. This group's recent work has centred on methods of donor heart preservation (Lacombe et al., 1967).

In South Africa, in preparation for human heart transplantation, M. S. Barnard performed cardiac homografting in dogs, using Shumway's technique, though the donor was cooled by immersion in ice as described by Kondo. Barnard did not report the number of experiments carried out, nor the maximal survival time, but did note that "the donor heart supported a satisfactory circulation in 90% of the animals" (M. S. Barnard, 1967).

Several other workers have subsequently used the techniques described by Shumway and Willman in animal studies, among them being Hairston and Muller (1961), Sen et al. (1965), and Hardy et al. (1966), but in general their results have been disappointing.

**Prolongation of Graft Survival by Immunosuppression**

When many of the technical and physiological problems had been overcome research workers turned their attention to the problem of combating the immune response by chemotherapy, and thus prolonging graft survival. Reemtsma et al. (1962) used Mann's cervical transplantation technique to study the effect of Amethopterin (methotrexate), a folic acid antagonist. In a control series of untreated dogs a maximal survival time of 10 days' cardiac activity was obtained, whereas in the treated group maximal survival was 27 days. In a further series of 14 studies in which the recipient was given azathioprine (Imuran) the maximal survival period was 32 days (Reemtsma, 1964). In both series about 40% of the transplanted hearts functioned for more than 10 days. Drug toxicity was less severe in the azathioprine-treated group.

Blumenstock et al. (1963) used Amethopterin in treating a group of dogs that had undergone orthotopic transplants, and reported five dogs that survived from 12 to 42 days, though no control series was performed for comparison.

Lower et al. (1965) reported their experience with a combination of steroids (hydrocortisone or methylprednisolone) and azathioprine or mercaptopurine. A control group of 20 dogs survived from 4 to 21 days, with a mean of 7 days. A group of 12 immunosuppressed dogs survived from 6 to 35 days, with a mean of 17 days. Three dogs which lived at least one month were thought to have died from infection and drug toxicity. The complications that arose as a result of the daily
administration of these drugs were numerous and clearly prohibitive. Six dogs were therefore given immunosuppressant drugs only during threatened rejection, as determined by diminution of the R-wave voltage in all leads of the E.C.G., which had been found to accompany immune disease of the cardiac homograft. (E.C.G. changes after homologous canine heart transplantation have been studied particularly by Schuldt (1964), DePasquale et al. (1965), and Lower et al. (1966).) Five of the six dogs lived at least one month, with three surviving longer than three months. One dog was later reported to be alive more than one year after homotransplantation, and had received no drugs for the previous three months. The E.C.G. was relatively stable, and the animal ate well and took daily walks outdoors, but required almost weekly blood transfusions to combat severe pancytopenia (Shumway et al., 1966b). More recently Lower and Cleveland (1967) reported a bitch with a homograft heart which had successfully whelped a litter of six without difficulty.

Ono et al. (1967) obtained improved survival following irradiation of rat heart homografts; the work of Stansel and Terino (1965) using mercaptopurine has already been discussed.

Cardiac Transplantation in Man—First Attempt

By the mid-1960s there had been built up a considerable fund of knowledge regarding the subject of heart transplantation, particularly in the areas of surgical technique and physiology. The increasing success of experimental cardiac transplantation led Hardy and his colleagues, at the University of Mississippi Medical Centre, to consider heart transplantation in man. This group had considerable experience in the field of animal studies, both of cardiac transplantation (Webb and Howard, 1957a, 1957b; Webb et al., 1959, 1961; Lee and Webb, 1958, 1959; Hardy et al., 1964a; Kurrus et al., 1964) and of lung transplantation (Hardy et al., 1963a, 1963b; Alicant and Hardy, 1963), and had carried out iuung homotransplantation in man on one occasion (Hardy et al., 1963c).

In 1964 they reported in a detailed paper (Hardy et al., 1964b) their attempt on 23 January to transplant the heart of a large chimpanzee into the chest of a 68-year-old man. The patient had had medical treatment for hypertensive cardiovascular disease for many years, and had widespread atheroma. He developed gangrene of the lower left leg, probably the result of an embolus, and was admitted to his community hospital in a comatose and collapsed state with no detectable blood pressure. Vasopressor drugs in high dosage were required to
raise his blood pressure to a systolic level of 100 mm. Hg. The cause of his impaired mental state could not be fully determined. He was transferred to the University Hospital, where his left lower leg was amputated. Cardiological examination and studies revealed evidence of past anteroseptal myocardial infarction. Intensive treatment brought about no improvement in his condition.

It was decided that heart transplantation offered him his only possible chance of survival. The patient's condition deteriorated suddenly and he passed into terminal shock. He was taken to the operating-theatre, his chest was opened, and he was supported by a pump-oxygenator just as effective heart action ceased. His heart was removed and subsequent dissection revealed gross atherosclerosis with about 90% occlusion of the coronary vessels.

It had been hoped to perform a homotransplantation, but no donor was available. Some of the members of the group had been impressed by the early results of kidney heterografts from chimpanzees to man reported by Reemtsma et al. (1964) and had taken the precaution of obtaining two chimpanzees as donors of hearts if no suitable human donor was available at the required time. The larger chimpanzee was therefore prepared and its heart excised and viability maintained by hypothermic retrograde coronary sinus perfusion. This animal weighed 96 lb. (43.5 kg.), considerably less than the recipient, but its cardiac output had been measured at 4.25 litres per minute. The cardiac output of the prospective recipient was 3.6 litres per minute before his terminal collapse. This output was not at a normal level for a man of his size, but had been sufficient to sustain a systolic blood pressure level of 90–110 mm. Hg.

Shumway's technique was used to insert the donor organ, which when defibrillated beat regularly and forcefully at a rate of about 80 per minute. It soon became apparent that the smaller heart would not be able to handle the large venous return unless its rate were increased. Pacemaker leads were sutured to the left ventricle and the heart was paced at 100 per minute, and at this rate was able to maintain a blood pressure ranging from 60 to 90 mm. Hg. However, about one hour after the removal of the bypass catheters the heart was judged incapable of accepting the large venous return without intermittent decompression by manual cardiac massage. Further support was abandoned.

The authors discussed the information they had obtained from this attempt and the collateral issues that arose from it. They concluded that their experience supported the scientific feasibility of heart transplantation in man.
Much further experimental work remained to be carried out, however, and nearly four years elapsed before another attempt was reported. Barnard and his colleagues (C. N. Barnard, 1967), at the University of Cape Town, performed cardiac homotransplantation in man on 3 December 1967, and by so doing initiated the several further attempts which have been carried out in recent months in many parts of the world.

REFERENCES


Printed in Great Britain by J. Smethurst & Co. Ltd.,
TRANSPLANTATION OF THE HEART AND BOTH LUNGS

I. HISTORICAL REVIEW

BY

D. K. C. COOPER


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LONDON

BRITISH MEDICAL ASSOCIATION

TAVISTOCK SQUARE, W.C.1
Transplantation of the heart and both lungs

I. Historical review

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Transplantation of the heart and both lungs is being considered as a clinical possibility in several surgical centres today. This paper reviews the experimental development of surgical techniques in this field. In the 1940s Demikhov in the U.S.S.R. succeeded in developing a technique by which he could carry out cardiopulmonary transplantation without the use of any artificial means of recipient support during the procedure. He obtained survivors for up to six days; late deaths occurred mainly from pulmonary complications. The techniques and results of subsequent workers using hypothermia or pump-oxygenator support are reviewed. Recent work has focused on the problem of the return of spontaneous respiration following denervation of the lungs which, of necessity, occurs during this procedure.

The very early work on heart transplantation was carried out by Carrel and Guthrie (1905), Carrel (1907), and Mann, Priestley, Markowitz, and Yates (1933), the donor heart being placed in the neck of the recipient dog, but it was not until Demikhov began his extensive studies in the 1940s that the heart and both lungs were transplanted into their orthotopic locus (Demikhov, 1949).

Demikhov's work embraced many aspects of transplantation and involved the study of many types of organs, but his studies on the heart and heart-lung preparation are particularly notable (Demikhov, 1950b, 1951, 1952, 1956, 1959, 1962). He developed 24 different methods of providing a recipient dog with an accessory heart within the chest (Demikhov, 1950a), using most of the available major vessels within that cavity, with survival for as many as 32 days. He subsequently (1951-55) went on to carry out orthotopic transplantation of the heart, though only two of 22 attempts were considered successful, the heart functioning for 'a few hours' (Demikhov, 1962). Much credit must be given to his achievements, particularly when it is remembered that neither in these experiments nor in his heart-lung transplantations was he able to use any form of recipient support, for example, hypothermia or the pump-oxygenator.

On 20 October 1946, Demikhov transplanted the heart and lungs of a dog, and the recipient survived for two hours without its own organs, but it was not until 1949 that more prolonged survival was obtained.

The technique used was ingenious (Fig. 1) as it enabled the blood supply to the brain to be maintained continuously throughout the operation, with the exception of two to three minutes at one critical stage. Demikhov took care to dissect out the phrenic and vagus nerves of the recipient with the intention of preserving the innervation of those structures, particularly the diaphragm, below the region of the heart and lungs. At this stage the right lung was removed to facilitate later parts of the operation.

FIG. 1. The completed operation of orthotopic heart-lung transplantation, using Demikhov’s technique, after anastomosis of the donor tissues (white) to the recipient tissues (shaded).
After preliminary mobilization, the donor heart-lung preparation was removed from the animal by clamping and dividing the thoracic aorta, the inferior vena cava, brachiocephalic and subclavian arteries, and the superior vena cava. During transfer the donor heart-lung was kept viable by its own closed-circuit circulation, blood from the left ventricle being pumped into the arch of the aorta, from whence it passed through the coronary vessels supplying the myocardium and into the right atrium, the right ventricle, and the lungs; oxygenated blood was returned to the left atrium. This form of heart-Jung preparation (Demikhov, 1950c) differed considerably from those described earlier by Pavlov and Christovitch, and by Starling (Demikhov, 1962), and subsequently has been found to be a promising means of transporting and temporarily preserving the heart and lungs (Longmore, Cooper, Hall, Sekabunga, and Welch, 1969).

The transferred heart and lungs, still functioning, were placed over the recipient's heart and lungs, and the brachiocephalic arteries of the two dogs were quickly connected over a tube, after which the superior vena cava of the transplant was sutured to the superior vena cava of the recipient. This procedure normally took only two or three minutes, thus preventing any undue stasis of venous blood in the brain. Consequently, the blood supply to the head was maintained by the transplanted heart and lungs, while the blood supply to the lower half of the body was still maintained by the recipient's heart and left lung. The two inferior venae cavae were then sutured together followed by anastomosis of the two aortae in the region of the arch. At this stage the transplanted heart and lungs provided the blood supply to the entire animal. During the inferior vena caval anastomosis the blood supply to the lower half of the body was temporarily interrupted for 15 to 20 minutes. Demikhov noted dilatation of the vessels of the lower half of the body as a result of this temporary occlusion, resulting in a sharp but easily correctable fall in blood pressure.

The tracheas of the transplant and recipient were then connected, either by means of a special tube or by silk sutures, using a technique which avoided interference with respiration. In later attempts tracheostomy was carried out prophylactically to prevent asphyxia from laryngeal spasm following damage to the recurrent laryngeal nerve.

Demikhov attempted this very considerable procedure on 67 occasions; of these, 23 were concluded with the recipient still on the operating table. Thirty dogs survived for less than 24 hours, six less than 48 hours, six less than four days, and one less than six days.

In those dogs which died soon after operation technical problems and thrombosis at the anastomoses, particularly of the brachiocephalic artery, were the common causes of death. Longer-term survivors died of bronchopneumonia which was noted to be confined to the lower lobes of the lungs. Demikhov felt that this local inflammatory process in the transplanted lungs could be explained by the anatomical properties of the lower lobes. He su that if the inflammation in the transplanted lungs resulted from tissue incompatibility it must have affected both upper and lower lobes. In all cases the heart appeared to show normality at necropsy.

Those dogs which did recover from the operative effects of the operation appear to have done quite well for the few days until their deaths. Respiration was generally slow, in the region of 12 per minute, and the pulse rate variable, frequently fast. Certain dogs appeared to react remarkably well, walked about their cages, drank water, ate meat 'with a good appetite', and reacted briskly to their surroundings. One dog was even sent by train from Rymryz to Moscow on the fourth post-operative day on arrival at its destination 'ran up the stairway to heaven'.

Several important observations and conclusions have resulted from Demikhov's pioneering experiments. Most significant is the fact that followin replacement of the heart and both lungs of these dogs did breathe spontaneously and appear to be the result of respiratory insufficiency unless caused by bronchopneumonia. A particularly important finding and one confirmed by all subsequent workers. Secondary respiratory rate was variable; one dog on the second post-operative day following operation had a respiratory rate of 20 per minute. On the second post-operative day another dog drank some water and ate meat but noted to have a pleural effusion. Atten asphyxia this led to vomiting for five minutes which the dog was dysnmonic and the respiratory rate rose to 135 per minute. Four and a half hours later the rate had returned to 12 per minute. Thirdly, the transplanted heart was able to maintain an adequate circulation for six days, despite the fact that it was totally denervated and neither atrium had been left in situ, it also
considerable variation in an individual dog, depending on the dog and its environment.

Demikhov's contribution to this experimental field has been considerable. Most subsequent workers have been unable to obtain survivors of more than a few hours, the failure of respiratory function being the major problem. A study of the authorized translation of Demikhov's own book (Demikhov, 1962) does not enlighten one as to why his results were better than other people's, neither his technique nor management differing in any crucial way from those of later workers.

During the period of Demikhov's studies, other workers, notably Sinitsyn (1948) and Marcus, Wong, and Luisada (1951; 1953), were studying the effects of cervical implantation of the transplanted heart. Sinitsyn (1945; 1951), working in the U.S.S.R., had also made studies on the transplanted frog's heart. Marcus et al. in the United States of America used similar techniques but also developed a technique for transplanting the heart and both lungs into the abdomen, thus giving the recipient two sets of heart and lungs.

The purpose of this latter experiment was to determine whether the heart and lungs as a pump-oxygenator unit could deliver self-oxygenated blood to a delimited part of the host's body. The heart-lung preparation was interposed either into the aorta-vena caval circulation just above their bifurcations (Fig. 2) or into the common iliac circulation. Blood was brought from the host's inferior vena cava to the right atrium of the transplant, passed through its own lungs, which were attached to an artificial respiration apparatus for oxygenation, and returned via the pulmonary veins to the left heart, which supplied part of the host's body and its own myocardium. This hypothesis was tested by (1) connexion of the host's trachea to a source of nitrogen only, (2) temporary ligation of the host's main pulmonary artery, providing an empty left atrium and ventricle, and (3) occlusion of the tricuspid valve in an attempt to empty both sides of the host's heart.

Eight heart-lung preparations were transplanted. Attached to its own ventilator such a heart-lung transplant lived for 75 minutes after the heart of the host animal had died. On one occasion the heart beat and pumped oxygenated blood to a portion of the host's body for 61 minutes after the host's lungs were purple by virtue of inhalation of 100% nitrogen. On another occasion the heart-lung preparation lived within the abdominal cavity of the recipient for over nine hours, during which time it was necessary to defibrillate it three times.

On two occasions, when the heart-lung preparation was working in the aorta-vena caval circulation, the chest of the host was entered and the main pulmonary artery was temporarily occluded to empty the left side of the heart. The host's heart was then opened through the left atrial appendage and manipulations of the mitral valve were made under direct vision. After seven minutes the atrium was closed and the appendage amputated. The pulmonary artery remained occluded once for 15 minutes and once for 48 minutes and was then released. The coronary arteries, in the meantime, had been supplied with oxygenated blood from the heart-lung transplant in the abdomen.

Among the difficulties that Marcus and his associates met were disturbances of rhythm of the donor heart by reflex or mechanical causes. The former, they felt, could usually be prevented by means of bilateral cervical vagotomies in the heart donor at the beginning of the experiment, and the latter cause was usually consequent upon varying degrees of overdistension of the right heart, which they therefore took steps to prevent.

In an attempt to minimize tissue incompatibility, the recipient and the donor animal's blood were crossmatched in many cases, but maximal survival time of the transplanted organs did not increase.
Among the conclusions on their work, the authors suggested the possibility of using a heterologous heart-lung preparation as an extracorporeal pump during intracardiac procedures. They also commented that the transplanted heart might act as an accessory pump to decrease the work load of the central heart, even if only temporarily.

With the advent of supportive techniques total heart-lung excision and replacement became more feasible. In 1953, Neptune, Cookson, Bailey, Appler, and Rajkowski used hypothermia to sustain life in the recipient while the transplantation was proceeding. They estimated that, by cooling the recipient to 21–24°C rectally, they allowed themselves approximately 15 minutes to perform the critical stages of the transplantation.

It is of interest to note that they obtained this low body temperature by placing the recipient in an ordinary beverage cooler.

Their technique involved rapid anastomosis of the ascending aortae by suture, and of the superior vena cavae over a polythene tube, after which the cerebral circulation could be resumed. The inferior vena cavae were then joined similarly, allowing restoration of the complete circulation.

When the heart was thought to be in good condition, as evidenced by E.C.G. studies, the tracheas were anastomosed end-to-end and the cavae were individually clamped, the polythene tubes removed and suture anastomosis performed. The animal was then rewarmed to normal body temperature.

This technique has similarities to that used by Demikhov, particularly in the need for early resumption of the circulation to the brain. The importance of the phrenic nerves was again realized and care was taken to preserve them.

The authors pointed out that by keeping the heart and lungs together the problem of the pulmonary circulation was solved, and, in addition, by keeping this circulation intact, the coronary circulation was maintained throughout the procedure. In fact, Neptune and his colleagues, like Demikhov before them, had realized the potential of this heart-lung preparation as a means of retaining cardiac viability during the transfer of the heart from donor to recipient.

Only three attempts to perform this procedure were reported by these authors. The first resulted in poor cardiac action which was maintained for 30 minutes. On the second occasion cardiac action was good, though there were E.C.G. irregularities, and there was a return of pupillary and knee reflexes, but the dog died after four hours as a result of haemorrhage from the posterior suture line of the aorta. In our own experience (Longmore et al., 1969), this is a crucial region, and one that is more likely to bleed if the anastomosis is made hurriedly, as was necessary when hypothermia was the only available means of support of the recipient.

The third dog had normal cardiac action, as evidenced by the E.C.G., and had return of reflexes and spontaneous respiration, an observation of great importance. Normal body temperature was restored and the animal did well for six hours, at which time it died. The cause of death was not fully explained, other than probable shock.

Matejček in 1956 briefly reported a study of the transplantation of the heart and right upper lobe of the lung into the chest; the recipient's heart and lungs (except for the right upper lobe) remained intact and continued to function. The donor heart was placed in position in such a way that its left atrium filled with oxygenated blood from its own left pulmonary vein. The coronary arteries were filled by the action of its own left ventricle. The transplant aorta was anastomosed to the central or distal end of the host brachiocephalic vessel. The donor right heart received desaturated blood through an anastomosis between the donor superior vena cava and the superior vena cava or azygos vein of the host, and emptied through the right pulmonary artery to the attached right upper lobe, and via the left pulmonary artery, which was either anastomosed to the host superior vena cava or donor pulmonary vein. In some cases an interatrial septal defect was created. The bronchus of the donor right upper lobe was anastomosed to that of the recipient after right upper lobectomy. The single lobe was thought to provide sufficient oxygenation for the coronary blood of the transplant; both sides of the donor heart were functioning.

Matejček carried out his procedure in 21 dogs and noted survival up to five days. No details of results were reported.

The first attempts at cardiopulmonary replacement with the animal maintained on mechanical pump-oxygenator support were by Webb and Howard (1957). They used a technique similar to that of Neptune et al., suturing the aortae, but using couples to anastomose the cavae. They differed in causing 'inflow and outflow stasis' of the donor heart, which was then 'perfused' with lactated Ringer's solution to wash all blood from the coronary and capillary beds, thus negating
the value of the heart-lung preparation as a means of maintaining cardiac viability.

Webb and Howard reported six such procedures, and found that it was possible to restore the heart to relatively normal function with acceptable E.C.G. patterns. These animals lived from 75 minutes to 22 hours. The early deaths were caused by continued oozing from the extensive raw surfaces developed during the dissection. In none of these animals was there a return to spontaneous respiration.

The authors also described a second series of experiments in which autotransplantation of the heart and both lungs, in four cases, and of the heart alone, in three cases, was attempted, and a third series in which the heart and one lung were homografted.

In the autotransplant experiments the heart-lung preparation was not perfused but was allowed to perfuse itself. The technique in the four autotransplants of the heart and both lungs was as in the homograft series just described. In two of these the coronaries were occluded by the aortic clamp with resulting fibrillation which could not be corrected, but in the other two, normal cardiac function was obtained for as long as four hours. It was again observed that these animals were unable to breathe spontaneously. Where the heart alone was transplanted there was an immediate return to spontaneous respiration.

In the series of heart and unilateral pulmonary transplants the donor heart and left lung were perfused with lactated Ringer's solution, excised, and then refrigerated at 4°C in Tyrode's solution with 10% serum during preparation of the recipient animal. These organs were exteriorized for two to four hours before their function was restored by suture anastomosis of the aortae and couplings of the cavae and right pulmonary artery and veins. Cardiac action was restored prior to the bronchial anastomosis. To prevent blood flow through the functionless left lung, the left pulmonary artery was clamped until the bronchus was re-anastomosed.

The authors do not state how many such operations they performed but note that satisfactory cardiac function was usually restored in all, though in one case a twisted inferior venae cava caused complete obstruction and proved fatal before it could be corrected. Another died early from oozing. The other dogs recommenced spontaneous breathing as soon as the chest was closed and artificial respiration discontinued.

As they were unable to obtain restoration of normal respiratory function in the presence of total denervation of the lungs, Webb and Howard concluded that transplantation of the heart and both lungs was not practicable, as denervation resulted in respiratory paralysis. They did suggest that while transplantation of the heart with one lung was technically more difficult, the technique might be feasible for use in cases with pulmonary hypertension. Such transplantation circumvented the respiratory paralysis by leaving one innervated lung in the recipient.

In a later study Webb, together with de Guzman and Hoopes (1961), reported a study of the autotransplanted heart. The heart and lungs were completely dissected free from all mediastinal attachments except the trachea, superior and inferior venae cavae, and the aorta. The major vessels were then divided one by one, and rejoined by nylon couples, each procedure being performed quickly enough to allow the entire operation to be accomplished without the use of cardiopulmonary bypass. The trachea was transected at the carina and immediately anastomosed, much care being taken to leave undisturbed the tissues around the proximal trachea. Respiration was maintained throughout this procedure.

Four animals successfully underwent reimplantation of the heart and both lungs. Each was able to return to the maintenance of a normal blood pressure and apparently normal cardiovascular function. In spite of a well-innervated lower tracheal segment, respiratory insufficiency was apparent in all, with none living longer than 14 hours.

Four further dogs were subjected to the preliminary dissection requisite for cardiac transplantation. The phrenic nerves were carefully dissected off the pericardium, and the vagal branches to the heart and lungs were divided. The dissection was continued until only the trachea, aorta, and venae cavae supported the heart and lungs. The trachea was divided immediately proximal to the carina and Anastomosed without interruption of ventilation at any time. As the cough reflex was abolished, tracheostomies were performed for frequent endotracheal aspiration.

None of these dogs was able to maintain the continuing adequate ventilation necessary for permanent survival; the animal surviving longest breathed spontaneously for only 16 hours postoperatively. One dog was able to make only gasps during the post-operative period. The authors came to the conclusion that continuing respiration was dependent on feedback afferents from the respiratory system, and felt that their studies indicated that respiratory paralysis was not due

**Transplantation of the heart and both lungs**
to phrenic nerve damage, excessive vagal or sympathetic dissections, or periods of shock accompanying the extensive dissection and trauma of the actual transplant. Accordingly they concluded that transplantation of the heart combined with both lungs was probably a physiological impossibility.

In 1958 Blanco, Adam, Rodriguez-Perez, and Fernandez reported eight attempts at orthotopic homotransplantation of the heart and lungs, maintaining the recipient on a pump-oxygenator. The surgical technique used was that described by Neptune et al. Two such attempts were terminated during the procedure because of technical difficulties. In the other six, cardiac contractions supported the circulation for one-half to four-and-a-half hours, with an average survival time of only two-and-a-half hours. In two dogs spontaneous respirations appeared and corneal reflexes were active at that time. Death followed a period of weakened cardiac contractions and ventricular fibrillation.

In the late 1950s several groups were attempting transplantation of the heart alone into the orthotopic locus. Notable among these were Golberg, Berman, and Akman (1958), Webb, Howard, and Neely (1959), and Cass and Brock (1959), but in no case was long-term survival achieved. In 1960, however, Lower and Shumway reported the first really significant breakthrough in the technical aspects of cardiac homotransplantation, and since that time they and their co-workers have contributed massively to our knowledge of this subject. They have reported one study of transplantation of the heart together with both lungs (Lower, Stofer, Hurley, and Shumway, 1961).

In 1961 this group reported the resumption of spontaneous respiration in six dogs after complete homograft replacement of the heart and both lungs. The actual number of operations attempted is not mentioned. During preparation of the donor the phrenic and vagus nerves were carefully preserved and the bronchial vessels ligated. The donor organs were immersed in saline solution at approximately 4° C. for 10 minutes and, after being cooled in this manner, were placed in the recipient, and anastomoses of the aorta, trachea, and venae cavae were carried out with fine silk sutures. After rewarming, the animal was electrically defibrillated.

In the six dogs who respired spontaneously post-operatively, it was observed that oxygenation remained adequate but the respiratory pattern was altered in that tidal volume was increased and the respiratory rate diminished. There was an occasional marked prolongation of the expiration phase. Four of the animals died of surgical complications 5-24 hours after the onset of tenuous breathing.

The remaining two animals recovered except for temporary gastro-intestinal ileus, supposedly due to vagus nerve injury during incision and ligation of the bronchial vessels. They were ambulatory, active, and eating until the fourth post-operative day when the arterial oxygen content of the animal was 14-21 vol. % with an oxygen saturation of 18-19 vol. % (78.2 % saturation). The CO₂ content was 48.8 vol. % (method of Slyke and Neill). Both animals died (of respiratory insufficiency) on the fifth post-operative day.

At necropsy the lungs of the dogs were heavy and firm. Microscopically there were considerable atelectasis, but the most striking finding was an extensive infiltration of mononuclear cells which appeared to be lymphocytes. Grossly the heart appeared normal microscopically showing it showed infiltration of the myocardium with mononuclear cells, chiefly plasma-cell variety. These changes of the myocardium were less extensive than the aorta observed previously with cardiac homotransplantation.

Lower and his colleagues felt that they confirmed earlier work by others (Juvenell-Wiles, and Stewart, 1951; Neptune, Redolf Bailey, 1952; Portin, Rasmussen, Stewart, Andersen, 1960), that the bronchial arteries to the lungs could be sacrificed without necrosis, but that the question of the possibility of prolonged survival after pulmonary denervation remained unanswered. It was evident that the sacrifice of peripheral innervation, which was sarly accompanied by pulmonary transplant resulted, in the cases reported, not in respiratory paralysis but in an altered respiratory pattern which resembled that observed after cervical vagotomy. The adequacy of this for normal ventilation after prolonged periods for the maintenance of respiratory equilibrium under various stresses, remained to be determined.

The authors concluded that complete homograft replacement of the heart and lungs was feasible, and that spontaneous respirations altered pattern remained which would suggest a pattern of homograft rejection supervened.

Sen and his colleagues in India have described several techniques of cardiac and coronary transplantation (Sen, Parulkar, D. K. C. Cooper...
and Kinare, 1965). Using the technique originally described by Neptune et al., they carried out five homologous cardiopulmonary transplants, and succeeded in obtaining survivors of 5-12 hours in which the systemic blood pressure was maintained at levels of 80-120 mm Hg. Three of these five animals breathed spontaneously post-operatively; but two required ventilatory support. The causes of death were not discussed by the authors, but haemorrhage from the suture lines was found to be a major problem, as it has been in most reported series.

De Bono, working in England but reporting his studies in a French journal (De Bono, 1966), also used Neptune’s technique. He obtained six survivors in whom spontaneous respiration of an apparently normal pattern occurred for periods of from two to 10 hours, at which times the dogs were sacrificed. Although no conclusion regarding prolonged survival could be drawn from these studies, De Bono felt that adequate spontaneous respiration was certainly possible following heart-lung transplantation, but it was rarely achieved because pulmonary oedema, caused by a number of factors, diminished the ventilatory capacity and compliance of the lungs. Damage to the lymphatic drainage of the lung might also bring about significant changes in lung function. It was suggested that the innervation of the chest wall and trachea might be sufficient to furnish an afferent respiratory stimulus following cardiopulmonary transplantation.

In his paper, De Bono also reported a type of anaphylactoid reaction (immediately following transplantation) which he had observed in a number of cases.

In a study of the preservation of cardiac viability using the heart-lung preparation, Robiček, Lesage, Sanger, Daugherty, Gallucci, and Bagby (1967) performed orthotopic transplantation of the heart and both lungs on 12 occasions, using Demikhov’s original technique. Eight of the 12 recipients lived longer than one day, with a maximal survival time of 37 hours. Better results were obtained with transplants of the heart alone. The authors considered that the main problems of their experiments in which both heart and lungs were grafted were respiratory rather than haemodynamic. The animals maintained satisfactory heart function, but their breathing appeared to be inadequate and had to be assisted by a respirator. Attempts to discontinue artificial respiration were followed by cardiopulmonary arrest.

As can be seen from the foregoing review, the major physiological problem is whether or not the totally denervated lungs will function spontaneously. That the denervated heart will function satisfactorily is a well-established fact, and our own studies (Longmore et al., 1969) confirmed that a technically successful procedure leads to a satisfactorily functioning heart able to maintain an adequate circulation.

A great deal of work has been carried out on transplantation of the lung and the problems of denervation of that organ. Hardy and Alican (1966) have reviewed these subjects thoroughly in a recent paper. It is sufficient to say here that the majority of evidence leads one to conclude that in the dog, denervation or transplantation of one lung only does not affect the spontaneous respiration of the animal, but denervation or transplantation of both lungs (or removal of the second lung) generally leads to cessation of spontaneous respiration within hours, though a number of exceptions to this generalization have been reported. At least some dogs have spontaneously respired adequately for several months following denervation or autotransplantation of one lung and contralateral pneumonectomy. The problem with much of the reported work lies, however, in not knowing fully the extent of what each author terms ‘denervation’. Total removal from the body and reimplantation of the lung is the only means by which one can be sure that full denervation has been carried out.

Nakae, Webb, Theodorides, and Sugg (1967) have recently followed up Webb’s earlier work on heart-lung transplantation, and have made further studies of the effects of denervation of the lungs. They carried out four types of procedure. First, they performed cardiopulmonary autotransplantation by piecemeal division and reanastomosis of the major vessels and trachea, and division of all other tissues connecting the heart and lungs with the surrounding structures. All 10 dogs operated on resumed some type of spontaneous respiration, but with extremely slow respiratory rates and abnormal respiratory patterns. Arterial O₂ saturation and CO₂ tensions rapidly fell and rose respectively, and apnoea occurred within 4 minutes unless the dog was mechanically ventilated. If artificial ventilation was stopped, apnoea again occurred in all cases. One dog died when a ligature slipped off the inferior vena cava, but all other nine died from respiratory failure.

In the second group of experiments mediastinal denervation and tracheal transection were carried out, but the major vessels were not divided. The results were as for autotransplantation. A third group of dogs underwent mediastinal denervation...
without tracheal transection. They all recovered slow, gasping respirations which lasted from 40 minutes to 6 hours, but all ultimately died from respiratory failure.

A final group of dogs underwent only division of the major vessels (superior vena cava, inferior vena cava, and ascending aorta) which were anastomosed by vascular couplings, without pulmonary denervation. They all resumed spontaneous respirations and maintained normal respiratory patterns, surviving for three to 14 days, and dying from occlusion of the couples by thrombosis.

Nakae and his colleagues repeated the second procedure in cats, with the same results, and in monkeys. It is a very important finding that the monkeys resumed a relatively normal pattern of spontaneous respiration and maintained normal blood gases, in contrast to the findings in the dogs. Only one out of six survived for more than 24 hours, but none required respiratory assistance, all deaths resulting from intra-thoracic bleeding or air-leakage.

This work lends some degree of confirmation to the earlier work of Haglin, Telander, Muzzall, Kiser, and Strobel (1963) that total denervation of both lungs does not prevent a return of adequate spontaneous respiration in primates, though it does in dogs.

Although the experimental work in dogs has been disappointing, with few breathing adequately after heart-lung transplantation, much valuable information has been gained from these studies in regard to the technical and physiological problems involved. The encouraging results obtained following denervation of the lungs in primates lead one to hope that man will tolerate this procedure also. Some of the many lessons learned on the dog will then prove of value in overcoming the problems that man will undoubtedly provide.

REFERENCES


(1950b). Experimental homotransplantation of the heart and lungs. Ibid., no. 5.


(1948). Transplantation of the Heart as a New Method is Experimental Biology and Medicine. Medgiz.


Trends in cardiac surgery at the University of Cape Town, 1971 - 1981

M. M. W. PARRY, D. K. C. COOPER, C. N. BARNARD

Summary

A review has been undertaken of the number and types of surgical procedures performed in the Department of Cardiac Surgery of the University of Cape Town during the 11-year period 1971-1981, together with data on associated mortality.

A yearly average of 560 operations was carried out, of which 75% were for acquired and 25% for congenital heart disease. The number of patients treated continued to rise, from 434 in 1971 to 690 in 1981. There has been a slight reduction in the overall average yearly mortality, from 6.1% between 1971 and 1975 to 5.0% between 1976 and 1981.

There was a significant increase in the number of valve replacements in 1975 and 1976 and a more recent, continuing increase in operations for ischaemic heart disease, which now form 19% of the total operations. In both these groups the average yearly mortality has fallen during the period of study. The number of operations each year for congenital heart disease has remained fairly constant, but disappointingly, there has been no significant reduction in mortality.

Clinical material

A total of 6161 cardiac operations were performed during the 11-year period (mean 560 per annum), of which 4618 (75%) were for acquired heart disease and 1543 (25%) for congenital heart disease.

Our unit is the most active cardiac surgical centre in the Cape Province, although both open and closed cardiac surgical procedures are also performed at Tygerberg Hospital in Cape Town and closed procedures at other centres in the Province, such as East London and Port Elizabeth.

The majority of our patients therefore come from the Cape, but a significant percentage hail from elsewhere in the RSA and abroad. The number of patients from outside Africa, however, slowly declining and will continue to do so, for it has become our policy not to accept patients for routine cardiac surgical procedures from outside Africa. The countries of origin of adults and children operated on during 1975 (chosen for the accessibility of accurate records) and 1981 are shown in Table I and the ethnic groups in Table II. Whereas Coloured patients made up 36% of the total in 1975, by 1981 they constituted 44%; this increase was seen in both sexes and in all age groups. Black patients made up only 13% of the total in 1975, and still constituted only 17% by 1981, the major increase in numbers being in women and children. The percentage of White patients fell from 51% to 40% over this period.

The Department of Cardiac Surgery of the University of Cape Town carries out open and closed cardiac surgical procedures on adults at Groote Schuur Hospital and on children at the Red Cross War Memorial Children's Hospital.

Cardiac surgery on a routine basis at the University of Cape Town Medical School began in Groote Schuur Hospital in 1951. The early work of the department, between the years 1951 and 1965, has been reported previously.1,2

We have reviewed the number and types of surgical procedures performed in our department during the 11-year period 1971-1981 inclusive, together with data on the associated mortality. This is not a detailed analysis of the indications for surgery or of the causes of postoperative death, but is intended as an overall view of the changes in workload in cardiac surgery in our institutions, and of the mortality associated with the major operative procedures. It gives an indication of the trends in cardiac surgery in South Africa with regard to the number and types of procedures being undertaken at present and the related mortality figures.

Table I: Countries of origin of patients operated on in 1975 and 1981

<table>
<thead>
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<tbody>
<tr>
<td>RSA and SWA</td>
<td>250 (75%)</td>
<td>152 (79%)</td>
<td>478 (94%)</td>
<td>214 (90%)</td>
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<td>Other African countries</td>
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<td></td>
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<tr>
<td>Angola</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>7</td>
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<tr>
<td>Botswana</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Kenya</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Malawi</td>
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<td>3</td>
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<td>3</td>
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<td>Mozambique</td>
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<td>1</td>
<td>7</td>
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<tr>
<td>Zambia</td>
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<td>3</td>
<td>3</td>
<td>2</td>
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<td>Zimbabwe</td>
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<td>2</td>
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<tr>
<td>Other countries</td>
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<td>Greece</td>
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<td>3</td>
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<td>2</td>
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<td>Turkey</td>
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<td>UK</td>
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<td>USA</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Foreign countries</td>
<td>85 (25%)</td>
<td>40 (21%)</td>
<td>30 (6%)</td>
<td>24 (10%)</td>
</tr>
</tbody>
</table>

GHS = Groote Schuur Hospital; RXH = Red Cross War Memorial Children's Hospital.

Department of Cardiac Surgery, Groote Schuur Hospital and University of Cape Town

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Date received: 30 March 1982


6-year period, despite a considerable increase in the number of men undergoing operative treatment for ischaemic heart disease (Table III).

**Overall workload and mortality**

The total number of patients (including both adults and children) operated on each year during the 11-year period under review varied from 434 in 1971 to a maximum of 690 in 1981 (Fig. 1). The significant rise in the number of operations performed in 1975 and 1976 was related to an increase both in valve replacement and operations for ischaemic heart disease. The fall in the overall number of operations performed during 1980 was entirely related to abnormal local factors, including a temporary closure of the operating theatres at Red Cross Hospital for renovation work over a period of 9 months; this necessitated a greatly reduced number of operations, all performed at Groote Schuur Hospital, on patients of all ages.

Operations for ischaemic heart disease continue to increase steadily, although there has been a slight decline in the number of valve replacements performed during the last 3 years. Operations for congenital heart disease have varied little in number throughout the 11-year period, with a minimum of 185 in 1972 and a maximum of 241 in 1978.

Although the number of operations for valve disease has risen over the 11 years, they have formed a fairly constant proportion of the totals — 45% in 1971 and 39% in 1981. Operations for ischaemic heart disease, on the other hand, provided only 4% of the total in 1971, but had increased to 19% in 1981. The percentage of operations for congenital heart disease showed a decline from 44% in 1971 to 30% in 1981, although the actual annual number of procedures has changed little throughout the period; the fall in percentage can be explained by the increase in operations for ischaemic heart disease and valve disease rather than by any actual decline in the number of operations for congenital heart disease. Other procedures (e.g. aortic aneurysmectomy, pericardiectomy, and heart transplantation) make up only a relatively small number each year, and formed 6% of the total in 1971 and 6% in 1981, with a minimum of 4% (1972) and a maximum of 12% (1976). The number of heart transplants performed each year is shown in Fig. 2.

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**TABLE II. ETHNIC GROUPING AND SEX OF PATIENTS OPERATED ON IN 1975 AND 1981**

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<td>RXH</td>
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<tr>
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</tr>
<tr>
<td>Male</td>
<td>89</td>
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<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Female</td>
<td>83</td>
<td>25</td>
<td>54</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
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<td>51</td>
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<tr>
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<tr>
<td>Female</td>
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<td>18</td>
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<tr>
<td>Total</td>
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<tr>
<td>Male</td>
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<tr>
<td>Female</td>
<td>18</td>
<td>5</td>
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<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>12</td>
<td>30</td>
<td>16</td>
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</table>

**TABLE III. ETHNIC GROUPING AND SEX OF PATIENTS OPERATED ON FOR ISCHAEMIC HEART DISEASE, 1977 - 1981**

<table>
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<tr>
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<tr>
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</tr>
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<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>100</td>
<td>94</td>
<td>100</td>
<td>91</td>
</tr>
</tbody>
</table>

*No Black patients underwent coronary artery bypass grafting.

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**Fig. 1. Total number of cardiac operations performed, 1971-1981, and related hospital mortality.** The numbers of operations for valvar, ischaemic and congenital heart disease are also shown.
Surgery for valve disease

A more detailed analysis of operations for valve disease in adults is shown in Fig. 3. The total number of open-heart procedures rose markedly during the years 1975 (189) to 1978 (284). The fall during 1980 was due to the local factors already discussed. This increase involved procedures on the mitral valve, the aortic valve, and two or more valves; the actual number of prosthetic valves inserted increased by a similar percentage.

Although it remains the policy of our unit to perform closed mitral valvotomy whenever this is deemed suitable on clinical grounds, there has been a fall in the number of these operations carried out in recent years. A maximum of 59 closed mitral valvotomies in 1972 fell to a minimum of 7 in 1978. This may reflect more stringent criteria for selection for closed valvotomy, or more confidence on the part of the surgeon in obtaining a better result by an open procedure. During the first 5 years of this study, the yearly average was 47 valvotomies, which represented 20.5% of the total number of valve operations; during the second 6-year period it had fallen to 24 (10.2%).

Rheumatic valve disease remains a major problem in the Cape Province, particularly in the lower socio-economic classes. The increased number of cases is due to a continuing referral of new patients together with a steady return of patients requiring replacement of previously implanted and now malfunctioning prostheses. Malfunction of bioprostheses (e.g. porcine xenografts) results largely from calcification, leading to stenosis or regurgitation; with mechanical prostheses, malfunction is usually related to thrombosis which may be accompanied by embolism.

There has been a substantial reduction in the hospital mortality associated with valve surgery, from a maximum of 8.3% in 1972 to 2.2% in 1980. During the period 1971-1975 the average yearly mortality was 7.6%; between 1976 and 1981 it had fallen to 4.7%. This reduction in mortality is probably related in part to improved methods of myocardial protection during valve insertion, namely the use of cardioplegia.

The mortality associated with closed mitral valvotomy remains low; there were only 4 deaths in 380 cases (1.1%) during the 11-year period, only 1 of these since 1973.

Surgery for ischaemic heart disease

There has been a dramatic increase in operations for ischaemic heart disease, beginning in 1975 (Fig. 4). Before 1975 few procedures for ischaemic heart disease were performed, these consisting mainly of the Vineberg operation and left ventricular aneurysmectomy. Vineberg operations are no longer performed and the incidence of aneurysmectomy remains constant at between 1 and 6 per year. The great increase has been due entirely to the advent of coronary artery bypass grafting using the saphenous vein, and the increase continues. In the Cape ischaemic heart disease affects mainly the White male population, but is being seen increasingly in the White female and Coloured male groups (Table III).

The mortality associated with these procedures has shown a marked fall; coronary artery bypass grafting, in particular, is associated with a low mortality. The higher mortality in the first 5 years of this study (mean yearly mortality 7.3%) was partly associated with the type of operation performed, but was greatly influenced by the small number of such procedures; one death greatly increases the percentage mortality. The average yearly
mortality during 1976-1981 was 3.5%; this included patients undergoing myocardial revascularization with and without aneurysmectomy and patients with poor left ventricular function from previous myocardial infarction. Myocardial revascularization is undoubtedly the 'growth area' of cardiac surgery at the present time.

**Surgery for congenital heart disease**

The total number of operations (both open and closed) for congenital heart disease per year has remained relatively constant (Fig. 5), averaging 205 per year between 1971 and 1975 and 216 per year between 1976 and 1981. The numbers of operations on adults and infants (children under the age of 1 year) have remained basically constant, although there has been a slight increase in the number of operations performed on children under the age of 15 years.

Simple congenital lesions were the first cardiac disorders amenable to correction by surgery; the absence of a significant increase in the number of operations for congenital heart disease over the past 11-year period would suggest that presentation of such patients in the Cape is static. There is almost certainly a large pool of untreated patients in many African countries, but as yet these patients are not being referred to South Africa in large numbers.

The proportion of closed to open surgical procedures has remained fairly constant. The percentage of open procedures per year varied in adults from 69% to 91%, in children (under 15 years) from 61% to 90%, and in infants from 46% to 75%. No obvious trend towards performing more or fewer open procedures in each age group is noticeable. An increase in the number of open-heart procedures performed in infancy and a coincident fall in closed palliative procedures might have been expected, as there has been much debate in recent years among surgeons about performing corrective operations at an early age in certain specified conditions; no significant trend has been reflected in our figures.

Mortality in the various age groups has fluctuated widely (Fig. 6). The numbers of patients in the adult and particularly in the infant groups are relatively small, and therefore one death can affect the percentage mortality markedly. There would, however, appear to be a slight increase in mortality in the later part of the 11-year period in the under-15 age group; the reason for this remains obscure. This group includes infants, but mortality in the infant group would not appear to have changed significantly, the annual mortality rate of 25% being identical between 1971 and 1975 and 1976 and 1981.

Cardiac surgery, whether palliative or corrective, is only performed in infancy in life-threatening conditions; both open and closed operations carry a high mortality. Closed operations were associated with an average yearly mortality of 26% in the first 5 years of this study and 20% during the next 6 years, although the number of operations involved was very small (average 10 per year). There was, however, a marked rise in the number of closed procedures in infants to 26 during 1981 with an associated mortality of 11%; the reasons for this significant increase in the number of closed operations in this age group remain uncertain, but would appear to be related to greater referral of Black infants from rural areas. Open operations carried a mortality of 26% during both the periods 1971 - 1975 and 1976 - 1981.

The lack of improvement in the mortality rate among patients of all ages undergoing surgery for congenital heart disease is disappointing, particularly as it does not reflect a more ambitious approach on the part of surgeons attempting correction of complex conditions previously deemed inoperable (Fig. 7); there has, in fact, been a slight fall in the number of complex forms of congenital heart disease operated on during the period of study.
Surgery in children

The majority of cardiac surgical procedures in children (under the age of 15 years) involved correction or palliation of congenital lesions, operations for valve disease forming only 7-15% of the yearly total (Fig. 7).

A small increase in the total number of operations performed on children, from an annual average of 173 between 1971 and 1975 to 194 between 1976 and 1981, was largely due to an increase in operations for 'simple' congenital conditions such as atrial and ventricular septal defects, patent ductus arteriosus, coarctation, and pulmonary and aortic valve stenosis. The number of operations for Fallot's tetralogy and transposition of the great arteries remained fairly constant, and procedures for the more 'complex' congenital lesions, such as total anomalous pulmonary venous drainage, complete atioventricular canal, single ventricle, pulmonary and aortic valvular disease, remain a major cause of death despite improved and pulmonary and aortic valvular disease. The general state of the patient at the time of operation is also an important factor in postoperative morbidity and mortality; emergency procedures on moribund patients obviously carry a very high risk.

Myocardial infarction in both valve and ischaemic heart disease patients, thromboembolism following valve replacement, and respiratory conditions such as adult respiratory distress syndrome and pulmonary infection remain significant causes of mortality. Complications of operative technique or judgement (e.g. failure to relieve pulmonary outflow tract obstruction in Fallot's tetralogy, thrombosis of pulmonary-systemic shunts in infants and children) have become relatively infrequent.

The authors acknowledge and thank the many members of the medical, nursing and laboratory staff who have cared for these cardiac surgical patients. They also thank Sister Elma Steensma and Miss Jenny Boisman, who prepared the figures.

REFERENCES


Causes of hospital mortality

Causes of operative and postoperative death have not been analysed in detail. There would, however, appear to be no significant change in factors relating to mortality throughout the 11-year period.

Postoperative myocardial failure in patients with advanced valve disease and in children with complex and congenital lesions remains a major cause of death despite improved methods of peroperative myocardial protection and postoperative circulatory support. This problem is most commonly related to patients who have presented themselves very late for consideration of surgical treatment; myocardial function is frequently extremely poor as a result of long-standing, unrelieved valve disease. The general state of the patient at the time of operation is also an important factor in postoperative morbidity and mortality; emergency procedures on moribund patients obviously carry a very high risk.

Myocardial infarction in both valve and ischaemic heart disease patients, thromboembolism following valve replacement, and respiratory conditions such as adult respiratory distress syndrome and pulmonary infection remain significant causes of mortality. Complications of operative technique or judgement (e.g. failure to relieve pulmonary outflow tract obstruction in Fallot's tetralogy, thrombosis of pulmonary-systemic shunts in infants and children) have become relatively infrequent.

The authors acknowledge and thank the many members of the medical, nursing and laboratory staff who have cared for these cardiac surgical patients. They also thank Sister Elma Steensma and Miss Jenny Boisman, who prepared the figures.

REFERENCES


Epidural and intramuscular pethidine — a pharmacokinetic study

K. A. PAYNE

Summary

Epidural preservative-free pethidine hydrochloride 0.75 mg/kg is rapidly absorbed into the blood. At 1.5 mg/kg the plasma levels reached are similar to those achieved by intramuscular preservative-free pethidine hydrochloride, as is the time course. Plasma levels fall more rapidly after epidural pethidine. Since the plasma levels lag behind the analgesic effects, they are unlikely to be of importance as regards clinical analgesia.

SA MEDIESE TYDSKRIF DEEL 63 5 FEBRUARIE 1983 193

Department of Anaesthetics, Tygerberg Hospital, Parowvallei, CP


Date received: 10 April 1982.

Reprints requests to: Dr K. A. Payne, 16 Elizabeth Avenue, Pinelands. 7405 RSA.

Epidural opiates such as pethidine have been shown to provide effective postoperative analgesia, their site of action being the substantia gelatinsa of the spinal cord. The epidural space contains many lymphatics and venous plexuses, and systemic absorption can therefore be rapid; as doses of pethidine equal to intramuscular doses have been used epidurally, blood levels may play a part in their action.

Serial measurements of CSF pethidine levels in patients undergoing routine operations pose ethical problems, but plasma samples are easily and painlessly taken. It was therefore decided to compare plasma levels of pethidine after epidural injection of 0.75 mg/kg and intramuscular injection of 1.5 mg/kg. These were the doses used in a previous study showing that epidural pethidine provided superior quality analgesia but was not associated with an increase in the incidence of side-effects. This study would indicate to what extent absorption and transport via the blood influenced the actions of epidural pethidine.

Methods

Consent of the hospital ethical committee was obtained, and the patients gave written informed consent. They were all fit, non-
Changes in indications for heart transplantation

An additional argument for the preservation of the recipient's own heart

The 1 year survival rate after heart transplantation has improved since 1967 from ±30% to ±70%, and the 5 year survival rate is now ±50%. This improvement has brought renewed interest in this procedure, now done in about twenty centers in eight countries, and increased confidence has widened the indication to patients who are less than terminally ill, to restore quality of life. This trend is illustrated by the Cape Town series, which can be divided into two parts: 10 patients treated by orthotopic heart transplantation (OHT); from 1967 to 1973, and 40 patients treated by heterotopic heart transplantation (HHT), from 1974 to 1981. The HHT group was younger (mean 37 ± 10 years versus 51 ± 9 years, p < 0.001), had been ill for a shorter length of time (mean 3.6 ± 0.7 years versus 6.6 ± 1.4 years, p < 0.001), and were in a lower New York Heart Association (NYHA) class (mean 3.43 ± 0.11 versus 3.9 ± 1.0, p < 0.005). The improved survival is linked to patient selection, progress in management, and switch to HHT, but not to progress in matching between donor and recipient. Since there is no means to predict tolerance of the donor heart, HHT limits the risks from unforeseeable mismatch. The recipient's heart is a built-in assist device, maintaining life when the donor heart fails acutely at operation or during acute [three cases] or chronic [two cases] rejection. Had these patients undergone OHT they would have died. Comparing the 10 oldest HHT patients with the OHT series, no difference in pretransplant parameters was found. However, survival of HHT recipients was longer during the critical post-HHT period: at 3 months, p < 0.011; at 6 months, p < 0.05. Larger series will separate the effects of progress in management from the intrinsic advantages of HHT. Retaining the recipient's heart is logical and has brought few complications. Survival rate of 40 HHT patients was 73% at 6, 65% at 12, and 51% at 36 months; 85% of survivors are in NYHA Class 1. In patients in less than desperate condition, but who refuse to remain cripples, HHT eliminates the growing ethical problem of removing a recipient's heart that may still support the patient.

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The great improvement in survival and rehabilitation of heart transplantation recipients has improved the image of this procedure and widened its indication. This is no longer a merely experimental operation aimed at prolongation of life at any cost; it is a rational therapy that returns a cardiac cripple to normalcy. Nevertheless, some controversy remains about the availability of donors and finances,1,2 and some discrepancy is required as to who should be accepted about progressively from greater selectivity of recipients, more finesse in management, and improved di-

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agnosis and monitoring of acute rejection brought about by transvenous endomyocardial biopsy.9-11

The impact of any one factor is difficult to assess, but the magnitude of the changes in selection criteria can be evaluated by comparing patients operated upon in Cape Town or Stanford during the early years (1967 to 1973) with those operated upon more recently. A simple look at the ages (Fig. 2) and other pertinent information (Fig. 3) of these populations illustrates that the indication for heart transplantation now defines a population of patients who are younger, have had a shorter illness, and are in better general condition, but who nevertheless are in terminal cardiac failure with a limited life expectancy. Strikingly, the number of patients with car-
Fig. 3. Comparison of preoperative parameters between the orthotopic and the heterotopic heart transplant series in Cape Town. NYHA, New York Heart Association. (Probabilities calculated by Fisher’s randomization test: analysis of 22 cases.)

Table I. The orthotopic heart transplantation series, 1967-1973, in Cape Town

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yr), sex</th>
<th>Diagnosis</th>
<th>Date of TX</th>
<th>Survival time</th>
<th>NYHA Class (postop.)</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54, M</td>
<td>IHD</td>
<td>12/2/67</td>
<td>19 days</td>
<td>—</td>
<td>Infection</td>
</tr>
<tr>
<td>2</td>
<td>59, M</td>
<td>IHD</td>
<td>1/2/68</td>
<td>426 days</td>
<td>1</td>
<td>Chronic rejection</td>
</tr>
<tr>
<td>3</td>
<td>53, M</td>
<td>RHD</td>
<td>9/7/68</td>
<td>623 days</td>
<td>1</td>
<td>Neoplasm</td>
</tr>
<tr>
<td>4</td>
<td>63, M</td>
<td>AVD</td>
<td>4/11/69</td>
<td>65 days</td>
<td>—</td>
<td>Infection</td>
</tr>
<tr>
<td>5</td>
<td>36, F</td>
<td>RHD</td>
<td>4/17/69</td>
<td>12 yr</td>
<td>1</td>
<td>Chronic rejection</td>
</tr>
<tr>
<td>6</td>
<td>44, M</td>
<td>IHD</td>
<td>5/10/71</td>
<td>10 yr</td>
<td>1</td>
<td>Alive</td>
</tr>
<tr>
<td>7</td>
<td>62, M</td>
<td>CM</td>
<td>11/13/71</td>
<td>12 days</td>
<td>—</td>
<td>Pulmonary embolism</td>
</tr>
<tr>
<td>8</td>
<td>41, M</td>
<td>IHD</td>
<td>1/23/72</td>
<td>61 days</td>
<td>1</td>
<td>Acute graft failure</td>
</tr>
<tr>
<td>9</td>
<td>52, M</td>
<td>IHD</td>
<td>8/15/72</td>
<td>1 day</td>
<td>—</td>
<td>Right heart failure</td>
</tr>
<tr>
<td>10</td>
<td>46, M</td>
<td>IHD</td>
<td>12/2/73</td>
<td>13 days</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

*Patient had pulmonary embolism before transplantation.
†Patient had severe pulmonary hypertension, cardiac cachexia, and ascites.

Diastolic dysfunction has paralleled these changes, rising from 10% (Cape Town series 1967 to 1973) and 19% (Stanford series 1968 to 1971) to well over 40% of all patients undergoing transplantation (Stanford series 1972 to 1981: 49%; Cape Town series 1974 to 1981: 42%).

Despite these changes in selection criteria and major progress in transplantation immunology, it remains impossible to predict the outcome of a heart transplantation and to compare it on an individual basis to the natural history of a patient’s condition. Although the retrospective statistics of a population of recipients may improve year after year, they cannot answer all the concerns of the potential candidate about his own future and balance his present degree of disability with the risks of going through a heart transplantation.

Heterotopic heart transplantation (HHT), initially developed to overcome the threat of fatal donor right ventricular failure following orthotopic heart transplantation (OHT) in patients with excessively high pulmonary vascular resistance, offers some solution to the doubts of when heart transplantation may be indicated. By retaining the patient’s own heart intact, HHT reduces the ethical constraints that still prevent heart transplantation from being offered to a larger group of people incapacitated by myocardial destruction. A brief review of the Cape Town experience with 51 heart
transplantations from 1967 until 1981 will illustrate these changes in indication and the advantages of HHT over OHT.

OHT

The first man-to-man heart transplantation was performed in December, 1967, at the Groote Schuur Hospital in Cape Town, and over the next 6 years a total of 10 patients underwent this procedure. There were nine men and one woman, the mean age was 51 ± 9 years, and all patients were in Class IV of the New York Heart Association classification with massive heart failure and only a few weeks or months to live (Table I). The selection criteria mainly relate to the desperate condition of the patient and the inability of conventional medical or surgical treatment to offer any relief. However, the experimental nature of the procedure prevented application of this radical treatment to any patient who still had a chance to survive months or years. On the basis of current selection criteria that were established through the extensive experience of the Stanford group, most of these patients would not be accepted today as candidates for heart transplantation. Six of the 10 were beyond 50 years of age, two beyond 60, one had a recent episode of pulmonary embolism and four had pulmonary resistance levels higher than 8 Wood units. Only one of these patients would fulfill today's criteria for heart transplantation, and she survived 12½ years. Five patients (50%) were discharged from the hospital in NYHA Class I with excellent hemodynamic improvement (Fig. 4), and four patients survived beyond 1 year. From the nine "unsuitable candidates," 33% survived more than 1 year and one of them is alive and asymptomatic 11 years after transplantation. Both long-term survivors were under 45 years of age at the time of the procedure.

HHT

Forty-four HHTs were performed in 40 patients from November, 1974, to December, 1981. The indications were terminal heart failure from ischemic heart disease (53%), from cardiomyopathy (42%), and from rheumatic heart disease (5%). There were 35 men and five women, their mean age being 37 ± 10 years. Overall survival rates with a functioning graft since 1974 were 63.2%, 54.5%, 41.5%, and 38% at 6 months, 1, 2, and 5 years, respectively (Table II).

Hemodynamic improvement was excellent; over 85% of all HHT patients were discharged from the hospital in NYHA Functional Class I. Acute rejection episodes were more frequent and severe during the first 3 postoperative months and became rare beyond the first year. Infection was the most common cause of death (42%), and lethal episodes could be related to a recent instance of acute rejection that had necessitated high doses of immunosuppressive drugs. Infection unrelated to rejection was usually controllable by medical means. Chronic rejection was responsible for 28% of the deaths (Table III). Three patients died of acute rejection and related complications. Retransplantation was performed in four cases, with no operative deaths and satisfactory early results. However, two patients died of infection in the months after operation. In a third case, the patient's system acutely rejected the second graft, and this patient appears to be unsuitable for further allografting because of sensitization to transplantation antigens. The fourth patient is alive and asymptomatic 8 months after retransplantation.

Comparison of OHT and HHT

Comparing the Cape Town patient population receiving OHT and HHT reveals obvious changes, with sig-
difficult to compare because of series heterogeneity and heterotopic well-tolerated allografting in the small number of cases. However, the restoration of normal hemodynamics was equally the rule following reduced cardiac indices and ejection fractions. The extent of heart failure was similar. The operative and immediate postoperative morbidity and mortality are significant differences for age, duration of pretransplant illness, and functional capacity (Fig. 3). Preoperative hemodynamic studies demonstrated that HHT patients had a lesser degree of right heart disease (Fig. 5) than OHT patients, probably reflecting the more recent onset of their cardiopathy, but all had similarly elevated left ventricular end-diastolic pressures and severely reduced cardiac indexes and ejection fractions. The extent of heart failure was similar. The operative and immediate postoperative morbidity and mortality are difficult to compare because of series heterogeneity and the small number of cases. However, the restoration of normal hemodynamics was equally the rule following well-tolerated allografting in the orthotopic or heterotopic position (Figs. 4 and 6).

The increased survival (in the number of patients and length of survival) after HHT, when compared to the Cape Town OHT series, is similar to that observed during the same period at Stanford. The increase in survival of the HHT recipients was most significant at 3 months (p < 0.005), at 6 months (p < 0.001), and at 1 year (p < 0.05). The potential advantages of HHT are not manifest from major differences between the OHT and HHT series although they have been illustrated by specific cases. The advantages will be demonstrated only when large series of both procedures have been performed in centers with similar selection policies and the results have become available for statistical analysis. One potential advantage of HHT is that the procedure is

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Table II. The heterotopic heart transplantation series, 1974-1981, in Cape Town

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yr), sex</th>
<th>Diagnosis</th>
<th>Date of TX</th>
<th>Survival time</th>
<th>NYHA Class (postop.)</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59, M</td>
<td>IHd</td>
<td>12/25/74</td>
<td>111 days</td>
<td>—</td>
<td>Infection + CVA</td>
</tr>
<tr>
<td>2</td>
<td>46, M</td>
<td>RHD</td>
<td>12/31/74</td>
<td>7 yr.</td>
<td>1</td>
<td>Alive</td>
</tr>
<tr>
<td>3</td>
<td>39, M</td>
<td>IHd</td>
<td>5/4/75</td>
<td>120 days</td>
<td>—</td>
<td>Infection - CMV</td>
</tr>
<tr>
<td>4</td>
<td>31, M</td>
<td>IHd</td>
<td>12/29/75</td>
<td>6 yr.</td>
<td>1</td>
<td>Alive</td>
</tr>
<tr>
<td>5</td>
<td>25, M</td>
<td>CM</td>
<td>1/3/76</td>
<td>122 days</td>
<td>—</td>
<td>Infection - CMV</td>
</tr>
<tr>
<td>6</td>
<td>45, M</td>
<td>IHd</td>
<td>1/31/76</td>
<td>6 yr.</td>
<td>1</td>
<td>Alive</td>
</tr>
<tr>
<td>7</td>
<td>46, M</td>
<td>IHd</td>
<td>5/8/76</td>
<td>117 days</td>
<td>—</td>
<td>Infection - CMV</td>
</tr>
<tr>
<td>8</td>
<td>24, M</td>
<td>CM</td>
<td>10/4/76</td>
<td>74 days</td>
<td>—</td>
<td>Infection - CMV</td>
</tr>
<tr>
<td>9</td>
<td>34, M</td>
<td>IHd</td>
<td>3/31/77</td>
<td>56 days</td>
<td>1</td>
<td>Chronic rejection</td>
</tr>
<tr>
<td>10</td>
<td>34, M</td>
<td>IHd</td>
<td>4/19/77</td>
<td>3 yr.</td>
<td>1</td>
<td>Chronic rejection</td>
</tr>
<tr>
<td>11</td>
<td>42, M</td>
<td>IHd</td>
<td>5/29/77</td>
<td>96 days</td>
<td>1</td>
<td>Chronic rejection</td>
</tr>
<tr>
<td>12</td>
<td>42, M</td>
<td>IHd</td>
<td>6/7/77</td>
<td>4 yr.</td>
<td>1</td>
<td>Chronic rejection</td>
</tr>
<tr>
<td>13</td>
<td>37, M</td>
<td>IHd</td>
<td>8/5/77</td>
<td>988 days</td>
<td>1</td>
<td>Chronic rejection</td>
</tr>
<tr>
<td>14</td>
<td>23, M</td>
<td>CM</td>
<td>8/25/77</td>
<td>268 days</td>
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</tr>
<tr>
<td>15</td>
<td>23, M</td>
<td>CM</td>
<td>9/4/77</td>
<td>3 yr.</td>
<td>1</td>
<td>Acute rejection</td>
</tr>
<tr>
<td>16</td>
<td>40, M</td>
<td>IHd</td>
<td>9/12/77</td>
<td>21 days</td>
<td>—</td>
<td>Suicide</td>
</tr>
<tr>
<td>17</td>
<td>34, F</td>
<td>CM</td>
<td>10/2/77</td>
<td>69 days</td>
<td>—</td>
<td>Acute rejection</td>
</tr>
<tr>
<td>18</td>
<td>46, M</td>
<td>IHd</td>
<td>12/24/77</td>
<td>403 days</td>
<td>1</td>
<td>Chronic rejection</td>
</tr>
<tr>
<td>19</td>
<td>44, M</td>
<td>IHd</td>
<td>1/11/78</td>
<td>594 days</td>
<td>1</td>
<td>Chronic rejection</td>
</tr>
<tr>
<td>20</td>
<td>44, M</td>
<td>IHd</td>
<td>7/7/78</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>


*This patient's system acutely rejected the allograft 3 months after transplantation. His own heart had significantly improved. Three and one-half years later, again in severe heart failure, he died refusing retransplantation.

†Because of acute rejection of the allograft, these patients underwent retransplantation. In Patient 22 the second graft was rejected 8 days after the operation, and he survived on his own heart.

‡This patient died during the early postoperative period of an accidental rapid potassium infusion.

††This patient was in NYHA Class I but complaining of severe sexual impotence.

‡‡This patient survived chronic rejection of the allograft for approximately 4 months.

¥This patient survived chronic rejection of the allograft for approximately 13 weeks.
less radical, less of a last, no-return step. In the present state of our knowledge about histocompatibility matching in cardiac allografting and because of the limited donor availability, the choice of a given organ for a given recipient cannot be determined on much hard data that are predictive of a successful heart transplantation. It is only a posteriori, after the recipient has survived the first 6 or 12 months uneventfully, that it becomes clear that a good match and suitable candidate were selected.

HHT, which preserves the recipient’s own heart as a built-in assist device, is logical and reduces the stress of having one’s heart removed (patient’s point of view) and of removing the diseased heart (surgeon’s point of view). A second significant benefit of HHT is encountered at the time of operation, when the donor heart fails. Acute failure may result from damage sustained during the accident or death of the donor. The recipient’s heart will then offer some support during recovery if the damage is reversible or will maintain life until another graft can be transplanted. Operative deaths resulting from failure of the donor heart have been re-

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yr.)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Date of IX</th>
<th>Survival time</th>
<th>NYHA Class (postop.)</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>214</td>
<td>24, F</td>
<td></td>
<td>CM</td>
<td>10/16/78</td>
<td>2 days</td>
<td>-</td>
<td>Iatrogenic</td>
</tr>
<tr>
<td>221</td>
<td>25, M</td>
<td></td>
<td>CM</td>
<td>2/16/79</td>
<td>3 yr.</td>
<td>IV</td>
<td>Alive</td>
</tr>
<tr>
<td>232</td>
<td>45, M</td>
<td></td>
<td>JHD</td>
<td>3/7/79</td>
<td>54 days</td>
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<td>Alive</td>
</tr>
<tr>
<td>24</td>
<td>36, M</td>
<td></td>
<td>JHD</td>
<td>9/16/79</td>
<td>962 days</td>
<td>1</td>
<td>Alive</td>
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<tr>
<td>251</td>
<td>34, M</td>
<td></td>
<td>CM</td>
<td>11/11/75</td>
<td>561 days</td>
<td>1</td>
<td>CHA</td>
</tr>
<tr>
<td>26</td>
<td>29, F</td>
<td></td>
<td>CM</td>
<td>11/18/79</td>
<td>720 days</td>
<td>1</td>
<td>CHA</td>
</tr>
<tr>
<td>27</td>
<td>38, M</td>
<td></td>
<td>CM</td>
<td>11/29/79</td>
<td>234 days</td>
<td>1</td>
<td>CHA</td>
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<tr>
<td>281</td>
<td>52, M</td>
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<td>JHD</td>
<td>12/23/79</td>
<td>417 days</td>
<td>1-IV</td>
<td>Chronic rejection</td>
</tr>
<tr>
<td>29</td>
<td>14, M</td>
<td></td>
<td>CM</td>
<td>11/5/80</td>
<td>833 days</td>
<td>1</td>
<td>Alive</td>
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<tr>
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<td>47, F</td>
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<td>IHD</td>
<td>11/17/80</td>
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<td>Infection</td>
</tr>
<tr>
<td>201</td>
<td>46, M</td>
<td></td>
<td>AR</td>
<td>1/27/80</td>
<td>24 days</td>
<td>1</td>
<td>Infection</td>
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<tr>
<td>31</td>
<td>34, M</td>
<td></td>
<td>IHD</td>
<td>9/26/80</td>
<td>526 days</td>
<td>1</td>
<td>Infection</td>
</tr>
<tr>
<td>321</td>
<td>45, M</td>
<td></td>
<td>IHD</td>
<td>10/24/80</td>
<td>36 days</td>
<td>1</td>
<td>Infection</td>
</tr>
<tr>
<td>33</td>
<td>41, M</td>
<td></td>
<td>CM</td>
<td>11/2/80</td>
<td>28 days</td>
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<td>Pancreatitis</td>
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<tr>
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<td></td>
<td>IHD</td>
<td>11/9/80</td>
<td>225 days</td>
<td>1</td>
<td>Neoptasia</td>
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<tr>
<td>322</td>
<td>31, M</td>
<td></td>
<td>AR</td>
<td>11/29/80</td>
<td>93 days</td>
<td>1</td>
<td>Infection</td>
</tr>
<tr>
<td>35</td>
<td>51, M</td>
<td></td>
<td>CM</td>
<td>12/2/80</td>
<td>416 days</td>
<td>1</td>
<td>Infection</td>
</tr>
<tr>
<td>251</td>
<td>36, M</td>
<td></td>
<td>AR</td>
<td>5/26/81</td>
<td>901 days</td>
<td>1</td>
<td>Alive</td>
</tr>
<tr>
<td>36</td>
<td>34, M</td>
<td></td>
<td>CM</td>
<td>6/13/81</td>
<td>35 days</td>
<td>1</td>
<td>Infection</td>
</tr>
<tr>
<td>37</td>
<td>14, M</td>
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<td>CM</td>
<td>7/31/81</td>
<td>274 days</td>
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<td>Alive</td>
</tr>
<tr>
<td>38</td>
<td>32, M</td>
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<td>CM</td>
<td>9/16/81</td>
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<td>Chronic rejection</td>
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<td>39</td>
<td>49, M</td>
<td></td>
<td>CM</td>
<td>10/11/81</td>
<td>211 days</td>
<td>1</td>
<td>Chronic rejection</td>
</tr>
<tr>
<td>221</td>
<td>28, M</td>
<td></td>
<td>AR</td>
<td>10/4/81</td>
<td>3 yr.</td>
<td>1</td>
<td>Alive</td>
</tr>
<tr>
<td>40</td>
<td>39, F</td>
<td></td>
<td>CM</td>
<td>10/29/81</td>
<td>183 days</td>
<td>1</td>
<td>Alive</td>
</tr>
</tbody>
</table>

Table II. Heterotopic heart transplantation: Causes of death

<table>
<thead>
<tr>
<th>Causes of death</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections</td>
<td>12</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>3</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>1</td>
</tr>
<tr>
<td>Escherichia coli</td>
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<td>Pseudomonas aeruginosa</td>
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<tr>
<td>Salmonella B</td>
<td>1</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>1</td>
</tr>
<tr>
<td>Aspergillus aliger</td>
<td>1</td>
</tr>
<tr>
<td>Pneumocystis jirovecii</td>
<td>1</td>
</tr>
<tr>
<td>Cytomegalic virus</td>
<td>1</td>
</tr>
<tr>
<td>Hepes virus</td>
<td>1</td>
</tr>
<tr>
<td>Acute rejection</td>
<td>3</td>
</tr>
<tr>
<td>Chronic rejection</td>
<td>8</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>5</td>
</tr>
<tr>
<td>Suicide</td>
<td>1</td>
</tr>
<tr>
<td>Cerebrovascular accident</td>
<td>1</td>
</tr>
<tr>
<td>Acute pancreatitis</td>
<td>1</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>1</td>
</tr>
<tr>
<td>Iatrogenic accident</td>
<td>1</td>
</tr>
</tbody>
</table>

Total | 28 |

Table III. Heterotopic heart transplantation: Causes of death

<table>
<thead>
<tr>
<th>Causes of death</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections</td>
<td>12</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>3</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>1</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>8</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>5</td>
</tr>
<tr>
<td>Salmonella B</td>
<td>1</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>1</td>
</tr>
<tr>
<td>Aspergillus aliger</td>
<td>1</td>
</tr>
<tr>
<td>Pneumocystis jirovecii</td>
<td>1</td>
</tr>
<tr>
<td>Cytomegalic virus</td>
<td>1</td>
</tr>
<tr>
<td>Hepes virus</td>
<td>1</td>
</tr>
<tr>
<td>Acute rejection</td>
<td>3</td>
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<tr>
<td>Chronic rejection</td>
<td>8</td>
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<tr>
<td>Miscellaneous</td>
<td>5</td>
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<tr>
<td>Suicide</td>
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<tr>
<td>Cerebrovascular accident</td>
<td>1</td>
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<td>Acute pancreatitis</td>
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<tr>
<td>Pulmonary embolism</td>
<td>1</td>
</tr>
<tr>
<td>Iatrogenic accident</td>
<td>1</td>
</tr>
</tbody>
</table>

Total | 28 |
corded, and the frequency of such events may increase with long-distance transportation of isolated organs. If hyperacute rejection occurs, the recipient’s heart again will be life-saving. Further, in the immediate postoperative period the modest contribution of the recipient’s heart has significantly reduced the requirements for assisted ventilation, intravenous inotropic agents, and cardiac pacing as compared to the previous OHT series. In a like manner, during acute rejection that may be life threatening and cause arrhythmias and failure, the recipient’s heart will be supportive. Fewer instances of heart failure were observed during episodes of acute rejection in HHT recipients than in OHT recipients. The management of acute rejection becomes less urgent; diagnostic tests yielding questionable results can be repeated, and treatment can be given more progressively. This advantage was illustrated by the history of the fifth HHT recipient of the Cape Town series, who survived acute rejection 8 months after transplantation (an episode of donor heart fibrillation), with eventual full recovery. This patient is presently asymptomatic 6 years later.

Increased long-term survival has brought to the forefront the complications related to chronic rejection—disease of the graft’s coronary arteries probably produced by a humor-mediated immune response. These coronary stenoses may cause myocardial infarction with lethal arrhythmias or heart failure. The human allograft remaining denervated, the disease progresses symptomlessly, the infarction is silent, and sudden death has been reported in patients with OHT. However, the recipient’s heart, if functioning, will once more maintain life and make retransplantation a matter of lesser urgency. In the Cape Town HHT series there has not been any instance of sudden death. To prevent the complications of chronic rejection, the Stanford group advocates yearly coronary angiographies to monitor the progress of the allograft’s coronary artery disease: they suggest retransplantation if a 70% or greater lesion is found in one of the major coronary arteries. This policy is logical but drastic, particularly because retransplantation yields a significantly lower survival rate than the first transplantation and therefore may be questionable. On a routine evaluation in 1976, a long-term survivor of the Cape Town OHT series (Case 5), was found to have significant lesions in all three coronary artery systems, but she survived an additional 5 years asymptptomatically.

The preservation of the recipient’s own diseased heart has occasionally been responsible for morbidity (11.3%) and possibly for one death (2.2%). The dilated heart of the recipient may contain thrombus in the ventricles, produced by low output, blood stagnation, and previous infarction. This common postmortem finding...
in patients dying of severe heart failure can be the source of systemic embolism. To prevent this complication, all HHT patients are permanently given anticoagulants. Despite this precaution, three patients experienced an episode of cerebral embolism, fortunately leaving only minor residual unilateral weakness in two but responsible for the death of one patient.

The blood stagnation in the recipient’s heart may also favor bacterial or fungal endocarditis. The second patient of the Cape Town HHT series had a prosthetic aortic valve in his own heart. Staphylococcus aureus endocarditis developed, unresponsive to medical treatment, and the prosthetic valve had to be removed. At operation the aortic root was obliterated with the anterior leaflet of the mitral valve used as a flap, and the free wall of the left ventricle was resected. The infection was controlled. This patient made a good recovery and has survived longest of all HHT recipients. Last, the original disease of the recipient’s heart may progress: Two patients operated upon for severe ischemic cardiomyopathy and untractable angina have had mild recurrence of symptoms, easily controlled by medical therapy. In two patients the recipient’s heart disease progressed to the extent that the heart ceased to function, without ill effects.

Conclusion

All the advantages of HHT derive from the occasional assistance provided by the recipient’s own heart, as long as it remains capable of ensuring a minimal output and maintaining life temporarily. This procedure has been demonstrated to reestablish normal cardiac function, to retain the recipient’s right ventricle that tolerates the high pulmonary resistance, and to carry few drawbacks from preserving the recipient’s diseased heart. It may be the operation of choice at this time, when encouraging results indicate heart transplantation for a larger number of patients in whom the aim is to restore a satisfactory quality of life rather than prolong survival. However, the limits of our knowledge and of our means to control the immune system justify that in this younger and less desperate population the patient’s own heart should be retained as a built-in assist device. Future progress in immunosuppression, bartered by Cyclosporin-A, in induction of tolerance, and in histocompatibility matching may someday render HHT obsolete. Pulmonary hypertension itself, the original motive to develop HHT, may soon be dealt with by heart-lung transplantation. However, in the interim, the preserved heart has been and will continue to be valuable.

We are grateful to the many members of the medical, nursing, and paramedical staff of Groote Schuur Hospital and the University of Cape Town Medical School who have contributed to the care of the patients reported in this paper.

REFERENCES

8 Lower RR, Szentpetery S, Quinn J, Thomas FT: Selec-
tion of patients for cardiac transplantation. Transplant Proc 11:293-295, 1979
28 Stuart FP, Veith FI, Crawford RE: Brain death laws and patterns of consent to remove organs for transplantation from cadavers in the United States and 28 other countries. Transplantation 31:238-244, 1981
34 Rose AG, Uys CJ, Cooper DRC, Barnard CN: Donor heart morphology twelve and a half years after orthotopic transplantation. Heart Transplant 1: . 1982
42 Reitz BA, Wallwork JL, Hunt SA, Pennock JL, Billingham ME, Oyer PE, Stinson EB, Shumway NE: Heart-

Discussion

DR. RAYMOND O. HEIMBECKER
London, Ontario, Canada

Congratulations to the authors on a very fine presentation and a very ingenious contribution to the technology of heart transplantation. Two questions arise, especially in the patients with severe pulmonary hypertension: First is the problem of the efficacy of a heterotopic graft in the presence of severe pulmonary hypertension. Do the authors have any data on the ejection fraction or the cardiac output? What is the capability of that transplanted heart to pump against a high fixed pulmonary vascular resistance?

Second are the problems of lymphoma. In our own London (Canada) experience with Drs. McKenzie, Kosuk, Stiller, Silver, and Painvin, we now have 3 years of experimental work and five clinical OHTs. The first has been followed-up over a year and we have seen no lymphoma. All patients received Cyclosporin-A and steroids. None received rabbit antithymocyte globulin or azathioprine (Imuran). The Pittsburgh experience appears quite similar, with no postoperative lymphoma as long as rabbit antithymocyte globulin is avoided.

The clinical response to our series of patients has been most rewarding, with relatively smooth postoperative courses and the total absence of rejection as confirmed by frequent serial endomyocardial biopsies. The clinical courses have also been excellent, with a return to all normal activities and exercise tolerance, as documented by normal myocardial function studies. Postoperative long-term monitoring has become simple, safe, and relatively inexpensive (total follow-up 800 patient-days).

Cardiac transplantation has now reached the stage of unquestionable patient benefit with acceptable costs to society.

Manuscript reviewer’s comment

I do not feel that Dr. Losman and colleagues have made a very strong case for expanding the use of HHT to patients with lesser degrees of cardiac disability than NYHA Class IV. There is no evidence presented to alleviate my concern that, like OHT, the procedure carries an unacceptably high morbidity and mortality for use in patients with Class III disease. Certainly we would conclude that if valve replacement carried such risks it would not be used in patients with comparable disability. A further concern is that with more centers becoming involved in cardiac transplantation, and this trend is appropriate, for therapy of suitable Class IV patients, the approach that Dr. Losman advocates would, if widely applied, further complicate the availability of donor hearts for those with the greatest therapeutic need.

It is my contention that there may be specific indications for HHT which include a high pulmonary resistance, although the exact limits have not been defined which would preclude OHT, and a small donor heart whose output might be insufficient to sustain the recipient safely in the early postoperative period. Again, precise limits have not been defined.

There are thus advantages and disadvantages to the use of HHT in place of OHT for Class IV patients undergoing transplantation for otherwise dismal survival, and the choice of procedure in this situation remains one for the clinical judgment of the surgical team. Until HHT is demonstrated to be clearly superior in morbidity and mortality, it will be used by most groups less frequently due primarily to greater difficulty in monitoring for rejection. Until the threat of rejection and safety of immunosuppression are significantly changed by new knowledge in management of the immune system in cadaver organ transplantation, it would appear to me to be unwise to subject Class III patients to such considerable risks.

Richard R. Lower, M.D.
Richmond, Va.

DR. LOSMAN (Closing)

The question raised about the indication of HHT in patients with high pulmonary vascular resistance is an important one, and some confusion exists in regard to the effects of this hemodynamic criterion. In 1972 to 1973, the Cape Town group noted acute right heart failure following OHT in patients with elevated pulmonary resistance. Dr. W. Beck suggested that in these patients only a left ventricle should be grafted, keeping the patient’s own hypertrophied right ventricle that copes with the pulmonary resistance. This led to heterotopic heart transplantation, a procedure that retains the patient’s heart and is indicated when severe pulmonary hypertension is present. In such patients acute failure of the normal right ventricle of the graft may be expected. When longstanding left ventricle failure has been responsible for acquired pulmonary hypertension, it may take days or weeks for the latter to drop, and OHT recipients with this degree of pulmonary hypertension have died at operation or during the early postoperative days. In patients with fixed pulmonary vascular resistance due to high-flow congenital lesions, multiple pulmonary emboli, or other forms of pulmonary hypertension, the indications are totally different. No progressive drop of the pulmonary resistance can be expected, and HHT will not be satisfactory. These patients are candidates for heart and lung transplantation, as has been recently demonstrated by Reitz and Shumway.

However, the initial rationale to perform HHT did lose its importance when other advantages became obvious. These advantages are even more persuasive now that the patients operated upon are younger, had a shorter illness, and are in better general condition. Dr. Lower’s point that heart transplantation should not be attempted in patients who are in less than Class IV is well taken, but we can only conclude from the data presented that the population of heart transplant recipients today is different from that of 10 years ago.
Indeed, there are advantages and disadvantages to the two models of heart transplantation and no data yet demonstrate the superiority of one model over the other. This will undoubtedly come when large series encompassing similar populations in similar programs will be available for analysis. Dr. Lower's observation about the difficulty to diagnose rejection following HHT differs from my own experience, careful monitoring in both models relying more than ever on endomyocardial biopsy.

The question about the risk of lymphoma in patients treated with Cyclosporin-A is difficult to answer presently. It is possible that Cyclosporin-A has carcinogenic properties or that the high incidence (± 20%) of lymphoma in the Stanford group's experience is related to the combined use of rabbit antithymocyte globulin and Cyclosporin-A. The larger renal transplant experience has not produced a similar incidence, and larger series over longer periods will give us in the future a solution to this problem.
Long-term survival after orthotopic and heterotopic cardiac transplantation

D K C Cooper, R G Charles, R C Fraser, W Beck, C N Barnard

Summary and conclusions

Five long-term survivors of heart transplantation were reinvestigated. Two patients had undergone orthotopic heart transplantation over 11 and 9 years earlier and constitute two of the world’s longest-surviving patients after this procedure. Three patients had undergone heterotopic heart transplantation (one left heart bypass alone and two biventricular bypass) four to six years earlier. Four of the five patients had had only one or no documented acute rejection episodes. Three had been given blood transfusions. None had had particularly good tissue matching in relation to the donor on HLA typing. All five patients were leading full and active lives. At review two patients had significant coronary artery disease, one severe, presumably due to chronic immune-complex deposition.

Heart transplantation remains a major undertaking, but it can offer the patient many years of good-quality life.

Introduction

From 1967 to 1973, 11 patients underwent orthotopic heart transplantation (10 men, 1 woman; age range 36 to 63 years,
average 49 years) at Groote Schuur Hospital. Four patients survived more than one year, and two patients remain alive 11 and 9 years after operation.

In the five and a half years from November 1974 to May 1980, 30 patients (27 men, 3 women; age range 14 to 52 years, average 37 years) underwent heterotopic heart transplantation. One patient underwent a second transplant 18 months after his initial operation. Fifteen (50%) were still alive six months to almost five and a half years after transplantation. One-year survival was 64%; three out of six patients have survived more than four years.

The two patients with orthotopic grafts represent two of the world’s longest survivors after this operation. The longest survivor of heterotopic heart transplantation, now in his sixth year, was one of only two patients who underwent left heart bypass alone. In this operation the anastomoses performed were donor-to-recipient left atria and aortae; donor heart coronary sinus return was channelled back to the recipient’s circulation by anastomosing the donor pulmonary artery to the recipient right atrium. In the two other patients with heterotopic grafts who have survived more than four years both right and left ventricles were supported by the donor heart. The donor heart was connected in parallel to the recipient heart by anastomoses between the two left atria, superior vena cavae, aortae, and pulmonary arteries (using a conduit of either donor aorta or Dacron); this has been the operation of choice in subsequent patients.

All five of these patients were recently admitted for investigation. All patients underwent routine right and left heart catheterisation via the transfemoral route in the supine position under light sedation. The zero reference was taken at the mid-thoracic level. Left ventricular ejection fractions were calculated from left ventricular cine-angiograms in the right oblique projection and in the posteroanterior projection for the donor hearts of cases 4 and 5. Selective coronary arteriography (Judkins) and donor right or left ventricular endomyocardial biopsy were performed in all cases.

Case 1

The longest surviving patient was a coloured woman with a long history of rheumatic heart disease, two previous mitral valvotomies, and subsequent mitral valve replacement. In April 1969, at the age of 37, she underwent orthotopic heart transplantation after being admitted in refractory cardiac failure with a normally functioning prosthesis. Her postoperative recovery was complicated by pneumonia,
hepatitis, leg myopathy, and oral and visual hallucinations. She has taken azathioprine and prednisolone for immunosuppression.

She suffered several early acute rejection episodes but has since enjoyed a full, active life. Major late complications have included avascular necrosis of the right humeral head and both tibias, presumably caused by steroid therapy, though it has not seriously limited her movement. She also required truncal vagotomy and pyloroplasty for acute perforation of a duodenal ulcer in 1978. Urinary tract infections, aseptic dysuria, and backache from osteoporosis have troubled her occasionally, and she has needed skin grafting for leg ulceration more than once. She also developed a cataract, mild glaucoma, and underwent cholecystectomy for gall stones in 1979.

At review she appeared thin with no obvious Cushingoid features and was in sinus rhythm with a blood pressure of 100/70 mm Hg. The venous pressure was raised 4 cm; the tip of the liver was palpable. The pulmonary second sound was palpable but there was no right ventricular lift. A soft apical fourth heart sound could be heard and the pulmonary second sound was prominent. In the second left interspace a short mid-systolic murmur was audible.

Electrocardiographic and radiographic features and the results of cardiac catheterisation are shown in the table. Histological examination of a right ventricular endomyocardial biopsy specimen showed minimal fibrosis with normal blood vessels and no other significant abnormality. Scored on the basis of the scoring system of Rose and Uys acute rejection activity was assessed as 0.

Her drug treatment included daily azathioprine 150 mg, methylprednisolone 10 mg, digoxin 0.25 mg, frusemide 60 mg, potassium and calcium supplements, and cimetidine.

Case 2

This Caucasian man underwent orthotopic heart transplantation for ischaemic heart disease in May 1971 at the age of 45 years. During induction of anaesthesia for transplantation he suffered repeated ventricular fibrillation; the operation itself was uneventful. He suffered no postoperative complications and has had no documented rejection episodes. Azathioprine and prednisolone have been continued since operation.

He returned to full-time employment within three months of his operation and has led a normal life. Backache in 1973 was related to compression of the 4th to 9th thoracic vertebrae due to osteoporosis. He had no other late complications. He has been mildly hypertensive for some years and took methyldopa; recently he developed intermittent claudication in both calves.

At review he was overweight, not obviously Cushingoid, and had no evidence of cardiac failure. His blood pressure was 140/90 mm Hg. Heart sounds were soft and there were no murmurs or added sounds.

There were no palpable pulses below the femoral arteries in either leg, but there was no overt evidence of leg ischaemia.

Electrocardiographic and radiographic features and the results of cardiac catheterisation are shown in the table. Endomyocardial biopsy
Investigations in two patients with orthotopic and three patients with heterotopic cardiac transplants

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Electrocardiogram</strong></td>
<td>SR, rate 98/min, axis +120°, incomplete RBBB</td>
<td>SR, rate 78/min, axis +20°, incomplete RBBB, minor ST and T-wave changes</td>
<td>Recipient</td>
<td>Recipient</td>
</tr>
</tbody>
</table>

| Chest radiograph | Slight pulmonary congestion | Unremarkable | Cardiac shadow showing donor heart and absence of recipient left ventricle | Cardiac and donor heart shadow, otherwise normal |

<table>
<thead>
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<th>Cardiac catheter pressures (mm Hg):</th>
<th></th>
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<th></th>
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<td><strong>Phasic</strong></td>
<td><strong>Mean</strong></td>
<td><strong>Phasic</strong></td>
<td><strong>Mean</strong></td>
<td><strong>Phasic</strong></td>
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<tr>
<td>Right atrial pressure</td>
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<td>32/8-12</td>
<td>32/8-12</td>
<td>34/2-8</td>
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<tr>
<td>Right ventricular pressure</td>
<td>44/2-8</td>
<td>31/17</td>
<td>31/17</td>
<td>34/22</td>
</tr>
<tr>
<td>Pulmonary artery pressure</td>
<td>23/44/22</td>
<td>24/32/17</td>
<td>22/34/22</td>
<td>22/34/22</td>
</tr>
<tr>
<td>Left ventricular pressure</td>
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<td>44/34/22</td>
<td>44/34/22</td>
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<td>Cardiac index (l/min/m²)</td>
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<td>Pulmonary vascular resistance (units)</td>
<td>210</td>
<td>210</td>
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<td>210</td>
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<tr>
<td>Systemic vascular resistance (units)</td>
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<td>180</td>
<td>180</td>
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<tr>
<td>Ejection fraction (%)</td>
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<td>45</td>
<td>45</td>
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</tr>
<tr>
<td><strong>Left ventricular angiogram</strong></td>
<td>Normal volume, interstitial dyskinesia</td>
<td>Normal volume, good contractility</td>
<td>Cardiac shadow showing donor heart and absence of recipient left ventricle</td>
<td>Cardiac and donor heart shadow, otherwise normal</td>
</tr>
</tbody>
</table>

| **Coronary angiogram** | RCA occluded, LAD empty, LCCA diffusely non-critical | Normal | Recipient: grossly dilated, global dyskinesia; donor: normal volume and contractility | Recipient: not done, donor: normal |

showed some hyaline thickening of the small vessel walls, but no other evidence of rejection (score 1-5).

His drugs were azathioprine 200 mg daily, prednisolone 5 mg twice a day, sulphinpyrazone 200 mg three times a day, methylldops 250 mg four times a day, and standard doses of clofibrate, Moduretic, and antacids.

Case 3

This 47-year-old Caucasian man had had a long history of rheumatic heart disease and despite two aortic valve replacements developed intractable left ventricular failure. In December 1974 a left ventricular bypass was performed using a cardiac allograft. A small aortic paraprosthetic leak was also repaired and the poppet of the University of Cape Town prosthesis replaced. His early recovery was uneventful. Only one significant acute rejection episode was documented at six months. He remained well for four months, when he experienced the first of several attacks of paroxysmal ventricular tachycardia of his own heart, requiring electrical defibrillation and drug treatment.

In May 1976 he was admitted with bacterial endocarditis due to Staphylococcus aureus. He then suffered an acute myocardial infarction in his own heart. Cardiac catheterisation showed that the prosthesis was not opening, probably because of a thrombus. The patient’s own left ventricle was not working. The prosthesis was excised, the aortic root closed with the anterior leaflet of the mitral valve, the mitral valve orifice closed, and left ventriculectomy performed in June 1976 in an effort to remove the source of the Staphylococcus aureus, excise the focus of the recurrent episodes of ventricular tachycardia, and remove a potential source of systemic emboli. One month later a bleeding peptic ulcer was successfully treated medically.

Since then he has remained well and active. At review he had no exercise limitation or other symptom but was overweight with minimal Cushingoid features. His pulse was regular at 90/min at rest, venous pressure raised 3 cm, blood pressure 120/80 mm Hg. He had some periorbital oedema and minimal ankle oedema. His apex beat was to the right of the sternum. Donor first and second heart sounds appeared normal; occasional added sounds from the recipient right heart were audible. Electrocardiographic features and findings on cardiac catheterisation are listed in the table. We could not perform full right and left heart catheterisation of the donor heart because there were no right-sided anastomoses between donor and recipient organs. Donor left ventricular endomyocardial biopsy showed minimal to mild acute rejection (score 2). The myofibres were basically well preserved, though some appeared vacuolated. There were occasional interstitial aggregates of mononuclear cells and some fibrosis.

His daily drug therapy consisted of azathioprine 250 mg, methylprednisolone 16 mg, sulphinpyrazone 600 mg, digoxin 0.25 mg, frusemide 80 mg, potassium supplements, cimetidine, and antidepressants.
Case 4

This Caucasian man with intractable failure from ischaemic heart disease underwent heterotopic heart transplantation with biventricular bypass at the age of 32 years in December 1975. Immunosuppression has been with azathioprine, methylprednisolone, and initially antilymphocyte globulin. On the eighth day after operation cardiac catheterisation showed severe failure of both donor and recipient right and left ventricles with right ventricular end-diastolic pressures of 32 mm Hg and a mean capillary wedge pressure also of 32 mm Hg. Donor right ventricular endomyocardial biopsy showed only mild oedema. Clinically he steadily improved, and this episode was never fully explained. He suffered no definite episodes of acute rejection.

His postoperative progress was complicated by a purulent, retrosternal wound infection which twice required exploration and drainage and prolonged antibiotic treatment. Late complications included sternal osteochondritis and dizzy spells and brief loss of consciousness of indeterminate cause in 1977, since when he has been taking both anticoagulants and phenobarbitone.

At review he was fit and extremely active, working as a fisherman, pulling in catches of up to 82 kg. Examination showed a normal appearance, an irregular pulse due to the two beating hearts, a blood pressure of 120/85 mm Hg, impalpable apex beats, and no signs of failure. Normal donor heart sounds could be heard with no murmurs; there was a recipient heart fourth sound with no murmurs.

Electrocardiographic, radiographic, and cardiac catheterisation findings are shown in the table. Biopsy sections of right ventricular myocardium showed evidence of minimal rejection, with occasional myofibre degeneration and mononuclear cells (score 2).

His drug treatment consisted of daily azathioprine 250 mg, methylprednisolone 14 mg, dipyridamole 400 mg, warfarin, phenobarbitone, and antacids.

Case 5

This Caucasian man developed a cardiomyopathy and underwent biventricular bypass with a heterotopic transplant in January 1976 when he was 25 years old. His early postoperative recovery was uneventful. Immunosuppression consisted of azathioprine, prednisolone, and antilymphocyte globulin. In October 1976 he discontinued his medication and underwent severe acute rejection; the donor heart deteriorated to the point of ventricular fibrillation, requiring electrical defibrillation and considerable antirejection treatment. He recovered completely.

Since then he has remained well and active. At review he looked normal and had a blood pressure of 120/80 mm Hg and no signs of failure. Normal donor heart sounds could be heard with no murmurs.

The electrocardiographic (fig 1) and radiographic (fig 2) features are detailed in the table, together with the results of cardiac catheterisation. Right ventricular endomyocardial biopsy showed no significant rejection changes. The myofibres were well preserved with only an
occasional mononuclear cell in the interstitium. The smaller coronary vessels had prominent media and there was some intimal thickening from cell proliferation (score 0.5).

His drug treatment consisted of daily azathioprine 200 mg, prednisolone 10 mg, dipyridamole 400 mg, digoxin 0.25 mg, warfarin, and antacids.

FIG 1—Case 5. Recent electrocardiogram of patient with biventricular heterotopic heart transplant. R = recipient QRS complex; D = donor QRS complex.

FIG 2—Case 5. Recent posteroanterior chest radiograph of patient with biventricular heterotopic heart transplant. The "button" is an atrial pacing electrode used in early postoperative period.
Discussion

All five patients have undoubtedly had their lives prolonged and have experienced a good quality of life since operation. The long survival of these patients may be related to several factors. The relative absence of acute rejection episodes in four cases is obviously important, although such episodes, particularly in the case of orthotopic grafts, were less well recognised at the time of the operations than they would be today. Of the two patients with orthotopic transplants, one (case 1), who had multiple acute rejection episodes, has advanced diffuse coronary artery disease, whereas the other, who had no rejection episodes, has minimal changes. If her heart were not denervated the first patient would probably now be troubled by angina. The risk of sudden cardiac arrest must be high, and we are therefore considering whether to advise her to undergo retransplantation, possibly with a heterotopic heart.

The first of the three patients undergoing heterotopic heart transplantation has fairly advanced coronary artery disease, but left ventricular function is well preserved. Tissue typing was relatively primitive at the time of the two orthotopic heart transplants, but none of the five patients had a particularly well-matched heart on HLA typing. One major series of orthotopic heart transplantation has found no correlation between tissue typing and survival.7 Before their transplantation two patients (cases 1 and 3) had undergone previous open heart surgery and one (case 4) had received a blood transfusion, and the Stanford group have reported improved survival in patients who have received previous blood transfusions.7

Three patients (cases 2, 3, and 5) showed no significant change in electrocardiographic voltage after the early postoperative period. In case 1 the electrocardiogram was stable and normal until the seventh year, when the patient developed right axis deviation and incomplete right bundle-branch block unrelated to any detectable clinical event; there has been no further change. Case 4 has shown a right bundle-branch block pattern in the donor heart since the episode of biventricular failure at the end of the first week after operation.

Haemodynamic function in these patients may be affected by the high steroid dose used for immunosuppression, the presence of donor heart denervation, and pre-existing pulmonary vascular changes.10 The mild increase in right heart pressures in two of our patients (cases 1 and 5) could have been related to pulmonary vascular changes, steroid-induced hypervolaemia, or chronic rejection, the functional effects of which may be predominantly right-sided.18 Left ventricular end-diastolic pressure (LVEDP) was moderately raised in case 1 because of the left ventricular
dysfunction and extensive coronary artery disease, but it was normal or only marginally raised in the others. Preoperative LVEDP at rest in case 4 was grossly raised at 38 mm Hg, reflecting severe left ventricular disease. At review the LVEDP in both recipient and donor left ventricles was equal and only slightly raised at 16 mm Hg. This is to be expected since both left ventricles are filled from a functionally common left atrium. Preferential filling of the healthy and presumably more compliant donor ventricle will occur, thus decompressing the diseased, non-compliant recipient ventricle and equilibrating the end-diastolic pressures.

In all three patients with heterotopic heart transplantation the recipient heart continued to function, albeit poorly. In the patient with a left heart bypass alone, only the right ventricle of his own heart remained, but this continued to maintain his pulmonary circulation. The help that the patient’s own heart can give, particularly during episodes of acute rejection, has been emphasised elsewhere and was life-saving in case 5, where the donor heart developed ventricular fibrillation during a rejection episode.

We believe that anticoagulation with warfarin is essential in patients with heterotopic grafts to diminish the risk of pulmonary or systemic emboli from thrombus which may form in the poorly contracting ventricles of the recipient heart. We also think that the presence of a prosthetic valve in the recipient heart is a contraindication to heterotopic heart transplantation as it may provide a focus for thrombus formation or infection, as in case 3. An orthotopic heart transplant should be carried out in such cases or the prosthetic material should be removed, though this will inevitably mean that a procedure such as left ventriculectomy is also needed.

The other late complications met in this small group were those associated with long-term steroid therapy. Nevertheless, although heart transplantation remains a major undertaking for both patient and surgical team, it can offer the patient many years of good quality life.

We thank the many members of the medical, nursing, and laboratory staff of Groote Schuur Hospital and the University of Cape Town Medical School who have contributed to the care of these patients.

References

(Accepted 16 September 1980)
Case Report

Donor Heart Morphology Twelve and a Half Years After Orthotopic Transplantation

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ABSTRACT — The autopsy findings in a patient who survived for 12½ years after orthotopic cardiac transplantation are reported. Death was due to chronic rejection with accelerated coronary arterial atherosclerosis. This patient's prolonged survival is a portent of the good results that could be achieved with cardiac transplantation.

At present there is a 65% one-year survival rate for orthotopic cardiac transplantation and 50% of the patients survive up to five years after transplantation.1 Prolonged survival2 of more than a decade following heart transplantation is, unfortunately, still an uncommon event despite improved results worldwide. The reasons for such prolonged survival are not altogether clear, but a study of these patients may provide clues. Chronic rejection has been studied in less detail than acute rejection. Chronic rejection with accelerated coronary atherosclerosis3,4 is one of the most important complications limiting long-term graft survival.

The number and extent of such complications in prolonged survivors are of special interest. These considerations prompted us to document the autopsy findings in a patient who lived for 12½ years following orthotopic cardiac transplantation.

Case Report

The patient (D.F.), 49 years old at the time of her death, had a history of rheumatic fever attacks and had undergone two closed mitral valvotomies prior to 1960. In 1968, at 36 years of age, her mitral valve was replaced with a porcine xenograft prosthesis. Her large left ventricle contracted poorly.

Orthotopic cardiac transplantation was performed on April 17, 1969, for intractable cardiac failure when she was 37 years of age. She was immunosuppressed with azathioprine and methylprednisolone; she received no anti-coagulants or antiplatelet agents. On the basis of changes in the amplitude of the electrocardiogram’s QRS voltage, it was believed that the patient had had several early acute rejection episodes. Catheterization of the donor heart in 1972 and 1973 showed good function and minimal coronary arterial narrowing, but a study in 1975 showed localized 50% narrowing of the right coronary artery. During this time the patient was treated intermittently by psychiatrists for depression, which had also been a problem before the transplant. Other problems included steroid-induced avascular necrosis of both tibiae and the right humeral head, a perforated peptic ulcer, and gallstones. Cardiac catheterization in June 1981 showed a poorly contracting left ventricle with inferoapical akinesia. The right coronary artery was occluded and the left coronary artery was diffusely irregular and narrow throughout its length.* Anticardiac failure therapy was given for one year before her death. On October 19, 1981, the patient collapsed at home and was pronounced dead on arrival at the hospital.

Autopsy Findings

The donor heart weighed 472 gm and had a thickened, focally calcified epicardium (Figures 1 and 2). The sutures linking the donor’s and recipient’s aortas and pulmonary arteries were still clearly visible on the intimal aspect. The atrial anastomotic sutures were less clearly visible due to a greater overgrowth of host tissue.

There was a striking difference in appearance between the two components of the patient’s left atrium (Figure 2). The portion of the left atrium that was derived from her own heart showed numerous organized thrombi, many of which had been converted into calcified plaques. Some organizing thrombi were also seen. At the anastomotic line there was an abrupt transition to the smoothly lined atrial component of the donor heart which was free from thrombi. None of the heart valves was abnormal.

The left anterior descending coronary artery showed total occlusion by rejection-induced accelerated atherosclerosis plus recanalized thrombus (Figure 3). Its diagonal branch had grade 2 (25% to 50%) narrowing, as did the circumflex branch of the left coronary artery.

The right coronary artery demonstrated grade 4 (75% to 100%) atherosclerotic narrowing and was totally occluded by an organized thrombus 2 mm from its aortic origin (Figure 4). Organization of a propagated thrombus led to conversion of most of the distal portion of the vessel into a fibrous cord.

Patchy subendocardial fibrosis could be seen in the left ventricle and the papillary muscles were fibrosed. Much of the left ventricle was occupied by an antemortem molded stasis thrombus which extended from the apex and intruded into the orifice of the mitral valve. The thrombus did not appear to be organized. To the naked eye the right ventricle showed extensive myocardial fibrosis as well as focal endocardial sclerosis. No thrombi were present in the right-sided chambers.
Characteristics of chronic rejection were seen microscopically. There was massive replacement fibrosis in the right ventricle (Figure 5), while sections of the left ventricle (Figure 6) confirmed subendocardial fibrosis. The small coronary arteries in both ventricles showed intimal cell proliferation and mural fibrosis with resultant severe luminal narrowing (Figures 7-9). There were areas of myocardial fibrosis in both atria. Nowhere did the myocardium exhibit acute rejection changes nor were Aschoff bodies seen in the recipient's atrial remnants.

Findings in the other organs may be summarized as follows: Lungs, liver, and spleen showed evidence of chronic venous congestion. A small calculus in one of the calices of the left

FIGURE 4. Donor's right coronary artery shows total luminal occlusion due to severe rejection, accelerated atherosclerosis, and central recanalised thrombus. (Elastic van Gieson.)

FIGURE 5. Outer portion of right ventricular wall shows extensive replacement fibrosis of the myocardium. Chronic rejection has led to severe narrowing of a small branch of the right coronary artery in the fibroed epicardium. (Elastic van Gieson.)

FIGURE 6. Subendocardial replacement fibrosis of the left ventricle. (Hematoxylin and eosin.)

FIGURE 7. Small coronary artery luminal narrowing due to intimal fibrosis from chronic rejection. (Elastic van Gieson.)

FIGURE 8. High-power view of another small intramyocardial artery shows that the intima is much thicker than the media due to myofibroblastic proliferation. (Elastic van Gieson.)
kidney led to the formation of a small abscess in the adjacent renal medulla. Culture yielded a mixed growth of organisms, including Proteus mirabilis, Staphylococcus epidermidis, Escherichia coli, and Clostridium welchii. Both kidneys also bore scars of healed pyelonephritis. Bone marrow infarcts in the right femur were probably due to steroid therapy. A left-sided hydrosalpinx was present. The aorta showed moderate atherosclerosis. The brain appeared normal. The autopsy diagnosis of the cause of death was chronic rejection with terminal cardiac failure.

Discussion

This patient, despite several complications related to immunosuppression, led a full and active life for more than 11 years following transplantation. Only during the last 12 months of her life did her exercise tolerance significantly deteriorate.

The following factors may have contributed to this patient's prolonged survival. First, she was under 40 years of age at the time of transplantation which, in our series, is the single most important factor related to survival. This is almost certainly due to the increased tolerance of a younger patient to the debilitating effects of immunosuppressive therapy. Patients 40 years of age or older at the time of transplantation have a statistically higher mortality rate, particularly during the first postoperative year. Second, the patient had undergone three previous cardiac operations and had, therefore, received numerous blood transfusions. In our series, this is associated with improved survival, though it reaches statistical significance only in three-year posttransplantation survivors.

Finally, although tissue typing was not well developed in 1969, there was compatibility between the patient and donor at one HLA-A and one B locus. This, when compared with the survival of recipients who were incompatible at all HLA-A and B loci, reaches statistical significance only in the one-year posttransplantation survivor group.

Transvenous endomyocardial biopsies of the donor's right ventricle performed in 1970, 1975, 1976, 1978, and 1980 did not show significant evidence of acute rejection. However, by 1978 there was biopsy evidence of chronic rejection—induced myocardial fibrosis. The latter appears to be secondary to rejection-accelerated atherosclerosis of the major epicardial coronary arteries and intimal thickening of the small coronary arteries. Such coronary arterial disease is the major complication presently limiting long-term survival of cardiac allografts.

We have observed on occasion severe accelerated coronary atherosclerosis in rejected donor hearts (eg, 81 days posttransplantation in one patient and one year later in another).

Some evidence of chronic rejection can usually be seen microscopically by 30 days posttransplantation, and one of our patients showed mild coronary arterial intimal thickening within 20 days. Fourteen of the 29 transplanted hearts we examined pathologically showed evidence of chronic rejection. One of the eight hearts transplanted orthotopically demonstrated mild chronic rejection. Of 21 heterotopic transplants, chronic rejection was mild in 4 patients, moderate in 2 patients, and severe in 7 others.

The myocardial fibrosis of chronic rejection appears to be attributed primarily to narrowed epicardial coronary arteries. Narrowing of the intramyocardial arteries and healing of acute rejection episodes may contribute to this fibrosis. The ischemia produces no painful symptoms since the donor heart is denervated.

Without doubt, the cardiac transplantation significantly prolonged and improved the quality of this patient's life. She was one of the longest surviving heart transplant patients in the world, providing ample justification for cardiac transplantation. How similar results can be achieved with other cardiac transplant patients, as well as how vascular lesions of chronic rejection (which led to her death) can be prevented, is an un-
solved challenge. Future laboratory studies of mechanisms of the graft enhancement and of factors controlling host mechanisms of graft tolerance will hopefully provide some answers.

References


Transplantation of the heart and both lungs
Experimental and clinical experience and review of the literature

D. NOVITZKY, D. K. C. COOPER, W. N. WICOMB, A. G. ROSE, B. REICHART

Summary

Transplantation of the heart and both lungs is the only therapy that can be offered to certain patients with end-stage pulmonary vascular disease. Our experimental experience with the baboon is presented. Fourteen allotransplants were performed, 12 recipients (inadequately immunosuppressed with cyclosporin A and azathioprine) surviving between 4 and 29 days. In 11 cases death resulted from acute rejection which predominantly involved the lungs, the heart being spared in 10 cases; the remaining death was from bronchopneumonia. Two autotransplanted baboons survived until sacrificed at 6 months.

Indications for the operation, selection of both the recipient and the donor, and recent results at other centres are briefly reviewed. It would seem that this operation is recommended in selected patients with idiopathic pulmonary hypertension or Eisenmenger's syndrome whose condition is deteriorating and in whom no other form of therapy is applicable.

Transplantation of the heart and both lungs is the only therapy that can be offered to certain patients with end-stage pulmonary vascular disease. In recent years the introduction of cyclosporin A as an immunosuppressive agent, an understanding of the blood supply of the trachea and bronchi, and the observation that the heart and lungs are rejected simultaneously have led to improved results with this procedure.

The early experimental work in this field has been reviewed elsewhere. In the early 1950s Demikhov achieved survival of animals for up to 6 days, deaths resulting from rejection and pulmonary infection. In this and other early studies the site of the airway anastomosis was at the level of the bronchi. Simplified techniques were introduced by Lower et al in 1961 and Longmore et al in 1969. Results in the dog were poor, and there is evidence that this animal requires the presence of afferent nerve pathways from the lungs to maintain spontaneous breathing, after transplantation of the heart and both lungs respiratory amplitude progressively decreases, and death results from hypercapnia and hypoxia.

However, primates appear to tolerate total denervation of the lungs, and spontaneous respiration is preserved.

Transplantation of the heart and both lungs in the baboon — an experimental study

Donor and recipient were matched for AB blood group and mass (25 - 27 kg), although a donor with a slightly smaller chest volume than the recipient was chosen in order to avoid the possibility of donor lung atelectasis.

Donor operation

Under general anaesthesia, after a median sternotomy and heparinization, the superior vena cava was ligated, the inferior vena cava clamped, and the tip of the left atrial appendage transected to decompress the left side of the heart. After cross-clamping the aorta, cardiopulmonary bypass was initiated; the animal was cooled to 20°C. The pleuropneumothorax was incised on each side from the sternum to the hilum of the lung, each hilum was dissected free, and the dissection extended posteriorly to mobilize the left atrium. The aorta was transected at the level of the brachiocephalic artery, and the trachea superior to the carina. The donor heart and lungs were then removed as a single block of tissue (Fig. 1). These organs were placed in a bowl of cold saline (4°C). The heart was prepared for insertion into the recipient by incising the right atrium from the inferior vena cava to the base of the right atrial appendage (as for orthotopic heart transplantation) (Fig. 1).

Recipient operation

Sternotomy and heparinization were followed by cannulation of the aorta and superior and inferior vena cavae, and cardiopulmonary bypass was initiated; the animal was cooled to 20°C.

Excision of the recipient organs

The aorta was clamped proximal to the arterial cannula. Both ventricles were excised as for orthotopic heart transplantation, leaving the posterior walls of both atria in situ (Fig. 2). Unnecessary dissection of the posterior mediastinum was therefore avoided. The right and left pleural cavities were entered through incisions made in the pleuropneumothorax posterior to the phrenic nerve, great care being taken to avoid damage to these structures. Each lung was then withdrawn through its corresponding pleuropneumothorax incision into the pericardial cavity, and the right and left pulmonary arteries were divided, a cuff of pulmonary artery tissue being left in situ under the arch of the aorta to prevent possible damage to the left recurrent laryngeal nerve (Fig. 2). The pulmonary
Fig. 1. Insertion of the donor heart and lungs into the recipient thorax. The remnants of the recipient right (RA) and left (LA) atria can be seen. The arrows indicate the route of insertion of the lungs into their respective pleural cavities. Note that both lungs pass posterior to the phrenic nerves and that, in addition, the right lung passes posterior to the recipient right atrium.

Fig. 2. View of recipient pericardial cavity after resection of the recipient heart and lungs, showing the remnants of the right (RA) and left (LA) atria, aorta and pulmonary artery. The incisions in the pleuropericardium posterior to the right and left phrenic nerves are indicated (SVC = superior vena cava; IVC = inferior vena cava).

Veins were transected close to the posterior wall of the left atrium. The trachea was transected above the carina, a minimum of mobilization having been carried out. The two lungs were then removed from the chest cavity.

Implantation of the donor organs

The donor heart and lungs were introduced into the pericardial cavity, each lung being passed through the respective opening in the pleuropericardium posterior to the phrenic nerve, the right lung being passed posterior to the remnant of the recipient right atrium (Fig. 1). Anastomoses of the recipient and donor tracheas, right atriun, and finally the aortas were performed (Fig. 3). Protection of both myocardial and pulmonary viability was maintained throughout the operation by the continuous or intermittent topical application of cold (4°C) saline. After removal of air from the cardiac chambers the aortic cross-clamp was released. Most of the hearts started beating spontaneously or required only a single electrical shock to achieve defibrillation. The animal was then rewarmed and cardiopulmonary bypass discontinued. Cardiopulmonary bypass time varied between 1 hour 30 minutes and 1 hour 50 minutes, the total ischaemic time of the donor organs being ± 45 minutes. Subsequent procedures were as for any open heart operation, culminating in chest closure. Ventilation was continued for 4 to 6 hours until the animal was breathing spontaneously and maintaining normal blood gas levels, at which time it was returned to its cage.

Postoperative monitoring and maintenance immunosuppression

Monitoring consisted of daily recording of the ECG, rectal temperature, arterial blood gas values, full blood count and liver function test results. Chest radiographs were taken at intervals in selected animals. Elevation of the temperature, a fall in the partial arterial oxygen pressure (Pao₂) and patchy shadowing on chest radiographs were characteristic findings in animals undergoing acute lung rejection.

The baboons were divided into three groups (A, B and C) (Table I); cyclosporin A was given daily either orally (group A), by a single intravenous injection (group B), or by twice-daily intravenous injection (group C). Cyclosporin trough levels in whole blood were found to be extremely low (below 100 ng/ml) in group A. The intravenous injection of a single daily dose (group B) resulted in a high peak level of the drug with rapid disappearance during the next 2 hours. In group C two high peaks were achieved but disappearance was equally rapid.

Results

Fourteen allotransplants and 2 autotransplants were performed. The 2 baboons with autografts survived for periods of 6 months, at which time they were sacrificed and studied. In both cases the tracheal suture line showed no dehiscence or stenosis (Fig. 4), and the histological appearance of the heart and lungs was normal.

Of the 14 allotransplanted animals, 1 died at the time of operation, showing features of progressive lung failure with hypoxaemia, hypercapnia and finally metabolic acidosis, 1 died...
TABLE I. IMMUNOSUPPRESSIVE REGIMENS AND SURVIVAL IN THREE GROUPS OF CHACMA BABOONS WHICH UNDERWENT TRANSPLANTATION OF THE HEART AND BOTH LUNGS

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Cyclosporin A</th>
<th>Azathioprine</th>
<th>Methylprednisolone</th>
<th>Allograft survival (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>16 mg/kg/d orally</td>
<td>2 - 4 mg/kg/d orally for first 2 weeks</td>
<td>2 mg/kg/d from beginning of 3rd week</td>
<td>1, 1, 6, 6, 19, 29 (mean 10)</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>18 mg/kg/d by single IV injection</td>
<td></td>
<td></td>
<td>7, 12, 19, 20 (mean 15)</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>18 mg/kg/d by twice-daily IV injection</td>
<td></td>
<td></td>
<td>4, 5, 20, 28 (mean 14)</td>
</tr>
</tbody>
</table>

IV = intravenous.

Fig. 4. Well-healed tracheal anastomosis after transplantation of the heart and both lungs in the baboon.

24 hours after surgery from a pneumothorax resulting from an air leak from the membranous portion of the trachea, and the remaining 12 baboons survived for between 4 and 29 days (Table I), death resulting from acute rejection in 11 and from bronchopneumonia in 1. Length of survival did not differ significantly between the 3 groups.

Anatomical considerations

The arterial blood supply to the lower trachea, carina and bronchi comes from the bronchial arteries, which originate from the aorta (Fig. 5). The carina and both bronchi also receive small but important arteries which arise from the major coronary arteries, predominantly the left. Although in the normal subject they play but a small role in the blood supply of the trachea and bronchi, once the donor trachea has been transected for heart and lung transplantation they become essential (Fig. 6). In single-lung transplantation, or where the bronchi are used as the site of airway anastomosis in transplantation of the heart and both lungs, this coronary artery

Fig. 5. Posterior view of the heart and trachea, showing blood supply to the trachea, carina and bronchi (LV = left ventricle).

Fig. 6. Donor trachea and bronchi, with transection above the carina, showing disruption of the blood supply to the carina and bronchi from the bronchial arteries, and retention of the blood supply from the coronary arteries.
Diagnosis and management of acute rejection

Since corticosteroids delay wound healing, centres performing this operation have excluded these drugs from the immunosuppressive regimen during the first 2 weeks to facilitate tracheal healing. During this period the patient receives cyclosporin A (10 - 12 mg/kg/d) and azathioprine (1.5 - 2 mg/kg/d). During the 3rd week azathioprine has been discontinued and replaced by maintenance steroids (0.2 mg/kg/d).10,11

In the majority of well-immunosuppressed patients it has been found that when acute rejection is occurring in the lungs, it is also occurring in the myocardium,10 and therefore methods of diagnosing acute rejection in the heart can be employed. It is therefore important to perform endomyocardial biopsy13 or use another reliable method of detecting rejection, such as radionuclide scanning14 or immunological monitoring,15 at frequent intervals. In some cases histological examination of the lung tissue may not be helpful since it can be difficult to differentiate acute rejection from infection; this is a major disadvantage of single-lung transplantation. The treatment of an acute rejection episode is identical to that for patients with heart transplants alone;16,17 intravenous boluses of methylprednisolone 1 g are administered daily for 3 days, together with antithymocyte globulin in severe cases.

Clinical experience

Single-lung transplantation

The results of transplantation of a single lung have been extremely disappointing.18 In 1963 Hardy et al.18 were the first to carry out this operation clinically. By 1980 38 such single-lung transplants had been performed, with a mean survival of only 8.5 days; 2 patients survived for 6 and 10 months.18 The high mortality was related to dehiscence of the bronchial anastomosis and to pneumonia. There is evidence that a major limiting factor preventing success of single-lung transplantation is an inadequate blood supply to the bronchial stump.20 The diseased lung is left in situ, and is therefore a potential source of infection to the transplanted organ. The diagnosis both of acute rejection and of infection may be extremely difficult.

Transplantation of the heart and both lungs

This operation was first performed by Cooley et al.21 in 1968 and by Lillehei22 in 1969; their 2 patients survived for only 14 hours and 8 days respectively.

The third operation was performed at Groote Schuur Hospital in July 1971 on a 49-year-old man with chronic respiratory failure, bronchiectasis and pulmonary hypertension.23,24 The bronchi, rather than the trachea, were chosen as the site of anastomosis of the air passages, since it was believed at that time that this would preserve both the blood supply to the carina and the cough reflex at the carinal area more satisfactorily. The patient initially did well but on the 8th postoperative day developed a right bronchopleural fistula, which was repaired. By the 18th day the patient showed features of a right pneumonia with recurrence of the pneumothorax, necessitating pneumonectomy. He died on the 23rd day of a Klebsiella pneumonia and septicemia.

It was not until another 10 years had elapsed that a fourth transplant of heart and lungs was performed, on this occasion at Stanford University, California.12

The availability of an improved immunosuppressive regimen including cyclosporin A, better understanding of the reimplantation syndrome, the availability of endomyocardial biopsy to diagnose acute rejection (since both organs are rejected simultaneously), and an improved anatomical understanding of the blood supply of the trachea and both bronchi, resulted in the first long-term survival of such a patient.10

At Munich University, 2 patients were operated upon by one of us (B.R.), one in February 1983 and the other in May 1984. Both men, aged 28 and 21 years, had disabling primary pulmonary hypertension with severe dyspnoea, being unable to walk the length of the ward floor without becoming deeply cyanotic. Both were already in a cachectic state. The first patient also had severe tricuspid valve insufficiency, with massive enlargement of the liver, ascites, and congested kidneys and gastro-intestinal tract. Although he was able to breathe...
satisfactorily as early as the first postoperative day, he soon showed signs of severe liver dysfunction, became comatose, and died on the 11th day. Autopsy revealed acute atrophy of the liver. This complication was thought to be the result of a combination of severe pre-operative and cyclosporin-induced liver damage. There were no signs of severe liver dysfunction in the second patient and the tricuspid valve remained competent. Although the postoperative course was complicated by acute pancreatitis and renal failure, this patient has survived and continues to do very well, enjoying life without any restrictions (Fig. 8).

Fig. 8. Anteroposterior chest radiograph taken in the early postoperative period of the second patient who underwent transplantation in Munich.

### Indications for heart and lung transplantation

Although patients with a variety of pulmonary and cardiac disorders might be considered for heart and lung transplantation (Table II), at the present time the operation is restricted to those with severe irreversible pulmonary vascular disease, either primary idiopathic or secondary to congenital heart disease (Eisenmenger's syndrome). Emphysematous patients are usually chronically infected, and there is a high risk of postoperative mediastinal sepsis.

### Selection of the recipient

The potential recipient must clearly have a severely restricted exercise tolerance and a life expectancy of from less than 6 months to 1 year; the \( Pao_2 \) will be less than 50 mmHg. He must fulfill the requirements for any patient being considered for heart transplantation, with the exception that a recent pulmonary infarction is not a contraindication for heart and lung transplantation. The presence of tricuspid incompetence with hepatomegaly, syncopal episodes, or oxygen dependence indicates a poor prognosis.

### Recent results after clinical heart-lung transplantation

The Stanford group has the most extensive experience with patients undergoing transplantation of the heart and both lungs. Seventeen patients underwent this procedure between March 1981 and December 1983. Five patients died within the first few postoperative weeks, and 12 patients remain alive between 2 and 35 months after operation (8 patients have survived for more than 1 year, and 4 for more than 2 years). Of the 12 remain well, although 4 have demonstrated progressive respiratory impairment 1 year after operation. In 2 patients this functional deterioration has been associated with radiological findings compatible with bronchiectasis and in 1 with the picture of diffuse fibrosis, and the remaining patient has both radiological findings. Bronchiectasis may result from the effects of denervation of the lung, leading to large-airway sensory loss or impaired mucociliary clearance. Fibrosis may be a result of chronic rejection, pulmonary vascular changes, or a toxic effect of cyclosporin. The Stanford group believes that good long-term function in cardiopulmonary transplants may be limited by these changes.

### Comment

The potential technical complications of this operation are numerous. In the initial Stanford series, 3 of the first 6 patients required exploration for postoperative bleeding. Haemorrhage may result from the posterior mediastinal dissection necessary in the recipient during excision of the heart and lungs, but also from bleeding points on the donor organs which have been implanted. Retention of the posterior wall of...
400 ng/ml). The for absolute freedom from pulmonary infection, and by the transplanted, this shortage becomes accentuated by the need for recipient operations described. 17 enabled bean and Jung transplantation to be performed with considerable success. Although the recent report from the Stanford group of late pulmonary failure in some patients is rapid excretion of the drug by the liver. Recent work in our intestinal tract absorption is impaired and/or that there is severe rejection of the lung was not associated with rejection of the heart (S. W. Jamieson — personal communication). Even the twice-daily administration of intravenous cyclosporin in relatively high doses was insufficient to maintain whole-blood trough levels in the therapeutic range (200 - 400 ng/ml). The low blood level achieved by the oral administration of this drug is probably species-related, since most other animal species, including man, achieve good blood levels after oral administration; in the baboon it seems likely that gastrointestinal tract absorption is impaired and/or that there is rapid excretion of the drug by the liver. Recent work in our laboratory has shown, however, that adequate trough levels can be attained by the daily intramuscular injection of cyclosporin A administered in a mixture of alcohol and Intralipid; this development will greatly facilitate transplantation studies in the baboon. In clinical series, both of heart and heart-lung transplantation, no difficulty has been found in achieving satisfactory levels of this drug after oral administration. Our own experience after heart transplantation has been described. 17

In summary, the introduction of cyclosporin has clearly enabled heart and lung transplantation to be performed with considerable success. Although the recent report from the Stanford group of late pulmonary failure in some patients is disappointing, it would seem that this operation is recommended in selected patients with idiopathic pulmonary hypertension or Eisenmenger's syndrome, who are deteriorating, and in whom no other form of therapy is applicable. The major logistical problem is that of donor organ supply. There is already an acute shortage of donor hearts for transplantation, particularly in South Africa. When the lungs are also to be transplanted, this shortage becomes accentuated by the need for absolute freedom from pulmonary infection, and by the lack of any satisfactory means of storing lungs for even a few hours, necessitating simultaneous, co-ordinated donor and recipient operations in adjoining theatres.

We wish to thank Miss Jenny Bosman and her colleagues in the Department of Clinical Photography of Groote Schuur Hospital for the illustrations, and MTP Press Limited, Lancaster, England, for permission to reproduce them from Heart Transplantation (edited by D. K. C. Cooper and R. P. Lanza). We also thank S. T. P. Ahrends, F. Barndes, Mrs J. Kloppe, P. Madlingozji, J. Rossouw and F. Snyders for skilled technical assistance. The experimental work was supported by the Chris Barnard Fund, the University of Cape Town, and the Cape Provincial Administration.

REFERENCES

Reversal of Acute Rejection by Cyclosporine in a Heterotopic Heart Transplant

DIMITRI NOVITZKY, CHRISTIAAN N. BARNARD, DAVID K. C. COOPER

ABSTRACT—A 47-year-old man with dilated cardiomyopathy underwent heterotopic heart transplantation (HHT). He received a conventional immunosuppressive regimen of azathioprine, methylprednisolone, and rabbit antithymocyte globulin (RATG). On the fifth postoperative day he developed a severe acute rejection episode. By the seventh day, the donor heart's function had markedly deteriorated and the recipient's own heart was carrying the major load of the circulation. Antirejection therapy, consisting of large intravenous doses of methylprednisolone and RATG, as well as a short course of cyclophosphamide, did not control the rejection process. On day 13, cyclosporine was added to this patient's immunosuppressive regimen. Conventional immunosuppressive therapy was continued for a further seven days. From day 20, immunosuppression was maintained with only oral cyclosporine and methylprednisolone. The acute rejection crisis was successfully reversed, and from day 25 after the transplantation, the donor's heart function recovered satisfactorily.

This report presents the case of a 47-year-old man, weighing 65 kg, who had a two-year history of progressive cardiac failure due to dilated cardiomyopathy. He was in atrial fibrillation and presented all the features of severe cardiac failure unresponsive to maximal therapy with digoxin, diuretics, and afterload reducing agents. The left ventricle ejection fraction (LVEF), measured by radionuclide scan, was reduced to 27%. The patient was accepted in the Groote Schuur Hospital transplantation program and on March 26, 1983, underwent a heterotopic heart transplant. The operation and immediate postoperative recovery were uneventful. The immunosuppressive regimen consisted of azathioprine (3.0 to 4.5 mg/kg body weight/day, orally), intravenous methylprednisolone during the first five days (600 mg/day, then a dose reduced by 100 mg every day), followed by oral maintenance prednisone (1 mg/kg body weight/day), and intravenous RATG at a dose that maintains the absolute number of T-lymphocytes between 50 and 150/mm³.

Monitoring Tolerance of the Cardiac Graft

Daily, or twice daily evaluations were made to detect evidence of acute rejection and included electrocardiograms (ECG), peripheral pulse wave traces, circulating T-lymphocyte counts, and a hemodynamic study of both hearts. Using radionuclide scans following injection of technetium-labeled red cells, the left ventricle's volume, ejection fraction, and output were measured. Endomyocardial biopsies, performed at intervals of a few days, completed the transplant patient's postoperative follow-up.

The pulse wave recordings, made over a large peripheral artery (e.g., carotid or femoral) provided information from both the donor and recipient hearts. When the hearts were out of phase, the area under the pulse wave of a donor and a recipient beat was measured and compared. This yielded an estimate of the relative contribution of each heart to the total cardiac output. However, this method did not give absolute values of the hearts' respective outputs. The results of this pulse wave area ratio (PWAR) evaluation correlated well with those of other monitoring methods, such as angiography, radionuclide scanning, endomyocardial biopsy, and the clinical condition of the patient. 99mTc-radiouclide scans also provided information that correlated well with the evidence of acute rejection obtained from the endomyocardial biopsy (D. Novitzky, unpublished data).
The Acute Rejection Episode

On the fifth and sixth postoperative days, the QRS voltage of the donor heart, measured from the ECG, fell dramatically by 50%. The donor/recipient pulse area ratio (PWAR), which had been 1.22, dropped to 0.66, and then fell to nothing two days later, indicating severe loss of function of the donor heart (Figure 1). Radionuclide scans also showed a dramatic fall in donor heart LVEF from 66% on day 3 to 27% on day 7, while stroke volume (SV) and cardiac output (CO) decreased (see below, Figures 1 and 2):

A chest radiograph on day 7 showed large distended donor and recipient hearts (Figure 3A), and despite large doses (<5.5 mg/kg/day) of RATG, the absolute number of T-lymphocytes rose to 1,000/mm³. With such obvious evidence of severely impaired donor heart function, the diagnosis of rejection was indisputable and an endomyocardial biopsy was unnecessary. The immunosuppressive therapy was immediately increased: "pulses" of methylprednisolone (1,000 mg intravenously) were given twice daily for four days in combination with large doses of rabbit ATG (<7.7 mg/kg/day, intravenously), the latter to reduce the number of circulating T-lymphocytes to immunosuppressed levels. Azathioprine was also increased (5.4 mg/kg/day). On the sixth and twelfth postoperative day, cyclophosphamide (1.5 mg/kg/day, orally) was added to the regimen. In this institution, cyclophosphamide has been found of value in controlling acute rejection in several other patients.

On day 9 the first endomyocardial biopsy was performed and it showed evidence of severe acute rejection, which was unresponsive to the

<table>
<thead>
<tr>
<th>Before rejection</th>
<th>Acute rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>QRS voltage</td>
<td>100% - 50%</td>
</tr>
<tr>
<td>PWAR</td>
<td>1.22 - 0.00</td>
</tr>
<tr>
<td>LVEF</td>
<td>66% - 27%</td>
</tr>
<tr>
<td>LV SV</td>
<td>28 mL - 6 mL</td>
</tr>
<tr>
<td>CO</td>
<td>2.1 L/min - 0.5 L/min</td>
</tr>
</tbody>
</table>

**Figure 1. Chart of patient’s progress.**

ECG: donor heart electrocardiogram; VR: right precordial lead V₃₃ + V₄₃ + V₅₃; STD: standard lead; Arterial pulse-donor % gives the donor heart contribution calculated from the PWAR; SV: donor heart stroke volume; IV pulses MP: pulses of intravenous methylprednisolone; CyA: cyclosporine; MP: maintenance methylprednisolone; AZA: azathioprine; CPP: cyclophosphamide; RATG: rabbit antithymocyte globulin.
increased immunosuppressive therapy. The biopsy's histological score was 5.5 on the scale proposed by Uys and Rose. During this period (from day 7 to day 13), the donor heart did not improve: PWAR remained at zero, and donor SV and CO stayed reduced. On day 13, an endomyocardial biopsy confirmed the ongoing severe acute rejection process (Uys and Rose score = 4.5).

At this stage it was thought that the donor heart was probably irreversibly damaged, so consideration was given to discontinuing the immunosuppressive therapy in order to limit the high risk of infection that accompanies antirejection therapy. An early retransplant was envisaged while the recipient's own heart was still adequate to support the circulation.

The Cyclosporine Regimen

At this time, however, cyclosporine became available at the Groote Schuur Hospital and so an attempt to reverse this acute rejection episode was made using the new drug. On day 13 cyclosporine was started at an initial dose of 18 mg/kg/day, which was reduced four days later to 12 mg/kg/day because of high cyclosporine blood levels (trough levels >1,000 ng/ml). The dose was further tapered, and by the fortieth day after transplantation the patient was receiving cyclosporine, 6 mg/kg/day. Pulsed methylprednisolone (500 to 1,000 mg, intravenously) was given between days 14 and 19, while oral steroid therapy was continued at 1 mg/kg body weight/day. The dose of azathioprine was reduced from day 13 to day 16 to less than 1 mg/kg body weight/day, and was discontinued on day 23. RATG, administered on days 16 to 18, completed this antirejection therapy.

On day 17, pulse wave recordings showed some donor heart contribution to the circulation, although this might have been in response
to the deterioration of the recipient's own heart, the donor SV and CO remaining at +15% of their prerejection values. The endomyocardial biopsy continued to show evidence of severe acute rejection (score = 4.0). However, three days later, an endomyocardial biopsy had an improved score of 3.0, but it also showed major myofiber damage and persistent cellular infiltration: the slight improvement in score was due to the absence of edema. At this time, pulse wave recordings, and donor SV and CO measurements did not suggest functional recovery. Nevertheless, on day 22, the chest radiograph showed considerable reduction in the size of both hearts (Figure 3B).

During the next five days the function of the donor heart improved significantly, its SV rising to 13 mL and its CO to 1.2 L/min. The endomyocardial biopsy score dropped to 2.5, showing evidence of moderate rejection. By day 34, the donor heart function had recovered its prerejection values (SV = 25 mL, CO = 2.0 L/min), but its electrocardiographic QRS voltage remained at ±50% of the prerejection value throughout this period.

Since this severe rejection episode, the patient has been well and, subsequently, did not develop any significant feature of acute rejection. The PWAR rose steadily to 9.0, as well as the donor heart's SV to 32 mL, and its CO to 2.9 L/min. The oral steroid therapy was slowly reduced to 0.6 mg/kg body weight/day by the 40th posttransplant day, and the patient was discharged from the hospital on day 51.

Four months after the transplant, the endomyocardial biopsy showed no evidence of acute rejection (score = 1). The patient remained on maintenance therapy consisting of cyclosporine (5 mg/kg body weight/day) and methylprednisolone (0.3 mg/kg body weight/day).

Discussion

This patient suffered from a severe, prolonged episode of acute rejection, with temporary, significant loss of the donor heart function. The rejection process proved to be resistant to standard immunosuppressive therapy. In such an instance, the patient would undoubtedly have died if he had undergone an orthotopic heart transplant. In a number of cases, the presence of the recipient heart maintained life when acute rejection led to significant donor heart failure. The history of this patient is also remarkable by the extent of donor heart recovery after a prolonged period of negligible function that lasted almost 20 days. Although this patient received a conventional antirejection immunosuppressive therapy that was continued for a number of days after cyclosporine administration began, the introduction of cyclosporine appears to be responsible for reversing the severe acute rejection episode. A similar observation was made in six patients who had renal transplants at our hospital. All were initially receiving conventional immunosuppression with azathioprine and methylprednisolone, and all developed severe acute graft rejection unresponsive to an antirejection therapy consisting of pulses of intravenous methylprednisolone (1,000 mg, for a total of 6 pulses). Rejection was reversed in all cases following the introduction of oral cyclosporine (12.5 to 18 mg/kg body weight/day), given in addition to the conventional therapy. The cyclosporine treatment was continued for 7 to 27 days until the acute rejection episode was controlled, at which time cyclosporine was discontinued.

From the current knowledge of cyclosporine's pharmacokinetics, the drug would not be expected to reverse acute rejection, its mode of action being thought to be a blocker of T-cell production and release of interleukin-2, as well as a blocker of macrophage production of interleukin-1. Thus, once T-lymphocyte cell precursors are activated into cytotoxic T-lymphocytes, the administration of cyclosporine would be ineffective. The observation reported here suggests that cyclosporine might act on the rejection phenomenon through other modalities.

Acknowledgments. The authors are indebted to the many members of the medical, nursing, and paramedical staffs of Groote Schuur Hospital and the University of Cape Town Medical School who have contributed to the care of this patient. And to Sandoz Ltd. for making cyclosporine available.

References

Ex vivo functional evaluation of pig hearts subjected to 24 hours’ preservation by hypothermic perfusion


Summary
A system has been developed for the ex vivo functional testing of isolated hearts. Three groups, each of 10 pig hearts, have been studied: group 1 — freshly excised hearts; group 2 — hearts hypothermically perfused for 20 - 24 hours with Krebs-Henseleit solution; group 3 — hearts similarly perfused with a clear fluid hyperosmolar solution. Group 2 hearts performed poorly on functional testing and would clearly have been unsuitable for transplantation. Haemodynamic observations on group 3 hearts showed little statistical difference from those of group 1, suggesting good preservation. The value of the ex vivo testing system as a reliable means of assessing myocardial function is discussed, and has been confirmed by subsequent successful orthotopic transplantation of baboon hearts stored under identical conditions.

Methods
Thirty healthy pigs weighing 18 - 25 kg were used as heart donors. These animals were starved overnight, premedicated with ketamine hydrochloride (5 mg/kg), and naaesthetized with intravenous thiopentone sodium (2,5 mg/kg) and morphine (1,5 mg/kg). Intravenous alcuronium chloride (0,5 mg/kg) was used as a muscle relaxant. After endotracheal intubation, ventilation was maintained by a Bird Mark 8 ventilator with a closed circuit, which recycled oxygen 3 l/min and nitrous oxide 6 l/min. Ventilation was monitored by repeated determination of arterial Pco2, Po2 and pH. All pigs received methylprednisolone (Solu-Medrol; Upjohn) 125 mg intravenously 30 - 60 minutes before heart excision.

Cold cardioplegic arrest and excision of the heart
A median sternotomy was performed, and the pericardium incised. The superior vena cava (SVC), inferior vena cava (IVC), ayzygos vein, and aortic arch were mobilized. Intravenous heparin (1000 U/kg) was given. The brachiocephalic artery was ligated distally and cannulated proximally, the tip of the cannula lying in the aorta. The SVC, ayzygos vein and both right pulmonary veins were each doubly ligated and divided. The aorta was cross-clamped distal to the origin of the brachiocephalic artery, the IVC (after clamping at the diaphragm) and both left pulmonary veins were divided, followed immediately by the infusion of 500 ml cold (4°C) cardioplegic solution (Table 1). The heart was also bathed in 500 ml cold (4°C) normal saline. The heart stopped within 15 - 20 seconds of the beginning of the infusion (which was completed within 1 - 3 minutes), by which time myocardial temperature had fallen to approximately 18°C.

Excision of the heart was then completed, and the latter immersed in cold (4°C) normal saline for 1 minute. While immersed, the bridge of tissue between the orifices of the left superior and inferior pulmonary veins was excised and a continuous suture placed around this opening, for subsequent ligation around the left atrial cannula of the testing apparatus. The heart was then weighed and transferred to the perfusion apparatus, the total time from initiation of infusion of cardioplegic solution being approximately 10 minutes.

Continuous hypothermic perfusion for 20 - 24 hours
The non-pulsatile perfusion apparatus (Fig. 1) was based on that originally described by Proctor and Parker. It consists of two perfusion circuits, one delivering perfusate from the lower reservoir to the aorta, and the second sucking coronary venous return from the right ventricle and returning it to the lower reservoir, into which a 95% oxygen and 5% carbon dioxide mixture was continuously bubbled to maintain the perfusate pH between 7,2 and 7,4. One circuit incorporated a 0,8 µm filter, and the system was primed with 2 litres of perfusate.
The heart was perfused at 8-10 cm H$_2$O pressure for 20-24 hours at 6°-8°C. Myocardial temperatures stabilized after 1-2 hours at approximately 8°C.

Perfusate pH was measured at 30-minute intervals and adjusted by increasing or decreasing the gas mixture flow. All perfusate entering the right heart was by coronary venous return; coronary flow could therefore be readily collected and measured. After the period of perfusion the heart was weighed and functionally evaluated.

**Ex vivo functional testing**

The system is shown in Fig. 2. Oxygenated blood, matched for group, was supplied by a perfusor pig to which deoxygenated blood was returned. Initially, the aorta was retrogradely perfused as a Langendorff system supplying blood to the coronary arteries of the isolated heart. This allowed a period of recovery, usually continued for 5-15 minutes. When the myocardial temperature reached 37°C the heart was deairified and the model converted into the working heart mode. Blood was then directed into the left atrium of the isolated heart, converting the role of the heart from a passive to an active one by 'challenging' the left ventricle with blood from the left atrial reservoir. If myocardial preservation had been good, the left ventricle pumped blood through the aortic balloon past the non-linear resistance to a height of 100 cm H$_2$O, after which the blood was returned to the left atrial reservoir. Preload and afterload could be adjusted, but were kept constant at 11 cm H$_2$O and 80 mmHg respectively during the period of testing.

The following pressures were measured using a Statham P23H transducer: left ventricular pressure (LVP), left ventricular end-diastolic pressure, aortic pressure and aortic end-diastolic pressure. Cardiac output was obtained by a timed collection of aortic output plus coronary venous return. All hearts were tested for 1 hour during which time these parameters were recorded. The hearts were then reweighed and biopsied for histological examination by light microscopy.

We have evaluated 21 different perfusates by this method; the results in 3 groups, each of 10 hearts, are reported here:

- **group 1** — freshly excised hearts immediately suspended from the functional testing apparatus after cardioplegic arrest. (With no major ischaemic interval, these hearts were considered as controls with which the performance of preserved hearts could be compared);
- **group 2** — hearts perfused for 20-24 hours with Krebs-Henseleit solution (Table II);
- **group 3** — hearts perfused for 20-24 hours with a clear fluid hyperosmolar solution (Table II).
models of looking at structures, compositions, and functions, would require something in the order of 10^6 experiments. Orthotopic transplantation is therefore impossible as a routine method of evaluation of cardiopлегic agents, perfusates and perfusion systems, since the choice of perfusate is similarly almost unlimited.

Ideally any such test of organ viability should be simple, rapid and reproducible. The search for such a test has explored two main routes. The first of these is a simple, single measurement or observation that confirms that the tissues under study are not irreversibly damaged; this may take the form of, for example, a visual assay of a myocardial enzyme system, a histochemical change, or the monitoring of a fundamental metabolic event such as anaerobic glycolysis or lactic acid production. The second is a functional evaluation of the isolated heart.

Functional testing has been widely used as a means of illustrating the efficacy of a method of preservation. Many such methods, however, involved possible damage to the heart being tested, such as the insertion of a purse-string suture into (or even excision of) the mitral valve leaflets for the housing of a soft latex balloon in the left ventricle. The system used in the present study has the great advantage that a left ventricular balloon is not necessary; this allows for the measurement of cardiac output as well as for pressure determinations.

An interpretation of the dynamics of the system used in the present study is difficult and complex. However, by comparing the performance of preserved hearts with that of freshly excised hearts, we believe we have been able to gain valuable information about the preservation systems, cardiopлегic agents, and perfusates which we have used. Hearts shown to be well-preserved by functional evaluation in our laboratory have been able to support the circulation after orthotopic transplantation. We believe that the method is sufficiently reliable to permit quantitative screening of various preservation techniques.

The extremely poor performance of the group 2 hearts on functional testing could be related to the different cardiopлегic agent used, but we believe is more likely related to the different perfusate. The minor functional differences noted between the hearts of group 1 and 3 must be related to the 24-hour perfusion, as these hearts received the same cardiopлегic agent. Further studies in our laboratory suggest that the cardiopлегic agent used in group 1 provides a more beneficial effect than that used in group 2, a higher coronary flow (and lower coronary resistance) being measured at the onset of the perfusion period in hearts being preserved.

The modified Krebs solution used in the group 2 experiments was clearly unsatisfactory. The hyperosmolar solution used in the group 3 experiments gave haemodynamic functional results comparable to those of freshly excised hearts (group 1). Mean cardiac output in group 3 hearts was not significantly different from that in group 1 hearts. However, the LVSP and aortic pressure were significantly reduced in group 3 compared with group 1 (Table III). There were no other significant differences between these two groups.

The perfusate used in the group 3 experiments contained agents included for their osmotic effect, agents included for their vasodilator effect, and agents included for metabolic inhibition and/or membrane stabilization. A high concentration of potassium was avoided in the cardiopлегic as well as the preservation solution to reduce any possibility of activation of the slow inward calcium current which may increase the tone of the heart during preservation.

Furthermore, it has been reported that a high potassium concentration results in increased renal vascular resistance at concentrations greater than 12 mmol/l, and its effect on the coronary vessels in this respect is unclear. The role of glycerol was twofold, namely to slow down the process of oedema formation and to impart some cryoprotective effect to the myocardium, although ice formation was not anticipated as 6°C to 8°C. Glycerol may be important in preventing damage by sudden temperature change; it has been shown that both rapid and slow cooling can be harmful, depending on the type of tissue which is being cooled. 11 Taurine constitutes about 50% of the free amino acid pool found in the mammalian species and is concentrated particularly in heart and skeletal muscle; there is also experimental evidence of numerous protective biological actions including a reduction in cellular potassium loss, and a protective effect on calcium binding and ATPase activity in the sarcoplasmic reticulum. 12, 13 Certainly in our own studies, the addition of taurine alone to Krebs-Henseleit solution brought about a dramatic reduction in the release of lactate dehydrogenase from the myocardium of the isolated hearts during the 24-hour period of perfusion. (W. Wicomb — unpublished data.)

The ideal pH at which to maintain the heart during the period of hypothermic perfusion remains uncertain, and this has been fully discussed elsewhere.

A possible explanation for the decreased LVSP noted in group 3 hearts when compared with those of group 1 is increased left ventricular wall stiffness due to considerable oedema formation. The problem of oedema formation remains controversial. In the development of a satisfactory perfusate for 24-hour preservation we have evaluated numerous solutions designed to reduce oedema formation, containing, for example, Dextran 40, mannitol or albumin. However, we have found no definite correlation between the degree of oedema and the haemodynamic performance of the heart on ex vivo testing or after orthotopic heart transplantation. What appears to be of greater importance is whether the oedema is reversible. The inclusion of many small yet osmotically active agents such as ionic phosphate and sulphate, non-ionic glucose, sucrose and glycerol as in the group 3 perfusate, led to improved haemodynamic results, though with very considerable oedema formation. The observation that group 3 hearts lost 16% of their post-preservation weight after only 1 hour's functional testing shows that the oedema is, at least in part, reversible. A longer period of reperfusion of the perfused hearts might well lead to a further loss of oedema and even to a return of the pre-preservation heart weight.

The histological appearance of the myocardium on light microscopy after 24 hours' hypothermic perfusion and 1 hour's re-perfusion is clearly a poor indicator of the functional state of the heart. There was little difference between the histological features of the group 2 hearts, which were clearly functionally non-viable, and the group 3 hearts, which functioned almost as well as freshly excised hearts.

We have subsequently confirmed that the perfusion system and perfusate used in group 3 led to consistently successful 24-hour preservation by orthotopically transplanting 6 similarly preserved baboon hearts. All animals survived with good myocardial function until rejection, with a mean survival period of 197±17 days. These results also lend support to the value of our functional testing system in the assessment of preserved hearts, as good results following orthotopic transplantation had been predicted by previous ex vivo functional testing.

We wish to thank P. Barends, Sister D. Kerr, P. Madlingozi, Miss J. Martin, S. Patel, J. Rousrouw, Miss S. Smit and F. Snayers for skilled technical assistance. This work was supported by the Chris Barnard Fund, the University of Cape Town, and the Cape Provincial Administration.

REFERENCES

### TABLE I. CONSTITUTION OF THE PERFUSATES

<table>
<thead>
<tr>
<th>Group 2</th>
<th>Group 3</th>
</tr>
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<tbody>
<tr>
<td>g/l</td>
<td>mM</td>
</tr>
<tr>
<td>1. NaCl</td>
<td>6.93</td>
</tr>
<tr>
<td>2. NaHCO₃</td>
<td>2.10</td>
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<tr>
<td>3. KCl</td>
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<tr>
<td>4. KH₂PO₄</td>
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<tr>
<td>5. CaCl₂·2H₂O</td>
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<tr>
<td>6. MgSO₄·7H₂O</td>
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<tr>
<td>7. Glucose</td>
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<tr>
<td>8. Sucrose</td>
<td>—</td>
</tr>
<tr>
<td>9. Glycerol</td>
<td>—</td>
</tr>
<tr>
<td>10. Taurine</td>
<td>—</td>
</tr>
<tr>
<td>11. Procaine HCl</td>
<td>—</td>
</tr>
<tr>
<td>12. Chlorpromazine</td>
<td>—</td>
</tr>
<tr>
<td>13. Phenoxylbenzamine</td>
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</tr>
</tbody>
</table>

Osmolality 275 mOsm/l
pH at 8°C 7.2 - 7.4

### Results

**Observations during the period of hypothermic perfusion**

In group 2 hearts coronary flow at the beginning of the perfusion period was low (mean 0.077 ml/min/g) and became steadily less over the 24 hours; in 5 of the 10 hearts no coronary flow could be recorded after 24 hours and in the remaining 5 hearts flow was less than 0.045 ml/min/g. Coronary resistance has not been calculated in this group.

In group 3 hearts initial coronary flow was 0.158 ml/min/g and fell to 0.087 ml/min/g at the end of the perfusion period; coronary resistance rose from an initial 55.38 cm H₂O/g/min/ml to 147.88 cm H₂O/g/min/ml at the end of perfusion.

**Observations during functional testing**

**Group 1.** These hearts quickly resumed sinus rhythm with forceful contractions. Haemodynamic measurements are listed in Table III. The mean cardiac output was 2.81/min, LVP 131 mmHg, and aortic pressure 114 mmHg. Heart weight did not change significantly during the 60 minutes of functional testing. Microscopic examination revealed no significant abnormalities.

**Group 2.** These hearts were oedematous at the end of the preservation period with a mean increase in weight of 21%. After reperfusion with blood and functional testing for 1 hour a further 2% weight increase occurred. The functional response was always poor. Contractions were weak and no cardiac output could be measured in 6 of the 10 hearts tested (Table III). The mean LVP of these hearts was 72 mmHg, not enough to overcome the afterload of the system. With such a poor functional response no further parameters were determined. At the end of the testing period the hearts felt hard to the touch; histological examination, however, showed interstitial oedema but no other significant changes.

**Group 3.** These hearts were markedly oedematous with an 82% increase in weight at the end of the perfusion period, but this accumulation of fluid was at least partly reversible upon reperfusion with blood as there was a mean loss of weight of 16% after 1 hour's functional testing. Haemodynamic observations are listed in Table III. Histological examination revealed interstitial oedema, but the myofibres and nuclei appeared well-preserved. Myofibrillar degeneration and scanty haemorrhage were present on occasions. No subendothelial haemorrhage or coagulative necrosis was observed.

### Discussion

The heart is unique as a transplanted organ in that it must be capable of full function immediately after its insertion into the recipient. The need for a test of functional viability of the organ is therefore essential if even short-term storage of the heart is to become a clinical reality. The ultimate measure of viability of the preserved heart is its capacity to fully support the circulation after orthotopic transplantation. Ethically this cannot be a test of function, and ideally the viability should be known throughout the period of storage and certainly at the time of transplantation.

Similarly, orthotopic transplantation as a means of testing all cardioplegic agents and storage perfusates is logistically impossible. Katz has pointed out that the large number of individual ingredients of cardioplegic solutions in various concentrations, volumes, temperatures, pressures, and rates of injections, and in all their possible combinations, according to the effect of each on the heart by all the possible means and

### TABLE III. HAEMODYNAMIC PARAMETERS (MEAN) ON FUNCTIONAL TESTING

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (N=10)</th>
<th>Group 2 (N=10)</th>
<th>Group 3 (N=10)</th>
<th>Statistical comparison</th>
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</thead>
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<tr>
<td>Cardiac output (l/min)</td>
<td>2.82</td>
<td>0.59</td>
<td>2.71</td>
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<tr>
<td>Coronary flow (ml/min)</td>
<td>258</td>
<td>122</td>
<td>334</td>
<td>NS</td>
</tr>
<tr>
<td>Stroke volume (ml/beat)</td>
<td>17</td>
<td>—</td>
<td>18</td>
<td>NS</td>
</tr>
<tr>
<td>Left ventricular pressure (mmHg)</td>
<td>131</td>
<td>72</td>
<td>114</td>
<td>P&lt;0.01</td>
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<tr>
<td>Left ventricular end-diastolic pressure (mmHg)</td>
<td>5.3</td>
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<td>3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Aortic pressure (mmHg)</td>
<td>114</td>
<td>—</td>
<td>94</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Aortic end-diastolic pressure (mmHg)</td>
<td>70</td>
<td>—</td>
<td>67</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = not statistically significant (P > 0.1).
Orthotopic transplantation of the baboon heart after 20 to 24 hours' preservation by continuous hypothermic perfusion with an oxygenated hyperosmolar solution

Baboon hearts were rapidly excised after being flushed with 500 ml of cardioplegic solution at 4°C and then immersed in cold 4°C saline or cardioplegic solution for 2 minutes. The hearts were then perfused at 8 to 10 cm H2O pressure for 20 to 24 hours under refrigeration with a hyperosmolar clear fluid perfusate at 6 to 8°C, through which 95% oxygen and 5% carbon dioxide were continually bubbled to maintain the perfusate pH between 7.2 and 7.4. Myocardial temperature remained at approximately 6 to 8°C. The hearts were then orthotopically transplanted into recipient baboons matched for size and AB blood group. Two groups (A and B) were studied, differing significantly only with respect to the constitution of the cardioplegic solution and perfusate used. The cardioplegic agent used in Group B contained a higher concentration of magnesium than that used in Group A and included the calcium antagonist verapamil. Perfusion B had higher osmolality than perfusate A, largely due to the inclusion of sucrose. A preliminary group of 10 baboons in Group A received no immunosuppression. Five of the remaining six immunosuppressed baboons in this group survived more than 48 hours to rejection or until killed at 2 to 29 days. All six of the baboons in Group B survived to rejection between 6 and 33 days, with mean survival 19.5 days. Cardiac catheterization was performed in six surviving baboons (Group A, four; Group B, two) between postoperative days 6 and 10 and showed good hemodynamic function. Histologic examination of hearts after death has shown only minor ischemic changes in those hearts which functioned well.


One of the limitations of heart transplantation is the availability of donor organs. Storage of the donor heart for periods of even 24 hours would allow transport of the heart to a suitable recipient or vice versa, and the emergency nature of the operation would be diminished. Successful preservation of hearts for such periods has been reported in the literature, but has not yet been transferred to the clinical situation. The aim of the studies presented here was to preserve functional viability of baboon hearts stored for 20 to 24 hours and to confirm such viability by orthotopic transplantation.

A review of the literature suggested that, to obtain successful 24 hour preservation, both hypothermia and continuous perfusion were needed.\(^1\)\(^-\)\(^4\)

Two combinations of cardioplegic solution and perfusate have been assessed; this paper is not primarily intended to compare these two studies, but rather to present and discuss a method of successful preservation of the isolated heart for periods of up to 24 hours.
Methods

Two groups (A and B) of outbred chacma baboons (Papio ursinus) have been studied. The two groups differed significantly only in the constitution of the cardioplegic solution and the perfusate used during 24 hour preservation; in almost all other respects the experimental method has been identical. Sixteen experiments were performed in Group A and six in Group B. Six animals in Group A and all six animals in Group B were given immunosuppressive agents after transplantation.

Cold cardioplegic arrest and donor heart excision. Baboons weighing 10 to 20 kg were premedicated with ketamine hydrochloride, 100 mg intramuscularly (IM). Each baboon was given pancuronium, 2 mg intravenously (IV); atropine, 0.3 mg IV; and morphine, 7.5 mg IV. The animal was intubated endotracheally and ventilated with oxygen (4 L/min) and nitrous oxide (6 L/min). Cephalothin sodium,* 1 gm IV, and methylprednisolone, 125 mg IV (in Group A only), were given. The heart was not excised until blood gases and acid-base balance were within normal limits.

Through a median sternotomy the azygos vein was doubly ligated and divided. Heparin (1,000 U/kg IV) was given. The superior vena cava (SVC) was then doubly ligated cephalad to the azygos vein and divided. The brachiocephalic artery was ligated distally and cannulated for subsequent cardioplegic infusion, the tip of the cannula lying in the arch of the aorta.

The aorta was cross-clamped distal to the cannula, followed by immediate division of the inferior vena cava (IVC) and one or more pulmonary veins. Then 500 ml of cardioplegic solution (Table I) at 4° C was infused, the infusion pressure not being allowed to rise above 100 mg Hg. Cessation of heartbeat occurred within 10 to 15 seconds if both right and left sides of the heart were adequately decompressed; the infusion of 500 ml of solution was completed within 1 to 3 minutes, by which time myocardial temperature had fallen to approximately 17° C.

Excision of the heart was then completed by division of the pulmonary veins, the pulmonary artery at its bifurcation, the aorta proximal to the brachiocephalic artery, and the mediastinal tissue posterior to the heart. The heart was then serially immersed for 1 minute in each of two bowls containing cold 4° C normal saline (Group A) or cardioplegic solution (Group B), myocardial temperature falling to approximately 12° C.

The heart was then weighed and transferred to the perfusion apparatus. The time interval between aortic cross-clamping and the initiation of perfusion under refrigerated conditions was approximately 10 to 12 minutes.

Continuous hypothermic perfusion for 20 to 24 hours. The heart was then perfused at 8 to 10 cm H2O pressure for 20 to 24 hours under refrigerated conditions (6 to 8° C) with a hyperosmolar clear fluid perfusate (Table II), through which 95% oxygen and 5% carbon dioxide were continuously bubbled to maintain

---

Table I. Constitution of the cardioplegic agents

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th></th>
<th>Group B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm/l</td>
<td>mM</td>
<td>gm/l</td>
<td>mM</td>
</tr>
<tr>
<td>NaCl</td>
<td>6.54</td>
<td>111.9</td>
<td>6.00</td>
<td>102.0</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>2.10</td>
<td>25.0</td>
<td>0.38</td>
<td>4.0</td>
</tr>
<tr>
<td>KCl</td>
<td>0.82</td>
<td>11.0</td>
<td>0.75</td>
<td>10.0</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>0.16</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCl2·2H2O</td>
<td>6.15</td>
<td>1.1</td>
<td>0.15</td>
<td>1.1</td>
</tr>
<tr>
<td>MgSO4·7H2O</td>
<td>0.29</td>
<td>1.2</td>
<td>3.50</td>
<td>14.0</td>
</tr>
<tr>
<td>Procain hydrochloride</td>
<td>0.27</td>
<td>1.0</td>
<td>0.27</td>
<td>1.0</td>
</tr>
<tr>
<td>Insulin</td>
<td>40 U/L</td>
<td>26 U/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>2.00</td>
<td>11.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td></td>
<td>50</td>
<td></td>
<td>278</td>
</tr>
<tr>
<td>Verapamil hydrochloride</td>
<td></td>
<td></td>
<td>1.5 mg</td>
<td></td>
</tr>
<tr>
<td>Osmolarity</td>
<td>300 mOsm</td>
<td>320 mOsm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH at 4° C</td>
<td>7.4</td>
<td></td>
<td>7.4</td>
<td></td>
</tr>
</tbody>
</table>

Table II. Constitution of the perfusates

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th></th>
<th>Group B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm/l</td>
<td>mM</td>
<td>gm/l</td>
<td>mM</td>
</tr>
<tr>
<td>NaCl</td>
<td>6.76</td>
<td>115.7</td>
<td>6.76</td>
<td>115.7</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>2.10</td>
<td>25.0</td>
<td>2.10</td>
<td>25.0</td>
</tr>
<tr>
<td>KCl</td>
<td>0.81</td>
<td>10.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KH2PO4</td>
<td>0.16</td>
<td>1.2</td>
<td>1.12</td>
<td>8.0</td>
</tr>
<tr>
<td>CaCl2·2H2O</td>
<td>0.16</td>
<td>1.1</td>
<td>0.16</td>
<td>1.1</td>
</tr>
<tr>
<td>MgSO4·7H2O</td>
<td>0.29</td>
<td>1.2</td>
<td>3.48</td>
<td>14.4</td>
</tr>
<tr>
<td>MgCl2·6H2O</td>
<td>3.00</td>
<td>14.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procaine hydrochloride</td>
<td>0.27</td>
<td>1.1</td>
<td>0.27</td>
<td>1.1</td>
</tr>
<tr>
<td>Insulin</td>
<td>40 U/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>2.00</td>
<td>11.1</td>
<td>2.00</td>
<td>11.1</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td></td>
<td>2.50</td>
<td>7.0</td>
</tr>
<tr>
<td>Glycerol</td>
<td>12.60</td>
<td>136.0</td>
<td>12.60</td>
<td>136.0</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.50</td>
<td>4.0</td>
<td>0.30</td>
<td>4.0</td>
</tr>
<tr>
<td>Osmolarity</td>
<td>330 mOsm</td>
<td>385 mOsm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH at 8° C</td>
<td>7.2-7.4</td>
<td></td>
<td>7.2-7.4</td>
<td></td>
</tr>
</tbody>
</table>

*Kellin, Lilly Laboratories, SA (Pty.) Ltd.
the perfusate pH between 7.2 and 7.4. Myocardial temperature stabilized after 1 to 2 hours at approximately 8°C.

The nonpulsatile perfusion apparatus (Fig. 1) was based on that originally described by Proctor and Parker and subsequently used by Copeland and associates. It consisted of a cylindrical Perspex 4 L chamber in which the heart was suspended by the aorta, through which it was perfused. The two perfusion circuits consisted of sterile polyvinyl tubing and roller pumps. One circuit, incorporating a 0.8 µ filter delivered perfusate from the lower reservoir to the aorta; the second circuit sucked coronary venous return from the right ventricle and returned it to the lower reservoir, where the oxygen—carbon dioxide mixture was bubbled into it. The system was primed with 2 L of perfusate.

Perfusate pH was measured at 30 minute intervals and adjusted by increasing or decreasing the gas mixture flow. All perfusate entering the right ventricle was coronary venous return; coronary flow could therefore be readily collected and measured. At the beginning and end of the perfusion period, coronary flow and coronary arteriovenous oxygen uptake were measured and coronary resistance was calculated.

**Technique of orthotopic transplantation.** Baboons, weighing 10 to 20 kg and matched for AB blood group with the donor baboons, were premedicated, anesthetized, and ventilated as were the donors.

Double the doses of pancuronium, atropine, and morphine were given, and these doses were repeated after opening the chest. Cephalothin sodium, 1 gm IV, was given and repeated every 8 hours for 3 days. Methylprednisolone, 25 mg IV, was given and was repeated at intervals during the operative and early postoperative periods until a total of 125 mg had been administered (or 10 mg/kg body weight in those baboons to be immunosuppressed). Vitamin K, 20 mg IV, was also given.

The technique of recipient heart excision and of donor heart insertion followed the description by Lower and Shumway, Lower, Stofer, and Shumway, and Stinson and associates, incorporating the modifications suggested by Barnard. Only when the recipient heart had been excised was the donor heart removed from the perfusion apparatus; the donor heart was weighed. At 20 minute intervals throughout the period of insertion, saline at 4°C was injected by syringe into the pericardium to cover the heart to maintain a low myocardial temperature. Myocardial temperature rose to approximately 20°C during the period of insertion.

It has been our policy to continue pump-oxygenator support for 60 minutes following the release of the aortic clamp. Spontaneous coordinated contractions, rarely, ventricular fibrillation requiring electrical defibrillation, occurred. Before discontinuation of cardiopulmonary bypass, an isoproterenol hydrochloride infusion (1 mcg/min) was set up; if urine output was low, furosemide, 3 to 5 mg IV, was administered.

**Postoperative management.** The baboon was weaned off ventilator support over the next few hours.

---

**Fig. 1. Diagram of nonpulsatile perfusion apparatus used for 24 hour hypothermic preservation.**
Table III. Changes in measured parameters during the period of continuous perfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean heart weight (gm)</th>
<th>Mean % change in heart weight</th>
<th>Coronary flow (ml/min/gm)</th>
<th>Mean % change in coronary flow</th>
<th>Coronary vascular resistance (cm Hg/ml/min/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At beginning of perfusion</td>
<td>97.0 (8.4)†</td>
<td>+32% (10%)</td>
<td>0.14 (0.012)</td>
<td>-1% (4.3%)</td>
<td>68.4 (9.8)</td>
</tr>
<tr>
<td>At end of perfusion</td>
<td>116.4 (5.0)</td>
<td>p &lt; 0.001</td>
<td>0.12 (0.010)</td>
<td>p &lt; 0.01</td>
<td>76.6 (10.8)</td>
</tr>
<tr>
<td><strong>Group B:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At beginning of perfusion</td>
<td>70.5 (7.3)</td>
<td>+82% (6%)</td>
<td>0.22 (0.015)</td>
<td>-25% (6.0%)</td>
<td>37.0 (2.6)</td>
</tr>
<tr>
<td>At end of perfusion</td>
<td>126.0 (9.1)</td>
<td>p &lt; 0.001</td>
<td>0.17 (0.018)</td>
<td>p &lt; 0.01</td>
<td>51.1 (6.6)</td>
</tr>
<tr>
<td><strong>Comparison, Groups A/B:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At beginning of perfusion</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>NS</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>At end of perfusion</td>
<td>NS</td>
<td>p &lt; 0.05</td>
<td></td>
<td>p &lt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Legend: AV, Arteriovenous. NS, Not statistically significant.
†Using initial weight of the heart.
*Figures in parentheses denote standard error.

Results

Observations during the period of hypothermic perfusion. Hearts in Group A had, by chance, an initial greater mean weight than those in Group B. All hearts increased in weight during the period of perfusion owing to edema formation. The mean increase in weight was 32% in Group A and 82% in Group B (Table III).

Initial mean coronary flow was significantly greater in Group B than in Group A. Coronary flow fell or remained unchanged in all hearts during the perfusion period, the mean percentage fall being greater in Group B, although this difference was not statistically significant. The coronary flow at the end of the perfusion period remained, however, significantly greater in Group B hearts than in Group A.

Initial mean coronary vascular resistance was significantly greater in Group A than Group B and rose during the perfusion period in all cases except one. Coronary vascular resistance at the end of perfusion was similarly significantly greater in Group A, but the actual increase in resistance from the beginning to the end of perfusion was not statistically significant in Group A, although it was in Group B. However, the mean percentage increase in resistance in Group B hearts was not significantly different from that in Group A hearts.

There was no consistent pattern of change in coronary arteriovenous oxygen uptake in either group, some hearts showing an increase and some a decrease in oxygen uptake during the perfusion period. Overall, mean
Orthotopic transplantation of baboon heart

<table>
<thead>
<tr>
<th>Mean % change in coronary vascular resistance</th>
<th>Coronary AV oxygen uptake* (mm Hg/min/gm)</th>
<th>Mean % change in coronary AV oxygen uptake*</th>
</tr>
</thead>
<tbody>
<tr>
<td>+15% (6.5%)</td>
<td>0.21 (0.078)</td>
<td>+43% (29%)</td>
</tr>
<tr>
<td></td>
<td>0.27 (0.050)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>+37% (10.5%)</td>
<td>0.27 (0.060)</td>
<td>+49% (45%)</td>
</tr>
<tr>
<td></td>
<td>0.35 (0.080)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Oxygen uptake rose in both groups during the perfusion period, the rise not being significant.

Results of orthotopic transplantation. Twenty-two experiments have been performed (Group A, 16; Group B, six) (Table IV). In the preliminary series of 10 experiments technical problems contributed toward a number of deaths; immunosuppression was not used and rejection occurred early. Of the immunosuppressed animals in Group A, five of six survived longer than 48 hours until rejection or until killed for histologic examination. In Group B all six baboons survived until rejection, with a mean survival period of 19.5 days.

Immediately before the baboon was returned to its cage (6 to 12 hours after discontinuation of cardiopulmonary bypass), mean arterial pressure in both groups had risen to 90 to 140 mm Hg with mean central venous pressures of 1 to 5 cm H2O, without inotropic support. In all animals surviving longer than 48 hours the electrocardiogram was normal; occasional flattening or inversion of the T wave in the left chest leads has been noted, but we have found this to be a not uncommon finding in healthy baboons.

Good hemodynamic function has been confirmed at cardiac catheterization in six immunosuppressed animals (Table V); virtually normal pressures were obtained. The slight pressure gradient between the right ventricle and distal pulmonary artery was accounted for by some narrowing occurring at the pulmonary artery suture line, confirmed at necropsy.

The myocardium was examined histologically in most cases (Table IV). Those baboons which died from technical complications in the early postoperative period generally showed a well-preserved myocardium with some interstitial edema, but no features of major ischemic injury. Those baboons killed between days 2 and 10 showed minor features of ischemic damage, notably myofibrillar degeneration (contraction band necrosis and loss of cross-striation). Those baboons which survived until rejection showed the features of severe acute rejection with mononuclear cell infiltration, edema, and myofiber damage.¹⁰

Only in those baboons where myocardial function was obviously poor throughout the postoperative period from inadequate preservation have there been microscopic features of severe ischemic injury to the myocardium. In Group A (1) baboon No. 7 showed focal myofibrillar degeneration, early myocytolysis, plus focal coagulative necrosis. Interstitial hemorrhage was also present in areas. These findings suggested a reperfusion type of myofiber necrosis; the heart failed within 48 hours.

Discussion

The preservation of myocardial viability during periods of ischemia has been a major concern of cardiac surgeons for several years, although most interest has been directed toward the relatively short periods of anoxia experienced following aortic occlusion during cardiac operations. However, several groups have attempted preservation of hearts for longer periods, though few have assessed the efficacy of their preservation systems by the ultimate test of orthotopic transplantation; the early research in this field has been fully reviewed.¹¹,¹²

Survival of animals receiving orthotopically placed donor hearts preserved for 24 hours has rarely been for more than a matter of hours. Kondo and his colleagues¹³ reported 5 day survival in one of 29 puppies after donor heart preservation by hyperbaric oxygenation at 3 to 4 atmospheres. Hyperbaric oxygenation and hypothermic perfusion together have been employed with survival of one of six animals for 8 days.¹⁴

The studies of Proctor, Mathews, and Archibald¹⁵ suggested that hearts preserved by hypothermic perfusion for as long as 72 hours retained viability, but this group did not attempt to obtain long-term survival after orthotopic transplantation, discontinuing assessment of the heart after 1 ½ to 2 hours’ function. Using a preservation system based on that of Proctor, Copeland and his associates¹ reported four of 30 dogs surviving longer than 4 days to rejection. Hearts suspended, but not perfused or oxygenated, in a hypothermic, intracellular, high potassium solution for up to 26 hours have been successfully transplanted, though only four of 20 survived to rejection, the longest survival being 5½
Table IV. Results of orthotopic transplantation following 20 to 24 hours' storage of the donor heart

<table>
<thead>
<tr>
<th>Baboon</th>
<th>Survival</th>
<th>Cause of death</th>
<th>Myocardial histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (i) (not immunosuppressed):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6 hr</td>
<td>Persistent acidosis</td>
<td>Well preserved</td>
</tr>
<tr>
<td>2</td>
<td>1 hr</td>
<td>Air emboli</td>
<td>Well preserved</td>
</tr>
<tr>
<td>3</td>
<td>6 days</td>
<td>Rejection</td>
<td>Rejection</td>
</tr>
<tr>
<td>4</td>
<td>6 days</td>
<td>Rejection</td>
<td>Rejection</td>
</tr>
<tr>
<td>5</td>
<td>3 days</td>
<td>Unclear</td>
<td>No sample taken</td>
</tr>
<tr>
<td>6</td>
<td>10 days</td>
<td>Killed</td>
<td>Myofibrillar degeneration/early rejection</td>
</tr>
<tr>
<td>7</td>
<td>1 day</td>
<td>Cardiac failure</td>
<td>Myofiber necrosis</td>
</tr>
<tr>
<td>8</td>
<td>4 days</td>
<td>Killed (gangrene of foot)</td>
<td>No sample taken</td>
</tr>
<tr>
<td>9</td>
<td>7 days</td>
<td>Rejection</td>
<td>Rejection</td>
</tr>
<tr>
<td>10</td>
<td>1 day</td>
<td>Respiratory failure (pulmonary atelectasis)</td>
<td>No sample taken</td>
</tr>
<tr>
<td>Group A (ii) (immunosuppressed):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10 hr</td>
<td>Unclear (cardiac arrest following NaHCO₃ infusion)</td>
<td>Well preserved</td>
</tr>
<tr>
<td>2</td>
<td>26 days</td>
<td>Rejection</td>
<td>Rejection</td>
</tr>
<tr>
<td>3</td>
<td>2 days</td>
<td>Killed</td>
<td>Interstitial edema</td>
</tr>
<tr>
<td>4</td>
<td>6 days</td>
<td>Killed</td>
<td>Myofibrillar degeneration</td>
</tr>
<tr>
<td>5</td>
<td>29 days</td>
<td>Rejection</td>
<td>Rejection</td>
</tr>
<tr>
<td>6</td>
<td>3 days</td>
<td>Killed (brain damage)</td>
<td>Myofibrillar degeneration</td>
</tr>
<tr>
<td>Group B (immunosuppressed):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>27 days</td>
<td>Rejection</td>
<td>Rejection</td>
</tr>
<tr>
<td>2</td>
<td>33 days</td>
<td>Rejection/emphyema</td>
<td>Rejection</td>
</tr>
<tr>
<td>3</td>
<td>12 days</td>
<td>Rejection</td>
<td>Rejection</td>
</tr>
<tr>
<td>4</td>
<td>8 days</td>
<td>Rejection</td>
<td>Rejection</td>
</tr>
<tr>
<td>5</td>
<td>6 days</td>
<td>Rejection</td>
<td>Rejection</td>
</tr>
<tr>
<td>6</td>
<td>31 days</td>
<td>Rejection</td>
<td>Rejection</td>
</tr>
</tbody>
</table>

Table V. Data obtained at cardiac catheterization 6 to 10 days after orthotopic transplantation*

<table>
<thead>
<tr>
<th>Group A (i)</th>
<th>Group A (ii)</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 1)</td>
<td>(n = 2)</td>
<td>(n = 2)</td>
</tr>
<tr>
<td>Right atrium</td>
<td>7.5</td>
<td>11.5 (1.3)</td>
</tr>
<tr>
<td>Right ventricle (systolic)</td>
<td>30.0</td>
<td>46.0 (4.6)</td>
</tr>
<tr>
<td>Main pulmonary artery (systolic)</td>
<td>17.0</td>
<td>31.7 (1.5)</td>
</tr>
<tr>
<td>Pulmonary capillary wedge</td>
<td>8.0</td>
<td>11.2 (0.6)</td>
</tr>
<tr>
<td>Left ventricle (systolic)</td>
<td>95.0</td>
<td>97.8 (6.3)</td>
</tr>
<tr>
<td>Left ventricular end-diastolic</td>
<td>6.0</td>
<td>6.0 (2.1)</td>
</tr>
<tr>
<td>Aorta (systolic)</td>
<td>91.0</td>
<td>91.7 (7.9)</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>Not measured</td>
<td>1.4</td>
</tr>
<tr>
<td>Max. dp/dt (mm Hg/sec) (n = 2)</td>
<td>Not measured</td>
<td>2.090</td>
</tr>
</tbody>
</table>

*Except where stated, figures refer to mean pressure in millimeters of mercury. Figures in parentheses indicate standard error in millimeters of mercury.

definitely survived for longer than 5 days. The present study, therefore, constitutes a considerable advance on previous reports, in that 14 of 22 baboons survived to rejection or were put to death after a minimum of 6 days after transplantation, five baboons dying from rejection at 26, 27, 29, 31, and 33 days, respectively. All six animals in Group B survived to rejection, with a mean survival time of 19.5 days.

We believe that the rapid cessation of function brought about by the metabolic inhibition and cooling effect of a cold cardioplegic agent is an important first step in successful preservation. In Group B the cardioplegic agent included a higher concentration of magnesium sulfate and the calcium antagonist verapamil in a low dose. This solution, possibly through the inclusion of verapamil, would appear to maintain a lower coronary vascular resistance and allow a greater coronary flow with no significant change in arteriovenous oxygen uptake during the period of preservation when compared with the cardioplegic solution used in Group A.
Orthotopic transplantation of baboon heart

The authors wish to express their thanks to Dr. F. I. Thandroyen and Mr. S. T. Boyd, B.Sc. (Hons.), for performing the cardiaca catheter studies, and to F. Barends, Sister D. Kerr, P. Madlingozi, Miss J. Martin, S. Patel, J. Roussouw, Miss S. Smi, and F. Smyders for skilled technical assistance.

REFERENCES


17 Raison JK, Lyons JM: Hibernation. Alteration of mio-

acids found in the myocardium in tested mammalian species, and there is experimental evidence that it reduces cellular potassium loss and has a protective effect on magnesium adenosine triphosphatase. 18, 19 Certainly in our preliminary studies, the addition of taurine alone to Krebs solution brought about a dramatic reduction in the release of lactic dehydrogenase from the myocardium of the isolated heart during the 24 hour period of perfusion (Wicomb: Unpublished data).

Although, by chance, the hearts in Group A were initially significantly heavier than those in Group B, the considerably increased edema formation in Group B hearts resulted in a much greater weight gain during perfusion. It is clear from our results that edema formation does not prevent subsequent and adequate myocardial function. Studies in our laboratory with pig hearts similarly preserved have shown a return toward normal weight from loss of edema within the first few hours of reperfusion. 10 We believe that this greater edema formation in Group B hearts was related to the presence in the perfusate of a greater number of osmotically active molecules of low molecular weight, such as phosphates, sulfates, and sucrose. Rapid diffusion of these small molecules back into the vascular compartment follows reperfusion with blood.

A marked increase in the coronary vascular resistance during the period of perfusion was generally considered a herald of relatively poor hemodynamic function after transplantation, but this conclusion was by no means reliable. This is in general agreement with the observations of Proctor and Jones, 20 though these authors emphasized that the pattern of resistance change was as valuable as the final resistance as a guide to subsequent performance after transplantation. We have not found the resistance pattern or final resistance to be such a reliable indicator.

Microscopic observations on the myocardium of the preserved hearts in this study have confirmed good histologic preservation of hearts in which good hemodynamic function was noted. The myofibrillar degeneration seen in some hearts suggests a relatively mild degree of hypoxic cellular damage. 4, 21, 22 In the one heart in which myocardial performance was quite obviously inadequate, there were histologic features suggesting severe damage. In animals surviving more than a few days the appearances were those of rejection. To avoid the intervention of rejection, we have recently embarked on a program of autotransplantation in which, during the 24 hour period of heart storage, the baboon’s circulation is maintained by an orthotopic cardiac allograft; at the end of this period the baboon’s own heart, preserved ex vivo for 24 hours, is replaced.
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Chondrial membranes as a requisite for metabolism at low temperature. Proc Natl Acad Sci 68:2092-2094, 1971
TWENTY-FOUR-HOUR PRESERVATION OF THE PIG HEART BY A PORTABLE HYPOTHERMIC PERFUSION SYSTEM

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A portable hypothermic perfusion system for storage of hearts has been developed. The system uses the airlift pump principle, whereby the flow of gas maintains circulation of the perfusate through the heart; no other energy source is required. Performance on ex vivo functional testing of 10 pig hearts stored for 20 to 24 hr using this system (group 3) was compared with that of freshly excised hearts (group 1) and hearts stored simply in the perfusate under hypothermic conditions, but not perfused (group 2). Group 2 hearts performed less well on functional testing than those of groups 1 and 3 which showed little statistical difference, suggesting good preservation by hypothermic perfusion. This has been confirmed by orthotopic transplantation of similarly preserved baboon hearts with survival until rejection at a mean of 27 days. The importance of the various constituents of the perfusate and the significance of weight gain during the storage and reperfusion periods are discussed.

One of the major limiting factors to the number of heart transplant operations performed each year in our own unit is the availability of donor organs (1). A successful portable method of storing donor hearts for 24 hr would ease this problem by enabling hearts to be "harvested" at distant centers and transported over long distances, and would also facilitate the logistics of transplantation by allowing for tissue typing, preparation of the recipient, and the convenient timing of the operative procedure.

We have recently reported preservation of pig hearts for 20 to 24 hr by ex vivo hypothermic perfusion; functional testing of these hearts after the period of preservation showed hemodynamic parameters indistinguishable from freshly excised hearts (2). Furthermore, orthotopic transplantation of baboon hearts, preserved for 20 to 24 hr by an identical technique, was followed by consistent survival of the recipient baboon until rejection occurred at a mean of 19.5 days (3).

Preservation in the above experiments was obtained using cardiopulmonary arrest of the donor heart followed by continuous perfusion at 8 to 10 cm of H₂O pressure at 6 to 8 °C with a hyperosmolar fluid perfusate, through which 95% O₂ and 5% CO₂ were bubbled to maintain the pH between 7.2 and 7.4. The nonpulsatile perfusion apparatus used was based on that originally described by Proctor and Parker (4); it incorporated two roller pumps and storage in a refrigerated room.

We have subsequently greatly modified and simplified the system to make it entirely and easily portable; in particular, we have done away with the need for any form of mechanical pump to maintain circulation of fluid through the heart.

We here describe the portable system and the results that we have obtained at functional testing of pig hearts, each preserved ex vivo for 20 to 24 hr.

MATERIALS AND METHODS

Healthy pigs, weighing 15 to 25 kg, were used as heart donors. Three groups of experiments were performed.

Group 1: freshly arrested hearts. After overnight starvation and premedication with ketamine hydrochloride (5 mg/kg), the animal was anesthetized with i.v. thiopentone sodium (2.5 mg/kg) and morphine (1.5 mg/kg). Alcuronium chloride (0.6 mg/kg i.v.) was used as a muscle relaxant. Endotracheal intubation was carried out and ventilation maintained with oxygen (3 liter/min) and nitrous oxide (6 liter/min).

Cold cardiopulmonary arrest and excision of the heart. A median sternotomy was performed. Heparin (1,000 units/kg, i.v.) was given. The brachiocephalic artery was ligated distally and cannulated proximally, the tip of the cannula lying in the aorta. The superior vena cava, azygos vein, and both right pulmonary veins were each doubly ligated and divided. The aorta was cross clamped distal to the origin of the brachiocephalic artery; the inferior vena cava (after clamping at the diaphragm) and both left pulmonary veins were divided, immediately followed by the infusion of 500 ml of cold (4 °C) cardioplegic solution (Table 1) through the brachiocephalic artery cannula, the infusion pressure not being allowed to rise above 100 mm Hg. The heart was also bathed in 500 ml of normal saline at 4 °C. Arrest of the heart occurred within 15 to 20 sec of the beginning of the infusion (which was completed within 1 to 3 min), by which time the myocardial temperature had fallen to approximately 18 °C.

Excision of the heart was then completed, and the heart immersed in cold (4 °C) normal saline for 1 min. While immersed, the bridge of tissue between the orifices of the left superior and inferior pulmonary veins was excised and a continuous suture placed around this opening, for subsequent ligation around the left atrial cannula of the testing apparatus.

The heart was then weighed and transferred to the functional testing apparatus, the total time for initiation of infusion of cardioplegic solution taking approximately 10 to 14 min.

Group 2: hearts stored in ice. Preparation, anesthesia, cold cardiopulmonary arrest, and excision of the heart were identical to those of group 1 pigs. The excised heart was then placed in a plastic bag containing a cold (4 °C) storage solution (Table 2) and packed in ice. Temperature of the solution was maintained between 1 and 6 °C for 20 to 24 hr. The heart was then taken out of the solution, weighed, and transferred to the functional testing apparatus.

Group 3: hypothermically perfused hearts. Preparation, an-
coronary arteries at a pressure of approximately 8 to 10 cm of H₂O the right atrium returned to the lower reservoir by gravity the ascending aorta of the suspended heart, perfusing the drainage via the inferior vena cava or pulmonary artery orifices. the upper chamber from filling completely with fluid.

ured at a partial pressure of approximately 1,000 mm Hg when approximately 60 to 120 ml/min. Perfusate P0₂ has been measured and maintained during this period of perfusion by the air-life pump principle (5).

A mixture of 97 % air, 3 % CO₂ was bubbled through a sterile gas filter into the perfusate in the lower reservoir through an air-ejector port inserted into the delivery tube, by which system gas was returned to the storage solution used in group 2 (Table 2), was sterilized and preserved for 20 to 24 hr.

Continuous hypothermic perfusion for 20 to 24 hr. The perfusion circuit is shown in Figure 1. The perfusate, identical to the storage solution used in group 2 (Table 2), was sterilized by being passed through a 0.8-μm filter, and the pH adjusted to an initial value of 7.2 by bubbling a 97 % O₂-3 % CO₂ mixture through it.

The perfusate was both oxygenated and circulated throughout this period of perfusion by the air-lift pump principle (5). A mixture of 97 % O₂ and 3 % CO₂ was bubbled through a sterile gas filter into the perfusate in the lower reservoir through an air-ejector port inserted into the delivery tube, by which system the fluid was transported to the upper chamber (reservoir) through a Cobe 20-μm filter. At a gas flow of approximately 500 ml/min, this gas solution maintained perfusate pH at 7.0 to 7.8 and maintained perfusate flow into the upper chamber at approximately 50 to 120 ml/min. Perfusate P0₂ has been measured at a partial pressure of approximately 1,000 mm Hg when determined at 30 C.

From the upper chamber, perfusate flowed by gravity into the ascending aorta of the suspended heart, perfusing the coronary arteries at a pressure of approximately 8 to 10 cm of H₂O, depending on aortic length. Coronary venous effluent to the right atrium returned to the lower reservoir by gravity drainage via the inferior vena cava or pulmonary artery orifices. From the lower chamber, the perfusate was recirculated. An overflow pipe between upper and lower reservoirs prevented the upper chamber from filling completely with fluid.

Two and one-half liters of perfusate were initially added to the lower chamber; the flow up the delivery tube could be increased or decreased simply by adding to or subtracting from this initial fluid load, enabling a constant gas flow to be maintained, and thus avoiding major fluctuations in pH which would follow changes in gas flow.

The perfusion apparatus was then placed in a stainless steel container insulated with polystyrene and perspex and packed with ice to maintain the desired temperature of 4 to 10 C throughout the 20- to 24-hr preservation period. At the onset and end of the perfusion period, coronary flow and myocardial oxygen uptake were measured; coronary vascular resistance was calculated. After the period of perfusion the heart was weighed and functionally tested.

Ex vivo functional testing (groups 1 to 3). The functional testing apparatus is shown in Figure 2. Oxygenated blood, matched for group, was supplied by a perfusor pig, to which deoxygenated blood was returned. Initially, the aorta was retrogradely perfused as a Langendorff system, supplying blood to the coronary arteries of the isolated heart. This allowed a period of recovery, usually continued for 5 to 15 min, during which the coronary venous blood and myocardial temperatures rose to 37 C and approximately 32 C, respectively, at which time the heart was defibrillated if spontaneous contractions had not already occurred. Blood was then directed into the left atrium of the isolated heart, converting the role of the heart from a passive to an active one by "challenging" the left ventricle with blood from the left atrial reservoir. The left ventricle pumped blood through the aortic balloon, past the nonlinear resistance, to a height of 100 cm/H₂O, after which the blood was returned to the left atrial reservoir. Preload and afterload could be adjusted, but were kept constant at 11 cm/H₂O and 80 mm/Hg, respectively, during the period of testing.

The left ventricular and left ventricular end-diastolic pressures were measured using a Statham P23H transducer. Cardiac output was obtained by a timed collection of aortic output plus

<table>
<thead>
<tr>
<th>Table 1. Constitution of cardioplegic solution</th>
<th>g/liter</th>
<th>mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NaCl</td>
<td>6.00</td>
<td>102.0</td>
</tr>
<tr>
<td>2. NaHCO₃</td>
<td>0.98</td>
<td>4.0</td>
</tr>
<tr>
<td>3. KCl</td>
<td>0.75</td>
<td>10.0</td>
</tr>
<tr>
<td>4. CaCl₂-2H₂O</td>
<td>0.15</td>
<td>1.1</td>
</tr>
<tr>
<td>5. MgSO₄-7H₂O</td>
<td>3.50</td>
<td>14.0</td>
</tr>
<tr>
<td>6. Procaine HCl</td>
<td>0.27</td>
<td>1.0</td>
</tr>
<tr>
<td>7. Insulin</td>
<td>20 U/l</td>
<td>—</td>
</tr>
<tr>
<td>8. Dextrose</td>
<td>50</td>
<td>278</td>
</tr>
<tr>
<td>9. Verapamil HCl</td>
<td>1.5 mg</td>
<td>—</td>
</tr>
<tr>
<td>pH at 4 C</td>
<td>7.4</td>
<td>—</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Constitution of the storage solution (group 2) and perfusate (group 3)</th>
<th>g/liter</th>
<th>mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NaCl</td>
<td>6.75</td>
<td>115.7</td>
</tr>
<tr>
<td>2. NaHCO₃</td>
<td>2.10</td>
<td>25.0</td>
</tr>
<tr>
<td>3. KH₂PO₄</td>
<td>1.12</td>
<td>8.0</td>
</tr>
<tr>
<td>4. CaCl₂-2H₂O</td>
<td>0.16</td>
<td>1.1</td>
</tr>
<tr>
<td>5. MgSO₄-7H₂O</td>
<td>3.48</td>
<td>14.4</td>
</tr>
<tr>
<td>6. Glucose</td>
<td>2.00</td>
<td>11.1</td>
</tr>
<tr>
<td>7. Sucrose</td>
<td>2.50</td>
<td>7.0</td>
</tr>
<tr>
<td>8. Glyceral</td>
<td>12.60</td>
<td>136.0</td>
</tr>
<tr>
<td>9. Taurine</td>
<td>0.56</td>
<td>4.0</td>
</tr>
<tr>
<td>10. Procaine HCl</td>
<td>0.27</td>
<td>1.1</td>
</tr>
<tr>
<td>11. Chlorpromazine</td>
<td>0.005</td>
<td>—</td>
</tr>
<tr>
<td>12. Phenoxybenzamine</td>
<td>0.01</td>
<td>—</td>
</tr>
<tr>
<td>pH at 8 C</td>
<td>7.2-7.4</td>
<td></td>
</tr>
</tbody>
</table>

esthesis, cold cardioplegic arrest and excision of the heart were as before. The excised heart was then transferred to a perfusion apparatus and preserved for 20 to 24 hr.

Continuous hypothermic perfusion for 20 to 24 hr. The perfusion circuit is shown in Figure 1. The perfusate, identical to the storage solution used in group 2 (Table 2), was sterilized by being passed through a 0.8-μm filter, and the pH adjusted to an initial value of 7.2 by bubbling a 97 % O₂-3 % CO₂ mixture through it.

The perfusate was both oxygenated and circulated throughout this period of perfusion by the air-lift pump principle (5). A mixture of 97 % O₂ and 3 % CO₂ was bubbled through a sterile gas filter into the perfusate in the lower reservoir through an air-ejector port inserted into the delivery tube, by which system the fluid was transported to the upper chamber (reservoir) through a Cobe 20-μm filter. At a gas flow of approximately 500 ml/min, this gas solution maintained perfusate pH at 7.0 to 7.8 and maintained perfusate flow into the upper chamber at approximately 50 to 120 ml/min. Perfusate P0₂ has been measured at a partial pressure of approximately 1,000 mm Hg when determined at 30 C.

From the upper chamber, perfusate flowed by gravity into the ascending aorta of the suspended heart, perfusing the coronary arteries at a pressure of approximately 8 to 10 cm of H₂O, depending on aortic length. Coronary venous effluent to the right atrium returned to the lower reservoir by gravity drainage via the inferior vena cava or pulmonary artery orifices. From the lower chamber, the perfusate was recirculated. An overflow pipe between upper and lower reservoirs prevented the upper chamber from filling completely with fluid.

Two and one-half liters of perfusate were initially added to
coronary venous return; stroke volume was calculated. Coronary flow was similarly measured by a timed collection of coronary venous return. Myocardial oxygen uptake was measured in groups 1 and 3. All hearts were tested for 1 hr during which time these parameters were recorded at regular intervals. The hearts were then reweighed and biopsied for histological examination by light microscopy.

RESULTS

Ten hearts were tested in each of the three groups.

Observations during the Period of Hypothermic Perfusion (Group 3)

Mean initial coronary flow was $15.9 \pm 1.6 \times 10^{-2}$ ml/min/g and dropped to $10.3 \pm 0.7 \times 10^{-2}$ ml/min/g at the end of the perfusion period ($P < 0.05$). Mean coronary vascular resistance rose from an initial $54 \pm 7$ cm H$_2$O/g/min/ml to $80 \pm 6$ cm H$_2$O/g/min/ml at the end of the perfusion ($P < 0.05$). Mean myocardial oxygen uptake was initially $4.3 \pm 0.9 \times 10^{-2}$ mmol/hr/g and rose to $5.6 \pm 0.8 \times 10^{-2}$ mmol/hr/g ($P > 0.1$).

Observations during Functional Testing (Groups 1 to 3)

Group 1. The results in these hearts have been reported previously (2). After approximately 10 min of passive perfusion, vigorous ventricular fibrillation had occurred; all hearts required electrical defibrillation. They then quickly resumed sinus rhythm with forcible contractions. Hemodynamic performance is documented in Table 3. The mean cardiac output was 2.8 liter/min and left ventricular pressure was 131 mm Hg. A ventricular function curve is shown in Figure 3. Heart weight did not change significantly during the 60 min of functional testing. Light microscopy revealed no significant abnormalities.

Group 2. Mean percentage weight gain during the period of storage was 6.8% and by the end of 60 min of reperfusion with blood and functional testing, there was an additional increase of 72.9%. These hearts, on rewarming, quickly resumed spontaneous sinus rhythm and, in the main, did not require defibrillation. While still being passively perfused, initial contractions were forcible, but in all cases deterioration occurred over the first 10-min period during which the hearts were expected to support a workload. Hemodynamic measurements, made after switching to the working mode, are listed in Table 3. Cardiac function showed only little recovery after the initial deterioration. Rhythm disorders were common in this group. Microscopy showed interstitial edema with capillary disruption in areas.

Group 3. These hearts were markedly edematous with a 79.2% increase in weight at the end of the preservation period, but there was no further accumulation of fluid during reperfusion with blood; in fact, there was a mean loss of weight of 7.5% after 1 hr of functional testing. The pattern of recovery was similar to that of group 1 hearts, namely, initial ventricular fibrillation requiring defibrillation, being followed by sinus rhythm with good contractions. There was a slow but steady improvement in performance over the course of the 1-hr testing. Hemodynamic observations during the “active” phase are listed in Table 3. Rhythm disorders were not seen in these hearts. A ventricular function curve is shown in Figure 3. Histological examination revealed interstitial edema, but the myofibers and nuclei appeared to be well preserved. Myofibrillar degeneration and scanty hemorrhage were present on occasion. No subendocardial hemorrhage or coagulative necrosis were observed.

DISCUSSION

The need for a reliable test of functional viability of an organ about to be transplanted has been discussed previously (2, 6), as has the necessity for a system of testing the efficacy of storage systems, cardioplegic agents, and/or perfusates (7).

The functional testing system used in the present study has the advantage that a left ventricular balloon is unnecessary, allowing the measurement of cardiac output as well as pressure determination. An interpretation of the dynamics of the system is difficult and complex. However, by comparing the performance of preserved hearts with that of freshly excised hearts, we believe we have been able to gain valuable information about the preservation systems, cardioplegic agents, and perfusates that we have used. Hearts shown to be well preserved by functional evaluation in our laboratory have been able to support the circulation after orthotopic transplantation (3). We believe that the method is sufficiently reliable to permit quantitative screening of various preservation techniques.

Hearts stored in ice (group 2) in several respects performed less well on functional testing than hearts freshly excised (group 1). Group 2 hearts gained weight during the reperfusion with blood, the rate of weight gain being inversely related to hemodynamic performance, i.e., a rapid increase in weight was associated with poor hemodynamic function. It would appear that these stored hearts had sustained damage to cell membranes, allowing an accumulation of intracellular fluid during the period of reperfusion; as this occurred, myocardial function deteriorated. We did not feel that these hearts would have been capable of supporting the circulation after orthotopic transplantation. In a subsequent study of only three hearts, the addition of dipyradromol and methylprednisolone to the storage solution brought about some improvement in myocardial function on subsequent testing; rhythm disorders were less common and

**Table 3. Hemodynamic performance on functional testing**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (n = 10)</th>
<th>Group 2 (n = 10)</th>
<th>Group 3 (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output (liter/min)</td>
<td>2.8 (0.22)</td>
<td>1.4 (0.16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coronary flow (ml/min)</td>
<td>258 (32.50)</td>
<td>296 (45.60)</td>
<td>NS</td>
</tr>
<tr>
<td>Stroke volume (ml/beat)</td>
<td>17.4 (1.27)</td>
<td>12.7 (1.24)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Left ventricular pressure (mm Hg)</td>
<td>131 (4.42)</td>
<td>102 (3.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mm Hg)</td>
<td>5.3 (0.30)</td>
<td>5.9 (1.34)</td>
<td>NS</td>
</tr>
<tr>
<td>Myocardial oxygen uptake (mmol/hr/g)</td>
<td>0.68 (0.11)</td>
<td>1.00 (0.23)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, SE.

* NS, not statistically significant ($P > 0.05$).
cardiac output reached a mean of 1.7 liters/min. Hemodynamic performance, however, still did not reach the levels of the freshly excised or hypothermically perfused hearts.

Hearts in group 3, by comparison, demonstrated a slight loss of weight during reperfusion, hemodynamic function showing no signs of deterioration throughout this period and being comparable to that of freshly excised hearts (group 1). Under the conditions of the experiments detailed in the present study, therefore, continuous hypothermic perfusion would appear to result in better myocardial protection when compared with simple storage in ice. The superiority of a continuous hypothermic perfusion system over simple cold storage is clearly a poor indicator of the functional state of the heart. There was little difference between the hemodynamic performance, however, between the degree of edema incurred during hypothermic perfusion and the hemodynamic performance of the heart on in vivo testing or after orthotopic heart transplantation. What appears to be of greater importance is whether the edema is reversible. The inclusion of many small yet osmotically active agents such as ionic phosphate and sulfate, monionic glucose, sucrose, and glycerol, as in the group 3 perfusate, led to improved hemodynamic results, though with very considerable edema formation. After transplantation, with continuing coordinated myocardial activity for several hours or days, loss of edema has been clearly documented; three preserved baboon hearts orthotopically transplanted showed a mean weight loss of 42% after 48 hr.

The importance or otherwise of the various constituents which make up the perfusate have been clarified in other experiments not detailed here. An absence of calcium led to no myocardial function whatsoever, and double the concentration of calcium to ischemic contracture (stone heart). When procaine hydrochloride was omitted, either a poorly contracting or a "stone" heart resulted. Lignocaine, substituted for procaine, led to improved myocardial function, though with very considerable edema formation. After transplantation, with continuing coordinated myocardial activity for several hours or days, loss of edema has been clearly documented; three preserved baboon hearts orthotopically transplanted showed a mean weight loss of 42% after 48 hr.

The hypothermic perfusion apparatus (Fig. 1) used in the group 3 experiments is simple in that no mechanically or electrically powered pump is required to circulate the perfusate up the delivery tube is effected by several factors: (1) the rate of gas flow; (2) the height (hydrostatic pressure) of the fluid in the lower chamber; and (3) the partial pressure of the gas in the lower chamber. The partial pressure of gas in the lower chamber increases during the first 3 to 4 min of perfusion, during which a state of equilibrium develops. An increase in gas pressure in the upper chamber is prevented by the presence of the breather port. A gas flow of 500 ml/min delivers 100 to 120 ml of perfusate into the upper chamber per minute. By using pig hearts of the size in the present series of experiments, a perfusate flow of only 20 to 40 ml/min was required.

Apart from this major change in circulatory energy source, a number of other small differences exist between the perfusion apparatus described above and that used in our original studies (2, 3). Originally, a 0.8-μm filter was included in the circuit, whereas only a 20-μm filter has been used in the present work. The pH was carefully maintained at between 7.2 and 7.4 in our earlier work, but was allowed to vary between 7.0 and 7.5 in the experiments reported here; in fact, no attempt was made to "correct" the pH in the present studies. Neither of these changes appears to have had any deleterious effect. The perfusate used, however, remains identical.

The solution-perfusate used in groups 2 and 3 contained agents included for their osmotic effect, agents included for their vasodilator effect, and agents included for metabolic inhibition and/or membrane stabilization (Table 2); the preservative effects of its constituents have been discussed previously (2, 3).

A possible explanation for the decreased mean left ventricular pressure noted in group 3 hearts when compared with that of group 1 is increased left ventricular wall stiffness attributable to the considerable edema formation incurred during preservation. The problem of edema formation remains controversial (9, 10). In the development of a satisfactory perfusate for 24-hr preservation, we have evaluated numerous solutions designed to reduce edema formation, containing, for example, Dextran 45, mannnitol, or albumin. We have found no definite correlation, however, between the degree of edema incurred during hypothermic perfusion and the hemodynamic performance of the heart on in vivo testing or after orthotopic heart transplantation: There was little difference between the histological features of the hearts of any of the three groups.

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The solution-perfusate used in groups 2 and 3 contained agents included for their osmotic effect, agents included for their vasodilator effect, and agents included for metabolic inhibition and/or membrane stabilization (Table 2); the preservative effects of its constituents have been discussed previously (2, 3).

A possible explanation for the decreased mean left ventricular pressure noted in group 3 hearts when compared with that of group 1 is increased left ventricular wall stiffness attributable to the considerable edema formation incurred during preservation. The problem of edema formation remains controversial (9, 10). In the development of a satisfactory perfusate for 24-hr preservation, we have evaluated numerous solutions designed to reduce edema formation, containing, for example, Dextran 45, mannitol, or albumin. We have found no definite correlation, however, between the degree of edema incurred during hypothermic perfusion and the hemodynamic performance of the heart on in vivo testing or after orthotopic heart transplantation: There was little difference between the histological features of the hearts of any of the three groups.

The hypothermic perfusion apparatus (Fig. 1) used in the group 3 experiments is simple in that no mechanically or electrically powered pump is required to circulate the fluid. Flow of the perfusate up the delivery tube is effected by several factors: (1) the rate of gas flow; (2) the height (hydrostatic pressure) of the fluid in the lower chamber; and (3) the partial pressure of the gas in the lower chamber. The partial pressure of gas in the lower chamber increases during the first 3 to 4 min of perfusion, during which a state of equilibrium develops. An increase in gas pressure in the upper chamber is prevented by the presence of the breather port. A gas flow of 500 ml/min delivers 100 to 120 ml of perfusate into the upper chamber per minute. By using pig hearts of the size in the present series of experiments, a perfusate flow of only 20 to 40 ml/min was required.

Apart from this major change in circulatory energy source, a number of other small differences exist between the perfusion apparatus described above and that used in our original studies (2, 3). Originally, a 0.8-μm filter was included in the circuit, whereas only a 20-μm filter has been used in the present work. The pH was carefully maintained at between 7.2 and 7.4 in our earlier work, but was allowed to vary between 7.0 and 7.5 in the experiments reported here; in fact, no attempt was made to "correct" the pH in the present studies. Neither of these changes appears to have had any deleterious effect. The perfusate used, however, remains identical.

The solution-perfusate used in groups 2 and 3 contained...
dioplegic solution and perfusate would appear to be important. At one stage in the development of this system, we unwittingly used procaine hydrochloride made up in 0.5% phenol; this resulted in inferior cardiac output when compared with hearts in group 3 in which the procaine hydrochloride was made up in 0.1% chioro-cresol. The hemodynamic performances of these two groups of hearts on testing are shown in Table 4; it should be stressed that the only difference in the two groups was the preservative used with regard to the procaine in both cardioplegic solution and perfusate.

Observations made during the period of hypothermic perfusion (Table 5) did not suggest that subsequent myocardial function would be significantly different between these two groups, as, rather surprisingly, the "phenol" group showed significantly greater coronary flow and lower coronary vascular resistance initially (after 1-hr perfusion), features which we had come to believe indicated satisfactory cardioplegic arrest and preservation, although the rather high myocardial oxygen uptake might have been interpreted as indicating inadequate metabolic inhibition.

The phenol hearts, however, gained weight (mean 1.6%) during the 1-hr period of reperfusion of functional testing, in contradistinction to the "cresol" group, where there was a mean weight loss of 7.5%. (Six of seven baboon hearts preserved identically in a phenol solution and subsequently orthotopically transplanted failed to support a circulation for more than 12 hr, despite inotropic support.)

We are unable to give a definite explanation for the mechanisms by which 0.5% phenol altered the effect of the cardioplegic agent and perfusate, although its damaging effect on tissues is well known. It may be that the phenol in the cardioplegic agent damaged the protein of the cell membranes, so that, on reperfusion, integrity was lost, allowing fluid to enter the cells. Any weight gain during reperfusion, even 1 or 2%, would appear to be a sign of inadequate preservation. We have subsequently confirmed that the perfusion system and perfusate used in group 3 led to consistently successful 20- to 24-hr preservation by orthotopically transplanting eight similarly preserved baboon hearts. All animals survived with good myocardial function until electively killed (three animals) or until rejection (five animals); in this latter immunosuppressed group mean survival was 27 days.

Acknowledgments. We wish to thank Professor A. G. Rose of the Department of Pathology, University of Cape Town Medical School, who performed the histological studies, and S. T. Boyd, who gave valuable assistance with the design of the preservation machine. We also wish to thank F. Sanders, S. Smit, J. Roussouw, J. Martin, P. Madlingozi, Sister E. Hanekom, and F. Barends for skilled technical assistance.

LITERATURE CITED


Received 25 December 1981.
Accepted 10 February 1982.
Orthotopic Allotransplantation and Autotransplantation of the Baboon Heart following 24-hr Storage by a Portable Hypothermic Perfusion System

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INTRODUCTION

The ability to store a heart after excision from the donor, even for periods of a few hours, has many obvious benefits which have been outlined previously (1, 2).

We have reported preservation of pig hearts for 20–24 hr by ex vivo hypothermic perfusion; functional testing of these hearts after the period of preservation showed hemodynamic parameters indistinguishable from those of freshly excised hearts (1). Furthermore, orthotopic transplantation of baboon hearts preserved by an identical technique was followed by consistent survival of the recipient baboon until rejection occurred at a mean of 19.5 days (2).

The nonpulsatile perfusion apparatus used in these studies was based on that originally described by Proctor and Parker (3); it incorporated two roller pumps and storage in a refrigerated room. We have subsequently greatly modified and simplified the system to make it entirely and easily portable; in particular, we have done away with the need for any form of electrically powered pump to maintain circulation of fluid through the heart. Using this simple, portable system we have reported preservation of pig hearts for 20–24 hr followed by hemodynamic function comparable with that of freshly excised hearts (4).

We here describe the results we have obtained following orthotopic allotransplantation and autotransplantation of baboon hearts preserved for 20–24 hr by this portable system.

METHODS

Two groups (A and B) of outbred chacma baboons (Papio ursinus) were studied.

Group A: Orthotopic Allotransplantation

Eleven experiments were performed. Baboons weighing 10–20 kg were premedicated with intramuscular (im) ketamine hydrochloride 100 mg (Ketalar; Parke Davis Laboratories Ltd.). The baboon was given pancuronium 4 mg intravenously (iv), atropine 0.6 mg iv, morphine 15 mg iv, and cefalothin sodium (Keelin; Lilly Laboratories, S.A. Ltd.) 1 G iv.

(1) Cold cardioplegic arrest and donor heart excision. Through a median sternotomy the azygos vein was doubly ligated and divided. Heparin (1000 units/kg iv) was given. The superior vena cava (SVC) was then doubly ligated cephalad to the azygos vein and divided. The brachiocephalic artery was ligated distally and cannulated for subsequent cardioplegic infusion, the tip of the cannula lying in the arch of the aorta. The aorta was cross-clamped distal to the cannula, followed by immediate division of the inferior vena cava (IVC) and one or more pulmonary veins, and 500 ml of cardioprotective solution was perfused through the bypass apparatus.
Cardioplegic solution (Table 1) at 4°C was infused. The pericardial cavity was filled with cold, normal saline (4°C) to externally cool the heart. Cessation of the heart beat occurred within 10–15 sec; the infusion of 500 ml of solution was completed within 1–3 min by which time myocardial temperature had fallen to approximately 12–15°C.

Excision of the heart was completed by division of the pulmonary veins, the pulmonary artery at its bifurcation, the aorta proximal to the brachiocephalic artery, and the mediastinal tissue posterior to the heart. The heart was immersed for 1 min in a bowl containing cold (4°C) cardioplegic solution. Myocardial temperature falling to approximately 10°C, after which it was weighed.

Nine of the hearts were transferred immediately to the perfusion apparatus. The time interval between aortic cross-clamping and the initiation of perfusion was approximately 10–12 min. Two hearts were selectively stored in the same cardioplegic solution in ice (at approximately 4°C) for 4 hr before being transferred to the perfusion apparatus; they were then perfused for 20 hr.

(2) Continuous hypothermic perfusion for 20–24 hr. The perfusion circuit is shown in Fig. 1. The perfusate (Table 2) was sterilized by being passed through a 0.8-μm filter, the pH being adjusted to an initial value of 7.2 by bubbling a 97% O₂; 3% CO₂ mixture through it.

The perfusate was both oxygenated and circulated throughout this period by the air-lift pump principle (5). A mixture of 97% O₂ and 3% CO₂ was bubbled through a sterile gas filter into the perfusate in the lower reservoir through an air ejector port inserted into the delivery tube, by which system the fluid was transported to the upper chamber (reservoir) through a Cobé 20-μm filter. At a gas flow of approx. 500 ml/min this gas solution maintained perfusate pH at 7.0–7.8, and maintained perfusate flow into the upper chamber at approximately 60–120 ml/min. Perfusion PO₂ has been measured at a partial pressure of approximately 1000 mm Hg when determined at 30°C.

From the upper chamber, perfusate flowed by gravity into the ascending aorta of the suspended heart, perfusing the coronary arteries at a pressure of approximately 8–10 cm H₂O, depending on aortic length. Coronary venous effluent to the right atrium returned to the lower reservoir by gravity drainage via the inferior vena cava and pulmonary artery orifices. From the lower chamber, the perfusate was recirculated. An overflow pipe between upper and lower reservoirs prevented the upper chamber from filling completely with fluid.

Two and a half liters of perfusate were initially added to the lower chamber; the flow
up the delivery tube could be increased or decreased simply by adding to or subtracting from this initial fluid load, enabling a constant gas flow to be maintained, and thus avoiding major fluctuations in pH which would follow changes in gas flow. The perfusion apparatus was then placed in a stainless steel container, insulated with polystyrene and perspex, and packed with ice to maintain the desired temperature of 4–10°C throughout the 20–24-hr preservation period.

At the onset and end of the perfusion period, coronary flow was measured, and coronary vascular resistance was calculated. After the period of perfusion the heart was weighed and transferred to the recipient.

(3) Technique of orthotopic allotransplantation. Baboons, weighing 10–20 kg and matched for AB blood group with the donor baboons, were premedicated, anesthetized, and ventilated as were the donors.

Double doses of pancuronium, atropine, and morphine were given, and these doses were repeated after opening the chest. Cephalexin sodium 1 g iv was given and repeated every 8 hr by intramuscular injection for 3 days. Methylprednisolone 25 mg iv was given and repeated during the operative and early postoperative period until a total dose of 100 mg/kg body weight had been given. Two baboons had been pretreated with cyclosporin A (50 mg/kg/day orally (Sandoz Ltd., Basel, Switzerland) for 4 days before operation.

The techniques of recipient heart excision and of donor heart insertion followed the description by Lower and Shumway (6), incorporating the modification suggested by Barnard (7). Only when the recipient heart had been excised was the donor heart removed from the overnight perfusion apparatus. At 20-min intervals throughout the period of insertion, saline at 4°C was poured into the pericardium to cover the heart to help maintain a low myocardial temperature, though this slowly rose to approximately 20°C.

Pump oxygenator support was electrically continued for 60 min following release of the aortic clamp. Spontaneous coordinated contractions or, rarely, ventricular fibrillation requiring electrical defibrillation, occurred. Before discontinuation of cardiopulmonary bypass, an isoproterenol hydrochloride infusion (1 μg/min) was set up and furosemide 3–5 mg iv administered. When hemodynamic function was stable, the chest was drained and closed.

(4) Postoperative Management. The baboon was weaned off ventilator support over the next few hours. The isoproterenol infusion was maintained electively for 1–2 hr and then slowly reduced. Once the baboon was hemodynamically stable without inotropic support and was maintaining satisfactory blood gases breathing spontaneously on air, the drains were removed, it was extubated and returned to its cage.

In 9 of the 11 baboons an immunosuppressive regimen consisting of azathioprine (Imuran; Wellcome Ltd.) 2.5 mg/kg/day and a decreasing dose of methylprednisolone (Solumedrol; Upjohn Ltd.) (until by the fourth postoperative day the baboon was receiving maintenance of 2 mg/kg/day) was given intramuscularly. No attempt was made
to treat acute rejection episodes by increased immunosuppressive therapy.

Two baboons received daily cyclosporin A (50 mg/kg/day orally) and the same dose of methylprednisolone as the above group, though this drug was also given orally in these two animals.

Three baboons were electively killed on the second or third postoperative days in order to weigh and histologically examine the heart. Of the remaining eight, six underwent cardiac catheterization under ketamine sedation 7–14 days after transplantation. This time interval was chosen as it allowed recovery from the operation but was not long enough to allow the development of severe acute rejection in most cases.

**Group B: Autotransplantation**

Three experiments were performed. Outbred chacma baboons weighing 10, 12, and 15 kg were used.

**Stage 1**

Premedication, anesthesia, and ventilation were as for the recipient baboons in group A.

A median sternotomy was performed. Total cardiopulmonary bypass was initiated after cannulation of the SVC, IVC, and distal ascending aorta. Cooling to 28°C was carried out. A left ventricular vent was inserted at the apex. A needle attached by sterile tubing to a 500-ml bag containing cardioplegic agent (Table I) (at 4°C) was inserted into the root of the aorta. The distal ascending aorta proximal to the aortic cannula was cross-clamped and 500 ml cardioplegic agent infused; an incision was made in the right atrium and coronary sinus return sucked to waste. The pericardial cavity was filled with normal saline at 4°C to externally cool the heart.

The heart was then excised, the line of excision being carried into the SVC and IVC, taking care to include the region of the sinus node with the heart; the aorta and pulmonary artery were divided approximately 2–3 cm distal to their respective valves. The heart was then weighed and transferred to the perfusion apparatus. (Continuous hypothermic perfusion of these hearts was identical to that used in group A.)

An orthotopic transplant was then performed using a heart excised from a donor baboon matched for AB blood group. The techniques of donor heart excision and orthotopic transplantation were basically as described in group A. Cardiopulmonary bypass was then discontinued, and, once donor heart function was deemed satisfactory, the chest was closed and the baboon returned to its cage. No immunosuppressive therapy was administered.

**Stage 2**

On the following day, when the excised heart had undergone continuous hypothermic perfusion for 20–24 hr the baboon was premedicated, anesthetized and ventilated as before. The sternotomy was reopened and cardiopulmonary bypass instituted. The ascending aorta was cross-clamped and the transplanted heart excised. The animal's own heart was then removed from the perfusion apparatus, weighed, and sutured into its original position. Postoperative management was as for group A.

**RESULTS**

**Group A**

(1) Observations during the period of hypothermic perfusion. All nine hearts perfused from the outset increased in weight during the period of perfusion due to the formation of edema, the mean increase being 80.3% (range 62.1 to 105.2%) (Table I). Coronary flow fell in all hearts during the perfusion period, the mean percentage fall being 30.9% (range 14.6 to 53.5%). Coronary vas-
Orthotopic allotransplantation and autotransplantation

Observations during 24 hr Hypothermic Perfusion

<table>
<thead>
<tr>
<th>Mean heart weight (g)</th>
<th>Mean % change in heart weight</th>
<th>Coronary flow (CF) (mL/min)</th>
<th>Mean % change in coronary flow</th>
<th>Coronary vascular resistance (CVR) (mm Hg/mL)</th>
<th>Mean % change in CVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthotopic allotransplantation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At beginning of perfusion</td>
<td>67.3 (7.6)</td>
<td>21.2 (3.0)</td>
<td>42.0 (4.6)</td>
<td>43.8 (5.5)</td>
<td></td>
</tr>
<tr>
<td>At end of perfusion</td>
<td>70.4 (12.6)</td>
<td>14.5 (2.6)</td>
<td>60.4 (6.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autotransplantation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At beginning of perfusion</td>
<td>65.3 (14.2)</td>
<td>16.0 (1.5)</td>
<td>44.0 (3.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At end of perfusion</td>
<td>61.1 (21.1)</td>
<td>12.1 (1.0)</td>
<td>62.3 (10.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Figures in parentheses denote standard error.

Cerical resistance rose by an average of 43.8% (range 16.9 to 60.0%). The change in the above three parameters (weight, coronary flow, and coronary vascular resistance) between the pre- and postperfusion states of the heart was in each case statistically highly significant (*P < 0.001*).

The two hearts stored in cardioplegic solution in ice for 4 hr before 20 hr of hypothermic perfusion showed slightly different patterns of change. No weight change occurred during the 4 hr of storage, though there was a subsequent mean weight gain of 90.0% during hypothermic perfusion. Both hearts were markedly vasoconstriicted at the onset of perfusion; coronary flow rose by approximately 200% during the first 2 hr of perfusion and then slowly fell by 30% over the remaining 18 hr. Coronary vascular resistance fell by 60% after 2 hr, rising only by 10% during the next 18 hr.

(2) Results of orthotopic transplantation. Eleven transplants were performed. Three baboons were electively killed after 48 or 72 hr. Almost half (mean 48.2%, range 44.0 to 68.0%) of the weight gained during hypothermic perfusion had been lost within this 2- to 3-day interval, indicating that although the hearts remained edematous this edema was steadily reducing. The loss of weight was significant (*P < 0.02*).

All eight remaining baboons survived to rejection; this group included the two hearts stored in cardioplegic solution for 4 hr before perfusion. The cause of death was confirmed by necropsy and histological examination of the myocardium by light microscopy. Survival of those immunosuppressed with azathioprine and methylprednisolone was 10, 13, 26, 49, 31, and 33 days (mean, 27.0 days), the latter two being those stored in ice for 4 hr before hypothermic perfusion. The two immunosuppressed with cyclosporin A and methylprednisolone lived 10 and 37 days, respectively.

Good hemodynamic function was confirmed by cardiac catheterization performed in six of these animals between 7 and 14 days after transplantation (Table 4). A slight pressure gradient between right ventricle and distal pulmonary artery was accounted for by some narrowing occurring at the pulmonary artery suture line, confirmed at necropsy.
TABLE 4
Data Obtained at Cardiac Catheterization 7-14 days after Orthotopic Transplantation (Group A)

<table>
<thead>
<tr>
<th></th>
<th>Pressure (mm Hg)</th>
<th>(a = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrium</td>
<td>7.3 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Right ventricle (systolic)</td>
<td>34.8 (2.9)</td>
<td></td>
</tr>
<tr>
<td>Main pulmonary artery (systolic)</td>
<td>22.6 (2.1)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary capillary wedge (mean)</td>
<td>6.5 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Left ventricle (systolic)</td>
<td>10.6 (1.8)</td>
<td></td>
</tr>
<tr>
<td>Left ventricle (end diastolic)</td>
<td>6 (0)</td>
<td></td>
</tr>
<tr>
<td>Aorta (systolic)</td>
<td>96.5 (6.5)</td>
<td></td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td>3.2 (1.0)</td>
<td></td>
</tr>
</tbody>
</table>

* Figures refer to pressure in mm Hg. Figures in parentheses denote standard error.

Group B

1) Observations during the period of hypothermic perfusion. The findings were similar to those in group A (Table 3).

2) Results of autotransplantation. Three autografts were performed.

Case 1. The first baboon made an excellent recovery and was active and obviously well until he was found dead on the morning of the 31st postoperative day. During the first week after operation he developed some edema of the scrotum for which he was given furosemide 5 mg on three occasions; the edema did not recur. Necropsy revealed that a false aneurysm, situated between the ascending aorta and sternum and communicating with the lumen of the aorta by a pin hole at the aortic suture line, had ruptured into the right pleural cavity, exsanguinating the animal. Unfortunately, postoperative cardiac catheterization had not yet been performed.

Histological examination by light microscopy of the myocardium showed a mild interstitial edema and scanty myocytolysis only; the small blood vessels were well preserved. Less than 5% of the myocardium in the sections studied was affected by myocytolysis.

Case 2. This baboon made an excellent recovery and remains well and active 8 months after autotransplantation. This animal also developed swelling of the lower abdomen and scrotum within the first few days after operation and required furosemide 5 mg on four occasions; again the edema did not recur.

Cardiac catheterization has been performed on three occasions at 1, 2, and 6 months (Table 5), and the data obtained differed little from that obtained from the same baboon before autotransplantation, except with regard to a small right ventricular-pulmonary artery gradient (as in group A baboons discussed previously).

An endomyocardial biopsy was obtained by the percutaneous transvenous route at 6 months; light microscopy (Fig. 2) revealed

TABLE 5
Data Obtained at Cardiac Catheterization before and after Autotransplantation of a Baboon Heart Preserved for 24 hr by Hypothermic Perfusion (Group B)

<table>
<thead>
<tr>
<th></th>
<th>Presautotransplantation</th>
<th>1 month</th>
<th>2 months</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrium (mean)</td>
<td>4</td>
<td>7</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Right ventricle (systolic)</td>
<td>28</td>
<td>32</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td>Main pulmonary artery (systolic)</td>
<td>28</td>
<td>30</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Pulmonary capillary wedge (mean)</td>
<td>3</td>
<td>8</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Left ventricle (systolic)</td>
<td>11.0</td>
<td>9.0</td>
<td>10.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Left ventricle (end diastolic)</td>
<td>2</td>
<td>3</td>
<td>2.5</td>
<td>3</td>
</tr>
<tr>
<td>Aorta (systolic)</td>
<td>116</td>
<td>95</td>
<td>105</td>
<td>110</td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td>4.9</td>
<td>6.02</td>
<td>-</td>
<td>6.46</td>
</tr>
</tbody>
</table>

* Figures refer to pressure in mm Hg.
Fig. 2. Section of right ventricular myocardial biopsy obtained 6 months after 24 hr storage and autotransplantation shows normal-looking transversely sectioned myofibers. (Hematoxylin and eosin. × 80.)

Fig. 3. Myocardial ultrastructure appears normal apart from dilatation of the tubules. Portion of a normal capillary containing an erythrocyte is seen at the bottom. (Electron micrograph. × 9000.)
a normal myocardium. Glycogen stores appeared adequate. Electron microscopy showed a normal myocardial ultrastructure apart from mild dilatation of the T tubules (Fig. 3). The microcirculation also appeared normal.

Case 3. In the early postoperative period the third animal developed dysentery from a severe enterocolitis which, despite therapy, progressed to peritonitis; death occurred on the seventh day. Because of sickness throughout this period, it was difficult to reliably assess his hemodynamic state; cardiac function appeared satisfactory. There was no dependent edema. Cardiac catheterization was not performed. Necropsy confirmed a grossly distended colon and peritonitis and pus.-culturing klebsiella and enterococci, but no anaerobic organisms.

Histological examination of the heart after death revealed focal coagulative necrosis of isolated myofibers and focal myocytolysis: as in case 1 only approximately 5% of the myocardium was involved by these changes, the remaining tissue being normal.

DISCUSSION

The number of experimental animals which have survived rejection following the orthotopic transplantation of hearts which had been stored for 24 hr is small (8-11), the most successful results being obtained by Guerraty (12, 13). In an earlier study using a nonportable system of hypothermic perfusion, but with identical cardioplegic agent and perfusate to those used in the present experiments, we reported six out of six animals immunosuppressed with azathioprine and methylprednisolone surviving to rejection with a mean survival time of 19.5 days (2). The results following orthotopic transplantation using the present portable system are comparable. Though mean survival of those baboons receiving azathioprine and methylprednisolone in the present study was 27 days, this is not significantly different from the earlier group.

The two experiments in which the heart was stored simply in cold cardioplegic agent in ice for 4 hr before undergoing approximately 20 hr of hypothermic perfusion confirmed that no deleterious effect occurs from combining these two forms of organ storage. Though the perfusion machine is easily portable, it may on occasion be convenient, or even necessary, to transport the heart simply in ice, and, if a longer period of preservation is required, then transfer it to the perfusion apparatus. We believe, however, that perfusion from the outset would be preferable as we have some evidence that it leads to better preservation of function than does storage in ice (4).

The three experiments involving autotransplantation proved difficult and only partially successful, but the one long-term survivor has provided considerable hemodynamic data regarding the function of a heart stored in this way and yet unaffected by acute rejection. The histological information has shown that no widespread cellular damage has resulted to date from the period of hypothermic perfusion.

The perfusion apparatus used in this study is simple in that no mechanically or electrically powered pump is required to circulate the fluid; it is therefore entirely and easily portable. It requires no attention other than to ensure that the gas flow continues. The mechanics of the apparatus (4) and the constitution of both the cardioplegic agent and the perfusate (1, 2) have been discussed previously.

Several previous workers have drawn attention to a correlation between edema formation, manifested as weight gain, in the isolated heart, with an increase in coronary resistance during the period of perfusion, and the resulting deterioration of subsequent myocardial performance (8, 13). Our own experience is markedly different. Though the system described in this report
preserves good myocardial function, edema formation is marked, and the heart remains significantly edematous for a period in excess of 48–72 hr. In the development of a satisfactory perfusate for 24-hr preservation we have evaluated numerous solutions designed to reduce edema formation, containing, for example, dextran 40, mannitol, or albumin. We have found no definite correlation, however, between the degree of edema formation incurred during hypothermic perfusion and the subsequent hemodynamic performance of the heart on ex vivo functional testing or after orthotopic transplantation. What appears to be of greater importance is whether the edema is reversible, and this may explain the discrepancy between our own studies and others previously reported. The inclusion of many small yet osmotically active agents such as ionic phosphate and sulfate, non-ionic glucose, sucrose, and glyceroi have led to improved hemodynamic results, despite considerable edema formation.

Moreover, under these circumstances, edema during storage may, in fact, play a beneficial role by diluting the intracellular content of calcium, which might otherwise reach dangerously high concentrations due to the passive, slow-channel, inward flow of calcium following the loss of control over calcium exchange which occurs in the hypothermic heart and which may result in ischemic contracture (“stone heart”).

We have subsequently used the perfusion system and perfusate described in this study to store four human donor hearts prior to heterotopic transplantation; this experience will be reported elsewhere (14).

SUMMARY

A nonpulsatile perfusion apparatus, based on the air-lift pump principle, has been developed. Circulation of the perfusate, as well as oxygenation and maintenance of acid-base balance, is provided by the flow of a mixture of 97% oxygen and 3% carbon dioxide. The system is easily and entirely portable.

Eleven baboons underwent orthotopic allotransplantation with donor hearts stored by continuous hypothermic (4–10°C) perfusion for periods of up to 24 hr. Three were electively killed after 2 to 3 days; the remaining eight, immunosuppressed with methylprednisolone and either azathioprine or cyclosporin A, survived to rejection at between 10 and 49 days. Cardiac catheterization performed in six animals 7–14 days after allotransplantation showed virtually normal hemodynamic data.

Three more baboons underwent heart excision and storage by hypothermic perfusion for 24 hr, and subsequent orthotopic autotransplantation, the circulation of the baboon being maintained in the interim by an allograft. One animal survives 8 months later with hemodynamic data at cardiac catheterization differing little from that obtained before autotransplantation. This perfusion system has subsequently been used to store four human donor hearts prior to heterotopic transplantation.

ACKNOWLEDGMENTS

We wish to thank P. Barends, Sister E. Hanekom, P. Muller-doozi, Miss J. Martin, S. Patel, J. Rossouw, Miss S. Smith, and P. Snyder for skilled technical assistance. This work was supported by the Chris Barnard Fund, The University of Cape Town, and the Cape Provincial Administration.

REFERENCES


Storage of the Donor Heart by a Portable Hypothermic Perfusion System: Experimental Development and Clinical Experience

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ABSTRACT—A nonportable perfusion system, a cardioplegic agent, and a perfusate were developed for storing hearts by hypothermic perfusion for a period of 24 hours. The system and solutions were tested by biochemical and histological studies of the myocardium, by functional testing of the preserved pig hearts, and by orthotopic transplantation in baboons. When satisfactory storage for 24 hours had been achieved, the perfusion system was modified to facilitate portability. An airlift pump was used to provide both oxygenation and circulation of the perfusate. Consistent survival after allotransplantation and autotransplantation in baboons confirmed the reliability of this compact system. Four patients received donor hearts preserved by this system. Periods of ischemia ranged from 6 hours 55 minutes to 16 hours 50 minutes. The details of this experience are reported here.

Introduction

The advantage of being able to store a donor heart for a few hours is obvious. There would be time to transport the donor or the isolated graft to the transplant center, prepare the recipient, and perform tissue typing. A much larger geographic donor pool could also be tapped.

At Groote Schuur Hospital the need for a large donor pool is acute. Although the number of patients referred to us annually for transplantation is not large, one-third of those accepted die before a suitable donor can be found. Geographically Cape Town is relatively isolated from the other major cities in South Africa and the distance to these cities is too great for donor hearts preserved by cardioplegic arrest and simple storage in ice to be transported to Cape Town. Where such techniques have been utilized elsewhere to transport donor hearts over distances, privately chartered jet aircraft have been available. But the expense is considerable and beyond our resources. Therefore, it was particularly important to develop a system for longer storage. The literature suggested that combined hypothermia with continuous perfusion would probably be successful.

Perfusion Unit

An apparatus for nonpulsatile continuous perfusion of the donor heart was set up, based on the work of Proctor and Parker (Figure 1). The coronary arteries were perfused continuously at 8 to 10 cm H₂O pressure for 20 to 24 hours under refrigerated conditions at 6° to 8° C. Two liters of perfusate were added to the lower chamber of the apparatus. A gas mixture of 95% O₂ and 5% CO₂ was bubbled continuously into the perfusate to maintain a pH of between 7.2 and 7.4; the pH was measured at 30-minute intervals and adjusted by increasing or decreasing the gas mixture flow.

The lower chamber consisted of a cylindrical plastic 4-liter chamber in which the heart was suspended by the aorta and through which it was perfused. The two perfusion circuits con-
sisted of sterile polyvinyl tubing and two peristaltic roller pumps. One circuit, incorporating a 0.8 µ filter to prevent occlusion of small coronary vessels by particulate matter, delivered perfusate from the lower reservoir to the aorta. The second circuit decompressed the right ventricle, aspirating all the coronary venous return into the lower reservoir. The coronary flow could, therefore, be collected and measured. At the beginning and end of the perfusion period the heart was weighed, the coronary flow and coronary arteriovenous oxygen uptake were measured, and the coronary vascular resistance was calculated.

Development of a Cardioplegic Agent and Perfusate

Experimental method

Rapid arrest and cooling of the heart is considered an essential prerequisite for storage. The donor animal heart is arrested with 500 ml of 4 °C cardioplegic agent after cross-clamping the ascending aorta and bathed in 500 ml of 4 °C normal saline. Arrest occurs within 15 to 20 seconds after infusion begins. The infusion is completed within 1 to 3 minutes, by which time the myocardial temperature has fallen to approximately 10° to 15° C. The heart is rapidly excised and immersed for 1 minute in both 4 °C normal saline and cardioplegic agent. In later experiments only immersion in the cardioplegic agent was used. The heart is then attached to the apparatus and perfused for 20 to 24 hours at 8° to 10° C. The total time from initiation of cardioplegic infusion to initiation of perfusion is 10 to 14 minutes.

Assessment of myocardial viability

Four methods were used to assess the effects of preservation. Freshly excised, unpreserved hearts served as controls. The biochemical, histological, and functional studies were performed with pigs; the orthotopic transplant series with baboons.

Biochemical estimations. During the hypothermic perfusion, serial studies evaluated myocardial metabolism and possible damage by measuring adenosine triphosphate, phospho-creatine, lactate, lactate dehydrogenase, creatine phosphokinase, and glucose. Selected hearts were serially biopsied and tissue pH and glycogen concentration measured. Oxygen consumption of isolate mitochondria determined the degree of coupling between phosphorylation and electron transport, and vesicular integrity was assessed by measuring the number of lysosomes, and the lysosomal acid phosphatase activity.

Histological examination. Light microscopy examination was performed on all hearts at the end of the storage period. Occasionally, electron micrographs were done. Light microscopy detected only gross injury caused by inadequate preservation, and was found not to be a sensitive method.

Functional testing. A system previously described evaluated the pig heart function (Figure 2). Matched oxygenated blood was supplied by an anesthetized, ventilated perfusor pig to which deoxygenated blood was returned. Arterial Po2, Pco2, and pH were monitored. Initially, the tested heart's aorta was retrogradely perfused as in a Langendorff system, supplying blood to the coronary arteries of the isolated heart. This period of recovery lasted 5 to 15 minutes.

When the myocardial temperature reached 37 °C, the heart was defibrillated and the circuit was converted into the working heart mode. Blood was directed into the isolated heart's left atrium from a reservoir challenging the left ventricle function. All hearts were tested for 1 hour, and hemodynamic parameters were recorded. The hearts were then weighed and biopsied for histological examination. Good preservation was demonstrated when the left ventricle pumped blood to a height of 100 cm through an aortic balloon and nonlinear resistance. This blood then returned to the left atrial reservoir. Preload and afterload were adjustable and kept constant at 11 cm H2O and 80 mm Hg, respectively.
Orthotopic transplantation. Orthotopic transplantation was performed using baboons. The grafts were stored exactly as those of the pigs.

Composition of the Cardioplegic Agent

Determination of an ideal cardioplegic agent goes beyond the scope of this study. An existing solution was chosen and a subsequently modified one was used for most of these studies (Table I). The modified solution attempted to prevent injury induced by cardioplegia from extending into the hypothermic perfusion period.

The cardioplegic effects of potassium, magnesium, and procaine are known, and insulin was included to facilitate the use of substrate until the contractile motion had ceased and to maintain glycogen stores. Calcium is not included in cardioplegic solutions used during surgery since it is supplied by noncoronary collateral flow. However, in the isolated heart, there is no collateral flow, and the absence of calcium results in a "calcium paradox" on reperfusion that may lead to extreme ischemic damage ("stone heart").

To prevent such myocardial contracture, the slow-channel calcium inhibitor verapamil was added in the final solution, acting through a conduction system-induced negative inotropic effect, reducing the work of the heart at the onset of ischemia, and delaying the rate of ATP consumption. Verapamil also has vasodilator properties.

Composition of Perfusionate

The first perfusate tried was the Krebs-Henseleit solution, originally used by Neely and colleagues in 1967 to preserve rat hearts at normothermia, which proved to be totally inadequate. The high energy phosphate stores became depleted and the release of lactic dehydrogenase was excessive. Eight different perfusates were then evaluated (Table II).

Certain constituents were included because of their effects on osmosis, membrane stabilization, vasodilatation, or metabolic inhibition. Their selection was based on the assumption that osmotically active molecules would lead to progressive extravascular swelling which would have a beneficial effect by gradually diluting the cellular calcium content; this calcium might otherwise reach a dangerously high concentration due to its passive, slow-channel, inward flow following the loss of calcium exchange.

<table>
<thead>
<tr>
<th>Table I. Composition of the two cardioplegic agents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solution 1</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><strong>gm/liter</strong></td>
</tr>
<tr>
<td>NaCl</td>
</tr>
<tr>
<td>NaHCO₃</td>
</tr>
<tr>
<td>KCl</td>
</tr>
<tr>
<td>KH₂PO₄</td>
</tr>
<tr>
<td>CaCl₂ • 2H₂O</td>
</tr>
<tr>
<td>MgSO₄ • 7H₂O</td>
</tr>
<tr>
<td>Procaine HCl</td>
</tr>
<tr>
<td>Insulin</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Dextrose</td>
</tr>
<tr>
<td>Isotopin</td>
</tr>
<tr>
<td>Osmolality</td>
</tr>
<tr>
<td>pH at 4°C</td>
</tr>
</tbody>
</table>
control occurring in the hypothermic heart, which is responsible for ischemic contracture ("stone heart"). Evidence for this phenomenon was provided by the gradual edema formation in the well-preserved hearts, with a weight gain of 80% during the 24-hour period of preservation. By comparison, hearts preserved in the same solution by simple storage in ice gained no weight, and had poor hemodynamic performance and frequent rhythm disturbances.

Previous researchers reported that the accumulation of edema in a preserved organ reflected inadequate protection from ischemic damage followed by poor function. This is not necessarily the case if the edema is reversible. This could be ensured by including small osmotically active agents such as ionic phosphate and sulfate, nonionic glucose, sucrose, and glycerol.

Results of Orthotopic Transplantation

At all four stages in the development of the perfusate, orthotopic allotransplantation was attempted. The results from the first two stages showed inadequate myocardial function, even though biochemical and functional evaluation had been encouraging.

With the last two solutions, the results of orthotopic transplantation were good, with consistent survival of the immunosuppressed recipient baboons until rejection, and a mean survival of 20 days. Cardiac catheterization performed during the second postoperative week revealed normal pressures and cardiac output.

### Portable Perfusion System

Before clinical application of the preservation method, the system was made portable. The goals were simplicity, compactness, independence from any source of power, and absolute sterility.

A totally new system was developed based on the airlift pump principle (Figure 3). The perfusate was sterilized by filtering (0.8 µ filter), and the pH was adjusted to an initial value of 7.2 by bubbling a 97% O₂:3% CO₂ gas mixture through a sterile gas filter. The perfusate was both oxygenated and circulated by the gas flow into the lower reservoir via an air-ejector port inserted into the delivery tube, by which the fluid was then transported to the upper chamber or reservoir through a Cobe 20 µ filter.

### Table II. Composition of the eight perfusates

<table>
<thead>
<tr>
<th>Constituent (mM)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
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<tbody>
<tr>
<td>NaCl</td>
<td>118.5</td>
<td>118.5</td>
<td>92.90</td>
<td>92.90</td>
<td>92.90</td>
<td>92.90</td>
<td>115.7</td>
<td>115.7</td>
</tr>
<tr>
<td>KCl</td>
<td>4.75</td>
<td>4.75</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.20</td>
<td>1.20</td>
<td>1.20</td>
<td>1.20</td>
<td>1.20</td>
<td>1.20</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>1.20</td>
<td>1.20</td>
<td>1.20</td>
<td>1.20</td>
<td>1.20</td>
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<td>1.20</td>
<td>1.20</td>
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<tr>
<td>CaCl₂·2H₂O</td>
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<td>2.5</td>
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<td>2.25</td>
<td>2.25</td>
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<tr>
<td>MgCl₂</td>
<td>—</td>
<td>14.8</td>
<td>14.8</td>
<td>14.8</td>
<td>14.8</td>
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<tr>
<td>Glucose</td>
<td>11</td>
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<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Taurine</td>
<td>—</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>ATP</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Insulin</td>
<td>—</td>
<td>40 units/liter</td>
<td>40 units/liter</td>
<td>40 units/liter</td>
<td>40 units/liter</td>
<td>40 units/liter</td>
<td>40 units/liter</td>
<td>—</td>
</tr>
<tr>
<td>Procaine HCl</td>
<td>—</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dextran 40</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4 gm/liter</td>
<td>2 gm/liter</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>.005 gm/liter</td>
<td>.005 gm/liter</td>
<td>.005 gm/liter</td>
</tr>
<tr>
<td>Phenoxycobenzamine</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>.01 gm/liter</td>
<td>.01 gm/liter</td>
<td>.01 gm/liter</td>
</tr>
<tr>
<td>Glycerol</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>136</td>
<td>136</td>
<td>136</td>
</tr>
<tr>
<td>Sucrose</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7</td>
</tr>
<tr>
<td>Osmolality (mOsm/liter)</td>
<td>277</td>
<td>280</td>
<td>295</td>
<td>315</td>
<td>302</td>
<td>321</td>
<td>330</td>
<td>385</td>
</tr>
</tbody>
</table>

**Figure 3. Portable hypothermic perfusion apparatus.**
### Table III. Summary of the four clinical cases

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Underlying cardiac pathology</th>
<th>Date of transplantation</th>
<th>Total donor ischemic time</th>
<th>Initial donor heart function</th>
<th>Clinical outcome (as of 1/7/82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31/Male</td>
<td>Cardiomyopathy</td>
<td>9/17/81</td>
<td>12 hr 50 min</td>
<td>Sinus rhythm. Poor output for 19 hr.</td>
<td>Excellent donor heart function despite 3 acute rejection episodes. Returned to full-time employment. Ruptured deteriorated and died from chronic rejection at 5 mo.</td>
</tr>
<tr>
<td>48/Male</td>
<td>Cardiomyopathy</td>
<td>10/1/81</td>
<td>8 hr 7 min</td>
<td>Sinus rhythm. Poor output for 20 hr.</td>
<td>Excellent donor heart function despite 4 acute rejection episodes. Found to have toxoplasmosis of both hearts on biopsy; treated successfully. Developed tuberculous meningitis; remains very ill.</td>
</tr>
<tr>
<td>27/Male</td>
<td>Cardiomyopathy (failed transplant 1979—acute rejection at 5 wk)</td>
<td>10/4/81</td>
<td>16 hr 50 min</td>
<td>Sinus rhythm. Poor function.</td>
<td>Accelerated acute rejection with donor specific antibody formation. Donor heart function ceased after 5 days. Heart excised. Patient remains alive but exercise tolerance extremely limited (NYHA Class 4).</td>
</tr>
<tr>
<td>38/Male</td>
<td>Cardiomyopathy</td>
<td>10/29/81</td>
<td>6 hr 55 min</td>
<td>Sinus rhythm. Excellent function immediately.</td>
<td>One late acute rejection episode. Remains well with excellent donor heart function.</td>
</tr>
</tbody>
</table>

A 500 ml/min gas flow maintained the pH between 7.0 to 7.8, and a 60 to 120 ml/min perfusate flow into the upper chamber was achieved. Perfusate pO₂ at 30°C was measured at between 1,000 and 2,000 mm Hg.

From the upper chamber, the fluid flowed by gravity through the ascending aorta into the coronary arteries of the suspended heart at a pressure of 8 to 10 cm H₂O, depending on aortic length. Coronary venous effluent, collected in the right atrium, returned to the lower reservoir by gravity drainage via the inferior vena cava or pulmonary artery orifices. An overflow pipe between the upper and lower reservoirs prevented the upper chamber from filling above a set hydrostatic pressure, at which the myocardium was perfused.

Initially the lower chamber contains three liters of perfusate. (The flow up the delivery tube can be increased or decreased simply by adding or subtracting from this initial fluid load, enabling a constant gas flow to be maintained and thus avoiding major fluctuations in pH that would follow changes in gas flow.)

The perfusion apparatus was then placed in a stainless steel container, insulated with polystyrene and plastic, and packed with ice to maintain a 4°C to 10°C temperature throughout the preservation period. The coronary flow was measured at the beginning and end of the perfusion. Using the final cardioplegic agent and perfusate, edema formation in the donor myocardium was of the same order (80%) as that found when using the nonportable system. Functional testing of pig hearts confirmed satisfactory myocardial protection. Orthotopic transplantation in baboons was also followed by consistent survival until rejection (mean survival period of 26 days). Posttransplantation cardiac catheterization again revealed normal pressures and cardiac output.

### Autotransplantation

In an attempt to study the long-term effects of donor heart storage without encountering the problem of rejection, autotransplantation was performed in three cases. The recipient's heart was excised and transferred to the perfusion apparatus, and the baboon was supported...
for 24 hours by an orthotopic transplant from another baboon. The next day the transplanted heart was removed, and the baboon’s own heart, having undergone continuous hypothermic perfusion for 24 hours, was reimplemented.

All three autografts functioned satisfactorily postoperatively, although two of the baboons died at 7 and 31 days—one of enterocolitis with purulent peritonitis and the other of a ruptured false aneurysm of the aorta. The third animal remains alive and well some 8 months later. Cardiac catheterization and biopsy at 1, 2, and 6 months demonstrated normal hemodynamics and normal histology on light and electron microscopy.

Clinical Experience

Four patients received donor hearts preserved with the portable hypothermic perfusion system. The total periods of ischemia ranged from 6 hours 55 minutes to 16 hours 50 minutes, and in one case the heart was transported by air a distance of 550 miles. Heterotopic transplantation was performed in all four patients using the operative technique described by C. N. Barnard and J. G. Losman, and the details have been reported elsewhere (W. N. Wicomb, et al, unpublished data). The outcome in each case is presented in Table III.

One patient experienced accelerated acute rejection within 5 days with development of donor-specific antibodies. The function of the donor heart, stored for nearly 17 hours, was never good. The relative responsibility of preservation, technical problems of the operation, the possibility of some compression of the donor heart after the chest was closed, and damaging effects of accelerated rejection are difficult to dissociate.

In the remaining three cases, the donor heart’s good function was demonstrated by the progress of the patient, by cardiac catheterization and angiography. One patient had early chronic rejection. However, in the two other patients, satisfactory donor heart function was delayed for several hours, during which the recipient’s own heart, supported with inotropic agents, maintained adequate circulation. Pulse wave traces taken over the femoral artery (case 1) showed the recipient heart’s dominant pulse (Figure 4). Recovery of the donor heart occurred in a few hours, after which it generated most of the cardiac output. On both occasions, the donor heart returned to sinus rhythm soon after blood reperfusion without any further rhythm disturbances. Although this phenomenon was seen previously after transplanting freshly excised donor hearts, it has never been as marked as on these two occasions.

In our baboon studies, early function following orthotopic transplantation was the rule. However, these hearts were harvested, stored, and transplanted under ideal conditions from a healthy baboon. A human donor heart may have undergone some damage during periods of hypotension, inotropic support, and other drug
therapy. Possibly such insults are more damaging to hearts that subsequently undergo long periods of ischemic storage.

ACKNOWLEDGMENT: The experimental work presented here was carried out with the skilled technical assistance of F. Barends. Ms. J. Martin, P. Maclingoz, J. Ronsonow, Sister E. Hanekom, Miss S. Smith and F. Snyder, and it was supported by the Christian Barnard Fund, the University of Cape Town, and the Cape Provincial Administration. The authors wish to thank the many members of the medical, nursing, and paramedical staffs of Groote Schuur Hospital and the University of Cape Town Medical School who cared for the four patients presented.

References

5. Wicomb WN, Cooper DKC, Barnard CN: Twenty-four hour preservation of the pig heart by a portable hypothermic perfusion system. Transplantation (in press).

Discussion

Michael Bess, Richmond: I would first like to congratulate the authors for attempting such a daring clinical study. Using a similar perfusion system, Richard Lower and coworkers compared continuously perfused hearts with a series of hearts stored in a standard way for four hours in ice cold saline. They studied cellular ultrastructure and myocardial metabolism, and were able to transplant all these hearts successfully. Their function was well preserved, but the biochemical parameters were better maintained with 24-hour perfusion than with a four-hour cold storage. I think that the method presented should work satisfactorily.

David Cooper, Cape Town: I indeed agree with your conclusion. However, the results will depend on the type of perfusate used and on the characteristics of the perfusion system. In fact, I think that a single flush with a cold cardioplegic solution followed by storage in ice would also be satisfactory. In our hands, nevertheless, we had a high incidence of success with hearts perfused continuously.

Michael Kaye, Rochester, Minn.: We are using a totally different principle, perfusing the aorta of the graft retrogradely with diluted blood at 30° to 32°C, and we keep the heart beating uninterrupted. We have 100% success after reimplantation of these hearts and presently we are comparing the hemodynamic performance of a control series of non-perfused, acutely transplanted hearts with our perfused hearts series. So far we have found that there is only a very slight depression of function in the perfused series.

Jacques Losman, Chicago: I am very surprised by the degree of edema that developed in some of your perfused hearts and by the fact that this edema had no significant deleterious effect. This is rather in contradiction to the work of many groups and to my own experience.

Mitchell Goldman, Richmond: I would like to know why you used oxygen to aerate your perfusate. Do you think that you need all of that oxygen at low temperatures? The experience of kidney preservation is that you can perfuse it and oxygenate the perfusate with room air. At 7°C the amount of oxygen dissolved from room air is sufficient for the requirements of the kidney. I wonder if there are different requirements for the heart.

Dr. Cooper: You are probably right and we may give far too much oxygen. We believe that the heart needs some oxygen but we overdo it.

Winston Wicomb, San Diego: I am presently working with Dr. Geoffrey Collins on kidney preservation. I agree with Dr. Goldman—we could probably perfuse these hearts with a much lower oxygen content. However, the pump used in the Cape Town perfusion system depends on the gas flow. I believe we could aerate the perfusate with air and I plan to do so in the future.
FORTY-EIGHT HOURS HYPOTHERMIC PERFUSION
STORAGE OF PIG AND BABOON HEARTS

BY

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ABSTRACT

A system has been developed for the continuous hypothermic perfusion of isolated hearts using a clear fluid perfusate. Myocardial viability has been maintained after periods of storage of up to 48 hours. Pig hearts stored in this way showed almost normal haemodynamic performance on subsequent functional testing. Orthotopic allotransplantation or autotransplantation of baboon hearts stored for 24 and 48 hours was followed by good immediate and long-term function. Baboons receiving allotransplants survived until rejection. Three of four of those autotransplanted survived until electively sacrificed at 1, 3 and 12 months; all showed normal haemodynamic function on cardiac catheterization and normal myocardial histology.
INTRODUCTION

The benefits of being able to retain viability of the donor organ for periods of several hours are obvious. Experimental work in the field of storage of the heart has been reviewed fully elsewhere (1).

We have previously reported successful hypothermic perfusion for 24 hours in pigs and baboons using both non-portable (2,3) and portable (4,5) perfusion systems. Clinically, hypothermic perfusion storage of donor hearts has been carried out at our own institution for periods ranging from up to 7 to 17 hours (6,7). The dynamics of the non-portable perfusion system have recently been clarified (8).

We have subsequently modified the perfusion solution further (Table 1). Based on the hypothesis that a relatively low pH, strong buffering action plays an essential role in calcium homeostasis during hypothermic perfusion, the bicarbonate buffer used in our earlier perfusates was replaced by a potassium phosphate buffer (in a molar ratio of 5 dipotassium hydrogen phosphate to 1 potassium dihydrogen phosphate). Using this perfusate, storage periods of 48 hours have now become possible.

MATERIALS AND METHODS

Three experimental models were used.

1. FUNCTIONAL TESTING OF PIG HEARTS

Fourteen pigs, weighing 15 to 25 kg, were used as heart donors. Details of the anaesthesia of these animals and of our methods of cold cardioplegic arrest and excision of the heart have been reported previously (2,4).
Group A - Freshly arrested hearts \((n = 10)\)

After the infusion of 500 ml of cold \((4^\circ C)\) cardioplegic solution (Table 2) into the root of the aorta, and the application of cold \((4^\circ C)\) normal saline over the heart, arrest of the heart occurred within 15 to 20 seconds. Excision of the heart was then completed and the heart immersed in cold \((4^\circ C)\) normal saline for 1 minute. The heart was then transferred to the functional testing apparatus (Figure 1), details of which have also been described previously \((2,4)\), the total time from initiation of infusion of cardioplegic solution taking approximately 10 to 14 minutes.

Group B - Hearts hypothermically perfused for 48 hours \((n = 4)\)

Preparation, anaesthesia, cold cardioplegic arrest and excision of the heart were as for Group A. The excised heart was then transferred to a perfusion apparatus and preserved for 48 hours. The perfusion circuit is shown in Figure 2. The perfusate (Table 1) was sterilized by being passed through a 0.8 \(\mu\) filter. The perfusate was both oxygenated and circulated throughout the period of storage by the air lift pump principle \((8,9)\), 100% oxygen being used as the gas supply. A gas flow of approximately 500 ml/minute maintained a perfusate flow into the upper chamber of approximately 60 to 120 ml/minute. The perfusate pH remained within the range of 6.85 - 6.95. Other details of this perfusion system have been reported previously \((4-8)\). The entire perfusion system was packed in ice and maintained at a temperature of 4 to 10\(^\circ\)C throughout the 48 hour preservation period. At the onset and end of the perfusion period, the heart was weighed and coronary flow was measured. After the period of perfusion, the heart was functionally tested.

**Ex Vivo Functional Testing** (Groups A and B)

The functional testing apparatus is shown in Figure 1 and has been described fully elsewhere \((2,4)\). Oxygenated blood, matched for group, was supplied by a perfusor
pig, to which deoxygenated blood was returned. The left ventricular and left ven-
tricular end-diastolic pressures of the isolated heart were measured using a Statham
P23H transducer. Cardiac output was obtained by a timed collection of aortic out-
put plus coronary venous return. Coronary flow was similarly measured by a timed
collection of coronary venous return. All hearts were tested for one hour, during
which time these parameters were recorded at regular intervals. The hearts were
then biopsied for histological examination by light microscopy.

2. ORTHOTOPIC ALLOTRANSPLANTATION IN BABOONS

Two groups (X and Y) of outbred Chacma baboons (Papio ursinus) weighing 20 to 30
kg were studied.

Group X - Orthotopic allotransplantation following 24 hours storage (n = 2)

Details of anaesthesia, cold cardioplegic arrest and donor heart excision, our techni-
que of orthotopic allotransplantation, and postoperative management have been re-
ported previously (3,5). The cardioplegic solution, portable hypothermic perfusion
apparatus, and perfusate were identical to those described for the pig model (above).
Both baboons received a maintenance immunosuppressive regimen of azathioprine
2.5 mg/kg/day and methylprednisolone (10 mg/kg/day reducing by 2 mg/kg/day until
a maintenance dose of 2 mg/kg/day was achieved). No attempt was made to treat
acute rejection episodes.

Group Y - Orthotopic allotransplantation following 48 hours storage (n = 4)

The experimental method was identical to that in Group X above, except that sto-
rage was continued for 48 hours. Three of the four baboons were given the immu-
nosuppressive regimen outlined above, but the fourth received cyclosporin A 18 mg/
kg/day given by intramuscular injection in a solution of intralipid and alcohol. This
pig, to which deoxygenated blood was returned. The left ventricular and left ventricular end-diastolic pressures of the isolated heart were measured using a Statham P23H transducer. Cardiac output was obtained by a timed collection of aortic output plus coronary venous return. Coronary flow was similarly measured by a timed collection of coronary venous return. All hearts were tested for one hour, during which time these parameters were recorded at regular intervals. The hearts were then biopsied for histological examination by light microscopy.

2. ORTHOTOPIC ALLOTRANSPLANTATION IN BABOONS

Two groups (X and Y) of outbred Chacma baboons (Papio ursinus) weighing 20 to 30 kg were studied.

**Group X - Orthotopic allotransplantation following 24 hours storage (n = 2)**

Details of anaesthesia, cold cardioplegic arrest and donor heart excision, our technique of orthotopic allotransplantation, and postoperative management have been reported previously (3,5). The cardioplegic solution, portable hypothermic perfusion apparatus, and perfusate were identical to those described for the pig model (above). Both baboons received a maintenance immunosuppressive regimen of azathioprine 2.5 mg/kg/day and methylprednisolone (10 mg/kg/day reducing by 2 mg/kg/day until a maintenance dose of 2 mg/kg/day was achieved). No attempt was made to treat acute rejection episodes.

**Group Y - Orthotopic allotransplantation following 48 hours storage (n = 4)**

The experimental method was identical to that in Group X above, except that storage was continued for 48 hours. Three of the four baboons were given the immunosuppressive regimen outlined above, but the fourth received cyclosporin A 18 mg/kg/day given by intramuscular injection in a solution of intralipid and alcohol. This
(Group B) than in the baboon hearts (Groups X, Y and Z). The release of LDH into
the coronary sinus effluent was significantly higher at both 24 hours and 48 hours
in the hearts of Group Y, and at 48 hours in the hearts of Group Z.

**Ex-Vivo Functional Evaluation of Pig Hearts (Groups A and B)**

The left ventricular pressure generated by the 48 hour stored hearts (Group B) was
significantly less than that of the freshly excised hearts (Group A). Cardiac output,
coronary flow, and left ventricular end-diastolic pressure did not differ significantly
between the two groups (Table 4).

**Orthotopic Transplantation of Baboon Hearts Following 24 hour Storage (Group X)**

Both hearts reverted to spontaneous sinus rhythm after warm blood reperfusion.
Inotropic support was minimal and the animals were weaned from the ventilator
within 4 hours. Survival was for 6 and 26 days, and death from acute rejection
(Table 5). These two baboons did not undergo cardiac catheterization following
transplantation.

**Orthotopic Transplantation of Baboon Hearts Following 48 hour Storage (Group Y)**

All 4 hearts required electrical defibrillation, which was followed by excellent myo-
cardial contractions. The animals immediately maintained mean arterial pressures
of between 80 and 100 mm Hg, with minimal inotropic support. They were returned
to their cages within 4 hours of discontinuation of cardiopulmonary bypass. Cardiac
catheterization data, obtained after transplantation, are tabulated in Table 6; hae-
modynamic performance of these hearts did not differ significantly from control
data obtained in these and other healthy baboons before operation. Mean survival
in the 3 baboons immunosuppressed with azathioprine and methylprednisolone was
20 days, death resulting from acute rejection (Table 5); the one animal immunosup-
pressed with cyclosporin A remains alive at 70 days.
(Group B) than in the baboon hearts (Groups X, Y and Z). The release of LDH into the coronary sinus effluent was significantly higher at both 24 hours and 48 hours in the hearts of Group Y, and at 48 hours in the hearts of Group Z.

**Ex-Vivo Functional Evaluation of Pig Hearts (Groups A and B)**

The left ventricular pressure generated by the 48 hour stored hearts (Group B) was significantly less than that of the freshly excised hearts (Group A). Cardiac output, coronary flow, and left ventricular end-diastolic pressure did not differ significantly between the two groups (Table 4).

**Orthotopic Transplantation of Baboon Hearts Following 24 hour Storage (Group X)**

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**Orthotopic Transplantation of Baboon Hearts Following 48 hour Storage (Group Y)**

All 4 hearts required electrical defibrillation, which was followed by excellent myocardial contractions. The animals immediately maintained mean arterial pressures of between 80 and 100 mm Hg, with minimal inotropic support. They were returned to their cages within 4 hours of discontinuation of cardiopulmonary bypass. Cardiac catheterization data, obtained after transplantation, are tabulated in Table 6; haemodynamic performance of these hearts did not differ significantly from control data obtained in these and other healthy baboons before operation. Mean survival in the 3 baboons immunosuppressed with azathioprine and methylprednisolone was 20 days, death resulting from acute rejection (Table 5); the one animal immunosuppressed with cyclosporin A remains alive at 70 days.
Autotransplantation of Baboon Hearts Following 48 hour Storage (Group Z)

When the recipient's own heart was replaced at the second operation, warm blood reperfusion and electrical defibrillation led to good ventricular contractions. The hearts took over the circulation satisfactorily, maintaining mean arterial pressures between 80 and 100 mm Hg with minimal inotropic support. One animal died at 8 days from an infected mediastinal haematoma. The remaining animals underwent cardiac catheterization within the first month. Two animals were sacrificed after one and 3 months respectively, the fourth animal remaining alive one year later; this animal has undergone cardiac catheterization on two subsequent occasions during the year. Cardiac catheter data throughout this study showed normal haemodynamics; the small pressure gradient between right ventricle and pulmonary artery seen in nearly all animals was due to narrowing at the pulmonary artery anastomosis, confirmed at necropsy (and seen in previous studies (3,5)). Histological examination of the myocardium appeared normal, except in one animal which showed scanty focal myocytolysis (Table 5).

DISCUSSION

Periods of preservation longer than 24 hours have met with only moderate success. In 1967, Manax et al (10) stored hearts by a combination of hypothermia and hyperbaric oxygenation for periods up to 72 hours; evaluation of viability, however, was based only on the number of hearts resuming regular ventricular beats for up to 80 minutes. Proctor and Parker (11) stored hearts by extracorporeal hypothermic perfusion for 72 hours, but evaluation of viability was again inadequate, the heart being passively perfused (viviperfusion) for a short period by attaching it to the vessels of another dog. This group later performed orthotopic transplantation after a similar storage period (12); immediate function was satisfactory, but technical problems prevented assessment for longer than 14 hours. In 1979, Toledo-Pereyra et al (13) preserved hearts for 48 hours by hypothermic pulsatile perfusion; 5 out of 6 animals
survived heterotopic transplantation (viviperfusion) to rejection or infection, donor heart failure occurring at a mean of 11 days.

Using storage in an intermediate host, by heterotopic transplantation (viviperfusion), Angell and his colleagues (14) stored hearts for 4 days; subsequent orthotopic transplantation was successful. This method would not seem practical for clinical use. Dupree et al (15) used a similar system of viviperfusion for the xenogeneic storage of primate hearts for periods of up to 7 days. Donor stump-tail monkey hearts were anastomosed to vessels in the abdomen of recipient baboons, whose immune response had been suppressed by whole body radiation 24 hours prior to receiving the donor hearts. This allowed survival of the host animal for 9 to 11 days, with nearly complete immunosuppression for 7 days. The donor monkey hearts showed negligible changes on electrocardiography, and, apart from minimal oedema in the interstitial spaces, histological sections remained essentially normal. Functional viability, however, was not tested. Such a xenogeneic storage system might prove to be a potential answer to short-term storage of human cadaver organs, though the risks of infection in the recipient animal, and therefore in the donor heart, remain unknown.

No group other than Angell et al, therefore, has previously reported consistent long-term survival after orthotopic transplantation or autotransplantation of animals in which the heart has been stored for 48 hours or more. We believe it is essential to be certain that the stored heart is capable of supporting the entire circulation long-term; this is only possible if orthotopic transplantation with effective immunosuppression or autotransplantation results in survival of the recipient animal for some weeks or, preferably, months.

In the present series of experiments comparison of the parameters measured during the period of hypothermic perfusion storage reveals that the pig heart appears to be
more sensitive to ischaemic injury than the baboon heart. Oedema formation (gain in mass) is more marked in the pig heart, and there is a greater reduction in coronary flow. Previous studies have shown that perfusate LDH levels rise more rapidly and reach a higher peak in stored pig hearts than in stored baboon hearts (2-5).

Functional testing of the pig heart stored for 48 hours using the perfusate detailed in the present study (Group B) showed no significant differences from hearts stored for only 24 hours with our previous perfusate (4), and only differed from the freshly excised hearts (Group A) in that the mean left ventricular pressure was slightly lower (Table 4).

The major perfusate modification incorporated in the present study, when compared with the previous perfusate developed in our laboratory (4,7), was in replacing sodium bicarbonate with potassium phosphate as the only buffer, bringing about buffering at the desired pH of 6.85 to 6.95. The sodium chloride concentration was increased, both to compensate for the fall in osmolality resulting from omission of sodium bicarbonate, and to increase the activity of the sodium-calcium exchange mechanism, driving calcium into the extracellular space.

These perfusate modifications were based on the hypothesis that calcium homeostasis plays a central role in myocardial protection during hypothermic perfusion preservation. In the presence of continued energy production, our aim was to limit energy expenditure by stringent control of cellular calcium fluxes; under hypothermic conditions it was believed that this could be achieved by lowering the perfusate pH from 7.4 to the "unphysiological" range of 6.85 - 6.95, using phosphate buffer. At high pH values (7.2 - 8.0), the calcium-dependent ATPase has maximum activity, but calcium accumulation in the sarcoplasmic reticulum is inhibited (16), leading to elevated intracellular calcium concentrations. At low pH values (less than 7.0) calcium transport and calcium ATPase activity remain coupled, accumulating calcium in the
sarcoplasmic reticulum (17), and maintaining a low intracellular calcium concentration. Low temperatures slow down sarcoplasmic reticulum calcium flux, with both calcium transport and calcium ATPase activity remaining coupled (18). Thus ATP hydrolysis is coupled with calcium uptake, with no energy wastage occurring. Uncoupled sarcoplasmic reticulum results in ATP hydrolysis with no net calcium uptake; free intracellular calcium increases, resulting in enhanced resting myocardial tone ("stone heart"). The presence of magnesium in the perfusate was also considered essential as it inhibits calcium efflux (19).

The perfusate sodium concentration was increased to maintain both the perfusate osmolality and to protect against intracellular calcium accumulation by accelerating the sodium-calcium exchange mechanism. Sodium competes with calcium for both the high affinity calcium receptor site of the sodium-calcium exchange mechanism and for the slow inward channel of the action potential plateau (20). By increasing the perfusate sodium concentration, more competition for these sites occurs, resulting in increased efflux of calcium by the sodium-calcium mechanism, and decreased calcium inflow via the slow inward channel, thus increasing the calcium concentration in the extracellular space and reducing it intracellularly. A high perfusate sodium concentration is, therefore, also a factor in preventing the intracellular calcium concentration from becoming too high.

Using the system of storage described in this study, the temperature at which the heart is maintained appears to be critical. Three pig hearts and one baboon heart (not included above), were subjected to temperatures below 1°C (but above 0°C) for part of the 48 hours storage period. These hearts showed no function whatsoever on functional testing or after transplantation. During long periods of perfusion storage, we now take care to ensure that perfusate temperature does not fall below 2.5°C, which we consider as the lowest limit of absolute safety. Simple storage in ice at
these low temperatures, however, does not result in injury. The different response between ice storage and continuous hypothermic perfusion is probably related to the effect of trauma caused by the continuous perfusion of membranous structures that have undergone solidification under extreme hypothermia. Hearts in simple ice storage survive at temperatures of 0°C, at least for periods less than 4 hours, because the brittle membranes are not subjected to the stress of perfusion. The threshold temperature of perfusion storage has not been finally determined, but recent work in our laboratory has demonstrated that a temperature of 2.5°C is not damaging to the myocardium.

Similarly, the purity of the perfusate constituents is also critical. We have previously reported poor results when the perfusate procaine hydrochloride was made up in 0.5% phenol rather than in 0.1% chloro-cresol (4). We have recently experienced failure of the system when the perfusate contained trace concentrations of iron greater than 1.2 uM (21); oxygen radical production would appear to be the cause of loss of viability of these organs. The damaging effects of such impurities may account for lack of reproducibility of perfusion systems from centre to centre, and hitherto do not appear to have been accorded sufficient attention by those interested in this field.

Although storage of the heart for 48 hours by the system used in the present study would allow long distance transportation, the routine use of extracorporeal hypothermic perfusion of donor hearts is still limited by the state of the myocardium at the onset of the preservation period. Functional and even structural damage can occur to the myocardium during the agonal period (22-24), and may be accentuated by therapy given to the donor to maintain an adequate haemodynamic status (23,24). Long periods of ischaemic storage have been shown to increase the depletion of myocardial energy stores, which have already been significantly reduced during the agonal period.
(23,24). Current work in our laboratory is focusing on methods of preventing depletion and even of replacing the myocardial energy stores of the donor before the heart is excised and stored. If this can be achieved we believe extracorporeal hypothermic perfusion storage of donor hearts for periods of 24 to 48 hours will become a clinical reality.
ACKNOWLEDGEMENTS

We thank Sister P Ahrends, F Barends, Mrs J Kloppers, P Madlingozi, J Rossouw, and F Snyders for skilled technical assistance. This work was supported by the Chris Barnard Fund, the University of Cape Town, and the Cape Provincial Administration.
REFERENCES


10. MANAX, W.G., EYAL, Z., LILLEHEI, R.C. Observations on the homotransplan-
ted canine heart following in vitro storage by hypothermia and hyperbaric

11. PROCTOR, E., PARKER, R. Preservation of the isolated heart for 72 hours.

12. PROCTOR, E., JONES, G.R.N. In vitro assessment of preserved dog hearts,
with some reference to preserved dog kidneys. Chapter in "Organ Preservation",
Page 216.

13. TOLEDO-PEREYRA, L.H., CHEE, M., LILLEHEI, R.C., JARA, F.M. Forty
eight hour hypothermic pulsatile perfusion of canine hearts before transplanta-

14. ANGELL, W.W., RIKKERS, L., DONG, E., SHUMWAY, N.E. Canine cadaver
heart procurement, resuscitation, and storage. Chapter in "Organ Perfusion
and Preservation" edited by J C Norman. Appleton-Century-Crofts, New York,

15. DUPREE, E.L., MILLS, M., CLARK, R., SELL, K.W. Xenogeneic storage of

16. TATE, C.A., CHU, A., MCMILLAN-WOOD, J., VAN WINKLE, W.B., ENTMAN,
M.L. Evidence for a calcium sensitive factor which alters the alkaline pH sen-
sitivity of sarcoplasmic reticulum calcium transport. J Biol Chem. 256, 2934-
2939, 1981.

binding and release by canine cardiac relaxing system (sarcoplasmic reticulum).

18. INESI, G., MILLMAN, M., ELETR, S. Temperature-induced transitions of function
and structure in sarcoplasmic reticulum membranes. J Mol Biol. 81, 483-504,


LEGENDS

TABLES

Table 1. Constitution of perfusate.

Table 2. Constitution of cardioplegic solution.

Table 3. Changes in heart mass, coronary venous effluent lactic dehydrogenase concentration, and coronary flow during 24 or 48 hours hypothermic perfusion storage of pig (Group B) and baboon (Groups X, Y and Z) hearts.

Table 4. Haemodynamic performance on functional testing of freshly excised pig hearts (Group A) and hearts stored by hypothermic perfusion for 48 hours (Group B).

Table 5. Length of survival, cause of death, and myocardial histology in baboons undergoing orthotopic allotransplantation (Group X and Y) or autotransplantation (Group Z) following storage of the heart for 24 (Group X) or 48 hours (Groups Y and Z).

Table 6. Data obtained at cardiac catheterization in control baboons and after orthotopic allotransplantation (Group Y) or autotransplantation (Group Z) of baboon hearts stored for 48 hours.
<table>
<thead>
<tr>
<th>Component</th>
<th>g/l</th>
<th>mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>7.98</td>
<td>143.80</td>
</tr>
<tr>
<td>MgSO&lt;sub&gt;4&lt;/sub&gt;· 7H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>3.48</td>
<td>14.40</td>
</tr>
<tr>
<td>CaCl&lt;sub&gt;2&lt;/sub&gt;· 2H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>0.16</td>
<td>1.10</td>
</tr>
<tr>
<td>KH&lt;sub&gt;2&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.24</td>
<td>1.73</td>
</tr>
<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;HPO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.11</td>
<td>6.34</td>
</tr>
<tr>
<td>Procaine hydrochloride</td>
<td>0.27</td>
<td>1.10</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>0.0031</td>
<td>-</td>
</tr>
<tr>
<td>Phenoxybenzamine</td>
<td>0.0025</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.00</td>
<td>11.10</td>
</tr>
<tr>
<td>Sucrose</td>
<td>2.50</td>
<td>7.00</td>
</tr>
<tr>
<td>Glycerol</td>
<td>12.60</td>
<td>136.00</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.50</td>
<td>4.00</td>
</tr>
<tr>
<td>Osmolality</td>
<td>410m0sm</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.85-6.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CONSTITUTION OF CARDIoplegic SOLUTION</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---------------------------------------------------</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>NaCl</td>
<td>6.54</td>
</tr>
<tr>
<td>2.</td>
<td>NaHCO₃</td>
<td>2.10</td>
</tr>
<tr>
<td>3.</td>
<td>KCl</td>
<td>0.82</td>
</tr>
<tr>
<td>4.</td>
<td>KH₂PO₄</td>
<td>0.16</td>
</tr>
<tr>
<td>5.</td>
<td>CaCl₂.  2H₂O</td>
<td>0.15</td>
</tr>
<tr>
<td>6.</td>
<td>MgSO₄.  7H₂O</td>
<td>0.29</td>
</tr>
<tr>
<td>7.</td>
<td>Procaine HCl</td>
<td>0.27</td>
</tr>
<tr>
<td>8.</td>
<td>Insulin</td>
<td>40 u/l</td>
</tr>
<tr>
<td>9.</td>
<td>Dextrose</td>
<td>50</td>
</tr>
<tr>
<td>10.</td>
<td>Verapamil HCl</td>
<td>0.0015</td>
</tr>
<tr>
<td></td>
<td>Osmolality</td>
<td>300 mOsm/l</td>
</tr>
<tr>
<td></td>
<td>pH at 4°C</td>
<td>7.4</td>
</tr>
<tr>
<td>Group</td>
<td>n</td>
<td>Period of Storage (hours)</td>
</tr>
<tr>
<td>-------</td>
<td>---</td>
<td>---------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Before (grams)</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>48</td>
</tr>
<tr>
<td>X</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>Y</td>
<td>4</td>
<td>48</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
<td>48</td>
</tr>
</tbody>
</table>

Figures in brackets refer to standard error.

*Student t test for paired data.
TABLE 4

HAEMODYNAMIC PERFORMANCE ON FUNCTIONAL TESTING OF FRESHLY EXCISED PIG HEARTS
(GROUP A) AND HEARTS STORED BY HYPOTHERMIC PERFUSION FOR 48 HOURS (GROUP B)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>CARDIAC OUTPUT (l/min)</th>
<th>CORONARY FLOW (ml/min)</th>
<th>LEFT VENTRICULAR PRESSURE (mm Hg)</th>
<th>LEFT VENTRICULAR END-DIASTOLIC PRESSURE (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2820(220)</td>
<td>258(33)</td>
<td>131(4.4)</td>
<td>5.3(1.03)</td>
</tr>
<tr>
<td>B</td>
<td>2249(365)</td>
<td>308(17)</td>
<td>112(6.7)</td>
<td>4.5(2.87)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

Statistical Comparison A vs B
LENGTH OF SURVIVAL, CAUSE OF DEATH, AND MYOCARDIAL HISTOLOGY IN BABOONS UNDERGOING ORTHOTOPIC ALLOGRAFTS (GROUPS X AND Y) OR AUTOGRAFTS (GROUP Z) FOLLOWING STORAGE OF THE HEART FOR 24 (GROUP X) OR 48 HOURS (GROUPS Y AND Z).

<table>
<thead>
<tr>
<th>Group</th>
<th>Survival (Days)</th>
<th>Cause of Death</th>
<th>Myocardial Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>26</td>
<td>Acute rejection</td>
<td>Acute rejection</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>16</td>
<td>Acute rejection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>Acute rejection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>Sacrificed</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>365</td>
<td></td>
<td>Normal on endomyocardial biopsy</td>
</tr>
</tbody>
</table>
### TABLE 6

**DATA OBTAINED AT CARDIAC CATHETERIZATION IN CONTROL BABOONS AND AFTER ORTHOTOPIC ALLOTRANSPLANTATION (GROUP Y) OR AUTOTRANSPLANTATION (GROUP Z) OF BABOON HEARTS STORED FOR 48 HOURS**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 4)</th>
<th>Group Y (n = 4) at 1-7 days</th>
<th>Group Z (n = 4) at 1-30 days</th>
<th>Group Z (n = 1) at 6 months</th>
<th>Group Z (n = 1) at 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressures (mm Hg):-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right atrium (mean)</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Right ventricle (systolic)</td>
<td>(1.5)</td>
<td>(1.0)</td>
<td>(1.2)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Pulmonary artery (systolic)</td>
<td>26</td>
<td>27</td>
<td>46</td>
<td>45</td>
<td>36</td>
</tr>
<tr>
<td>Pulmonary artery (systolic)</td>
<td>(0.67)</td>
<td>(3.5)</td>
<td>(7.5)</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Pulmonary capillary wedge (mean)</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Pulmonary capillary wedge (mean)</td>
<td>(3.6)</td>
<td>(2.2)</td>
<td>(1.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricle (systolic)</td>
<td>110</td>
<td>124</td>
<td>125</td>
<td>170</td>
<td>140</td>
</tr>
<tr>
<td>Left ventricle (systolic)</td>
<td>(8.3)</td>
<td>(6.1)</td>
<td>(5.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricle (end-diastolic)</td>
<td>2.0</td>
<td>2.0</td>
<td>3.5</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Left ventricle (end-diastolic)</td>
<td>(0.3)</td>
<td>(0.4)</td>
<td>(0.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td>2.6</td>
<td>2.7</td>
<td>2.7</td>
<td>3.9</td>
<td>3.2</td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td>(0.26)</td>
<td>(0.38)</td>
<td>(0.74)</td>
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</tbody>
</table>

Figures in brackets refer to standard error.
LEGENDS

FIGURES

Figure 1. System for functional testing of the isolated heart (HE = heat exchanger; F = filter; AoO = aortic output (for monitoring cardiac output); WK = windkessel; PR = non-linear resistance device; AoB = aortic balloon; AoP = aortic pressure monitoring transducer (mm Hg); ECG = needle electrodes; LAR = left atrial reservoir; LAP = left atrial pressure (cm of H_2O); P = roller pump; LVP = left ventricular pressure monitoring transducer (mm Hg); CBF = coronary venous return.

Figure 2. Portable hypothermic perfusion apparatus.
AN AIRLIFT PUMP DEVICE FOR LOW PRESSURE
PERFUSION STORAGE OF THE ISOLATED HEART

BY

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INTRODUCTION

At the present time, there is an inadequate supply of donor hearts, resulting in a high mortality in potential recipients awaiting transplantation (1). Storage of donor hearts for periods of up to 24 to 48 hours would enable transportation over long distances, and would therefore increase the donor supply available to any one centre. The majority of donor hearts used in clinical transplantation programmes to date has been preserved by simple storage in ice for periods of up to 4 hours, allowing transportation by chartered jet aircraft over quite long distances (2). Non-portable (2-4) and portable (2,5-7) hypothermic perfusion units for heart preservation have been designed and tested successfully in the experimental laboratory, allowing storage for up to 48 hours (8). Such a portable system has been used clinically on 4 occasions (7,9).

The essential features of the design of any portable storage system should be simplicity, compactness, independence from any external source of power, and absolute sterility. We describe here a portable preservation unit which we believe meets these requirements.

Cold Cardioplegic Arrest and Heart Excision

The technique of heart excision has been described fully elsewhere (3-9). Briefly, after heparinization, the right side of the heart is decompressed by inflow occlusion and division of the inferior vena cava, and the left side of the heart similarly decompressed by division of one or more pulmonary veins. The aorta is then cross clamped and cardioplegic solution* at 4°C infused through the coronary arterial system.

* Composition of cardioplegic solution: NaCl, 6 gm per liter (102 mmol); NaHCO₃, 0.38 gm/l (4 mmol); KCl, 0.75 gm/l (10 mmol); CaCl₂, 2H₂O, 0.15 gm/l (1.1 mmol); MgSO₄ 7H₂O, 3.5 gm/l (14 mmol); procaine hydrochloride, 0.27 gm/l (1 mmol); insulin, 20 units per liter, dextrose, 50 gm/l (278 mmol); verapamil hydrochloride, 1.5 mg/l; osmolality, 320 mOsm/l; pH at 4°C, 7.4.
The pericardial cavity is filled with cold (4°C) normal saline to externally cool the heart. Cessation of heart beat occurs within 10 - 30 seconds. The volume of cardioplegic solution infused depends on the mass of the experimental animal or patient, (15-20 ml/kg body weight), but infusion is usually completed within 1 to 3 minutes, by which time myocardial temperature has fallen to approximately 12 - 15°C.

Excision of the heart is completed by division of the pulmonary veins, pulmonary artery at its bifurcation, aorta proximal to the brachiocephalic artery, and the mediastinal tissue posterior to the heart. The heart is then immersed for 1 minute in a bowl containing cold (4°C) cardioplegic solution or saline, before being suspended from the preservation unit (described below) and continuously perfused.** The time interval between aortic cross clamping and the initiation of perfusion is approximately 10 - 12 minutes.

DESCRIPTION OF PRESERVATION SYSTEM

1. Apparatus

A diagram of the portable unit is shown in Figure 1. The unit consists of two vertically located chambers connected by a polyvinyl chloride pipe (T2), leading from the lower chambers (C1) to the upper chamber (C2). The fluid capacity of C2 is 250 ml and of C1 4 litres. The total volume of fluid perfused in the system is 3 litres. A fluid filter of 20 micron pore size is inserted along the pipe to remove any macromolecules which may have accumulated in the system. Compressed oxygen from a 4.5 kg gas cylinder, with a regulated flow rate, is supplied to the system at E. The

**Composition of current perfusate: NaCl, 7.98 gm per litre (143.8 mmol); MgSO₄ 7H₂O 3.48 gm/l (14.4 mmol); CaCl₂ 2H₂O 0.16 gm/l (1.1 mmol); KH₂PO₄ 0.235 gm/l (1.7 mmol); K₂HPO₄ 1.105 gm/l (6.3 mmol); Procaine hydrochloride 0.27 gm/l (1.1 mmol); Chlorpromazine 0.0031 gm/l; Phenoxybenzamine 0.0025 gm/l; Glucose 2.00 gm/l (11.1 mmol); Sucrose 2.50 gm/l (7.0 mmol); Glycerol 12.60 gm/l (136.0 mmol); Taurine 0.50 gm/l (4.0 mmol); Osmolality 410mOsm/l; pH at 4°C 6.85-6.95.
heart is suspended in C1 by the aorta, from an extension tube from C2. An over-
flow pipe leads from C2 into C1, though this can be closed by a simple regulator
mechanism (R). C1 is open to the atmosphere via a breather port containing ste-
rine cotton wool. With the regulator open, C2 is also at atmospheric pressure. The
preservation unit is maintained at a temperature below 10°C, but above 4°C, by
placing it in a stainless steel, insulated box, packed with ice. Storage of the heart
below 3°C by this system has been found to result in loss of viability (8). The
entire device, including preservation unit, ice and box, weighs approximately 25
kg when fully loaded.

2. **Energy Source**

Energy is provided to the system from a 4.5 kg pressurized oxygen cylinder contai-
ning 1.56 kg of liquid oxygen (1 kg = 755 l oxygen at 21°C). The cylinder is fitted
with a flow regulator which can regulate gas flow between rates of 0 and 1000 ml/
minute. Oxygen enters C1 through a pipe (T1) from the cylinder.

3. **Fluid-Gas Dynamics**

The design of the pumping device is based on the airlift principle used to lift water
from boreholes and wells (10). The flow of gas and fluid is illustrated in Figure 1.
A pressurized oxygen cylinder provides the driving force for the pumping mechanism.
When the gas enters the system at E through T1, the equilibrium between gas and
fluid in T2 is destroyed, as the density of the mixture of gas and fluid is less than
that of the fluid alone; fluid therefore flows into T2 from below, pushing the aera-
ted column up to restore equilibrium in the system. The displaced fluid passes from
C1 via the filter to C2. The fluid now perfuses through the heart at a flow rate
ranging from 10 to 100 ml/minute; the remaining fluid accumulates in C2. Factors
which influence flow rate through the heart include the size of the organ, the coro-
nary vascular resistance, and perfusion pressure, and will be discussed later. The
 perfusion pressure head to which the coronary arteries are subjected is approxi- mately 10 cm H$_2$O, depending on the length of aorta from which the heart is sus- pended. Once C2 is full to overflow, the fluid drains through the overflow pipe into C1. The overflow pipe is fitted with a volume overflow regulator (R), which may be used to control the fluid draining into C1. The fluid is continuously re- cycled between the two chambers throughout the period of preservation.

4. Special Features

Our portable system differs from the conventional airlift pump in both its dimen- sions and in the type of ejector nozzle (T1 at E) used. To obtain maximum effi- ciency in the conventional pump, it is desirable that the stream of gas emanating at E be broken up as much as possible in the form of small bubbles; this facilitates and therefore increases fluid displacement. In our device, however, production of small bubbles is minimized to avoid foaming. (Foam may result from protein re- lease from the myocardium into the perfusate). Thus the ejector nozzle at E has a single port gas outlet as opposed to the multiple port gas outlet needed in the conventional airlift pump.

The oxygen from the pressurized cylinder has 3 essential functions:- (i) it circu- lates and (ii) oxygenates the perfusate, and (iii) maintains sterility by creating a slight positive pressure in C2, ensuring a unidirectional outward gas flow through the breather port.

The advantages of the system are as follows:- (i) no electricity-dependent pump is required; (ii) there are no moving parts, and so the risk of malfunction is reduced; (iii) the fluid delivery pipe T2 can be bent at any angle with little change in the rate or volume of fluid delivered.
SYSTEM VARIABLES

Alterations in Effective Myocardial Perfusion Pressure

The pump can operate at three levels of efficiency, with each level depending on the pressure in the lower chamber Cl; these three levels will be discussed later.

The pressure in Cl depends on both the rate of fluid flow through the overflow pipe at R (an increase in flow through R increases the pressure in Cl) and the rate of fluid removal from Cl to C2 (a faster rate transiently lowers Cl pressure at any given volume of fluid delivered to C2). An increase in pressure in Cl leads to increased fluid displacement up T2 into C2. This in turn leads to further perfusate flow into Cl, increasing the pressure further. The radius of the overflow pipe limits the volume of the overflowing fluid. The fluid flowing through R into Cl creates a rising pressure in Cl; adjusting the regulator (R), and therefore reducing the overflow, lowers the pressure in Cl.

The effect of changes in oxygen flow on the pressure in Cl is shown in Figure 2, both with R open or closed. With R open, the pressure change with increased gas flow is less dramatic; for example, with R fully open, at a gas flow of 500 ml/minute, the pressure in Cl is +2.5 cm H₂O, but with R closed it is -5 cm H₂O.

Changes in the pressure in Cl have a direct effect on the effective myocardial perfusion pressure, thus influencing myocardial perfusion. Figure 3 illustrates the effect of two rates of gas flow on the effective perfusion pressure. At a gas flow of 500 ml/minute the pressure in Cl is increased to +2.5 cm H₂O by allowing maximum fluid inflow through R, decreasing the actual +10 cm H₂O perfusion head to an effective +7.5 cm H₂O. At the same gas flow, but after closing R, the pressure in Cl becomes negative to -5 cm H₂O, increasing the effective perfusion pressure to
+15 cm H₂O; myocardial perfusion will therefore be increased.

The effect of a reduced rate of gas flow of 400 ml/minute is to increase the pressure in C1 to +4.25 cm H₂O, thus reducing the effective pressure head to +5.75 cm H₂O; myocardial perfusion will be decreased. If R is closed, only a small negative pressure can be achieved in C1 (-0.5 cm H₂O), and thus effective myocardial perfusion is not increased greatly. Any further reduction in gas flow (less than 400 ml/minute) will result in a positive pressure in C1, thus reducing the effective myocardial perfusion further. The greatest effective myocardial perfusion pressure will therefore be achieved with the regulator closed and with a gas flow rate of 500 ml/minute or more (Figure 2).

In our laboratory, to maintain a viable myocardium, the minimum acceptable coronary flow is 10 ml/minute/100 g heart tissue. Thus a large 400 g human donor heart requires a minimum coronary flow of 40 ml/minute. At a gas flow of 500 ml/minute the absolute minimum fluid delivered to C2 is 33 ml/minute and the maximum 280 ml/minute (Figure 4).

Prolonged perfusion results in myocardial oedema and an increase in coronary vascular resistance, with a resulting reduction in myocardial perfusion. Thus to maintain a minimum effective myocardial perfusion, the effective myocardial perfusion pressure may have to be increased during the course of the storage period by partially or fully closing the regulator (R).

There are a number of advantages of being able to change the effective perfusion pressure of the myocardium. A low perfusion pressure conserves gas flow, which may be useful if a long preservation period is required, and results in less myocardial oedema formation. Higher perfusion pressures will ensure adequate myocardial
perfusion in hearts with a high coronary vascular resistance, which may be seen, for example, in hearts taken from brain-dead animals (11,12) or following a period of warm ischaemia, and as myocardial oedema increases following prolonged perfusion storage.

Changes in effective myocardial perfusion pressure are also accompanied by changes in the volume of perfusate delivered to C2; these are discussed below.

Alterations in Perfusate Flow

(i) C1 at atmospheric pressure

When the regulator R is open, C1 is in continuous contact with the atmosphere through C2 and the breather port. When the pressure in C1 is atmospheric, no additional effect is exerted on the volume of fluid circulated between C1 and C2. At gas flow rates of 400 and 500 ml/minute, the perfusate delivered to C2 is 100 ml and 200 ml/minute respectively (Figure 4). The myocardial perfusion pressure is equivalent to 10 cm H\textsubscript{2}O, assuming a constant length of aorta as was used in this study (Figure 3).

(ii) C1 below atmospheric pressure

With constant gas flow rates of either 400 or 500 ml/minute, partial or total occlusion of R results in a negative pressure in C1 (Figure 2). At a gas flow rate of 500 ml/minute and with R fully occluded, the pressure in C1 falls to -5 cm H\textsubscript{2}O (Figure 3). This increases the effective myocardial perfusion pressure, but decreases the delivered perfusate volume to C2 (Figure 5). A direct relationship exists between the pressure in C1 and the perfusate delivered to C2 (Figure 4), but an inverse relationship exists between the effective myocardial perfusion pressure and the perfusate delivered to C2; an increased perfusion pressure results in decreased perfusate
delivery to C2 (Figure 5). If for any reason coronary vascular resistance is high, a compensatory increase in perfusion pressure, resulting in maintenance of the myocardial perfusion flow, can be achieved by closing the regulator; this will lead, however, to a reduced (but still adequate) perfusate flow to C2.

(iii) Cl above atmospheric pressure

With R fully open, an increase in gas flow (greater than 500 ml/minute) will lead to a continuous stream of perfusate inflow into Cl from C2, raising the pressure in Cl above atmospheric. If gas flow is maintained at greater than 500 ml/minute, the fluid level in C2 will rapidly rise until it reaches the breather port and leaves the system. To prevent this, the oxygen flow can be reduced, yet an adequate perfusate flow from Cl to C2 can be maintained. The raised pressure in Cl (above atmospheric) decreases the gas bubble size, resulting in increased bubble velocity at the ejector port E, increasing the delivery of fluid to C2.

If the gas flow were discontinued at this point, the positive pressure in Cl would raise the fluid column in T2 to a height of 8 cm H₂O pressure under static conditions. If the total lift required under equilibrium conditions is 20 cm H₂O, then the lift required in the functioning mode is 20 - 8 = 12 cm. Thus, once the 8 cm head is established, less energy is required to displace the fluid, and less friction occurs, as the lift required is smaller. Under these circumstances, perfusate delivery to C2 therefore increases, allowing a higher fluid flow rate without an increase in gas flow rate.

When functioning at a gas flow rate of 500 ml/minute, a positive pressure of +2.5 cm H₂O in Cl is maintained (Figure 4) and the perfusate delivery to C2 is increased to 280 ml/minute (Figure 4); the perfusion pressure, however, is lowered to +7.5 cm H₂O (Figure 5).
The effects of opening or closing the regulator on the effective myocardial perfusion pressure and on the volume of perfusate delivered to C2 are summarised in Figure 6.

ENVIRONMENTAL VARIABLES

Air travel is the most practical form of transport if donor hearts are to be procured at long distances from the transplant centre. One disadvantage of the portable storage unit described above is that a rapid change in altitude results in reduced fluid delivery to C2. A rise in altitude leads to reduced atmospheric pressure, resulting in the changes described in (ii) above. With the regulator open, a decrease in environmental (atmospheric) pressure leads to a decrease in the fluid delivered from C1 to C2. In aircraft during ascent, therefore, it is necessary to compensate for the reduction in atmospheric pressure by frequent increases in gas flow, resulting in increased perfusate flow, until the cruising height of the aircraft is reached; under conditions of low atmospheric pressure, therefore, the oxygen supply is more rapidly consumed. At the time of descent, the reverse occurs, an increase in perfusate flow between C1 and C2 occurs (leading to a possible loss of fluid from the system from C2); oxygen flow rate must, therefore, be reduced, bringing about a compensatory reduction in perfusate flow.

In commercial aircraft, in which cabins are pressurized at a constant equivalent to flying at 10,000 feet, sudden changes in altitude will not alter the dynamics of the perfusion system, except on ascent and final descent, when the pressure in the cabin will be sequentially decreased or increased. In small aircraft, in which cabin pressure cannot be controlled, sudden changes in altitude may greatly alter the system dynamics; compensatory procedures must be initiated.
PRACTICAL ASPECTS

A portable unit has been designed in which perfusate flow can be increased or decreased, and in which effective myocardial perfusion pressure can be regulated by altering the overflow rate between two superimposed chambers. The volume of perfusate which can be delivered to a 400 g human donor heart is greater than five times its minimum requirements; loss of viability of the stored heart from a low fluid delivery rate is therefore highly improbable. As no moving parts are involved, mechanical failure of the system is also extremely unlikely. A small gas cylinder of 4.5 kg can reliably drive the pump mechanism for 20 to 30 hours at gas flow rates of 300 to 600 ml/minute. The storage time allowed by the sequential use of two such cylinders will allow transport of an organ between most of the world’s major cities.
SUMMARY

A portable apparatus for the continuous hypothermic perfusion of the isolated heart is described. The system has been used successfully to store pig and baboon hearts for periods of up to 48 hours, and to store human donor hearts for periods of 7 to 17 hours before being transplanted. The perfusate is both oxygenated and circulated by gas flow from a pressurized oxygen cylinder, using the air-lift pump principle. The apparatus has no moving parts and requires no electrical energy supply; malfunction is, therefore, extremely unlikely. A regulator has been incorporated which can be adjusted to increase or decrease the myocardial perfusion pressure. The system and environmental variables which can affect flow and pressure within the apparatus are discussed. The storage time allowed by this system will enable transportation of donor hearts between most of the world's major cities.
ACKNOWLEDGEMENTS

We wish to thank Mr N De Waal for help in interpretation of the dynamics of this perfusion system, and Sister P Ahrends, F Barends, Mrs J Kloppers, P Madlingozi, J Rossouw, and F Snyders for skilled technical assistance. This work was supported by the Chris Barnard Fund, the University of Cape Town, and the Cape Provincial Administration.
REFERENCES


LEGENDS

FIGURES

Figure 1. Portable hypothermic perfusion apparatus.

Figure 2. Effect of changes in gas flow on pressure in C1 chamber with the regulator closed or opened. An increase in the rate of gas flow results in a decrease in pressure in C1. Pressure reduction is greater with the regulator closed.

Figure 3. Effects of changes in pressure in C1 chamber on effective myocardial perfusion pressure at gas flow rates of 400 and 500 ml/minute. Opening the regulator results in an increase in pressure in C1 chamber and a decrease in the effective myocardial perfusion pressure. The fall in myocardial perfusion pressure is greater at the lower gas flow rate. With the regulator closed C1 pressure decreases, resulting in a higher effective perfusion pressure.

Figure 4. Effect of changes in pressure in C1 chamber on the volume of perfusate (perfu sate flow rate) delivered to C2 chamber at two different gas flow rates. An increase in C1 pressure results in an increase in the perfusate delivered to C2. High pressures in C1 (with therefore high perfusate flow rates) can only be achieved by opening the regulator. At any given C1 pressure a greater gas flow rate results in a greater perfusate flow rate.

Figure 5. The influence of perfusate flow rate (to C2) on the effective myocardial perfusion pressure at two different gas flow rates. At any given perfusate flow rate a higher gas flow rate will result in a higher effective myocardial perfusion pressure. An inverse relationship exists between the perfusate flow rate and myocardial perfusion pressure.
Figure 6. Demonstrates the effects of opening or closing the regulator on (i) the pressure in the lower chamber (C1), (ii) the perfusate flow to the upper chamber (C2), and (iii) the effective myocardial perfusion pressure, at gas flow rates of either 400 or 500 ml/min. A negative sign (-) indicates reduction in the pressure or flow, and a positive sign (+) indicates an increase.
Gas Flow = 500 ml/min

Effective Myocardial Perfusion Pressure (cm H2O)
Gas Flow = 400 ml/min

Perfusate Delivered to C2 (ml/min)

Pressure in G1 (cm H2O)
FIGURE 6

REGULATOR OPEN

\[
\begin{align*}
\text{EFFECTIVE MYOCARDIAL} & \quad (\cdot) \\
\text{PERFUSION PRESSURE} & \quad 400 > 500 \\
\text{PRESSURE IN C1} & \quad (\cdot) \\
500 > 400 & \quad 400 > 500 \\
\text{PERFUSATE FLOW TO C2} & \quad (\cdot)
\end{align*}
\]

REGULATOR CLOSED

\[
\begin{align*}
\text{EFFECTIVE MYOCARDIAL} & \quad (\cdot) \\
\text{PERFUSION PRESSURE} & \quad 500 > 400 \\
\text{PRESSURE IN C1} & \quad (\cdot) \\
400 > 500 & \quad 500 > 400 \\
\text{PERFUSATE FLOW TO C2} & \quad (\cdot)
\end{align*}
\]
LOSS OF MYOCARDIAL VIABILITY FOLLOWING HYPOTHERMIC PERFUSION STORAGE FROM CONTAMINATING TRACE METALS IN THE PERFUSATE

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INTRODUCTION

Hearts may be stored simply in ice or by continuous hypothermic perfusion (1). The compositions of both cardioplegic agents and perfusates are varied; many constituents remain controversial in regard to the degree of protection they offer the myocardium.

Myocardial injury occurring during ischaemic storage frequently becomes apparent during the reperfusion phase when reoxygenation of the ischaemic heart exacerbates damage which has occurred during the ischaemic period (2,3). Recent investigations have revealed that the injury occurring during reperfusion results from metabolites of oxygen ($O_2^-$, $OH$; and $H_2O_2$), which exert a cytotoxic effect (4,5). In normal respiring cells, though these highly reactive molecules are produced, they are continually scavenged by the enzymes superoxide dismutase (SOD) and catalase (CAT) (6,7). Following ischaemia, there is an imbalance between production of metabolites and the capacity of the scavengers to neutralize them.

We here propose that the arrested heart may be subjected to injury during hypothermic perfusion as a result of the production of cytotoxic metabolites of oxygen generated in the presence of contaminating trace metals in the perfusate. Our evidence is that hearts stored by continuous hypothermic perfusion for 48 hours suffered no major detectable biochemical or functional deterioration when the perfusate (the individual chemical components of which were highly purified with an assay purity exceeding 99.5%) contained only minimal trace concentrations of iron ($Fe^{++}$); a 382% increase in perfusate $Fe^{++}$, however, resulted in the development of enhanced resting tension of the heart (stone heart). Free $Fe^{++}$ is known to be toxic to tissues and, in the concentration used in the perfusate in the present experimental study, may be responsible for the generation of cytotoxic oxygen.
MATERIALS AND METHODS

Healthy baboons weighing 20 to 30 kg were used as heart donors and premedicated with intravenous (i.v.) ketamine hydrochloride 100 mg. After the administration of pancuronium 4 mg i.v., atropine 0.6 mg i.v., morphine 15 mg i.v., and cephalothin sodium 1g i.v., the animals were intubated endotracheally, and ventilated with oxygen (4ℓ/minute) and nitrous oxide (6ℓ/minute).

Cold Cardioplegic Arrest and Donor Heart Excision

Our technique of cardiac arrest and excision has been described fully elsewhere (8,9). After heparinization, arrest of the heart was induced by the infusion of 500 ml of cardioplegic solution (Table 1) at 4°C. Cold saline was poured over the heart. After arrest, the heart was rapidly excised.

Continuous 48 hour Hypothermic Perfusion

The perfusion circuit, which has been described in detail previously (8,9,10), is shown in Figure 1. The perfusate was both oxygenated and circulated throughout the storage period by the air lift pump principle (11). Pure compressed oxygen was bubbled through a sterile gas filter into the perfusate in the lower chamber through an air ejector port inserted into the delivery tube, by which system the fluid was transported to the upper chamber through a Cobe 20 micron filter. The perfusate flowed from the upper chamber by gravity into the ascending aorta of the suspended heart. Coronary venous effluent to the right atrium returned to the lower chamber by gravity drainage. Perfusate pH was maintained between 6.85 and 6.95 by phosphate buffer, and perfusate temperature between 4 - 10°C by encasing the apparatus in ice.

Before and after the period of hypothermic perfusion, the heart was weighed. Coronary flow (CF), coronary sinus effluent lactic dehydrogenase (LDH) and lactate levels
were determined after storage intervals of 1, 24 and 48 hours. The arterio-venous differences for oxygen ($\Delta pO_2$), carbon dioxide ($\Delta pCO_2$), and pH ($\Delta pH$) were also determined at similar intervals.

**Orthotopic Cardiac Allotransplantation or Autotransplantation**

After a period of 48 hours hypothermic perfusion storage the heart was either orthotopically allotransplanted into a recipient baboon or autotransplanted into the original baboon (which had been kept alive in the meantime by an allograft); the surgical techniques have been described elsewhere (9). Baboons receiving allografts were immunosuppressed with methylprednisolone (MP) and either azathioprine (AZA) or cyclosporin (CYA). No attempt was made to treat acute rejection episodes. All hearts were examined histologically after death or sacrifice of the baboon.

**EXPERIMENTAL GROUPS**

Thirteen experiments were performed (Group A-8; Group B-5). In both groups, baboon hearts underwent identical cardioplegic arrest, heart excision, 48 hours hypothermic perfusion, and allotransplantation or autotransplantation by the techniques outlined above. The only difference between the two groups was in the constitution of the perfusate (Table 2), and this was confined only to the concentration of Fe$^{++}$ and, to a lesser extent, lead (Pb) and arsenate (Table 3).

**Group A (n = 8)**

The perfusate was prepared using pure grade chemicals, in which the total calculated Fe$^{++}$ content was $36.46$ nM, Pb $54.36$ nM and arsenate $13.50$ nM (Table 3).

Orthotopic allotransplantation was performed in 4 experiments and autotransplantation in the remaining four.
Group B (n = 5)

The perfusate was prepared using lower graded chemicals in which the total calculated Fe$^{++}$, Pb, and arsenate concentrations were 175.6 nM, 74.4 nM and 24.2 nM respectively (Table 3). Orthotopic transplantation was performed in all 5 cases.

RESULTS

Observations During the Period of Hypothermic Perfusion (Table 4)

1. Heart mass (reflecting oedema formation)

There was a significant increase in mass in the hearts of both groups. Group A hearts showed a 48% (± 3.5%) increase in mass during the perfusion period; Group B hearts increased by only 34% (± 3.1%), which was significantly less (P<0.02).

2. Coronary flow (CF)

In Group A, the CF did not decrease significantly during the storage period. In Group B, it fell significantly at both the 24 hour and 48 hours storage intervals (P<0.001, P<0.01 respectively). CF was not significantly different between the 2 groups at one hour, but at 24 hours and 48 hours a significant difference had occurred (P<0.02, P<0.002 respectively).

3. Coronary sinus effluent lactate dehydrogenase (LDH)

In Group A, LDH increased significantly at 24 and 48 hours (P<0.02, P<0.0004 respectively). In Group B, although there were very considerable increases in LDH in all hearts at both 24 and 48 hours, the increase was significantly greater only at 48 hours (P<0.03). When the two groups were compared, LDH release was significantly greater at 48 hours in Group B than in Group A (P<0.04).
4. **Coronary sinus effluent lactate**

In Group A, the lactate concentration at the two later storage intervals was significantly lower than at 1 hour ($P<0.01$). In Group B, lactate similarly decreased at both later time intervals ($P<0.003$, $P<0.005$). Groups A and B differed significantly in lactate levels only after 48 hours preservation ($P<0.002$).

5. **Arterio-venous pO$_2$ difference ($\Delta$ pO$_2$)**

In Group A, $\Delta$ pO$_2$ remained relatively constant during the storage period, but in Group B it decreased significantly at the end of storage ($P<0.03$). A comparison between Groups A and B showed a significant difference in $\Delta$ pO$_2$ after 48 hours storage ($P<0.03$).

6. **Arterio-venous pCO$_2$ difference ($\Delta$ pCO$_2$)**

In Group A, $\Delta$ pCO$_2$ showed no significant change during storage, whereas in Group B significant decreases were seen at 24 and 48 hours ($P<0.02$, $P<0.01$ respectively). $\Delta$ pCO$_2$ did not differ significantly between Groups A and B, however, at any of the three time intervals.

7. **Arterio-venous pH difference ($\Delta$ pH)**

$\Delta$ pH showed no significant change during storage in either group, nor was there any statistical difference between either group at any time interval.

Results of Cardiac Allotransplantation and Autotransplantation

All 4 baboons in Group A which underwent orthotopic allotransplantation using donor hearts stored for 48 hours functioned well immediately after insertion and electrical defibrillation. Three animals immunosuppressed with AZA and MP survived until the
hearts were acutely rejected at a mean time interval of 20 days; all showed normal haemodynamic data at cardiac catheterization during the first post-transplant week (12). One baboon immunosuppressed with CYA and MP remains alive and well 90 days after orthotopic transplantation; haemodynamic function has been normal on cardiac catheterization on two occasions (12).

All 4 recipients which underwent autotransplantation also recovered immediate good function. One died at 8 days from an infected mediastinal haematoma, two were sacrificed electively for myocardial microscopic examination at 1 and 3 months, and one was electively sacrificed after one year. All 4 baboons showed normal heart function at cardiac catheterization before final sacrifice, and a well preserved myocardium on histological examination at autopsy. These findings have been detailed elsewhere (12).

None of the 5 hearts in Group B functioned after orthotopic allotransplantation. No contraction activity was visible, the heart showing the typical features of enhanced resting myocardial tension (stone heart). Cardiopulmonary bypass was prolonged for a further 60 minutes in each case, but despite the administration of increasing amounts of inotropic support, no significant recovery of myocardial function occurred. Atrial fibrillation and some incoordinated right ventricular muscular activity was observed and confirmed by electrocardiography, but no left ventricular activity was recorded and no heart could be successfully defibrillated. When cardiopulmonary bypass was discontinued, the animal immediately died. Histological examination showed features of myocardial damage, with contraction band necrosis and capillary disruption.

DISCUSSION

We have previously reported a cytotoxic effect exerted by trace concentrations of 0.5% phenol, when used as stabilizing molecules in the procaine hydrochloride solution which forms a constituent of our perfusate (8). Hearts stored for 24 hours
in such a solution failed to function satisfactorily. Use of chlorocresol as a stabilizing molecule for procaine hydrochloride in the same perfusate, however, resulted in excellent myocardial function after storage.

We believe that the perfusate used in the present study was rendered toxic to the myocardium during continuous perfusion for 48 hours by a concentration of the trace element Fe$^{++}$ of 175.60 nM. The exact maximum "safe" concentration of Fe$^{++}$ remain uncertain, but is presumably between 36.46 and 175.60 nM; (the concentration would clearly vary depending on the length of time of perfusion). To avoid this toxicity the perfusate chemicals required an extremely high degree of purity. Hearts perfused with solutions exceeding the threshold concentration of Fe$^{++}$ underwent a rapid biochemical deterioration during the first 24 hours, which progressed to an irreversible state of enhanced resting myocardial tension by 48 hours; the loss of viability was demonstrated by no post-transplant function and a total failure to support the circulation following orthotopic allotransplantation, and by observations made during the period of storage (outlined above).

As there was no other change in the system of storage nor in the perfusate except the higher concentration of Fe$^{++}$, changes in concentrations of Pb and arsenate were not considered significant and are discussed later, we have no other explanation for the loss of viability in Group B hearts and for the observed changes during the preservation period. This concentration of Fe$^{++}$ must be damaging if the myocardium is exposed to it for periods of 48 hours. (Pig hearts which have been stored with the perfusate used in the Group B baboons have remained viable, though with significant diminished functional capacity, when preserved for only 24 hours (unpublished data). It would seem that the additional 24 hour period of exposure to the perfusate leads to loss of viability).

The observations made during the period of hypothermic perfusion would strongly suggest that the damage occurs during this period and not during the reperfusion period. In previous experiments where the perfusate was inadequate, the 24 hour
period of ischaemic storage clearly led to damage, but the phenomenon of the stone heart did not occur until after warm reperfusion. In the present series of experiments, stone hearts were seen at the end of the storage period before any warm reperfusion occurred.

Though oedema formation during the perfusion period, manifest by an increase in mass, has been shown to lead to a reduction of CF in hearts stored by this system in previous experimental studies (8,9,12), the significant decrease in CF seen during the perfusion period in Group B hearts was an indication of the development of enhanced resting myocardial tension, and not a consequence of oedema formation alone, as CF did not decrease significantly in Group A hearts.

Although a marked reduction in CF occurred in Group B hearts, the release of the macromolecule LDH into the coronary sinus continually increased. If the effective LDH concentration is calculated by correcting for the decrease in CF, LDH concentration still increased significantly by the end of the preservation period. If this large molecule were released in significant quantities from the myocardium, then smaller molecules would be expected to be similarly released with even greater ease. This, however, did not occur in the case of coronary sinus lactate concentration, nor of hydrogen ions, illustrated by no change in $\Delta pH$. This implies that in Group B hearts there was a gradual decrease in aerobic metabolism in the myocardium, demonstrated by the significant reduction in myocardial oxygen uptake, without the intervention of anaerobic metabolism; the basal rate of lactate production by Group B hearts was reduced to negligible levels at the end of the storage period, suggesting inhibition of the anaerobic energy cycle.

It would appear that the decreased metabolic activity seen in Group B hearts was closely associated with cellular destruction, and the progressive development of
enhanced resting myocardial tension, which is particularly notable as it occurred in the absence of warm blood reperfusion. Enhanced resting myocardial tension is a not uncommon consequence of inadequate preservation, but is usually not seen until warm reperfusion has taken place (13-16).

$Fe^{++}$ is extremely toxic to cells (17); the degree of toxicity depends on the amount of free $Fe^{++}$ available (18). $Fe^{++}$ can exist in two oxidation states ($Fe^{++}$ or $Fe^{+++}$); in the presence of oxygen an interchange between these states results in production of superoxide anions. (Hydrogen peroxide can similarly react with trace concentrations of $Fe^{++}$ salts, forming another toxic species, the hydroxyl radical ($OH\cdot$) (17,19)). These reactive molecules can attack and destroy most cellular constituents (18,19). The active role of trace heavy metals (as contaminants of reagents) has also been considered responsible for the autoxidation of adrenaline, and this reaction is strongly inhibited by superoxide dismutase and the chelating agent ethylenediaminetetra-acetic acid (EDTA) (7,20).

A possible mechanism can be suggested for the sequence of events which may be occurring in these hearts and leading to myocardial death. During mitochondrial respiration, toxic metabolites of $O_2$ are generated (6,21). In the presence of trace concentrations of $Fe^{++}$, $O_2$ and $H_2O_2$ react together to form the hydroxyl radicals ($OH\cdot$). Tissue ischaemia has also been shown to generate these radicals (22), which affect calcium transport in the sarcoplasmic reticulum (23), and, by lipid peroxidation, destroy membrane integrity, with increased leakiness resulting in an increased intracellular calcium concentration (19). The endothelial cells, which are the first to come into contact with the perfusate $Fe^{++}$, are exposed to these toxic metabolites, resulting in damage, which may lead to spasm of the coronary arteries and result in diminished coronary flow. This in turn results in ischaemia of the myocardial cells, with calcium maldistribution, resulting in a gradual increase of myocardial resting
tension, manifest by reduction in heart size (on visual inspection), increased stiffness (on palpation), less oedema formation (manifest by gain in mass), and markedly reduced coronary flow; in an advanced state the myocardium is rendered non-viable.

The endogenous defence system protecting biological integrity from destruction by 
\( \cdot \cdot \) and \( \cdot \cdot \) includes the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), glutathione peroxidase, and vitamins E and C (7). SOD and CAT have been used successfully to protect organs during storage (5); these authors believe injury to occur at the time of reperfusion, when mitochondrial function is restored, thus generating large quantities of toxic oxygen metabolites. Readmission of oxygen to hypoxic hearts may result in \( \cdot \cdot \) production above the neutralizing capacity of SOD, decreasing enzyme activity (4). In hypothermically perfused, \( \cdot \cdot \) contaminated, oxygenated hearts, SOD would presumably be less effective because of overproduction of superoxide anions; the addition of SOD in preservation mediums should therefore have a beneficial effect, and this has been shown in some studies (3,24).

Pb has a toxic effect on various metabolic pathways by reacting with sulphydryl groups affecting crucial enzyme catalytic activity, resulting in disturbed haemoglobin synthesis and altered membrane function (25). A Pb concentration of 10 \( \mu \)M results in 50% inhibition of pyrimidine 5-nucleotidase, and even a concentration of 1 \( \mu \)M has a detectable inhibitory effect at normothermia (26). In the present study, Pb would not therefore appear to have played a destructive role as hearts in Groups A and B had Pb concentrations of only 54.36 nM and 74.38 nM respectively, which were both fractional compared with the minimal toxic concentration of 1 \( \mu \)M.

Other trace contaminants that may be injurious to organs in preservation media include arsenate, iron cyanide and potassium cyanide, which also occur in trace
concentrations in various laboratory chemicals. Arsenate appears to be involved in uncoupling of mitochondrial energy transduction and inhibition of ion transport (27). Arsenate also influences calcium binding and uptake by the sarcoplasmic reticulum. Concentrations of 5 mM arsenate partially inhibit binding, but completely inhibits uptake. These arsenate concentrations are, however, greatly in excess of the amounts normally found in preservation solutions. During hypothermia, however, arsenate may have a more potent effect on mitochondrial metabolism. This aspect is unresolved and confirmation requires further investigations.

The effect of cyanide is twofold: (i) it inhibits reperfusion injury following substrate-free anoxic perfusion, and thus protects from injury during this phase (14); (ii) it blocks essential mitochondrial function during energy transduction (28).

Ganote and his colleagues (14) showed cyanide at 5 mM was an effective inhibitor of biochemical reactions in the myocardium of rat hearts in vitro, and did not increase injury beyond that caused by anoxia alone. The contaminating trace concentration of cyanide in the preparation of perfusates is unlikely to exceed 20 nM; any toxic effect at this concentration has not been demonstrated, but clearly requires further investigation.

The hitherto unconsidered presence of contaminating trace metals in perfusates, and possibly even in cardioplegic solutions, may account for lack of reproducibility of experimental results between one centre and another. Greater attention perhaps needs to be paid to such detail than in the past, particularly if organs are to be continuously perfused, and thus exposed to the effects of the contaminant, for prolonged periods of time.
SUMMARY

Two groups (A and B) of isolated baboon hearts were preserved by continuous hypothermic perfusion storage for 48 hours using perfusates which differed only in the concentration of the contaminating trace elements iron, lead and arsenic. Storage with the perfusate containing the higher concentration of these elements (perfusate B) led to significantly less gain in heart mass, a greater reduction in coronary flow, coronary sinus effluent lactate, and myocardial arteriovenous oxygen difference and a greater increase in coronary sinus effluent lactate dehydrogenase, when compared with perfusate A. Group B hearts totally failed to support the circulation following orthotopic transplantation, whereas Group A hearts showed excellent function. Group B hearts had undergone the typical changes of enhanced resting myocardial tension during the storage period (before warm blood reperfusion); we propose that these changes were brought about by the production of superoxide anions and radicals by the high concentration of iron in perfusate B.
ACKNOWLEDGEMENTS

We thank Sister P Ahrends, F Barends, Mrs J Kloppers, P Madlingozi, J Rossouw and F Snyders for skilled technical assistance. This work was supported by the Chris Barnard Fund, the University of Cape Town, and the Cape Provincial Administration.
REFERENCES


19. HALLIWELL, B. Superoxide and superoxide-dependent formation of hydroxyl radicals are important in organ toxicity. Tibs. 1982; 7: 270.


LEGENDS

TABLES

Table 1. Constitution of cardioplegic solution.

Table 2. Constitution of the perfusate used in both groups (A and B).

Table 3. Perfusate constituents containing contaminating elements.

Table 4. Changes in heart mass, coronary flow, coronary venous effluent lactic dehydrogenase (LDH) and lactate concentrations, arterio-venous oxygen, carbon dioxide and pH differences during 48 hours hypothermic perfusion storage in Group A and Group B hearts.
<table>
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<th>mM</th>
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<tr>
<td>7.</td>
<td>Insulin</td>
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<td>8.</td>
<td>Dextrose</td>
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<tr>
<td>pH</td>
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<tr>
<td>CONTAMINANT (nM)</td>
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<td>LEAD</td>
<td>ARSENATE</td>
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<td>-----------------</td>
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<td>---------------</td>
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<td>PERFUSATE (GROUP)</td>
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<td>B</td>
<td>A</td>
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<td>1. NaCl</td>
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<td>-</td>
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<td>TOTAL CONTAMINANT (nM)</td>
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<td>54.36</td>
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<td>PARAMETER</td>
<td>GROUP (A:n=8) (B:n=5)</td>
<td>PERIOD OF HYPOTHERMIC PERFUSION STORAGE</td>
<td>STATISTICAL DIFFERENCE *</td>
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<tr>
<td>-----------</td>
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<td>----------------------------------------</td>
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<tr>
<td></td>
<td>1 hour</td>
<td>24 hours</td>
<td>48 hours</td>
</tr>
<tr>
<td>1. HEART MASS (g)</td>
<td></td>
<td>A 137 (9) { NS**</td>
<td>203 (16) { p&lt;0.02**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B 147 (13)</td>
<td>197 (18)</td>
</tr>
<tr>
<td>2. CORONARY FLOW (ml/min/100g)</td>
<td></td>
<td>A 17.4 (2.25) { NS</td>
<td>16.6 (1.87) { &lt;0.02**</td>
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<td></td>
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<td>B 16.8 (2.72)</td>
<td>8.0 (2.12)</td>
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<td>3. LDH (u/L)</td>
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<td>A 14.4 (3.59) { NS</td>
<td>27.4 (4.26) { NS</td>
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<td></td>
<td></td>
<td>B 10.0 (2.04)</td>
<td>56.0 (25.20)</td>
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<td>4. LACTATE (mg/100mL)</td>
<td></td>
<td>A 1.94 (0.282) { NS</td>
<td>1.07 (0.154) { NS</td>
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<td></td>
<td></td>
<td>B 2.31 (0.266)</td>
<td>1.18 (0.242)</td>
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<tr>
<td>5. ∆pO2 (mm Hg)</td>
<td></td>
<td>A 218 (57.0) { NS</td>
<td>263 (50)</td>
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<td></td>
<td></td>
<td>B 310 (57.0)</td>
<td>190 (24)</td>
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<tr>
<td>6. ∆pCO2 (mm Hg)</td>
<td></td>
<td>A 1.53 (0.596) { NS</td>
<td>3.00 (1.110) { NS</td>
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<td>B 3.28 (0.733)</td>
<td>1.56 (0.368)</td>
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<td>7. ∆pH</td>
<td></td>
<td>A 0.058 (0.0189) { NS</td>
<td>0.038 (0.0160) { NS</td>
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<td></td>
<td></td>
<td>B 0.061 (0.0060)</td>
<td>0.069 (0.0230)</td>
</tr>
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* Student t test for paired data
** Student t test for unpaired data.
LEGENDS

FIGURES

Figure 1. Portable hypothermic perfusion apparatus.
HAEMODYNAMIC AND MYOCARDIAL HISTOLOGICAL AND ULTRASTRUCTURAL STUDIES ON BABOONS FROM 3 TO 27 MONTHS FOLLOWING AUTOTRANSPLANTATION OF HEARTS STORED BY HYPOThERMIC PERFUSION FOR 24 OR 48 HOURS.

BY

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ABSTRACT

Haemodynamic and myocardial histological studies have been made in 4 baboons, each of whom had undergone heart excision and storage, followed by autotransplantation: The excised baboon heart was stored by continuous hypothermic perfusion for 24 or 48 hours and then replaced orthotopically, the baboon being maintained alive in the interim by an orthotopic cardiac allograft. Follow-up of the autotransplanted baboons has been from 3 to 27 months. Cardiac catheterization revealed normal function both early and late after the period of storage. In 3 animals, myocardial histology was normal on light microscopy, though some dilatation of the T-tubules was seen on electron microscopy. In the 4th baboon, whose heart had been stored for 48 hours with follow-up for 13 months, light microscopy revealed some variation of staining of the myofibres and mild interstitial oedema; ultrastructural studies showed mild intra-cellular oedema, local scanty loss of myofilaments, and a lack of dilatation of the T-tubular system. With this one possible exception, this study confirms that the system of hypothermic perfusion storage developed in our laboratory does not have any significant damaging effects on myocardial functional structure. There would appear, therefore, to be no contraindication to its use in clinical heart transplantation.
INTRODUCTION

We have reported 24 and 48 hours storage of the baboon heart by continuous hypothermic perfusion followed by orthotopic allotransplantation and autotransplantation (1-3). We here report haemodynamic and myocardial light and electron microscopy findings in four long-term surviving baboons following autotransplantation of the stored heart. Three of the 4 baboons reported in the present study were survivors from 2 previous experimental studies (2,3).

MATERIALS AND METHODS

Operative Technique and Hypothermic Perfusion Storage

Our methods of anaesthesia, cold cardioplegic arrest, and donor heart excision have been detailed previously, as has our system of continuous hypothermic perfusion storage (Figure 1) (2,3). Briefly, the donor heart was arrested with cold cardioplegic agent (Table 1), rapidly excised, weighed and transferred to the portable hypothermic perfusion apparatus, where it was perfused for a period of 24 or 48 hours. The perfusate used in baboon 1 (below) was slightly different from that used to perfuse the subsequent 3 hearts (baboons 2 - 4) (Table 2).

Following the period of storage, the heart was sutured back into the donor animal, using a standard technique of orthotopic transplantation (4). In the interim, between heart excision and replacement, the animal was maintained alive by a cardiac allograft from a baboon matched for AB blood group; after the period of isolated heart perfusion, the allograft was excised and replaced by the baboon’s own heart once again. This technique of autotransplantation therefore allowed long-term survival of the baboon, without the intervention of acute rejection, which might have occurred if allografting had been performed. Any deterioration
in cardiac function or any pathological changes on light or electron microscopy would be related solely to the period of storage, rather than to any other pathological process such as rejection. After operation, all 4 baboons remained clinically well, requiring no medication whatsoever, until electively sacrificed between 3 and 27 months later (Table 3). Two hearts had undergone hypothermic perfusion storage for 24 hours and two for 48 hours. All 4 baboons underwent cardiac catheterization during the post-transplant period on one or more occasions (Table 3). After sacrifice, the heart was excised, weighed, and sections of myocardium prepared for histological examination by both light and electron microscopy.

Preparation of Tissue for Histopathological Examination

Samples for light microscopy were fixed in 5% buffered formaldehyde solution and processed in the routine manner. Paraffin-embedded tissue sections were stained by the haematoxylin-eosin and periodic acid-Schiff stains.

Tissue for ultrastructural examination was immediately immersed in 5% phosphate-buffered glutaraldehyde. After several hours the specimen was removed from the glutaraldehyde and post-fixed in osmic acid (Palade's fixative) for one hour, dehydrated in graded acetone, and embedded in Spurr's resin. The embedded tissue was orientated for sectioning so that the myofibres were cut longitudinally. Ultrathin sections were cut and stained with uranyl acetate (1 minute), washed in distilled water, and then stained with Reynold's lead citrate (1 minute), followed by a further wash in distilled water.

RESULTS

Observations during the period of hypothermic perfusion have been reported previously (2,3).

In these 4 animals the mean percentage gain in body weight was 2% per month, and the mean percentage gain in heart weight was 8% per month between the time of original heart excision and of sacrifice.
**Baboon 1**

This baboon had undergone heart excision, storage for 24 hours using perfusate A, and autotransplantation 27 months before sacrifice. It had undergone cardiac catheterization on two occasions (Table 3); on both occasions the haemodynamic data obtained were basically normal. The small gradient recorded between right ventricle and pulmonary artery was related to a slight narrowing of the anastomosis at this point, and has been noted in several baboons undergoing orthotopic cardiac allotransplantation or autotransplantation (1,2). Light microscopy showed normal, well preserved myocardial appearances. Electron microscopical studies showed the myocytes to be well preserved; the myofilaments were not deficient and the mitochondria showed no significant abnormality (Figure 2). The T-tubules appeared somewhat dilated.

**Baboon 2**

Storage was for 24 hours using perfusate B; the baboon was sacrificed 12 months later. Cardiac catheterization findings are detailed in Table 3, and were again normal. Light microscopy revealed no significant myocardial abnormality, and ultrastructure showed a normal appearance apart from dilated T-tubules (Figure 3).

**Baboon 3**

Storage was for 48 hours using perfusate B, and sacrifice 3 months later. No significant abnormalities were noted on cardiac catheterization (Table 3), nor on light microscopy. Well preserved myocytes were seen on electron microscopy, which showed no abnormality apart from dilatation of the T-tubular system (Figure 4).
Baboon 4

Storage was for 48 hours using perfusate B, sacrifice being 13 months later. Cardiac catheterization findings were normal (Table 3); light microscopy revealed some variation of staining of the myofibres and mild interstitial oedema, with heightened eosinophilia of some myofibres, which may have been artifactual in nature. Ultrastructural studies showed mild intra-cellular oedema, focal scanty loss of myofilaments, and a lack of dilatation of the T-tubular system (Figure 5).

DISCUSSION

Previously reported experimental work from our laboratory has shown the system of hypothermic perfusion storage used in the present 4 experiments to be reliable and successful (2,3). Our most recent perfusate (perfusate B) would appear to be a significant advance on that reported previously (perfusate A). Using these perfusates for storage periods of 24 and 48 hours we have obtained consistent survival of recipient baboons following orthotopic allotransplantation (2,3). Though these baboons were immunosuppressed with azathioprine and methylprednisolone, no attempt was made to treat acute rejection episodes, and death has been from acute rejection, usually within the first month after transplantation; one baboon, immunosuppressed with cyclosporin A, remained alive and well until sacrificed 4 months after orthotopic allotransplantation (3).

Orthotopic allotransplantation has 2 major disadvantages:— (i) long-term survival is not assured, (ii) haemodynamic performance of the heart and histological examination of the myocardium may be altered by the presence of even low-grade acute or chronic rejection. To overcome this problem, we undertook autotransplantation in a number of animals. Autotransplantation is a major undertaking as it involves the baboon undergoing 2 heart transplant procedures within 24 or 48 hours. In our
first group of experiments, using perfusate A and a storage period of 24 hours, 3 autotransplants were performed (2); though good cardiac function was recorded in all 3 animals, 2 baboons died at 7 days (from enterocolitis progressing to peritonitis) and at 31 days (from a ruptured false aneurysm of the ascending aorta at the suture line) respectively. The third animal survived for 27 months until electively sacrificed (baboon 1 above).

In the second series of experiments, 4 autotransplants were performed using perfusate B (3); storage periods were for 48 hours. One baboon died after 8 days from a mediastinal abscess; the remaining 3 baboons survived long-term, being electively sacrificed at one, 3 (baboon 3 above) and 13 months (baboon 4 above). A further experiment (not reported previously) using the same perfusate, but with storage for only 24 hours, was followed by survival until sacrifice at 12 months (baboon 2 above).

The clinical course and cardiac catheterization data obtained from the 4 baboons in the present study confirms that myocardial function remains normal over periods as long as 2 years after storage of the heart using our system.

On light microscopy, histological features of the myocardium were normal in all 4 baboons except baboon 4 in which there were minor irregularities. Ultrastructural studies in this baboon also showed some abnormalities suggestive of degenerative changes (5). The remaining 3 baboons showed some dilatation of the T-tubules on electron microscopy, which can be a sign of left ventricular hypertrophy (5); we are unable to explain this finding. The myocytes in these 3 cases appeared well preserved.

With the one possible exception of baboon 4 (discussed above), this study confirms
that the system of hypothermic perfusion storage developed in our laboratory
does not have any significant damaging effects on myocardial function or struc­
ture. There would appear, therefore, to be no contraindication to its use in
clinical heart transplantation (6). The advantages of the system over simple
storage in ice (which at present is considered to allow a safety margin of approxi­
mately only 4 hours) are numerous, particularly in allowing transportation of donor
hearts over very long distances, and in obviating the need for the transplant pro­
cedure to be carried out as an extreme emergency.

The routine use of extracorporeal hypothermic perfusion of donor hearts is still
limited, however, by the state of the myocardium at the onset of the preservation
period. Functional and even structural damage can occur to the myocardium
during the agonal period, and may be accentuated by therapy given to the donor
to maintain an adequate haemodynamic status (7,8), or by a long period of ischaemi­
mic storage (8). Current work in our laboratory is, therefore, focusing on methods
of preventing or reversing the depletion of myocardial energy reserves which can
occur during the agonal period before the heart is excised and stored (9). If this
can be achieved, we believe hypothermic perfusion storage for periods of 24 to
48 hours will become a routine clinical procedure.
REFERENCES


Table 1. Constitution of cardioplegic agent.

Table 2. Constitution of perfusates A and B.

Table 3. Cardiac catheterization data following autotransplantation of 4 baboon hearts stored by hypothermic perfusion.
<table>
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pH at 4°C: 7.4
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<td>24 hr</td>
<td>48 hr</td>
<td>48 hr</td>
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<tr>
<td>Elective Survival Time (months)</td>
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<td>12 m</td>
<td>3 m</td>
<td>13 m</td>
</tr>
</tbody>
</table>

**Cardiac Catheterization Data**

(Time after autotransplantation (mths) | 1m | 3m | 26m | 1m | 12m | 3m | 3m | 11m | 13m |
---|---|---|---|---|---|---|---|---|---|
Right atrium (mean) | 7 | 4 | 2 | 4 | 1 | 5 | 2 | 4 | 2 |
Right ventricle (systolic) | 32 | 28 | 32 | 35 | 20 | 50 | 34 | 36 | 35 |
Main pulmonary artery (systolic) | 20 | 20 | 26 | 30 | 20 | 25 | 22 | 40 | 28 |
Pulmonary capillary wedge (mean) | 8 | 10 | 10 | 9 | 4 | 4 | 5 | 10 | 9 |
Left ventricle (systolic) | 90 | 110 | 120 | 140 | 120 | 130 | 100 | 140 | 125 |
Left ventricle (end-diastolic) | 3 | 1 | 2 | 6 | 6 | 4 | 2 | 6 | 5 |
Cardiac Output (l/min) | 2.16* | 2.32* | 2.30 | 2.07 | 1.82 | 1.90 | 2.50 | 2.10 |

*Cardiac output incorrectly reported in previous communications (2).
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<th>Perfusate B</th>
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<td>pH</td>
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<tr>
<td>Period of Storage (hours)</td>
<td>24 hr</td>
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<tr>
<td>Elective Survival Time (months)</td>
<td>27 m</td>
<td>12 m</td>
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**Cardiac Catheterization Data**

(Time after autotransplantation (mths)

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<th>Pressures</th>
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<td>30</td>
<td>20</td>
<td>25</td>
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<td>Pulmonary capillary wedge (mean)</td>
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<tr>
<td>Left ventricle (end-diastolic)</td>
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<td>6</td>
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</tbody>
</table>

**Cardiac Output (ℓ/min)**

|                | 2.16* | 2.32* | 2.30 | 2.07 | 1.82 | 1.90 | 2.50 | 2.10 |

*Cardiac output incorrectly reported in previous communications (2).
LEGENDS

FIGURES

Figure 1. Portable hypothermic perfusion apparatus.

Figure 2. Electron micrograph of myocardium (Baboon 1). The myofilaments are not deficient, the mitochondria normal, but the T-tubules appear dilated. (Original magnification, x 45,000).

Figure 3. Electron micrograph of myocardium (Baboon 2). Myocytes appear normal apart from dilatation of the T-tubules. (Original magnification, x 18,000).

Figure 4. Ultrastructure of myocytes of Baboon 3 shows no significant abnormality apart from dilatation of the T-tubular system. (Original magnification, x 24,000).

Figure 5. Ultrastructure of the myocytes of Baboon 4 reveals intracellular oedema, scanty myofilament loss, and lack of dilatation of the T-tubular system. (Original magnification, x 18,000).
CURRENT RESEARCH REVIEW

The Donor Heart
The Present Position with Regard to Resuscitation, Storage, and Assessment of Viability

D. K. C. COOPER, M.A., M.B., B.S., Ph.D., F.R.C.S.
Director of Studies in Medical Sciences, Magdalene College, Cambridge, England
Submitted for publication March 29, 1976

Clinical cardiac transplantation has stimulated considerable interest and further research into the outstanding problems of transplantation. Many of these problems are of immediate importance today to the cardiac surgeon. The morphological, biochemical, and hemodynamic changes occurring during periods of myocardial anoxia, simple techniques for measuring such changes, and methods of preventing or reversing them are all of interest both to the clinician today and to the transplant surgeon of the future. In particular, problems relating to donor heart storage are intimately associated with those of myocardial protection during periods of ischemic arrest during open-heart surgery. Studies of the effects of anoxia on the donor heart have provided information on such important problems as the etiology and possible prevention of ischemic contracture of the heart (stone heart), which is of immediate clinical relevance.

Specific problems of the transplant surgeon include investigation of methods for the resuscitation of the cadaver heart and for the prevention of cardiac viability in vitro for several hours or even days; the control of organ rejection still awaits a final solution.

This paper is concerned with reviewing and discussing the present situation in these fields of experimental and clinical endeavor.

Shumway and his colleagues [98] have emphasized that techniques for preserving the vast potential supply of human hearts must be devised if transplantation is to become a routine therapeutic procedure. Progress will really be made when methods become available for storing hearts and livers for extended periods outside the body so that organs can be transported from one hospital to another, or perhaps even from one country to another as required [79].

The essential gain from storing the isolated heart is time; this includes time to transport the heart from donor to recipient, time to do an elective operation, time to tissue-type the heart and find the best recipient, time to gain anonymity of the heart and thus reduce undesirable publicity, and time, possibly, to resuscitate a heart too damaged by antemortem changes to transplant immediately [87].

Before one can consider resuscitation or preservation of the cadaver heart, some knowledge of the effects of anoxia on the myocardium is essential.

THE EFFECTS OF ANOXIA ON THE MYOCARDIUM

An early distinction between the reversible changes of cellular ischemia and the irreversible changes of cell necrosis is essential to a determination of myocardial viability as a preliminary to cardiac transplantation or to elective anoxic arrest during open-heart surgery. Information is available on the biochemical, morphological, and functional changes resulting from myocardial anoxia.

Following interruption of the coronary circulation, there is a rapid depletion of myocardial energy-rich phosphates, such as adenosine triphosphate and creatine phosphate, and a transition from aerobic to
anaerobic metabolism. Lactic acid accumulates by an increase in intramyocardial carbon dioxide tension [10]. Creatine phosphate falls within 4 min to what is considered the critical level of 2 µmol/g; this period is increased to 10 min with cooling to 15°C, and to 30 min at 5°C [67].

Myocardial mitochondrial enzymes show significant changes during normothermic anoxic arrest [39]. α-Ketoglutaric dehydrogenase is markedly reduced, but returns to normal after 60 min of coronary reperfusion; malic dehydrogenase is similarly reduced but the change is irreversible; there are less significant changes in the other important enzymes during the early stages after periods of ischemia.

The major morphological changes occurring within the cell during the first few hours of anoxic arrest of the heart have been described in detail [14, 20, 30, 38, 52]. Conventional staining and light microscopy reveal minimal or no change from the normal within the first hour of anoxic arrest. Electron microscopy reveals definite changes in myocardial ultrastructure after 15 min of anoxia and increasing abnormalities after longer periods. The earliest changes seen are in the mitochondria, where focal swelling and a decrease in matrix density occur at 15 min. These changes become more uniform and widespread by 30 min, by which time obvious abnormalities are occurring in the nucleus and cytoplasm. The myofibrils and capillaries of the myocardium develop abnormalities at the same time.

Correlation of ultrastructural changes with biochemical data confirms the importance of catecholamine release and ionic shifts (loss of magnesium, potassium, and phosphate; influx of calcium, sodium, and water) in the early evolution of ischemic injury. An altered cellular metabolism induced by ischemia causes rapid depletion of glycogen and is followed quickly by alterations in the mitochondria, nucleus, and sarcotubular system; the myofibril is the organelle most resistant to hypoxia.

Postmortem autolytic changes mimic early ischemic change very closely. Significant hypoxic-autolytic changes may begin during the agonal state and are well established by 60 min. At present it is unrealistical to expect to obtain acutely ischemic human myocardium soon enough after death to be of value in the estimation of the degree of duration of ischemia by electron microscopic techniques.

Ultrastructural studies have shown that acute hypoxic changes appear to be completely reversible up to 20 to 30 min, with irreversible damage a constant occurrence after 60 to 90 min of oxygen deprivation. Irreversibility in cells begins at 20 min, 50% are dead by 40 min, and almost 100% by 60 min. Fifteen to 30 days after recovery from 30-min periods of normothermic anoxic arrest, only a few scattered areas of myocardial replacement fibrosis can be seen on light microscopy [15]. However, after 45 min of anoxia, extensive myocardial damage which is selectively localized to the left ventricular papillary muscles and subendocardium is seen.

Moderate hypothermia protects against the development or limits the severity of hypoxic changes. An ischemic cardiac arrest of 45 min at a temperature of 24°C still results in cardiac destruction, but after 45 min of arrest at a temperature of 18°C the ultrastructure of the heart tissue remains intact [52]. However, severe cold itself may damage the myocardium.

Electron microscopic techniques are only one way of assessing myocardial damage following periods of anoxia; studies of ventricular function have provided valuable information, which is possibly of more direct significance to the cardiac surgeon.

In dogs maintained on a pump-oxygenator, occlusion of the ascending aorta leads to an immediate and progressive fall in myocardial temperature to between 29 and 34°C at 30 min [90]. Such a period of aortic occlusion results in moderate depression of contractility and decreased compliance when these are determined 30 min after unclamping. Sixty minutes after unclamping, diastolic compliance decreases further but contractility returns to near control levels. When myocardial temperature was maintained at
37°C, 30 min of aortic occlusion caused severe and persistent depression of function. After 60 min of myocardial anoxia, left ventricular contractility is markedly reduced and takes between 2 and 2.5 hr to reach a new maximum, but even then remains at only 70% of control values [37]. This impairment of contractility increases slightly in the following 72 hr despite complete metabolic and circulatory support.

The earliest clinical experience of normothermic anoxic arrest appears to be that of Cooley and DeBakey [22] who, in successfully resecting the ascending aorta of a 50-year-old man, believed that they had the coronary orifices occluded for 31 min.

Myocardial ischemia of the canine heart for 10 min at 37°C, 15 min at 28°C, 30 min at 18°C, and 60 min at 10°C results in a less than 30% decrease of left ventricular work capacity [42]. Myocardial ischemia for longer periods at these temperatures is accompanied by pronounced depression of left ventricular function. The decline in myocardial oxygen availability is inversely proportional to the depth of cardiac hypothermia.

A heart may remain viable, that is, be able to support life, despite a considerable depression of left ventricular function. Angell and his colleagues [3, 4] stored donor hearts in saline at each of three temperatures (37, 24, and 15°C) for variable periods of time. Following the anoxic interval, coronary circulation was reestablished by perfusion of the aortic arch from the cannulated artery of a second dog. Viability of the heart was assessed by a large number of hemodynamic, histological, and serum enzyme studies, with orthotopic transplantation serving as the ultimate test. Hearts stored at 37°C remained viable for greater than 30 but less than 40 min, those at 24°C for greater than 90 but less than 140 min, and those at 15°C for greater than 180 but less than 280 min. These authors pointed out that hearts remaining anoxic at greater than 37°C suffer rapid irreversible damage, and under the usual conditions of organ procurement cannot be transplanted successfully; even moderate degrees of organ hypothermia significantly extend allowable anoxia time.

From such studies it would, therefore, appear that adequate myocardial function can be obtained after a period of anoxia of approximately 30 min at 37°C and after correspondingly greater periods as the degree of hypothermia is increased, though in all cases there is some loss of functional capacity. It is likely that in most cases a degree of irreversible myocardial damage has occurred despite the satisfactory support of the circulation.

Useful information regarding the etiology and prevention of the condition of ischemic contracture of the heart has arisen from some of the studies on the effects of myocardial ischemia.

Ischemic Contracture of the Heart ("Stone Heart")

The occurrence of spastic contracture of the human myocardium was recognized after prolonged periods of ischemic cardiac arrest, especially in conditions where oxygen consumption was presumed to be elevated, for example, in aortic stenosis [24]. In the experimental laboratory, stone heart was not seen in the hearts of anesthetized, asphyxiated normal dogs when the anoxic interval was less than 30 min, but was seen in six of eight hearts anoxic for periods longer than 60 min [26]. Serum electrolyte changes in these dogs suggested that ischemic contracture was associated with an imbalance in calcium-potassium metabolism, a suggestion which was supported by features of a further experiment in which contracture occurred in one dog after the administration of a large bolus of potassium chloride.

The onset of ischemic contracture has been defined precisely as the time when the slope of the left ventricular pressure-volume compliance curve changes abruptly [58]; this occurs in the previously normal dog heart after 68 ± 8 SD min of anoxia. It has been suggested that ischemic contracture is caused by anoxic paralysis of the calcium control mechanism, resulting in a drift of calcium ions into the myocardial sarcoplasm and their irreversible precipitation there as
Working on the hypothesis that contracture would, therefore, be delayed or prevented by ionic manipulation of the extracellular environment to control calcium drift, Kurkji and his colleagues [58] tested 10 different coronary system perfusates at a standard temperature (33°C) and pH (7.4).

Isooncotic, isotonic saline perfusion alone delayed the onset of stone heart over simple ischemic arrest (111 min versus 68 min). Adding magnesium to the saline was more effective (165 min), and a high potassium concentration in the perfusate had an even more striking effect (215 min). Combinations of the calcium, magnesium, and potassium had no further beneficial effect. That calcium plays a role in the genesis of stone heart was demonstrated by perfusing the coronary system with high calcium solutions (5 to 12.5 mEq/liter). These perfusates resulted in an immediate increase in myocardial tone without the loss of compliance, but within 15 min noncompliant contracture set in.

THE INFLUENCE OF THE AGONAL PERIOD

The mode of death of the agonal period has been shown to be at least as important for the postmortem metabolic state of the heart as the warm ischemia time [68]. Of the modes studied, asphyxia caused the most damage, followed by death from ether overdose, with death from exsanguination producing the least damage.

The periods of anoxia compatible with successful resuscitation of the heart-lung preparation by the simple technique of manual cardiac massage and positive-pressure ventilation with oxygen have been assessed [29]. Hearts from animals dying from exsanguination could be resuscitated satisfactorily after 45 to 60 min, whereas hearts from asphyxiated animals were generally irreversibly damaged after periods of anoxic arrest in excess of 30 min. In heart-lung preparations resuscitated after 30-min periods of circulatory arrest, observations on myocardial performance, in particular left ventricular contractility (max \((dp/dt)/P\)) showed a much more rapid improvement in the exsanguinated animals than in the asphyxiated.

These differences are probably explained by the observation that a heart undergoing death from asphyxia is forced to attempt to support an entire circulatory load while functioning in an increasingly hypoxic environment, whereas when exsanguination is occurring, the circulatory load is progressively diminishing as anoxia increases.

A prolonged interval of antemortem shock in combination with a brief period of postmortem anoxia adversely influences the viability of canine cadaver hearts [92]. Resuscitative perfusion prior to transplantation might be necessary for donor hearts suffering reversible antemortem or postmortem insults.

One other feature of the antemortem period which may affect the function of the donor heart requires brief mention. The major source of donor hearts has been, and would appear to continue to be, from persons dying of head injury or spontaneous intracranial hemorrhage. The adverse effect of brain dysfunction on the heart has been demonstrated [16, 35, 43, 49, 101]. Electrocardiographic changes have been reported clinically in association with subarachnoid hemorrhage, intracranial infections, and cerebral tumors. Subendocardial hemorrhage and even myocardial necrosis have been reported in association with intracranial lesions. Electrocardiographic changes can be produced in animals by midbrain stimulation, and chronic stimulation produces myocardial necrosis; excessive sympathetic discharge may be etiologically responsible.

Griepp and his colleagues [44], however, found no evidence of central nervous system mediated cardiac damage in a series of 22 patients evaluated as potential cardiac donors but stressed that continuing consideration of this possibility was necessary. In the selection of donor hearts, the presence and severity of "neurogenic heart lesions" should be assessed as far as possible. Such occult cardiac damage may conceivably contribute to the failure of some transplants and ob-
scure or complicate the histological manifestations of rejection or ischemia in others.

RESUSCITATION OF CADAVER HEARTS

Langendorff, in 1895 [61], Kountz [56], Demikhov [34], and Baker [5] were among the earliest to experiment with techniques of resuscitating cadaver hearts. Many of the earlier efforts, and some of the more recent, illustrate man’s ingenuity, but the degree of success obtained is frequently difficult to assess as the methods used to demonstrate the viability of the resuscitated heart were primitive. This still remains a major problem; orthotopic transplantation and successful support of the circulation to rejection remain the only final proof, though methods of measuring myocardial performance—both physiological and metabolic—and morphological observations give a great deal of information on the state of the resuscitated myocardium.

There is considerable overlap between methods of resuscitation and those of preservation; the crucial point in classifying a technique as a resuscitation method is that a period of warm ischemia of the heart has occurred before efforts to reconstitute function are made.

Wuerflein and Shumway, in 1966 [110], made one of the first attempts to resuscitate the cadaver dog heart and subsequently transplant it. They resuscitated the excised heart–lung preparation after 60 min of normothermic arrest, using positive-pressure ventilation and a pump-oxygenator. Attempts were made to transplant 10 of the successfully revived hearts, but there were no long-term survivors; four hearts were able to maintain an adequate circulation without supportive drugs for from 8 to 38 hr.

Coronary perfusion from an intermediate host animal has been used to resuscitate cadaver hearts after up to 60 min of anoxic arrest [1]. Cardiac action improved during the subsequent 24 hr, when the heart was removed and orthotopically placed in a third animal. Function was maintained for up to 21 hr. Angell and his colleagues [2, 3] have developed this intermediate host perfusion technique further, using cannulas to connect the donor heart to the host circulation. Orthotopic transplantation was subsequently performed in a number of animals. It was felt that 2 hr of perfusion was essential to optimal organ recovery, but perfusion for longer than 3 hr offered no further advantages within the limits of the anoxic intervals and temperatures tested.

It is possible that this technique will have clinical application; the excised donor heart could be perfused by cannulation of the femoral vessels of an intermediate human host, possibly a relative of the final recipient, while preparations are made for orthotopic transplantation. Longer storage periods could be obtained only by employing a heterologous intermediate host, and this would create considerable immunological problems. Whether any type of intermediate host technique would be aesthetically or ethically acceptable to the medical profession or to the general public is, of course, debatable.

Resuscitation of the heart with the aid of a pump-oxygenator and its conversion to an autoperfusing heart–lung preparation provided successful preservation for an average of 7 hr in hearts anoxic for 30 min, and diminishing periods of time in hearts anoxic for longer intervals [96, 103].

Resuscitation of the heart by whole body perfusion was used clinically by Barnard and his colleagues [6], who used a pump-oxygenator to perfuse a human cadaver to preserve the heart in preparation for successful transplantation. On a similar basis, closed-chest, regional, extracorporeal perfusion of the donor animal, involving perfusion of the upper half of the body for 2 hr, excision of the heart, and its transplantation into the neck of a recipient animal proved feasible [82]. When perfusion had been started within 40 min after death, 15 to 20 hearts were successfully transplanted. The two factors of time elapsing between clinical death and the onset of perfusion, and the restoration of a spontaneous beat as opposed to fibrillation during
the perfusion period, appeared to be important in influencing the results.

Koga and his colleagues [55] excised puppy hearts 30 min after clinical death, immersed the hearts in 5 to 10°C saline solution, and then perfused the coronary system with a pump-oxygenator through the aortic root. Resuscitation was achieved in all 23 cases attempted, but myocardial function deteriorated during the perfusion period. The use of diluted blood as the perfusate and the addition of a microfilter enhanced the probability of obtaining a graft in good condition.

Resuscitation of the canine cadaver heart by internal manual cardiac massage and positive-pressure ventilation of the lungs after isolation of the heart-lung preparation from the rest of the systemic circulation proved successful in 20 of 25 cases after periods of anoxic cardiac arrest ranging from 15 to 128 min (average, 35 min) [27]. An evaluation of the state of the myocardium in the postresuscitation period was made by a number of hemodynamic, electrocardiographic, blood chemistry, and histopathological observations, with particular note being taken of those relating to left ventricular contractility and to the hematoxylin-basic fuchsín-picric acid (HBFP) staining reaction. After 30 min of anoxic arrest, followed by 2 hr of autoperfusion, there was no difference in the absolute figures for max \( (dp/dt)/P \) between this group and a control group of autoperfusing isolated hearts in which there had been no period of anoxia. In both groups, however, max \( (dp/dt)/P \) fell to approximately 40% of the control levels after either the period of anoxia or conversion of the circulation to that of an autoperfusing preparation, though there was a steady improvement to 60% during the 2 hr of autoperfusion. This initial substantial deterioration in contractility reflects a considerable degree of myocardial damage. The HBFP test appeared to be a good indicator of the state of the myocardium and will be discussed more fully later.

In several of these reported studies, apparently satisfactory myocardial function was obtained after periods of arrest in excess of the 30 min which other workers have suggested is the longest period of normothermic anoxic arrest compatible with subsequent functional viability. This can partly be explained by the observation that at room temperature, particularly if the chest has been opened and the heart has been exposed or excised, the myocardial temperature falls fairly rapidly to temperatures well below 37°C, providing some degree of protection against rapid tissue damage.

Clinicians will note that longer periods of ischemic arrest are not infrequently satisfactorily reversed on the operating table. The temperature of the heart under these conditions does, however, vary between ambient and body temperature. Many of these hearts are suffering from diseased states which lead to a degree of compensatory anaerobic metabolism, and the hemodynamics of such hearts are nearly always improved by the operative procedure. These hearts are, therefore, better able to recover from a period of anoxia than normal hearts under rigid experimental conditions. Although clinicians observe adequate postoperative myocardial function, several of the experiments cited above reveal that these hearts are functioning well below optimum levels; recovery is slow and frequently incomplete.

**STORAGE OF THE HEART**

The problems of resuscitation and short-term preservation are closely linked. Some research workers have limited their studies to preservation of beating, "live" hearts or of freshly excised hearts. Organ preservation can be achieved in two fundamental ways:

1. By reducing the metabolic demands of the tissues, and thus increasing the organ's resistance to injury.

2. By maintaining or increasing the supply of vital substances such as oxygen and nutrients to the organ.

Several techniques combine a method of increasing resistance to injury with maintenance of a supply of vital substances to the organ.

It is desirable to prevent intravascular
solution significantly increases the survival of rat hearts preserved at 5°C [17].

Webb and Howard [107] were the first to study preservation by refrigeration and demonstrated that the heart could be maintained, viable and functional, for periods of at least 8 hr.

Local myocardial hypothermia induced by surface-cooling with isotonic saline has been used by the Stanford group to maintain viability of the cardiac transplant graft in both the experimental and clinical situations [102]. This group, in 1962, was the first to report successful orthotopic transplantation of the dog heart after 7 hr of preservation in cold saline [66].

Simple hypothermia would not appear to be likely to provide myocardial protection for more than a few hours.

Supercooling and freezing (cryopréervation). Techniques for the long-term storage of living cells and some simple tissues (spermatozoa, erythrocytes, lymphocytes, tissue culture cells, and bone marrow) have already been developed; these have all used techniques of cryopreservation, that is, storage at temperatures below 0°C in conjunction with agents which prevent freezing injury. The freezing of tissues without the benefit of a cryoprotectant results in a biochemical derangement known as "thaw rigor." During slow cooling, water is withdrawn from the cells, which therefore shrink, and this results in irreversible membrane damage. Cryoprotective agents, such as glycerol and dimethyl sulphoxide (DMSO), are able to reduce cell shrinkage and in this way prevent freezing injury, but their effectiveness varies with the concentration, and cooling and rewarming rates used, so that it is necessary to optimize between all three parameters to obtain maximal survival. Different cells have different optima. The application of this knowledge to whole organ preservation has proved to be an intractable problem [83].

Rat and hamster hearts frozen at -0.6 to -1°C for 5 to 30 min recovered after freezing [99]. Pieces of frog atria beat after cooling to -70°C [69]. Puppy hearts frozen at -2°C did not recover [7]. After perfusion with 15% glycerol some hamster hearts tolerated cooling to ~20°C, with subsequent restoration of function [100].

By supercooling, the rat heart may survive a temperature as low as -18°C after perfusion with 2.1 M DMSO, though preservation is only possible for 1 to 2 hr in this state [81]. A younger organ is more resistant to freezing damage than an adult organ, and this difference probably depends on the age rather than the weight of the organ; it is almost certainly related to a difference in resistance to increased sodium chloride concentration in the extracellular fluid.

Although a method of preservation based on these techniques may offer the prospect of successful long-term storage in the future, at the present time cryobiological techniques of preservation are not clinically applicable.

Hypothermia and hyperbaric oxygenation. Bloch and his colleagues [8] were the first to report some success in preserving the heart with a combination of hypothermia and hyperbaric oxygen. Thirty hearts preserved at 3.3 ATA (99% O₂; 1% CO₂) at 0 to 4°C for 24 hr resumed a coordinated ventricular beat after revascularization. Prolonged storage to 72 hr with subsequent defibrillation was possible by increasing the hyperbaria to 15.0 ATA at 2°C [72]. The authors concluded that hypothermia and hyperbaria acted synergistically to improve preservation, rather than by addition of individual effects.

Reacting to this work, Shumway and his colleagues [98] transplanted numerous heart grafts orthotopically after storage under hyperbaric oxygenation and hypothermia for 24 to 48 hr. Although such hearts would defibrillate and maintain a coordinated beat, regular maintenance of the circulation could not be assured.

Lacombe and his colleagues [59] also orthotopically transplanted puppy hearts after various periods of storage at 4 ATA and 4°C. The longest period of storage compatible with survival of the recipient animal was 4 hr 10 min. A few attempts were made to preserve hearts at pressures up to 10 ATA, but no improvement in the results was obtained. The same group has stressed that
thrombosis by maintaining large heparin levels, and edema should be minimized by maintaining an adequate colloid osmotic pressure or a low perfusion pressure, though recent work has suggested that neither of these features may be absolutely essential [29, 31].

Early research workers minimized damage (by anoxia) to the donor organ by incorporating it as rapidly as possible into the new circulatory system. This straightforward and simple method has been advocated in the clinical situation by various surgeons, notably by Cooley and his associates [23]. Most of those involved in clinical cardiac transplantation have, however, utilized simple hypothermia, the donor heart being excised as soon after death as possible, flushed with cold saline or similar infusion at 4°C, and maintained in this fluid until its insertion, which is performed as soon and as rapidly as possible [102]. The fact that for clinical purposes the simplest possible preservation technique is utilized reflects the lack of complete faith in any of the more ambitious, but usually more complex, methods at the present time; organ storage is still at the experimental stage, but recent advances have brought several methods to the verge of clinical application.

Preservation methods can be divided into two basic groups—those which utilize some form of perfusion system and those which do not. The success of all methods would be likely to be enhanced by "pretreatment" of the donor animal, if this were considered ethically acceptable.

Pretreatment

Studies on the rat kidney have shown that if the donor animal is treated variably with methylprednisolone, phenoxybenzamine, chlorpromazine, or heparin before the donor organ is removed, subsequent function after a period of warm ischemia can approximate that in control experiments [60]. The steroid, alpha-receptor blocker, and phenothiazine are believed to function by "stabilizing membranes," by which it is inferred that the cell and intracellular organelle membranes retain a greater degree of control over the exchange of ions between the intracellular and extracellular compartments; the alpha blocker and the chlorpromazine reduce agonal vascular spasm, and heparin prevents intravascular clotting. In addition, the phenoxybenzamine maintains both the intracellular concentration of adenosine phosphate and the electronmicroscopic structure.

From dog experiments it appears that, to obtain the best results, methylprednisolone should be given 2 hr before the kidneys are removed—a course which would raise a serious ethical problem. There are no studies of this nature in regard to preservation of the heart, but it is very likely that similar results would be obtained.

Nonperfusion Methods

Hypothermia. The oldest and simplest method of preservation is hypothermia, which has been used in renal transplantation since 1956 [9]. Much of the early work on preservation of the heart by simple hypothermia resulted from studies devoted to arrest of the heart during intracardiac surgical procedures [40, 41]; even today, myocardial protection by cooling is of great interest to the cardiac surgeon and transplanter, though the latter seeks longer periods of protection.

In a study of patients undergoing aortic valve replacement, there was no statistical difference in the efficacy of myocardial protection between coronary perfusion and mild hypothermia (32°C) and cold ischemic arrest (22-24°C) [97]. However, both groups showed a high incidence of myocardial necrosis and new infarction, suggesting that neither technique is perfect.

More encouraging is the report that a single aortic root flushed with 4°C Ringer's lactate has been shown to protect myocardial function during 60 min of ischemic arrest [106]; there was no drop in cardiac output, peak systolic pressure, and heart rate, and histological and histochemical techniques revealed only minimal damage.

The addition of phenoxybenzamine, chlorpromazine, lignocaine, or insulin to a flushing
progressive decompression after periods of hyperbaric preservation causes much less myocardial damage than abrupt, step-wise decompression; though histological examination revealed that even the progressively decompressed hearts were severely damaged [13].

From the results reported to date, it must remain doubtful whether the addition of hyperbaric oxygen produces more successful preservation of the heart than hypothermia alone.

**Metabolic inhibition.** During the early years of open-heart surgery, surgeons experimented with various methods of inducing cardiac arrest; of the chemical agents tested, potassium citrate is, perhaps, the best known [75]. Electron microscopic studies revealed, however, that although the cardiac arrest induced with potassium citrate led to a stabilization of the ultrastructure, after reanimation, heart muscle necrosis with dilatation of the heart occurred [48]. The search for chemical agents which inhibit metabolism, but do not irreversibly damage the heart, has been further stimulated by the need to preserve organs for transplantation.

The metabolic inhibition achieved by magnesium sulphate, used singly or in combination with chlorpromazine, has been shown to maintain the anoxic normothermic heart at virtually its antemortem functional state for at least 3 hr [78]. The combination of magnesium sulphate and hypothermia appears to be synergistic and extends more than twofold the effect of either alone in the preservation of the isolated rat heart [54].

By combining hypothermic (28°C), hypoxic cardiac arrest with asanguinous coronary perfusion containing 0.75% magnesium sulphate, only minimal depression of left ventricular function occurs after 90 min of total coronary artery ischemia; the acute survival rate of four dogs thus studied was 100%. The results were markedly better than when profound hypothermia (4°C) alone was used [77].

Potassium has been reinvestigated recently; hearts have been successfully stored at 4°C in intracellular-like solutions containing a high potassium content for periods of 18 to 26 hr without oxygen or perfusion [91]. Subsequent orthotopic transplantation was successful in 14 of 21 cases with survival for 8 hr to 5.5 days, with four dogs surviving to rejection. Twenty-six hours is, to date, the longest reported period of anoxic arrest which has been followed by indubitably viable heart function. High potassium solutions act as metabolic inhibitors by depolarizing cell membranes; intracellular solutions attempt to abolish all ionic gradients across the cell membrane, further reducing metabolic requirements and maintaining intracellular integrity.

Normothermic ischemic arrest leads to a marked reduction in high-energy phosphates (adenosine triphosphate (ATP) and creatine phosphate) in the myocardium and a poor functional recovery. Coronary perfusion with hypothermic solutions or solutions containing high concentrations of potassium induces arrest without depleting the ATP or creatine phosphate [47]. It appears important to maintain these myocardial high-energy phosphates during arrest; unfortunately, oligomycin, a selective ATPase inhibitor, which should have this effect, has not been found superior to simple hypothermia in the protection of the myocardium [11].

Dypiridamole (Persantin) has also been shown to increase tissue levels of ATP in the heart [57] and is successful in prolonging anoxic storage times when added to a cold flushing perfusate before storage of the rat heart [18].

The recent report of a 26-hr preservation of the heart in high potassium intracellular solutions followed by orthotopic transplantation with survival to rejection is most encouraging; the application of such techniques in the near future may well be possible in the clinical situation both as a means of myocardial protection during open-heart surgery, and as a means of preservation of the donor heart prior to transplantation.

**Perfusion Methods**

**Regional total body perfusion.** The donor heart can be preserved for short periods
of time in the cadaver by pump-oxygenator support. The work of Parulkar and his colleagues [82], using regional extracorporeal perfusion to resuscitate and preserve the cadaver heart, has already been mentioned.

In the first clinical transplant operation performed in South Africa the donor underwent total body perfusion after death in order to preserve both the heart and kidneys, which were to be used for transplantation [6]. The apparatus comprised of a Rygg-bag oxygenator, pump, and heat exchange unit. The patient was cooled until the midesophageal temperature dropped to 16°C, at which point the heart was excised and the perfusion was discontinued. During suture of the donor heart into the recipient, coronary perfusion was maintained by a line from the recipient heart-lung machine. The lack of a vent in the left ventricle would appear to be a hazard with such procedures, but this has not been commented on by the groups concerned.

Extracorporeal normothermic perfusion. The first successful attempt to maintain living organs outside the body for more than a few hours must be attributed to the development by Lindbergh in 1935 [63] of a self-contained, all-glass, pulsatile perfusion apparatus in which experiments could be carried out under sterile conditions. Carrel and Lindbergh, in 1938 [19], described this apparatus in more detail and reported on the results of over 1000 experiments. Histological examination of guinea pig hearts revealed that the general morphological architecture was preserved after perfusion for more than 24 hr.

The technical problems in this field remain, however, formidable. Factors contributing to increasing vascular resistance of the rat heart during perfusion at 37°C include lack of filtration of the perfusate, un-siliconized surfaces of the perfusion apparatus, perfusion pressure changes, any degree of air-perfusate contact, growth of microorganisms, and oxygen tensions which are too high or too low [50].

Lindbergh's original perfusion apparatus has been modified and has maintained monkey hearts in a pulsating state for periods of up to 72 hr, though there was a diminution in the contractions which may have been due to an exhaustion of the supply of nutrient in the medium or an accumulation of toxic by-products [84].

Various types of perfusate have been and are continuing to be studied in relation to the storage of hearts and other organs [80]. Of great interest is the work of Hutson and his colleagues [53], who have shown that emulsified fluorocarbon fluids and emulsified vegetable oils such as safflower oil can serve as red cell substitutes in the perfusion of the isolated dog heart. This may prove to have clinical application in the field of blood replacement.

Left ventricular performance has been measured in isolated hearts perfused with blood oxygenated by isolated lungs and has been compared with function when the blood was oxygenated by a membrane oxygenator [76]. When the membrane oxygenator was substituted for the isolated lungs there was invariably an abrupt increase in coronary flow and a decrease in ventricular performance; the increase in coronary flow could always be reversed by reinclusion of the lungs, although the decline in ventricular performance was sometimes irreversible.

The function of the lung as a filter of microemboli is almost certainly a major factor in its beneficial effect.

Following the original work of Hardy's group [46], retroperfusion of the coronary system via the coronary sinus during cardiac arrest has been explored by various investigators. An energy substrate such as glucose can be supplied by this route [64] and has been shown to exert a protective effect upon the heart during 30-min periods of anoxic arrest at normothermia.

The perfusion systems and solutions available at present do not appear to be sufficiently sophisticated to preserve the myocardium successfully at normothermia; the addition of hypothermia has brought about a significant improvement.

Extracorporeal hypothermic perfusion. Progress here has been substantial, and it holds out hope of early clinical application,
though the technical problems are considerable. In fact, retrograde coronary sinus perfusion using low pressure gravity flow of cooled oxygenated blood was used to maintain the viability of the donor heart in the first heart transplantation performed in man, in 1964, in which a chimpanzee heart was used as the donor organ [45].

Using a perfusion circuit which utilizes a membrane oxygenator, with filtered plasma as the perfusate, hearts have been preserved for 24 hr with no gain in weight, no rise in perfusion pressure, minimal ultrastructural changes, and excellent immediate function after homotransplantation [105]. In spite of these encouraging findings, the hearts failed 4 to 6 hr after orthotopic transplantation. Using filtered undiluted canine plasma as the perfusion medium, no significant difference in myocardial contractility was observed between control hearts and hearts preserved for from 3 to 24 hr, though after 24 hr there was a significant decrease in ventricular function and myocardial compliance in some hearts [62].

One of the first major advances in this field came from Proctor and Parker [89], who managed to preserve the isolated canine heart successfully in a viable state for 72 hr with hypothermic perfusion (5°C) using filtered, modified Krebs’s solution. Subsequent experiments resulted in the orthotopically transplanted heart supporting the circulation of the recipient dog for 8 to 14 hr, with death occurring from exsanguination rather than myocardial failure [88].

Using a technique based on that of Proctor, Copeland and his colleagues [31] have recently successfully preserved hearts for 24 hr followed by orthotopic transplantation, with three dogs surviving for 4 days or more and dying of rejection. These results cast serious doubts on the assertion by Belzer’s group [73] that preservation by hypothermic perfusion is universally unsuccessful due to the high intrinsic cold sensitivity of cation transport in the heart. The success of Copeland’s group is a significant advance.

Autoperfusion (biological oxygenation). The use of an autoperfusing heart-lung preparation as a means of short-term preservation of the heart during its transplantation from the donor to the recipient must be credited to Demikhov [34], who initially published his work in 1948. The essential feature of the Demikhov preparation is that the systemic circulation is represented by the coronary vessels alone. With artificial maintenance of respiration and temperature, it could function normally for many hours.

In 1959, Robicsek and his colleagues [93] developed a modification of this heart-lung preparation in which the coronary perfusion pressure was kept stable and the blood volume was self-adjusting. A “buffer” bag was incorporated to stabilize the pressure in the aorta and allow a second exit for blood not passing to the coronary artery circulation. Hearts have subsequently been transplanted into recipient animals following 1 to 12 hr of live storage at normothermia. Thirteen of 19 orthotopic grafts were successful, with the longest survival being 13 days [94, 95].

Biochemical and pathological studies of such an autoperfusing system suggest that integrity of the cell membrane is eventually lost in the myocardium, the lung parenchyma, the red cells, and the capillaries, and that at each level, dexamethasone exerts a favorable influence. Progressive metabolic deterioration occurs, with significant changes being detectable 6 to 12 hr after perfusion had been started; these changes are not due to lack of available oxygen in the blood [111].

Observations on the Demikhov preparation have revealed a very marked fall in the measurements of left ventricular contractility after the initial conversion of the normal canine circulation to that of the heart-lung preparation; a steady improvement occurred during the subsequent 2-hr period of autoperfusion, suggesting satisfactory myocardial perfusion [28]. There is considerable evidence that a heart preserved as a heart-lung preparation for a few hours is subsequently capable of supporting an entire circulatory load after orthotopic transplantation [34, 65, 94, 107, 109], but there seems little immediate hope that an autoperfusing system
will be able to maintain cardiac viability of practical clinical value for more than 4 to 6 hr.

Intermediate host perfusion (parabiosis). Parabiosis was probably first performed in 1862 by Paul Bert in Claude Bernard’s laboratory in Paris [38]. Viability of the organ is maintained by cross-perfusion from a host animal. The use of an intermediate host in the resuscitation of cadaver hearts has already been discussed.

Dupree and his colleagues [36] have used such a system for the xenogeneic storage of primate hearts. They have termed this technique “xenobanking” to distinguish it from homograft storage in intermediate hosts. Donor stump-tail monkey hearts were anastomosed to vessels in the abdomen of recipient baboons, whose rejection response had been immunosuppressed with a lethal dose of whole body radiation 24 hr prior to receiving the donor hearts. From previous studies, the dose of radiation given (800 rad) was known to allow survival of the host animal for 9 to 11 days, with nearly complete immunosuppression for 7 days. During this period, electrocardiograms from the donor monkey hearts in the immunosuppressed baboons showed negligible changes, and, apart from minimal edema in the interstitial spaces, histological sections remained essentially normal.

Working with baboons provides a number of technical and management problems, yet this technique has provided donor hearts apparently in good condition (though this has not been satisfactorily measured) after storage periods longer than those reported using any other method. Although it is difficult to see a homologous intermediate host storage system being used in man, xenogeneic storage, using an immunosuppressed animal, might prove to be a potential answer to short-term storage of the human cadaver organs. As with other aspects of this experimental field, such a procedure may not be aesthetically acceptable to many members of the lay and medical communities.

In summary, at the present time, simple hypothermia probably affords the most reliable means of myocardial protection of the donor heart. However, the potential of this method is very limited, and a combination of hypothermia with a perfusion system will probably enable considerably longer periods of storage of the donor organ in the near future. Such a perfusion system has not yet been conclusively developed though some encouraging advances have recently been made.

It is doubtful whether the addition of hyperbaric oxygen to hypothermia significantly prolongs the storage period. Preservation at temperatures below 0°C may prove feasible in the future, but recent work in the field of cryopreservation has proved almost uniformly disappointing.

Metabolic inhibition by a chemical agent is an attractive method of preservation, possibly associated with hypothermia or hypothermic perfusion. Recent successful orthotopic transplantation following 26 hr of storage after perfusion with solutions containing a high potassium concentration may prove to be a major advance of value not only to the transplant surgeon, but also in all cardiac operations where periods of myocardial ischemia are required.

Closed-chest, regional or total body perfusion would appear to be limited by the limitations of pump-oxygenators and the absence of left ventricular decompression. The use of the autoperfusing heart-lung preparation as a short-term storage system deserves further study, but its value as a really long-term system of storage of the heart seems unlikely at the present time. Xenobanking has been encouragingly successful in the experimental situation, but its clinical application will prove expensive and difficult.

THE ASSESSMENT OF MYOCARDIAL VIABILITY

The transplanted cadaver heart is unique in that it must be capable of full function immediately after its insertion into the recipient. The need for a test of functional viability of the organ is therefore essential if even short-term storage of the heart is to be
come a clinical reality. A simple, reliable, in vitro test of organ viability would similarly save a great deal in time and animals in the assessment of any new preservation technique. The ultimate measure of viability of the preserved heart is its capacity to fully support the circulation after orthotopic transplantation. Ethically this cannot be a test of function, and the viability should be known at the time of resuscitation, during storage, and at transplantation.

Ideally, any such test of organ viability should be simple, rapid, and reproducible. The search for such a test has explored two main routes. The first of these is a simple, single measurement or observation that confirms that the tissues under study are not irreversibly damaged; this may take the form of, for example, a visual assay of a myocardial enzyme system, a histochemical change, or the monitoring of a fundamental metabolic event such as anaerobic glycolysis or lactic acid production.

The second is a functional evaluation of the isolated heart. At present, this involves multiple hemodynamic and biochemical observations of myocardial function while the heart is perfused. Such methods are frequently elaborate and time-consuming, but it is possible that, with further experience, a single measurement will be found which will indicate the functional state of the myocardium.

The single, simple observation can be classified as a “tissue viability test,” whereas the hemodynamic and biochemical studies carried out on the isolated, perfused heart can be considered methods of “functional evaluation” of the organ. Whatever the method used, it is crucial that it can be assessed or compared at some stage with the function of the whole organ after storage and orthotopic transplantation.

**Tissue Viability Tests**

Most investigators in this field attempt to measure or define a change occurring during cell anoxia.

Tetrazolium bromide has been suggested as a possible screening agent for viability of both kidneys [70, 104] and heart [71]. The principle of this very simple and rapid test is a visual assay of the dehydrogenase enzyme system of an organ. A drop of solution is placed on a small piece of myocardium, and the presence of the enzyme produces a darkening of the tissue. The enzyme is produced only by living cells and is rapidly inactivated when cell death occurs [33]. Encouraging early results have not been reproduced [25], and in particular, difficulty has been found in estimating the exact end-point of the reaction.

Measurement of the surface pH of the myocardium is similarly rapid and simple and offers a means for monitoring anaerobic glycolysis and lactic acid production [32]. Subsequent work has suggested that the change in pH reflects only the blood flow of the myocardium and may rapidly return toward normal when blood flow recommences, even in infarcted, nonviable myocardium [21, 74]. Surface pH studies would therefore appear to be of no value in assessing myocardial viability.

Pitzele and his colleagues [85] attempted to judge viability by inorganic phosphate estimations, which appear to be related to the mechanical capacity of the perfused heart, but did not correlate this with in vivo function and have not reported further on this method.

The estimation of adenine nucleotide levels (ATP, ADP, and AMP), based on the hypothesis that the energy metabolism of the organ may relate to viability, has been correlated with in vivo function, and the results are encouraging; preliminary studies in the rat heart suggest that ATP alone is sufficient as a measure of organ viability [18]. This assay, however, takes approximately 1 hr to perform; it would, therefore, be acceptable in regard to the heart only if a satisfactory storage technique were being utilized, and not if an urgent assessment of a cadaver organ were necessary.

The hematoxylin-basic fuchsin-picric acid (HBFP) stain has been investigated as a test of myocardial viability [25]. This histochemical technique is simple and rapid to
perform as a routine frozen or paraffin section procedure and provides a clear and striking demonstration of early myocardial ischemia, the ischemic fibers staining a vivid crimson color in contrast to the light brown color of nonischemic or infarcted myocardium. The extent of the area of color change seen on a microscopic section allows for a quantitative assessment of the degree of ischemia. Preliminary results have been encouraging and show reversal of a “positive” response (after 30 min of anoxic cardiac arrest) to “negative” after successful resuscitation of the heart; these histochemical findings correlate well with functional studies on these hearts and with several ultrastructural studies reported in the literature [14, 30]. This method can also give information on the efficacy of perfusion systems as a means of preservation of the isolated heart; if the perfusion is inadequate, the appearance of patches of crimson-staining myocardium demonstrate the presence of ischemia.

Other histochemical methods have proved disappointing in predicting mechanical performance of the myocardium after cold preservation, presumably because cold permits histochemical appearances to be preserved when function is not [12].

Functional Evaluation of the Heart

Most of the investigations relating to myocardial function included in this group have been intended as illustrations of the efficiency of a method of preservation and not as a rapid assessment of a potential donor organ. However, such a functional viability test may yet evolve from the techniques of functional evaluation currently being developed.

A detailed and complex system of measuring various biochemical and hemodynamic parameters during storage of the isolated heart by hypothermic perfusion has been developed by Pitzele’s group [86]. The apparatus comprises two parallel circuits, one to support the myocardium of the isolated heart and a second to provide a work-load for the heart. That such a system is, as yet, not intended as a rapid assessment of a potential donor heart can be illustrated by listing some of the parameters measured: (a) blood chemistry—(i) hemoglobin, (ii) blood gas analysis, (iii) blood glucose, (iv) serum lactate, (v) pyruvate, and (vi) nonesterified fatty acids in plasma; (b) hemodynamic—(i) cardiac output, (ii) aortic pressure, (iii) total left ventricular work, and (iv) heart rate. These authors claim that the method permits quantitative screening of various storage techniques without the need for orthotopic transplantation of the preserved hearts, but admit that their conclusions based on experiments with isolated hearts have not yet been supported with experience from orthotopic transplantation after storage.

Levitsky and his colleagues [62] described another complex experimental method for the functional evaluation of the preserved heart which hopefully permits, during the preservation period, a prediction of the function which may be expected after subsequent transplantation. However, the system as described necessitates excision of the mitral valve leaflets and chordae tendinae for the insertion of a soft latex balloon into the left ventricle, for measurement of left ventricular pressure and left ventricular end-diastolic pressure, and would therefore be unsuitable in its present form for clinical application. A number of hemodynamic, blood chemistry, and histological observations are made to assess the state of the organ.

The state of left ventricular contractility, as indicated by max \((dp/dt)\) and its derivative, max \((dp/dt)/P\), has been emphasized as a major parameter in assessing the performance of the isolated heart in a number of studies [27, 28, 51, 62]. It is easy and quick to measure, reproducible, and gives an absolute figure as a result. Changes in myocardial contractility between the control state, the anoxic or ischemic state, and the resuscitated state during experiments involving resuscitation of the heart can be readily compared. Similarly, improvement or deterioration during a period of preservation can be assessed without difficulty.

Other workers have emphasized alternative individual parameters. In the hypo-
thermic perfusion system developed by Proctor, changes in coronary resistance have been found to be a reliable indicator of the subsequent functional capability of the myocardium [88]. The pattern of change of resistance and the final resistance value are both important; in general, the lower the final resistance, the greater the viability of the heart.

Isovolumic ventricular function tests form the basis of the studies of Brown and his colleagues [11, 12], though their technique necessitates the insertion of a purse-string suture in the mitral value to hold the balloon in place; the possibility of damage to the valve could be a hazard in the clinical situation.

Cohen and Folkman [20] have described an apparatus, based on the property of silicone rubber which allows a rapid diffusion of gases across it, for the direct measurement of oxygen tension and oxygen consumption of cells, tissues, and organs in vivo. The oxygen consumption of a perfused organ can be obtained without taking samples of perfusate, biopsies of tissue, or otherwise breaking sterility in the system and without removing the organ from the environment in which it is being maintained.

A successful outcome to the search for a simple, yet reliable, viability test would not only allow the rapid evaluation of a cadaver donor organ, but would also greatly facilitate the search for reliable methods of resuscitation and preservation; the necessity of demonstrating the efficiency of such a technique by orthotopic transplantation of the organ would be removed. To date, however, no single test has satisfied all of the criteria necessary, though the ATP assay and HBFP staining reaction are worthy of further study; parameters measuring ventricular contractility and coronary resistance also show promise.

There are still many problems which require solutions before clinical cardiac transplantation can become a routine procedure; many of these have been discussed in the preceeding pages. The problems, however, are no greater than those which faced the pioneers of open-heart surgery 20 years ago, of closed cardiac procedures 30 years ago, or of pulmonary surgery even earlier; those problems were solved. Let us hope that, given time, the ingenuity and dedication of the present generation of surgeons will solve the present generation of problems.

REFERENCES

13. Bui-mong-hung, V. M., Leandri, J., and Laurent, D. Influence of decompression procedure on heart...


61. Langendorff, O. Untersuchungen am überlebenden Stügetherieren. Pflügers Arch. 61:291, 1895.
76. Monroe, R. G., LaForge, C. G., Gamble, W. J., Honda, S., and Key, S. V. Ventricular performance and coronary flow of isolated hearts when perfused through isolated lungs and membrane oxygenators. In J. C. Norman (Ed.), Organ Perfu-


ELECTROCARDIOGRAPHIC, HEMODYNAMIC & ENDOCRINE CHANGES OCCURRING DURING EXPERIMENTAL BRAIN DEATH IN THE CHACMA BABOON


—Abstract—
In Cape Town, approximately 20% of donor hearts are unsuitable due to myocardial deterioration. An experimental model was designed to study changes occurring during and after brain death in 10 baboons. Brain death was produced by creating intracranial hypertension. A Foley catheter was introduced into the subdural space through a frontal burr hole in the skull which was filled with 20 to 30 mL of saline. Brain death occurred within 20 minutes. During a 24-hour period observations were made before inducing brain death at 5, 15, 60 minutes and at three-hour intervals after brain death, lest the experiment be terminated because of heart fibrillation. ECG, hemodynamic parameters, blood chemistry and hormone levels were recorded. The following sequence of ECG changes were seen in all animals: sinus bradycardia, sinus standstill, nodal rhythm, sinus tachycardia, ventricular ectopic activity, ventricular tachycardia multifocal in origin and a final episode of sinus tachycardia that gradually slowed as time progressed. Ischemic changes were present at the onset of ventricular tachycardia although these changes reverted to normal at a later stage. The blood level of catecholamines increased 5 minutes after the onset of brain death (adrenaline + 1100% noradrenaline + 300% and dopamine + 200%), then slowly returned to control levels and eventually to subcontrol levels during the course of the experiment. Thyroid hormone T3 and T4 levels fell significantly at six hours and became undetectable nine hours after brain death. Cortisol levels increased at five minutes, then declined progressively to subcontrol levels. Insulin levels also fell progressively to half-control values by three hours and continued to fall. Antidiuretic hormone fell significantly between the control and the last samples. Glucagon and ionized calcium showed no significant change from the control level. Mean arterial pressure rose significantly during the induction of brain death, remaining high for a variable period (up to three hours), when it stabilized at a mean pressure of 40 to 50 mm Hg. Cardiac output dropped markedly during the induction of brain death, rising when the heart rate stabilized. During the hypertensive period, the right atrial pressure rose two-to three-fold of its control value, and the pulmonary artery wedge pressure increased up to ten-fold. Systemic vascular resistance showed a marked elevation during induction of brain death but was subsequently followed by a statistically significant fall. Once stabilization of the hemodynamic parameters occurred, challenging the animal with iv fluids caused a disproportionate rise in the pulmonary artery wedge pressure compared with the right atrial pressure. Myocardial biopsies were taken in six animals at the end of the experiment to measure adenosine triphosphate, creatine phosphate, glycogen and lactate content. Animals that had received large amounts of iv fluid showed depletion of adenosine triphosphate and creatine phosphate stores and an increase in the lactate levels when compared with animals that received little or no fluids. These observations suggest modalities of donor management to prevent loss of myocardial energy stores and limit myocardial deterioration before donor heart excision and transplantation.

HEART TRANSPLANTATION IV, 63-69, 1984

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THE JOURNAL OF HEART TRANSPLANTATION / VOLUME IV, NUMBER 1 / NOVEMBER 1984

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INTRODUCTION

At Groote Schuur Hospital, approximately 20% of potential heart donors show progressive hemodynamic deterioration despite supportive measures. These hearts become unsuitable for transplantation. To investigate the effects of brain death (BrD) on the heart and circulation, experimental BrD was produced in baboons to simulate head trauma or subarachnoid hemorrhage.

Additional experiments were performed to elucidate the role of the vagai and sympathetic nerve supply to the heart, and of the catecholamines secreted by the adrenal glands in the circulatory changes observed during and after induction of BrD.

MATERIALS and METHODS

Chacma baboons (Papio ursinus), weighing 20 kg to 25 kg, were tranquilized with ketamine (10 mg/kg/1.M.). After endotracheal intubation, a mixture of oxygen and nitrous oxide was administered, the baboon breathing spontaneously. Blood gases and urine output were measured and a neurological examination confirmed the presence of all brain stem reflexes. Electrocardiogram (ECG), arterial pressure (AP), pulmonary artery wedge pressure (PAWP), and right atrial pressure (RAP) were continuously recorded, while stroke volume (SV), cardiac output (CO) and systemic vascular resistance (SVR) were measured and calculated at intervals. Circulating catecholamines (adrenaline, noradrenaline, dopamine), thyroid hormones (T3, T4), thyroid-stimulating hormone (TSH), cortisol, insulin, antidiuretic hormone (ADH), glucagon, and ionized calcium were also measured at intervals. Blood levels of myocardial lactic dehydrogenase (LDH) and creatine kinase (CK) were estimated in four animals. At the end of the experiment ATG, CP, glycogen and lactate were measured in the myocardium and the heart was examined histologically.

After baseline measurements, the anesthetic level was deepened (ketamine 2 mg/kg/1.1V.). A burr hole was drilled in the right frontal area of the skull, the dura mater was incised, and a Foley catheter was introduced into the subdural space. The rapid injection of 10 mL to 20 mL of saline inflated the balloon of the catheter, producing an acute increase in intracranial pressure with herniation of the parahippocampal gyri, compression of the midbrain, and paralysis of brainstem reflexes. Cerebellar herniation (coning) causes interruption of neurological pathways between the midbrain and the spinal cord. BrD occurred within 20 minutes (mins) in all animals established by neurological examination.1 When spontaneous respiration ceased, positive pressure mechanical ventilation was initiated; it was continued throughout the experiment and adjusted following blood gas measurements. Potassium chloride was administered to maintain the serum level between 3 mmol/L and 4.5 mmol/L; no inotropic or other drugs were given.

The baboons were divided into two groups of five. All underwent the same BrD protocol. Group A received no I.V. fluids and the RAP and AP were allowed to fall without support. Group B received a continuous I.V. infusion (a crystalloid solution containing 100 G/L of dextrose) to replace fluid losses and maintain the mean RAP (mRAP) ± 5 mm Hg and the mean AP (mAP) ± 60 mm Hg.

In additional experiments, BrD was induced as it had been in groups A and B. In one animal in each group, the protocol was supplemented by a procedure (i.e., bilateral vagotomy or adrenalectomy) or a pharmacologic agent (Table I).

RESULTS

Four Group A baboons were observed for periods of 16 hours to 24 hours. In Group B, three animals were observed for 24 hours, two arresting 12 and 14 hours after BrD, respectively. Five major stages could be identified during the observation period (Table II). ECG and hemodynamic changes were similar in both groups: stage I and II AP and PAWP are illustrated in Figure 1, and stage III ventricular arrhythmias are shown in Figure 2. Abnormal ECG features consisting of inverted T waves, ST elevation, or infarct pattern persisted throughout the observation period in three group A and two group B baboons (Table III). Circulating catecholamines increased significantly five mins after inflation of the Foley catheter balloon (Figure 3). Adrenaline concentration rose eleven-fold over baseline levels (p < 0.001), noradrenaline three-fold (p < 0.01), and dopamine two-fold (p < 0.05). Ten mins later, these levels returned to control values. By the third hour, catecholamine levels decreased further, below baseline values. However, only the noradrenaline drop was significant (p < 0.05). During the later stages of BrD the catecholamine levels were not measured. The thyroid hor-
### TABLE II: The five stages of electrocardiographic and hemodynamic change during the development of brain death.

<table>
<thead>
<tr>
<th>Stage</th>
<th>ECG</th>
<th>Hemodynamic</th>
<th>Mean Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Sinus bradycardia, A-V dissociation, sinus standstill, nodal rhythm</td>
<td>Slight fall in: MAP (Fig 1)</td>
<td>8 min</td>
</tr>
<tr>
<td>II.</td>
<td>SVT* (mean 180 bpm) (P &lt; 0.002)</td>
<td>Marked rise in: MAP (P &lt; 0.002) PCWP (P &lt; 0.01) PVR (P &lt; 0.02) CVP (P &lt; 0.01) Reduction in: SV (P &lt; 0.01)</td>
<td>5 min</td>
</tr>
<tr>
<td>III.</td>
<td>Multiple VPBs** (Figure 2) Infarct</td>
<td>Rises in: MAP PCWP PVR CVP 1</td>
<td>15 min</td>
</tr>
<tr>
<td>IV.</td>
<td>Return of SVT (mean 180 bpm)</td>
<td>Further falls in: MAP PCWP PVR CVP 2</td>
<td>156 min</td>
</tr>
<tr>
<td>V.</td>
<td>Regular rhythm (mean 50 - 60 bpm) Ischemic Changes (persistent in 5 animals [50%])</td>
<td>Group A: Low MAP (P &lt; 0.01) PCWP PVR (P &lt; 0.03) CVP 0.4-1 cm H2O</td>
<td>17 hours</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group B: Low MAP (P &lt; 0.002) PVR (P &lt; 0.003) CVP 4-5 cm H2O</td>
<td></td>
</tr>
</tbody>
</table>

* SVT = Sinus tachycardia
** VPB = Ventricular premature beats
*** VT = Ventricular tachycardia
1 Initially then slowly falling towards control levels
2 CVP and MAP supported in Group B animals by fluid infusion
3 All other parameters in both groups not significantly different from control levels

(bpm = beats/minute; MAP = mean arterial pressure; PCWP = pulmonary capillary wedge pressure; SV = stroke volume; CO = cardiac output; CVP = central venous pressure; PVR = peripheral resistance. * All P values refer to difference from control (before induction of brain death) levels. NS = no statistically significant difference from control value).

### TABLE III: Relationship of persistent ECG abnormalities in stage V and histopathologic features of myocardial damage following induction of brain death.

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal</th>
<th>ECG</th>
<th>Stage V</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>T wave inversion</td>
<td>VI - V6</td>
<td>Edema, Focal Necrosis, Mononuclear infiltrate</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>T wave inversion</td>
<td>VI - V6</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Normal</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Normal</td>
<td></td>
<td>Focal myofibre necrosis Mononuclear cell infiltrate</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>T wave inversion</td>
<td>VI - V6</td>
<td>Normal</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>T wave inversion</td>
<td>VI - V6</td>
<td>Contraction band necrosis Smaller QRS complex</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Normal</td>
<td></td>
<td>Focal contraction band necrosis</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Normal</td>
<td></td>
<td>Focal myofibre necrosis Mononuclear cell infiltrate</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Normal</td>
<td></td>
<td>Focal myofibre necrosis Mononuclear cell response</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Normal</td>
<td></td>
<td>Normal</td>
</tr>
</tbody>
</table>

FIGURE 1. Extreme example of changes in AP and pulmonary capillary wedge pressures during stages I and II. (Arrow marks the moment of inflation of the intracranial Foley catheter balloon).

FIGURE 2. ECG taken during stage III showing multifocal ventricular extrasystoles.
FIGURE 3. Changes in circulating adrenaline, noradrenaline and dopamine during the first three hours following induction of BrD. Statistical differences between levels taken at 5 minutes, 15 minutes, and 3 hours after intracranial Foley catheter balloon inflation and control levels are shown.

FIGURE 4. Changes in circulating cortisol in groups A and B following induction of BrD. Statistical differences between levels taken at 1 hour and 16 hours after intracranial Foley catheter balloon inflation and control levels are shown.

FIGURE 5. Changes in circulating insulin in groups A and B following induction of BrD. Statistical differences between levels taken at 1 hour and 13 hours after intracranial Foley catheter balloon inflation and control levels are shown.

FIGURE 6. Microscopic section of myocardium showing widespread contraction bands and edema.

FIGURE 7. Microscopic section of myocardium showing focal myocardial cell necrosis and mononuclear cell infiltration.
mones T3 and T4 fell sharply to 50% of control values (p < 0.04) by the end of the first hour; no circulating T3 or T4 was detectable 16 hours after induction of BrD (p < 0.0001). TSH showed no significant change from the baseline level throughout the experiment. Cortisol blood levels rose in all animals during the first five min., then declined progressively to 50% of the baseline values at one hour (p < 0.05) (Figure 4). By 16 hours, a further decline occurred (p < 0.0001). In group B baboons, slightly lower levels were obtained, presumably a dilutional effect. The circulating insulin also fell in all animals during the first five min. (Figure 5). In group B, which received I.V. fluid containing dextrose, the insulin level returned to baseline values 10 mins later, presumably as a response to the dextrose. Then, insulin levels fell again in both groups at one hour (p < 0.04), with a further fall at 13 hours reaching 20% of the baseline levels (p < 0.003). Antidiuretic hormone levels fell significantly in all animals, disappearing from the circulating plasma within six hours. In group A, the urine output ceased between the second and third hour of observation, by which time MAP had fallen to ± 45 mm Hg. The mean total urine output of group A baboons was 500 mL, while group B animals continued to pass large quantities of urine throughout the experiment, the mean total output reaching 8 L. Glucagon blood levels and ionized calcium showed no significant change from baseline values in either group.

LDH and CK serum levels were measured in four baboons of group B. Both levels rose after drilling the burr hole in the skull. This elevation was maintained in three animals; in the fourth, LDH and CK levels returned to baseline values after one hour. The isoenzyme CK-MB was positive in the only two animals in which it was measured. Myocardial ATP, CP, glycogen and lactate were measured at the end of the experiment (Table IV). In group A no significant change was observed, while significant depletion (p < 0.01) of ATP, CP and glycogen, and an increase in lactate (p < 0.01) were observed. Myocardial histology, studied at the termination of the experiment, showed abnormalities in two group A and four group B specimens (Table III). Contraction bands, focal myocardial cell necrosis, interstitial edema, and mononuclear cell infiltration around the myocytes showing contraction bands were present (Figures 6 and 7).

The additional experiments (Table I) modified the pattern of ECG and hemodynamic changes following BrD induction. Bilateral vagotomy at the base of the skull (baboon C) abolished the initial period of bradycardia and sinus standstill (stage 1) but did not modify either stages II or III. Bilateral adrenalectomy (baboon D) had no effect. Bilateral adrenalectomy performed on baboon E (which had a denervated heart following autotransplantation one week before the experiment) abolished tachycardia and arrhythmias of stages II and III, but did not abolish the initial MAP rise after BrD induction. When autotransplantation had been performed four months earlier (baboon F), by which time there is evidence of cardiac reinnervation (unpublished data), the effects of sympathetic activity were present (stages II and III) and bilateral vagotomy abolished stage I changes. Lastly, bilateral vagotomy and I.V. propranolol (10 mg/kg) abolished all ECG and hemodynamic changes of stages I to III in baboon G.

**DISCUSSION**

Irreversible myocardial damage might be due to sequelae of BrD, caused by a sudden increase in intracranial pressure, as took place in this study. A similar sequence of events is assumed to occur after head trauma or subarachnoid hemorrhage in potential organ donors. This study suggests that the duration of stage I is related to the rate of rise in intracranial pressure. The hemodynamic changes of stage II (tachycardia, hypertension) reflect a response of the body to compensate for the intracranial changes taking place during "coming". The increase in circulating catecholamines of stage II is associated with increased myocardial activity, the appearance of ventricular arrhythmias and ischemic changes that might evolve to infarction. Myocardial damage certainly occurs at this stage, as evidenced by persisting ischemic ECG changes and abnormalities found on histopathologic examination. The additional experiments suggest that the increased myocardial activity of stages II and III is due to an increase in circulating catecholamines. These amines do not appear to be released by either the adrenal glands or the myocardium. Cardiac denervation abolished the tachycardia and arrhythmias without suppressing the rise in blood pressure, while β blockade abolished both the tachycardia and the hypertensive response. The catecholamines might be released directly from the brain when intracranial hypertension is induced. All hemodynamic changes are completely abrogated by a high dose of a β blocking agent.

The histopathological findings were not unlike those seen in early acute rejection following cardiac transplantation which consisted of mononuclear cell infiltration, edema and focal cell necrosis. It might be that a diagnosis of rejection had been made on such findings when the donor heart had been damaged during the agonal donor phase. This study suggests that attempts to maintain the RAP and AP in physiological ranges after BrD may be responsible for acceptable postoperative function.
for myocardial function deterioration before transplantation and/or they may impair the heart's function after transplantation. A significant reduction in myocardial ATP, CP and glycogen stores, and an increase in lactate were seen in group B baboons who received fluid replacement, but were absent in group A baboons who did not receive fluids and in which both the RAP and the AP were allowed to fall. Whether ATP, CP and glycogen are rapidly replenished after transplantation remains unknown. The clinical experience at this institution suggests that periods of up to 24 hours are required for full donor heart recovery after transplantation. It is probable that acute donor heart failure within the first hours or days is due to myocardial damage that occurred during the agonal donor and BrD periods. The rise in LDH and CK seen in the four baboons certainly resulted from the trauma of drilling a burr hole. The presence of CK-MB isoenzyme in two baboons confirms the occurrence of myocardial damage. The lack of correlation between persisting ECG abnormalities and evidence of myocardial injury suggests that the ECG might not always be a reliable indicator of the state of the myocardium. Therefore, caution should be exercised when assessing the suitability of a donor heart for transplantation.

The significant fall in cortisol, insulin, and thyroid hormones may be factors in the deterioration of hemodynamic function seen in donors. A study is planned in which these hormones will be maintained in the normal range in BrD people. Although the TSH level did not change throughout the experiment and although histologic examination of the thyroid gland showed abundant stored colloid with inactive cells, T3 and T4 levels fell significantly. One could assume the presence of either a neural or hormonal T3 releasing factor. The disappearance of such a factor, because either the thyroid gland has lost its central nervous control or the damaged brain no longer releases it, prevents the normal response of the thyroid gland to TSH.

REFERENCES


SCIENTIFIC SESSION—DISCUSSION

Jack Copeland: This extensive evaluation of the donor condition is most interesting. A number of years ago Randall Griepp presented a paper in which he stated that the only contraindication to the use of a donor heart was the presence of Q waves on the ECG; all other types of changes could be seen, but all seemed to be reversible.

Keith Reemtsma: I would like to commend Dimitri Novitzky and his coworkers for their research in an area that has not received the appropriate attention. I would urge members of the Society to spend time and effort looking at the available donors, particularly when the harvesting is done in your own hospital, because that gives you the opportunity. In the United States most hearts are retrieved at distant hospitals, and therefore, it is more difficult to do these kinds of studies. The Society should perhaps develop some guidelines for donor maintenance which could be distributed among the various hospitals in this country.

Jack Copeland: There is a need to take better care of donors than is currently practiced, particularly in some area where many brain-death patients are wasted as potential donors.

Christian Cabrol: Almost all the donors at our hospital are obtained from local procurement. Indeed, donor management is very important. There is a difference between hearts retrieved through distant procurement and through local procurement. This is especially important for heart-lung transplantation. I would like to ask Novitzky if he has seen right bundle branch block (RBBB) in the baboon donor hearts after transplantation. This is seen rather often in our human allografts.

Dimitri Novitzky: We have not seen this either in our patients nor in the baboon grafts.

Denton Cooley: I think that Dimitri Novitzky's method of producing brain death is much more straightforward than that encountered in the clinical situation. In most of the patients brain-death is due to massive cerebral injury complicated by bodily injury. This study offers a pure evaluation of what a simple brain injury would do and how quickly it would affect cardiac function. In the clinical situation there are pulmonary injuries, gastrointestinal injuries, etc. I believe that the urgency of performing the transplant is greater than some institutions appreciate, and that when a suitable donor is available, the operation should not be considered an elective procedure that can be leisurely scheduled 12 or 18 hours later, at leisure. It should be done as quickly as can be accomplished.

Dimitri Novitzky: In 5 donors who were in a very poor hemodynamic status, totally depending on inotropic support, the administration of T3, cortisol, and insulin improved their condition to the point that isotropic support could be discontinued two hours later. These hearts, initially thought to be useless for transplantation, behaved well after transplantation.

Jack Copeland: How much T3 did you use in these donors?

Dimitri Novitzky: Every 15 mins, the donor receives 2 microG/I.V. of T3 while the blood gases are monitored. T3 immediately increases the pCO2 and the heart rate. As soon as these effects are apparent, isotropic support is tapered. We did a similar study using 2 baboons; one was treated with T3, the other not. The heart of the treated one had an excellent postoperative performance. The other baboon allograft from the donor that did not receive T3 had poorer function, and the recipient required prolonged ventilatory assistance. Eventually, the baboon died of heart failure. Myocardial ATP, CP and glycogen stores were significantly different in these two grafts.

Jack Copeland: In our institution, donor selection is based on the following criteria: males must be less than 35 years old,
and females less than 40. Inotropic support must not exceed 10 microG/kg/min of dopamine, sepsis must not be suspected, nor heart damage sustained if trauma was the cause of death. Have you established any additional criteria that would help in determining the suitability of a donor besides giving T3?

Dimitri Novitzky: I recommend strongly treating the donors with T3, however with caution, to avoid producing a hyperthyroid state. It should first be tried in an animal model. The measure of the donor heart ejection fraction (EF) and ventricular volume is also helpful. Donors who have EF less than 35% while receiving inotropic support are unsuitable for orthotopic transplantation. If heterotopic transplantation is to be performed, the recipient heart will support the patient until donor heart recovery.

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THE JOURNAL OF HEART TRANSPLANTATION / VOLUME IV, NUMBER 1 / NOVEMBER 1984 69
The Problem of the Presensitized Heart Transplant Recipient

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ABSTRACT—A presensitized patient experienced early severe rejection following heart transplantation despite a negative T-cell crossmatch. He developed strong multi-specific antibodies against T-lymphocytes after rejecting an HLA nonidentical heterotopic transplant in February 1979. In October 1981 a donor heart became available with which there was a negative T-cell lymphocytotoxic crossmatch; however, B-cell crossmatches were strongly positive at all temperatures. A second heterotopic heart transplant was performed after the donor heart had been preserved by hypothermic perfusion for 15 hours. Although the initial heart function was poor, it improved during the first three days, but by then features of a severe rejection episode had become apparent. Absence of donor heart function was suspected by external pulse traces on the sixth postoperative day, and this was confirmed by cardiac catheterization two days later. At that time a 32-fold increase in lymphocytotoxic antibodies against donor T-cells was demonstrated.

Introduction

Sensitization following rejection of a renal allograft appears to have a deleterious effect upon secondary graft survival.1,2 We are reporting on a case of accelerated rejection in a presensitized HLA nonidentical cardiac transplant recipient following retransplantation despite a negative T-cell crossmatch.

Case History

The patient, a 27-year-old man with a two-year history of cardiomyopathy, was in NYHA Functional Class IV in 1978. Following intensive drug therapy he improved, but his exercise tolerance remained extremely limited. On February 17, 1979, he underwent a heterotopic transplantation using the heart from an HLA nonidentical donor (Table I). The patient received 10 units of blood peroperatively. During the third postoperative week the patient suffered a severe, irreversible, acute rejection episode, which was manifested by a high fever, a fall in ECG voltage, and a rise in the percentage of rosetting T-lymphocytes. The episode culminated in graft failure two weeks later. The donor heart was excised because of systemic symptoms and fever.

By July 2, 1979, the patient, surviving with his own failing heart, had developed strong multispecific antibodies against 88% of the T-lymphocytes of a panel of 17 different known control cells. A 12-month effort was made to locate a second donor heart against which he would not have preexisting antibodies, but this search was unsuccessful. Approximately one year later (29 months posttransplantation), laboratory studies revealed that all of these antibodies had disappeared.

The patient was given a test transfusion of one unit of whole blood, and this was followed by the return of strong lymphocytotoxic antibodies to T-cells against 98% of a panel. Despite this finding, further efforts were made to obtain a second donor heart.

Manuscript received April 26, 1982
Manuscript accepted July 13, 1982
Two months later (October 3, 1981), an 18-year-old donor heart became available (Table I). The donor-specific T-cell lymphocyte crossmatches for all recipient sera taken from the time of the patient's first transplant until September 28, 1981 (date when the last sera were available), were negative at all temperatures when using both standard and extended time crossmatch tests. However, B-cell crossmatches were still strongly positive at all temperatures.

A heterotopic heart transplant was performed on October 4, 1981. The operation was difficult in view of the previous surgery. Total donor heart ischemic time was 16 hours and 15 minutes, which included 15 hours for transportation of the heart and for serological tests. During that time, the heart was preserved by hypothermic perfusion. During one hour and 15 minutes of operative ischemic time the heart was protected by intermittent topical cooling with saline at 4°C. The patient received 12 units of blood peroperatively.

The initial donor heart function was poor, possibly due to less than optimal myocardial protection and the degree of compression following closure of the chest caused by the noncompliant thoracic organs following the previous transplant operation. During the first three postoperative days, there was some improvement in donor heart function, but it was accompanied by indications of an early rejection episode, including a fall in ECG voltage and a marked rise in rosetting T-cells. Larger doses of immunosuppressive drugs, including intravenous methylprednisolone and rabbit antithymocyte globulin were administered without success. On the sixth postoperative day absence of donor heart function was suspected by external pulse traces and was confirmed by cardiac catheterization two days later. An endomyocardial biopsy showed significant cellular infiltration with areas of myocytolysis, thereby verifying the severity of the acute rejection episode.

At this time a strongly positive lymphocytotoxic antibody against donor T-cells, from the stored donor spleen, was demonstrated. On the eighth posttransplantation day, the serum had an antibody titer of 1 in 1024, a marked increase from the preretransplantation titer of 1 in 32. A B-cell titer could not be obtained due to the exceptionally high level of antibodies to T-cells that was already present.

High-level immunosuppression was maintained for an additional 48 hours but without any response, after which it was reduced to a low maintenance level in order to decrease widespread myocardial necrosis until the donor heart could be excised 10 days later. Excision of the donor heart was delayed until the patient had recovered from a chest infection and become hemodynamically stabilized following supportive therapy for his own heart. He made a slow recovery. Sinus bradycardia developed, necessitating the insertion of a transvenous endocardial sequential atrioventricular pacemaker.

The patient remains alive in NYHA Functional Class IV five months after the failure of the retransplantation; he is surviving once again only by his own heart, supported by considerable antifailure therapy.

**Discussion**

The difficulty of making a long-term prognosis for a patient with cardiomyopathy is well illustrated by the fact that this patient has survived more than three years since first advised to undergo heart transplantation. The quality of his life, however, has been extremely poor and not comparable to that which could be achieved after a successful transplantation.

One of the potential advantages of heterotopic over orthotopic cardiac transplantation is demonstrated here. The patient has undergone two transplant procedures, with rapid rejection in both cases, and yet remains alive. This sequence of events would probably not be possible after orthotopic transplantation, particularly in a geographic area where there are not many potential donors.

The initially poor function of the second donor heart was almost certainly due to the prolonged (15 hours) period of ischemia. Other factors such as periods of hypotension, anoxia, and acid-base disturbance can influence subsequent myocardial function, affecting the heart before its exci-

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**TABLE I. Recipient and donor HLA-A, B, C, DR phenotypes and blood group**

<table>
<thead>
<tr>
<th>Recipient</th>
<th>HLA antigens</th>
<th>Blood group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3, AW31; B40; BW7; BW3; CW6.</td>
<td>A+</td>
<td></td>
</tr>
<tr>
<td>First donor</td>
<td>A1, 1, B12, BW3</td>
<td>A+</td>
</tr>
<tr>
<td>Second donor</td>
<td>A3, AW31; B7, B27; CW7, DRW8; DRW6</td>
<td>O+</td>
</tr>
</tbody>
</table>
sion from the donor. Because of the patient's previous surgery, the operation was technically difficult and the heart suffered further ischemia (more than one hour) during insertion, at which time it was protected only by the intermittent topical application of a cold (4°C) saline solution. Finally, there was some compression of the donor heart after closure of the chest. All of these factors may have contributed to the poor myocardial function initially and to patches of coagulative necrosis that developed in the donor heart and were seen both on endomyocardial biopsy and after excision. It was our pathologist's opinion, however, that these changes were due to an accelerated rejection phenomenon.

From our experience with this patient and with a similar one who underwent renal transplantation on three occasions, it appears that whenever there is a history of presensitization there is a high risk of early severe rejection despite negative T-cell crossmatch.

The influence of preexisting lymphocytotoxic antibodies on the subsequent survival of a kidney allograft (when the donor-specific cytotoxicity crossmatch [CDC] is negative) remains uncertain. From early observations it seemed that patients with broadly reactive antibodies had impaired graft survival rates. More recently, crossmatching has been carried out using previously reactive sera (often having higher titer) as well as sera obtained at the time of transplantation. When more sensitive crossmatching techniques were used on these sera, no significant correlation between the degree of presensitization and subsequent graft survival was found. (Use of an insensitive CDC test or failure to test for antibodies in previously obtained sera that have high levels of cytotoxins may lead to higher rates of hyperacute and accelerated rejection within the first days to several weeks after transplantation.) There is, however, some evidence to suggest that, despite the use of more sensitive crossmatching techniques, sensitization following rejection of an allograft confers a less favorable prognosis. Our experience with the cardiac transplant patient reported here supports the latter conclusion; previously our understanding of this matter had been based exclusively on data from renal allotransplantation.

The role of B-cell lymphocytotoxins (BCLs) in clinical transplantation has received much attention following the first reports of a successful graft outcome in the presence of a positive B-cell crossmatch. Donor-specific BCLs, when present in a potential recipient before retransplantation, have been variously reported to: (a) lead to early graft rejection, (b) bear no relationship to the subsequent transplant outcome, and (c) correlate with improved graft survival. Furthermore, a distinction has been made according to the temperature at which the test is performed. Antibodies reacting at 37°C correlate with a poor prognosis, while those reacting at 5°C correlate with an improved prognosis.

It has been established, however, that preformed BCLs do not lead to immediate failure due to the hyperacute or accelerated graft rejection that occurs when preformed donor-specific HLA-A, B, and C cytotoxins are present in the recipient serum, although an isolated case has been reported.

It appears, therefore, that whenever there is or has been evidence of presensitization, there is a risk of early severe rejection associated with rapid development of strong multispecific antibodies against T-lymphocytes despite a negative T-cell crossmatch during transplantation.

References
EDITORIAL COMMENT* This article presents an interesting case report. The heart to be transplanted was reportedly stored by hypothermic perfusion for 15 hours and then failed soon after the procedure. Whether the heart failed simply because of ischemic or perfusion damage occurring during the prolonged storage time or because of an accelerated acute rejection is not clear. The only evidence offered here is the statement “It was our pathologist’s opinion . . . that these changes were due to an accelerated rejection episode.”

No statement is made regarding the presence or absence of mononuclear cell infiltrate in the specimens. The reader could be deluded into thinking that the problem was one of acute rejection, whereas there could have been a preexisting ischemic injury. The presence of strong B-cell antibodies that were found in the recipient’s blood should have precluded the use of this heart in this patient. Furthermore, the T-cell tests that were used were not the most sensitive tests; a more sensitive test would probably have resulted in positive T-cell testing. Additional information on the pathology of the rejected heart, as well as comments regarding transplantation in the face of strong B-cell antigens and justification of T-cell testing employed, will be of interest.

AUTHORS’ REPLY The statement that the transplanted heart “failed soon after the procedure” is an oversimplification of the sequence of events. The initial poor function was almost certainly related to the prolonged storage time. Improvement in cardiac function, however, occurred during the first 48 to 72 hours. A similar pattern was observed in two other transplanted hearts stored for shorter periods of time, with complete recovery. Deterioration did not begin until the third postoperative day, was coupled with other clinical and laboratory features of rejection outlined in the paper, and suggests that rejection rather than ischemic injury was the major factor in cessation of donor heart function. The full histopathologic report states: “The biopsy consists of small myocardial fragments, some of which show areas of coagulative necrosis with scanty neutrophils. The viable myocardium shows evidence of a prominent mononuclear cellular infiltrate in keeping with moderate acute rejection (see accompanying figure). Other mononuclear cells are accumulating within the lumen and walls of the small blood vessels. The biopsy shows evidence of significant cardiac rejection. This myocardial necrosis could be caused by acute rejection or by ischemia related to the preservation procedure. Either cannot be excluded.”

That “the presence of strong B-cell antibodies . . . should have precluded the use of this heart in this patient” remains uncertain and is at variance with much of the literature (some of which we cite). At least two patients have undergone renal transplantation at our institution in the presence of strong B-cell antibodies, and both have done well. Furthermore, a T-cell crossmatch at both 22°C and 37°C using the standard lymphocyte microtoxicity test, and a prolonged T-cell crossmatch at 22°C (serum and cells for one hour, followed by the addition of complement for another two hours) were carried out. Some leading centers would consider a prolonged crossmatch unnecessary. These same tests, negative before transplantation, demonstrated strong lymphocytotoxicity against donor T-cells on the eighth postoperative day, suggesting that these antibodies had been formed since transplantation. Such tests have been used in our laboratory in more than 500 kidney and 60 heart transplants without a case of hyperacute rejection. We would be pleased, however, to hear about more sensitive tests.

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*These comments represent elements of the discussions provided by the article’s reviewers and were sent to the authors for reply.

Toxoplasmosis of Donor and Recipient Hearts After Heterotopic Cardiac Transplantation

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Toxoplasmosis of both donor and recipient hearts was diagnosed by means of endomyocardial biopsy specimens after heterotopic cardiac transplantation for dilated cardiomyopathy. Before transplantation, the donor had raised antibody titers to Toxoplasma, and the recipient was negative. When toxoplasmosis was diagnosed on the basis of endomyocardial biopsy specimens, the recipient had a greatly elevated antibody titer of 1:1,027. This suggests that the infection could have been transferred with the donor heart. The mononuclear cell response elicited by disrupted toxoplasmic cysts interferes with the diagnosis of rejection in graft biopsy specimens. Electron microscopy is valuable in confirming a light microscopic diagnosis of toxoplasmosis. Drug therapy eradicated the toxoplasmosis, but the patient died later of tuberculous meningitis.

(Arch Pathol Lab Med 1983;107:368-373)

The majority of Toxoplasma infections have occurred in immunodeficient hosts, namely, fetuses and newborns, whereas their immunocompetent mothers usually show no symptoms. Several cases have been reported in immunodeficient adults; in some, the disease was confined to an immunodeficient organ such as the brain.

Infections are an ever-present problem in heart transplant recipients. Toxoplasmosis has been reported twice in such patients and occasionally in renal transplant recipients. Involvement of the donor myocardium by toxoplasmosis interferes with the assessment of rejection because the infection itself may produce a mononuclear cellular infiltrate. In our situation, both the donor and recipient hearts were simultaneously infected. The serological data suggested that the infection was transferred with the donor heart. A less likely possibility was that toxoplasmosis may have been the cause of the recipient's initial heart failure. A recrudescence infection in an immunosuppressed host was the third possibility. All of these aspects are discussed in this case report. Electron microscopy is valuable in confirming a histological diagnosis of toxoplasmosis.

REPORT OF A CASE

A 49-year-old man was well until May 6, 1981, when a viral-like illness developed.

His condition rapidly deteriorated, and shortness of breath and signs of congestive heart failure developed. There was some improvement with antiarrhythmic therapy, but symptomatically he remained in class IV (New York Heart Association classification) and was referred to Groote Schuur Hospital, Cape Town, South Africa, for further management.

On examination, he was tachypneic and had a pulse rate of 110 beats per minute and a sinus rhythm; the jugular venous pressure was just visible. The cardiac apex lies in the sixth left intercostal space in the anterior axillary line. There was a right ventricular precardial lift and a grade 1/6 murmur of mitral incompetence. The ECG showed evidence of left atrial enlargement and left bundle-branch block. He was treated with vasodilators. Cardiac catheterization, performed on Aug 19, showed features of a congestive (dilated) cardiomyopathy, with systolic hypotension (systolic pressure, 86/70 mm Hg), mild pulmonar hypertension (57/30 mm Hg), and a cardiac index of 2.63 L/min/sq m. The ejection fraction was 33%, and there was mild mitral incompetence. Heterotopic cardiac transplantation was performed on Oct 2, 1981, using the technique previously described. The immunosuppressive regimen consisted of azathioprine, methylprednisolone, rabbit antithymocyte globulin, and daclizumab. The dosages of these drugs were controlled by the recipient's lymphocytic T-rosette response and by graft endomyocardial biopsy.
Since it may be difficult on tissue sections to distinguish Toxoplasma from other less common organisms such as Sarcocystis, the patient's stored preoperative and fresh postoperative serum samples were serologically tested (dye test) for antibodies to T. gondii. The postoperative stored serum sample of the recipient was negative, while the serum freshly taken on Dec 19 had a raised titer of 1:1,927, confirming the histological diagnosis of toxoplasmosis. A myocardial biopsy specimen of the patient's own heart before transplantation showed no sign of toxoplasmosis.

A review of the seven earlier donor heart biopsy specimens demonstrated evidence of toxoplasmosis in two myofibers in the specimen of Nov 19 that had been missed on initial examination. Four of the five donor heart biopsy specimens taken after Dec 17 showed evidence of toxoplasmosis. A myocardial biopsy specimen of the patient's own heart before transplantation showed no sign of toxoplasmosis.

The five donor myocardial biopsy specimens that showed evidence of toxoplasmosis were also regarded as displaying signs of cardiac rejection. Rejection was diagnosed as mild in one specimen, moderate in two, and severe in three. One of the latter specimens had a total rejection score of 7, indicating very severe rejection (details of our biopsy scoring system for diagnosis of rejection are reported elsewhere). The inflammatory changes were diffuse and more severe than usually reported in toxoplasmosis.

Because of concern that the recipient's own heart might also be infected by Toxoplasma, a biopsy specimen was taken on Jan 3, 1982, at the time of routine donor heart sampling. Histological examination showed toxoplasmosis of the recipient heart too. In addition, a scanty toxoplasmic cyst within a few myofibers that elicited no inflammatory response (Fig 2), there were a few scattered foci of interstitial mononuclear cell infiltration. No parasites were recognizable within the latter zones.

Electron microscopy performed on an endomyocardial biopsy specimen of the donor heart's right ventricle showed the ultrastructural appearance of toxoplasmosis (Figs 3 through 6). The organisms were contained within a true cyst that had a wall of parasitic origin. A transversely sectioned myofiber showed about 63 individual Toxoplasma organisms in the plane of section (Fig 3). Observed details of these organisms confirmed most of the findings of Garnham et al and with regard to the presence within the cytoplasm of conve-

Routine serial posttransplantation transvenous endomyocardial biopsy specimens of the donor heart were obtained at about weekly intervals. The specimens showed evidence of mild to severe acute rejection. At one stage, severe neutropenia developed, necessitating stopping all immunosuppression. He also had a transmural, high-running temperature, but cultures of blood, sputum, and urine were all negative. A donor heart biopsy specimen examined on Dec 17, 1981, showed aggregates of numerous small parasitic organisms (Fig 1) within the cytoplasm of a few muscle cells. The appearance was that of infection by Toxoplasma gondii.
F.g

- Transversely sectioned myofiber is filled by cyst containing numerous Toxoplasma organisms. Arrows indicate outer limits of cyst wall (original magnification X7000).

luted tubules and secretory paired organs (Fig 4), as well as the conoid and polar ring (Fig 5). Each organism was surrounded by a double-layered cell wall (Fig 6).

- The peripheral fibrils described by Garnham et al were shown in our specimens to be clearly tubular (Fig 6). They have been called microtubules by others, and may have contractile properties. No microtubules were seen in our specimens.

At no time did the patient show any signs of systemic toxoplasmosis. Attempting to overcome the toxoplasmosis infection, we administered an initial loading dose of pyrimethamine, 100 mg twice daily on the first day, followed by 50 mg daily. Trisulphonamide (Sulphadiazine), 1 g every six hours, was also given. This combination therapy was continued for 3½ months.

A few graft biopsy specimens taken after therapy was instituted showed persistent Toxoplasma infection, but all specimens taken from both hearts more than 2½ months after institution of antitoxoplasmosis therapy were negative for Toxoplasma and inflammatory cells. The patient died with clinical features of tuberculous meningitis on Aug 14, 1982. Autopsy revealed focal areas of cerebral infarction, but no sign of active tuberculosis. No Toxoplasma organisms were seen.

**COMMENT**

Toxoplasmosis is due to infection by *T. gondii*, a minute coccidian parasite (toxo is the Greek word for bow or arc) that has a curved shape. The infection may cause a myocarditis or a lymphadenopathy. Many cases are asymptomatic, and *T. gondii* has been isolated from apparently healthy individuals. By 20 years of age, about 25% of the population have had the infection, as indicated by a significant elevation of antibody titers to *T. gondii*. These percentages vary in different areas of the world. We have no information regarding the relevant South African data. This intracellular protozoan often infects domestic animals (cats, dogs) and farm animals and has a worldwide distribution. Transmission occurs from the ingestion of oocysts from cat feces or fecally contaminated soil, or from undercooked meat.

In 1976, Frenkel stated that during acute Toxoplasma infection, rapidly multiplying tachyzoites (measuring 2 x 6 µm) form "groups" within intracellular vacuoles and the host cells are eventually destroyed. During chronic infection, the organisms (bradyzoites) multiply slowly and are tightly packed in cysts. The latter originate in intracellular vacuoles.

Infection in man usually involves the brain, heart, lungs, lymphatic system, and skin. Even in mild cases of adult toxoplasmosis, it is likely that the myocardium is involved. Myocarditis may be encountered in the acute, subacute, chronic, or relapsing forms of toxoplasmosis. Myocardial involvement may produce cardiomegaly, congestive heart failure, pericarditis, and arrhythmias, including bundle-branch block due to lesions in the
Fig 4.—Higher magnification shows details of Toxoplasma organisms. Longitudinally sectioned organism (top left) shows appearance of anterior end of organism (arrow). Within cytoplasm are convoluted tubules (star) and obliquely sectioned portions of paired organs (curved arrow). Nucleus lies within hollow of curved arrow (original magnification X24,000).

Fig 5.—In this field, plane of section passes transversely through anterior tip of two Toxoplasma organisms (center left and top right). Conoid (C) and polar ring (R) are clearly seen (original magnification X30,000).
conducting system. One in two infants with congenital toxoplasmosis will have myocardial involvement.14

Macroscopically, the heart appears to be dilated and may show petechial hemorrhages and grayish-yellow mottling due to focal necrosis. Focal fibrosis follows healing of the necrotic foci.

Histologically, toxoplasmosis produces focal interstitial infiltrates containing lymphocytes, plasma cells, histiocytes, and a few eosinophils. Because inflammation is not encountered in the vicinity of parasitized fibers, the inflammatory response is attributed to rupture of the infected myofibers and cyst disintegration, rather than to the presence of the parasites within the fiber.13,14

It should be noted that a light microscopical appearance histologically identical to Toxoplasma may be seen with Sarcocystis,12,13,15 which is found occasionally in man. Sarcocystis infection produces cysts limited to striated muscle and connective tissue; unlike toxoplasmosis, however, the cysts may eventually reach such a size as to be visible microscopically. Other differences are that the cysts of Sarcocystis may have internal septa and that the organisms, which are seen intracellularly only, are arranged so that mature forms lie centrally and immature forms (metrocystes) are found at the peripheral portions of the cyst. Sarcocystis organisms are larger (about 10 to 14 mm) and rounded at both ends. Toxoplasma parasites (about 7 mm) are either all at the same stage or there is an intermingling of young and adult forms. The numbers of raphtries or microtubules are not consistently different. While antibody response to Toxoplasma supports a diagnosis of toxoplasmosis, antibody titers to Sarcocystis would have to be measured to invoke or exclude the diagnosis of sarcosporidiosis.

Other organisms bearing a superficial resemblance to T. gondii include Leishmanias and aflagellate forms of Trypanosoma cruzi, but they are characterized by the presence of a prominent rod-shaped kinetoplast in their cytoplasm. They do not store PAS-positive material, as do Toxoplasma and Sarcocystis Nosema (Encephalitozoon) cuniculi16 usually has no nuclear structure, a clear cytoplasm, and a PAS-positive granule in the anterior end of the spore. Gram's stain shows up the entire spore.

In the late stages of cardiac involvement by toxoplasmosis, parasitized myofibers may not be found, and such patients dying of cardiac failure may be considered as having idiopathic cardiomyopathy. For example, in 1967, Durge et al17 described two men found at autopsy to have severely hypertrophied and fibrotic hearts and who had previously received the diagnosis of toxoplasmosis.18 Severe coronary artery disease was absent, and the authors believed that the cardiomyopathy in their two patients was caused by the previous toxoplasmosis. At autopsy, the heart may appear grossly normal or may show hypertrophy and dilatation.19 Areas of interstitial fibrosis are seen when the disease has been of long duration.

Our patient underwent heterotopic cardiac transplantation for intractable heart failure. The rapid onset of his initial illness and the associated progressive cardiac failure clinically suggested a viral myocarditis. Cardiac catheterization showed features of a dilated cardiomyopathy. It is unlikely that the Toxoplasma infection of the...
recipient occurred as a recrudescence of toxoplasmosis in an immunosuppressed host, since the recipient lacked pretransplantation Toxoplasma antibodies. It is more likely that the donor was infected with toxoplasmosis and that the infection was transferred with the donor heart.

Although 2% of the general population aged 20 to 39 years may have a Toxoplasma antibody titer (dye test) of up to 1:16, the titer found in the stored donor serum samples varied from 1:16 to 1:256. Since Beverley and Beattie2 regard a titer of 1:64 or greater in persons over 20 years of age as significant, it would suggest that the donor was infected with toxoplasmosis. A third, less likely possibility is that the patient’s idiopathic cardiac failure, which necessitated transplantation, may have been due to toxoplasmosis, immunosuppression allowing the persistent parasites to proliferate. The recipient’s lack of antibodies to Toxoplasma before transplantation and the raised titer after transplantation argue against the concept of a prior infection in the recipient. On the other hand, encysted Toxoplasma organisms have been recovered from tissue of patients with low or unmeasurable dye test titers. At no time did the latter patients show symptoms or signs of systemic toxoplasmosis.

The most important aspect of toxoplasmosis of the donor heart is the way it complicates interpretation of rejection in graft biopsy specimens. The interstitial mononuclear cell infiltrate that follows release of the organisms from infected myoblasts is very similar to that seen in acute rejection. This causes difficulty both in detecting cardiac rejection and in assessing its severity. Pyrimethamine does not appear to be helpful in distinguishing between cardiac rejection and infection with Toxoplasma. The cellular infiltrate in toxoplasmosis is said to be of a more mixed type and includes lymphocytes, plasma cells, histiocytes, and occasionally eosinophils. In acute rejection, the cellular infiltrate in the early stages consists almost entirely of activated lymphocytes. Later, transformation into plasma cells occurs. Cardiac histiocytes may appear to be activated, but they usually do not form a conspicuous part of the cellular infiltrate. A few eosinophils and neutrophils may be seen in acute rejection. Despite these theoretical differences, we know of no sure way of deciding which components of the mononuclear cell infiltrate in a patient’s donor heart are due to rejection and which are due to toxoplasmosis.

References

9. Cooper DXC, Fraser RC, Rose AG, et al: Technique, complications and clinical value of

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17. Melhorn H, Heydorn AD, Janitschke K: Light and electron microscopic study on sarcocystis from muscles of the rhesus monkey (Macaca mulatta), baboon (Papio cynocephalus), and tamarin (Saimiri (=Cebus) oedipus), Z Parasitenk 1977;53:160-178.
THE SIGNIFICANCE OF LEFT VENTRICULAR VOLUME MEASUREMENT AFTER HEART TRANSPLANTATION USING RADIONUCLIDE TECHNIQUES

Dimitri Novitzky¹, D. Cooper¹, J. Boniaszczuk², S. Isaacs³, R.C. Fraser⁴, P.J. Commerford ⁴, C.J. Uys⁵, A.G. Rose⁵, J.A. Smith² & C.N. Barnard¹

-Abstract—Multigated equilibrium blood pool scanning using Technetium 99m labeled red blood cells was used to measure left ventricular volumes in three heterotopic and one orthotopic heart transplant recipient(s). Simultaneously, an endomyocardial biopsy was performed and the degree of acute rejection was assessed by a histological scoring system. The scores were correlated to changes in ejection fraction and heart rate. Technetium 99m scanning data were pooled according to the endomyocardial biopsy score: (A) no rejection; (B) mild rejection; (C) moderate rejection, and (D) severe rejection. In each group, the median of the left ventricular volume parameters was calculated and correlated with the endomyocardial biopsy score, using a non-parametric one-way analysis of variance. A decrease in stroke volume correlated best with the endomyocardial biopsy score during acute rejection. A decrease in end-diastolic left ventricular volumes did not correlate as well. Changes in the end-systolic left ventricular volumes were not statistically significant, but using a simple correlation between end-systolic left ventricular volumes and endomyocardial biopsy the correlation reached significance. Changes in left ventricular volumes measured by Technetium 99m scanning may be useful to confirm the presence or absence of acute rejection in patients with heart grafts.

HEART TRANSPLANTATION IV, 206-209, 1985

INTRODUCTION

To date, the most reliable method of diagnosing acute rejection of the heart has been endomyocardial biopsy (EMB).¹ The histological features have been well defined and a grading system has been developed by Rose and Uys, based on (a) mononuclear cell infiltrate, (b) myocardial cell damage, (c) edema, (d) vascular change, and (e) Unna Pappenheim staining of the mononuclear cell cytoplasm.³ Each parameter can be graded from 0, no rejection; 1, mild changes; 2, moderate changes and 3, severe rejection. Theoretically, the score can range from 0 to 15. However, scores greater than 7 are extremely uncommon. A score of 0 signifies no rejection, 0.5 to 2 indicates mild, 2.5 to 4 moderate, and 4.5 or more severe acute rejection. EMB has, in our hands, a 4% complication rate, and in a further 4% an inadequate tissue sample is retrieved that does not allow a meaningful assessment.³ Other methods to diagnose acute rejection have been explored, but none are reliable. In this study we compared EMB data with Technetium 99m scan (Tc99m-scan) left ventricular volume (LVv) measurements in four heart allograft recipients.

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CLINICAL MATERIAL and METHODS

Three patients who underwent heterotopic (HHT) and one who underwent orthotopic heart transplantation (OHT) are included in this study (Table I). In HHT, the graft is positioned in the right pleural cavity. Anastomoses are performed between donor and recipient left atria, right atria, aortae, and pulmonary arteries, this last anastomosis requiring the interposition of a prosthetic tube graft (Figure 1).⁴ Multigated blood pool scanning (MUGA) illustrates

<table>
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<th>TABLE I. Demographic data</th>
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<tr>
<td>Patient</td>
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<td>JH</td>
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<tr>
<td>Age</td>
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<tr>
<td>Sex</td>
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<td>Pathology</td>
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<tr>
<td>Operation</td>
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<tr>
<td>Height (cm)</td>
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<tr>
<td>Body weight (kg)</td>
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<tr>
<td>BSA (m2)</td>
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<tr>
<td>Attenuation Factor</td>
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IHD = Ischemic heart disease
CM = Cardiomyopathy
HHT = Heterotopic heart transplantation
OHT = Orthotopic heart transplantation
BSA = Body surface area

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the HHT anatomy, showing the donor heart in the right pleural cavity, anteriorly to the right hilum, with its apex pointing towards the anterior or mid axillary line (Figure 2). The anatomical axis of the heart lies approximately parallel to the frontal plane of the body. The donor left ventricle (LV) forms the inferior chamber, with its base lying against the recipient heart and its apex directed towards the lateral chest wall. The right ventricle (RV) and its outflow tract lie above the LV. The interventricular septum lies in an oblique direction. Following HHT, the ECG records two QRS complexes, one from each heart. In order to obtain a donor ECG signal for MUGA acquisition, without recipient QRS complex interference, three electrodes are placed, one on the right shoulder and two in the V4R and V5R position. The donor heart R-R interval is divided into 28 equal time intervals, each accumulating 300,000 counts per image, using a standard anterior view. In the patient who underwent OHT, a 30° left anterior oblique position was selected.

In order to calculate LV, from MUGA recordings, an attenuation factor (AF) was calculated for each patient (Table I) using a modification of the technique described by Schwaiger and coworkers (Appendix 1). The Tc99m-scans were performed in the patient’s isolation room using a standard technique. The LV ejection fraction (EF) was calculated using the commercial MDS A² program with semi-automatic edge detection. A background region of interest was identified below the donor LV. Immediately after data acquisition, 4 mL of blood were withdrawn to measure Tc-99m activity of the blood (Appendix 2). The LV end-diastolic (ED) counts obtained from the MUGA were corrected for attenuation using the AF. The ED volume (EDV) was calculated as:

\[
EDV = \frac{\text{"Background Corrected ED Counts"}}{AF \times \text{counts /ml of blood}}
\]

Similarly, the end-systolic volume (ESV) was calculated and stroke volume (SV) derived.

Statistical Methods
Each variable calculated from the Tc99m-scans (SV, EDV, ESV, EF, and heart rate [HR]) was correlated separately with the EMB score. The 36 studies were divided into four groups depending on the EMB score.*

<table>
<thead>
<tr>
<th>EMB scores</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
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<tbody>
<tr>
<td>0 to 0.5</td>
<td>none</td>
<td>mild</td>
<td>moderate</td>
<td>severe</td>
</tr>
<tr>
<td>REJECTION</td>
<td>none</td>
<td>mild</td>
<td>moderate</td>
<td>severe</td>
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The values of the LV, EF, and HR were allocated into each EMB score group and the median value of each LV, for each group was calculated using the Kruskal

*This grouping did not correlate exactly with the scoring system described previously, but had the advantage because it distributed the number of studies evenly, taking into account the small number of EMBs with scores greater than 3.0.
non-parametric one-way analysis of variance. The significance of the difference between the groups was calculated. EF and HR data were directly correlated with the EMB score. Absolute values of LV$_v$ among patients were not calculated because of the differences in body surface area between donor and recipient and among the four recipients. However, the percentage of change in the LV$_v$, in relation to the change in the EMB score was analyzed for each patient to detect a correlation between these two parameters. The largest LV$_v$ measured at the time of the highest EMB score was graded as 100%, and all other measures were expressed as a fraction of this highest LV$_v$, and plotted against their EMB score (Figure 3 and 4).

**RESULTS**

There was no statistical difference between groups A and B regarding the median value of the SV, but there was one between groups A and C ($p < 0.05$), and groups A and D ($p < 0.0005$), (Figure 5). The median value of the EDV fell significantly between groups A and D ($p < 0.02$), (Figure 6). Changes in the median value of the ESV showed no difference ($p=NS$) between any of the four groups. Using direct correlation of the ESV with the EMB score, however, these changes became significant ($p < 0.05$). A simple correlation of the EF or HR with the EMB score showed no statistical significance.

**FIGURE 3:** Relationship of absolute stroke volume to endomyocardial biopsy score in four patients with heart transplants. The encircled stroke volume represents 100% against which the other volumes were compared.

**FIGURE 4:** Relationship of percentage change in stroke volume to endomyocardial biopsy score in four patients with heart transplants. These values are derived from the data presented in Figure 3.

**FIGURE 5:** Differences in median stroke volume between groups A, B, C and D; statistical differences are given.

**FIGURE 6:** Differences in median end-diastolic volume between groups A, B, C and D; statistical differences are given.
DISCUSSION

None of the techniques described for the measurement of LV by radionuclide methods are entirely satisfactory, and no single method correlates well with angiographic estimates of LV. However, when estimating LV in an individual patient, the method measures relative rather than absolute volume changes. Of all the parameters measured by radionuclide scanning, SV clearly correlates best with EMB scores, followed by EDV and, to a lesser extent, ESV. LV decreased during acute rejection as the LV wall and septum thickened with edema and cellular infiltration (Figure 7). During mild to moderate rejection, the EF was maintained or even increased, as the volume of blood in the LV was reduced. During severe rejection the EF fell.

This study shows that changes in SV and, to a lesser extent, EDV and ESV are sensitive parameters to assess the degree of acute rejection, and can provide valuable information during the course of treatment of rejection. In addition, radionuclide scanning has advantages over EMB, being a non-invasive procedure, and if further clinical experience demonstrates that MUGA can provide information as reliable as EMB, it is preferred to EMB.

Appendix 1: In this study, the attenuation factor (AF) was calculated in the catheterization laboratory at the time of the first EMB, five to seven days after the transplantation, using data from a MUGA performed on the third post-operative day. One millicurie (mCi) of Tc-99m was placed in front of the gamma camera*, and a count was recorded**. The camera was then positioned over the donor heart (anterior view) and 1 mCi of Tc99m was rapidly injected through a catheter positioned in the donor right ventricle. The ratio between the counts measured over the donor right ventricle and those measured over the 1 mCi was calculated, giving the AF for that patient. It was assumed that the AF was the same for all patients undergoing different degrees of acute rejection:

A—No rejection (normal ventricular volume);
B—Mild acute rejection;
C—Moderate acute rejection;
D—Severe acute rejection.

Note the decrease in left ventricular volume as rejection progresses.

Appendix 2: A vial containing 59.40 mM sodium pyrophosphate*** and 1.092 mg of stannous chloride, is reconstituted with 4 mL of sterile water and injected into the patient intravenously. Twenty minutes (min) later 15 mL of blood are withdrawn from the patient into a heparinized syringe and incubated with 25 mCi of Tc-99Pm for ten min. This mixture is then administered to the patient through a peripheral vein. A MUGA scan is then carried out over 10 to 15 min, the gamma camera being placed over the right chest to obtain an anterior view of the donor LV.

*** Atomic Energy Corporation

Acknowledgments: The authors thank the members of the medical, nursing and paramedical staff of Groote Schuur Hospital and of the University of Cape Town Medical School who have contributed towards the care of these patients.

REFERENCES

PHYSIOPATHOLOGICAL EPIDEMIOLOGY OF
TUMORS AFTER TRANSPLANTATION

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Running title: Tumors after Transplantation

Key words : Tumors, cancer, neoplasms, malignancies, transplantation.
ABSTRACT

There is a markedly increased incidence of de novo malignant tumors in patients who undergo organ transplantation. We have reviewed the neoplasms occurring in our own series of cardiac and renal allograft recipients, and compared our findings with neoplasia in the general population and at transplantation centers throughout the world.

Skin cancer and lymphomas are the most frequently encountered neoplasms in transplant recipients, accounting for 770% of 10 neoplasms at our own institution and for 105055% of 1897 neoplasms worldwide.

The physiopathological epidemiology of these malignant tumors is discussed, particularly with regard to the site distribution and pathologic types of tumors that arise de novo after organ transplantation, and to the probable etiologies and mechanisms involved in their development.
INTRODUCTION

It has been clearly established that immunosuppressed recipients of organ grafts have a markedly increased incidence of tumors (1-3). The incidence of de novo malignant tumors at major transplant centers in the United States, Europe and Australia varies from 1% to 16% (mean of 4%), an incidence on average approximately 100 times greater than that of the matched general population (3). There are also well documented epidemiological differences between tumors arising after transplantation and those arising in the general population (3).

Mechanisms involved in the etiology of the various tumors that arise in immunosuppressed transplant recipients may include impairment of surveillance mechanisms for neoplastic mutant cells (4-6), direct or potentiating actions of the immunosuppressive agents or other treatments administered to the patient (7-9), viral oncogenesis (10), or chronic antigenic stimulation by the donor organ (11, 12).

Virtually all of our knowledge of the physiopathological epidemiology of malignant neoplasms occurring after transplantation comes from cardiac and renal transplant recipients. We have reviewed the occurrence of de novo malignant tumors in our own series of cardiac and renal allograft recipients and compared our findings with those from recipients of donor organs throughout the world.

PATIENTS AND METHODS

Heart Transplants

Between December 1967 and January 1982, a total of 54 heart transplants (10 orthotopic and 44 heterotopic) were performed in 50 patients at Groote Schuur
Hospital (GSH), Cape Town, South Africa; living patients were followed up until January 1983. The 30 recipients who survived with a functioning cardiac allograft for at least three months have been reviewed.

The basic maintenance immunosuppressive therapy throughout this period was as follows:

1. Azathioprine administered at the highest tolerated level as judged by the absence of features of bone marrow and hepatic toxicity; the maintenance dosage for adults was between 1.5 and 4.5 mg/kg/day.

2. Methylprednisolone sodium succinate given intravenously (IV) on the day of operation (600 mg) and reduced by daily increments of 100 mg until discontinued. Oral dosages of 64 mg/day of methylprednisolone sodium succinate were administered, reducing steadily to approximately 32 mg/day at three months and 20 mg/day at one year, if the patient's progress permitted.

3. Between 1970 and 1979, equine anti-lymphocyte globulin (EALG) was given IV during the first month to maintain a low level of circulating T lymphocytes. From mid-1979, rabbit antithymocyte globulin (RATG) was used.

Acute rejection episodes were treated with three to five daily 1G IV pulses of methylprednisolone sodium succinate and short courses of RATG; actinomycin D was also administered in most cases.

Kidney Transplants

Between December 1967 and January 1982, 167 kidney transplant recipients at GSH survived with a functioning allograft for at least three months; all were followed up for a minimum period of one year.
The immunosuppressive regimen did not differ greatly from that used in the cardiac recipients (2), although methylprednisolone was given in larger doses during the first three months (initial dosage 86 mg/day, reducing by 2 mg/day until 32 mg/day was reached at approximately one month; thereafter, reduction was slow until an eventual maintenance dosage of 8 mg/day was achieved, usually during the second year. EALG or RATG, however, did not form a regular or substantial component of the immunosuppressive regimen as in the cardiac recipients.

RESULTS

Tumors developed in three (10%) of the cardiac allograft recipients and in 7 (4.2%) of the renal allograft recipients. The age at transplantation, type of tumor, time of diagnosis after transplantation, treatment, and outcome for each patient are given in Table 1. There was a greater than twofold increase in the overall incidence of neoplasia in the cardiac recipients when compared with the kidney recipients, and almost a sixfold increase in the incidence of visceral neoplasia ($p < 0.02, \chi^2$).

The average age of the 10 patients developing tumors was 41, and that of those who did not was 36 years. The average duration of immunosuppression of the ten patients prior to diagnosis of the malignant tumors was 34 months. The shortest periods after transplantation during which tumors developed were 10, 16 and 18 months, these all being Kaposi's sarcomas.

Heart Transplants

Adenocarcinoma of the stomach was diagnosed in a patient 19 months after orthotopic cardiac transplantation; he died two months later with abdominal and liver metastases. A cerebellar microglioma developed in a second patient, confirmed
by biopsy 61 months after heterotopic cardiac transplantation. The tumor responded to cranial radiotherapy, and the patient continues in remission 18 months later with only slight residual neurological dysfunction. Kaposi's sarcoma developed in a black recipient 10 months after heterotopic cardiac allografting; the tumors were localized mainly in the trunk. Immunosuppression was reduced in an effort to curtail the proliferation and dissemination of the tumors, but the patient died four months later with widespread dissemination of the neoplasm.

**Kidney Transplants**

Kaposi's sarcoma also developed in two of the kidney transplant recipients. Both were of mixed racial background (i.e. Cape Coloured). One of these patients died as a result of dissemination of the tumor 19 months after diagnosis, despite chemotherapy and complete withdrawal of immunosuppressive therapy. The other patient had tumors localized to the lower limbs and responded well to radiotherapy and withdrawal of immunosuppressive therapy with complete regression of the neoplasms. Two of the kidney allograft patients developed squamous cell carcinomas and one recurrent basal cell carcinoma of the skin. These lesions all developed on sun-exposed skin areas and responded well to local radiotherapy or excision. In another patient who died from septicemia, a localized sclerosing undifferentiated carcinoma of the thyroid was found at autopsy. The final kidney recipient died from an intraductal biliary carcinoma.

**DISCUSSION**

The malignant tumors occurring at our own institution are contrasted in Table 2 with those occurring in transplant patients throughout the world.

**Skin Cancer**

Skin tumors are the most frequently encountered neoplasms in transplant reci-
Patients, accounting for 38% of the neoplasms worldwide and 30% of the neoplasms at GSH. The incidence of skin cancer has been found to vary with the amount of exposure to the oncogenic effects of sunlight (3). In parts of the world with relatively little sunshine the incidence is approximately 4 to 7 times greater than that in the matched general population; in regions with high sunshine exposure the incidence rises to almost 21 times greater than the already high incidence found among the general population of these areas. In one Australian series where there was high sunshine exposure, skin tumors accounted for 87 (80%) of 109 neoplasms (13).

These cancers also differ qualitatively from those encountered in the general public (1). Although basal cell carcinomas usually far outnumber squamous cell carcinomas, this relationship is reversed after transplantation (51% squamous vs 28% basal worldwide and 2 squamous vs 1 basal at GSH). Transplant patients in whom these lesions occur are approximately 30 years younger than individuals in the public at large who develop similar malignancies.

The vast majority of the skin malignancies occurring worldwide after transplantation are low-grade and only 8% show lymph node metastases when first diagnosed (3). Five percent of the patients die from metastases (two-thirds from squamous cell carcinomas and one-third from malignant melanomas).

**Lymphoma**

Excluding skin cancers, lymphomas occur at the highest frequency in transplant recipients, comprising 17% of all neoplasms worldwide. This contrasts with the incidence of lymphoma in the general population where it represents only 3% to 4% of all tumors (1).
A striking feature is the marked incidence of central nervous system (CNS) involvement; 40% have CNS involvement, compared with an incidence of only 2% in the general population (9). In our own series the involvement of the brain by a microglioma - the only lymphoma other than Kaposi's sarcoma which occurred - is consistent with this observation. The weak immunological reactions of the CNS are thought to allow tumor cells to proliferate more readily than in other sites (14).

The predominant type of lymphoma is the reticulum cell sarcoma, which occurs in 49% of cases. Its incidence is approximately 350 times that found in the general population (9). By contrast Hodgkin's disease is a relative rarity in transplant recipients, accounting for only 10 (3%) of the 325 lymphomas worldwide. This is in sharp contrast with its incidence in the general population of 18% (15). The incidence of Hodgkin's disease in transplant recipients is therefore only one sixth that in the general population, whereas non-Hodgkin's lymphomas occur 28 times more often in transplant patients than in the remainder of the population (16).

This high incidence of lymphoma may reflect an abnormal immune response to the HLA antigens present on the transplanted tissue, since the neoplasms which occur usually show morphological features of antigen-activated lymphocytes (17). Although the etiology remains uncertain, oncogenic viruses are suspected of playing an important role. In four of 134 cyclosporin A (CYA) - treated transplant patients lymphoma occurred; Epstein-Barr virus (EBV) nuclear antigen was found in the tumor cells of one patient (18), and rising titres of antibody to EBV capsid antigen were present in three (19). EBV, of course, has clearly been associated with Burkitt's lymphoma and has been implicated in the pathogenesis of lymphoma in transplant recipients who did not receive CYA (20). In the case of CYA-treated patients, and possibly others, it is thought that the cell-mediated T cell
response to transformation and viral infection is inhibited by either primary in-
fection or reactivation of latent virus (21). The polyclonal proliferative B cell
response is thought to allow development of monoclonal lymphomas. The lympho-
mas developing patients immunosuppressed with CYA have thus far occurred re-
latively early after transplantation (approximately 6 months) (Israel Penn - per-
sonal communication).

Kaposi's Sarcoma

Kaposi's sarcoma is an unusual malignancy which occurred in a striking 3 (30%) of
the patients in our own series; this represents a significant increase over the
3% incidence in transplant patients worldwide (p<0.001). Before the recent
Acquired Immune Deficiency Syndrome (AIDS) epidemic, Kaposi's was seldom
seen in America or Europe (0.06% of all malignant neoplasms in the Chicago area
(22)).

Kaposi's sarcoma occurs relatively early after transplantation - in our own series
after an average period of 15 months (compared with 51 months for the other
tumors (p<0.05), and in the worldwide series after 17 months (compared with
56 months)).

All three Kaposi's sarcomas at GSH occurred in transplant recipients with Afri-
can ancestry. One occurred in a patient from central Africa (Kenya) where, in
the general population, Kaposi's sarcoma represents 9% of all malignant tumors
(22). That there was only a 3% chance of all three Kaposi's sarcomas developing
in patients with African ancestry would suggest that black patients living in
Africa who undergo transplantation may have a propensity for this complication
to develop. The relative rarity of transplant operations in this group would ex-
plain why this association has not been reported at other transplant centers.
In non-transplant patients a probable impairment of cellular immune mechanisms in the pathogenesis of the disease has been described (23). There is also evidence that Kaposi's sarcoma may be caused by viral infection (24). A genetic factor may well be important; apart from the high incidence in central African blacks referred to above, it has been shown that Kaposi's sarcoma occurs 400 to 500 times as often in patients of Jewish or Mediterranean origin compared with the matched population (25).

Other Tumors

Malignant tumors involving the colon and rectum, breast, uterine cervix, prostate, and bronchus do not show an increased incidence in transplant patients (3). In situ carcinomas of the body of the uterus, however, develop 14 times as frequently in transplanted patients as in the age-matched general population (26). Carcinomas of the vulva and perineum also show a much higher incidence in transplanted patients than in the population at large.

Possible etiology of tumors in transplant patients

The high incidence of malignant tumors in transplant recipients is almost certainly due to the heavy immunosuppression they receive. The more intensive immunosuppression of cardiac recipients may account for the significantly higher incidence of tumors found in our cardiac patients when compared with our renal patients. In a recent review of the long-term follow-up of the Stanford series of cardiac allograft recipients there was also a high incidence of tumors (13%) (27). This immunosuppression may lead to impairment of surveillance mechanisms for neoplastic mutant cells (4-6). Transformed cells arising by somatic mutation or viral infestation, which have the potential to become malignant, are more likely to escape elimination by the patient because of the attenuated efficacy of immunosurveillance.
Direct of potentiating actions of immunosuppressive agents may be causal; the Stanford cardiac transplantation group has reported the occurrence of tumor at the site of multiple RATG intramuscular injections in two patients (28). RATG causes immunosuppression by eliminating specific types of lymphocytes implicated in immune reactions and may indirectly lead to proliferation by failing to stimulate a proposed feedback mechanism, predisposing the lymphoreticular system to neoplasia (29). When used in conjunction with chemical carcinogens or oncogenic viruses, antithymocyte globulin has been shown to have a potentiating effect on cancer development (8, 30-32). Although no direct oncogenic effect has been demonstrated, azathioprine is known to cause chromosome breaks and nuclear abnormalities in both animals and man (33), and has been shown to potentiate the actions of oncogenic stimuli (7).

Chronic antigenic stimulation of the lymphoreticular system by the donor organ itself may be important in the development of neoplasia (11, 12). It has been suggested that partial immunosuppression fails to stimulate a feedback system which normally controls immune reactions and may allow viral activation and proliferation with subsequent tumor formation (29).

It is well-known that certain viruses can cause cancer. Since immunosuppressed transplant patients are highly susceptible to viral infections it certainly seems possible that transplant patients would be especially prone to viral oncogenesis. Oncogenic viruses have been thought to play an important etiological role in the development of lymphomas, cancers of the skin, and other types of malignancies encountered in transplant patients (10).
ACKNOWLEDGEMENTS

We thank Professor Israel Penn for providing us with data from the Cincinnati (formerly Denver) Transplant Tumor Registry. We also thank the renal transplant surgeons at Groote Schuur Hospital for permission to include their data.
REFERENCES


15. PENN, I. Lymphomas complicating organ transplantation. Transplant Proc, in press.


29. SCHWARTZ, R.S., BELDOTTI, L. Malignant lymphomas following allogenic disease: transition from an immunological to a neoplastic disorder. Science 149, 1511-1514, 1965.


TABLES

LEGENDS

Table 1. Malignant tumors occurring after transplantation at Groote Schuur Hospital, Cape Town.

Table 2. Incidence of malignant tumors in transplant patients worldwide and at Groote Schuur Hospital, Cape Town.
Comparison of patients with ischemic, myopathic, and rheumatic heart diseases as cardiac transplant recipients

Fifty-four human-to-human cardiac transplants (10 orthotopic and 44 heterotopic) in 50 patients were performed between December, 1967, and December, 1981. The underlying cardiac pathology was ischemic (IHD) in 29, cardiomyopathic (CM) in 17, rheumatic (RHD) in four, and mixed or other pathology in four. Patients with RHD survived for a mean period over three times as long as those with either CM (p < 0.02) or IHD (p < 0.05). Although CM patients were on average over a decade younger than those in other groups, they had a lower survival rate. There was a higher incidence of death from chronic rejection in patients with IHD, in whom there was also a higher incidence of thromboembolic episodes. Major infections were over twice as frequent in IHD patients as in CM patients (p < 0.01). Noncompliance with regard to adherence to instructions and therapy was a significant factor in morbidity and mortality, especially in CM patients. Our data suggest that survival and morbidity of recipients of heart transplants might be influenced to some extent by the nature of the underlying primary cardiac condition, RHD being considered a favorable survival factor when compared with IHD, and CM being particularly unfavorable. (Am Heart J 107:8, 1984.)

Cape Town, South Africa

Since 1967 over 500 cardiac transplants have been performed in patients with various types of terminal heart disease. Patients with ischemic heart disease (IHD) and cardiomyopathy (CM) have most frequently had transplants. Few studies have compared post-transplantation morbidity and mortality in relation to the underlying cardiac pathology. We have analyzed our own series of cardiac transplant recipients to ascertain whether the underlying disease necessitating transplantation influenced the outcome of the procedure.

METHODS

Between December, 1967, and December, 1981, 54 heart transplants (10 orthotopic and 44 heterotopic) in 50 patients were performed at Groote Schuur Hospital.

Heterotopic transplantation. Heterotopic heart transplantation involves the insertion of the donor heart into the right chest in parallel with the recipient heart; anastomoses are carried out between donor and recipient left atria, right atria, aortas, and pulmonary arteries. Blood flow depends on the relative compliance of recipient and donor ventricles. Normally, as the recipient ventricle is diseased, blood flow will be largely through the donor heart. At times of severe acute rejection, however, the recipient heart may receive a greater percentage of the venous return and will temporarily contribute increased assistance to the overall cardiac output.

Types of heart disease. Follow-up has been for a minimum of 5 months to a maximum of 12½ years. Twenty-nine of the transplants were carried out in patients with a clinical diagnosis of IHD, 17 in patients with CM, four with rheumatic heart disease (RHD), two with mixed pathology (RHD and IHD), and two with endomyocardial fibrosis.

Re-transplants. Four retransplant operations were performed in three patients with IHD and one with CM; all received heterotopic grafts at both operations.

Data analysis. The records of all patients in the IHD, CM, and RHD groups have been reviewed in detail. The four patients with either mixed pathology or endomyocardial fibrosis have been excluded from the study. Statistical analysis of the data was performed by means of the chi-square and Student’s t tests, and analysis of covariance where appropriate.
Table 1. Length of survival and causes of death and/or
graft failure in heart transplant recipients in relation to
the underlying primary cardiac pathology

<table>
<thead>
<tr>
<th></th>
<th>IHD</th>
<th>CM</th>
<th>RHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of transplants</td>
<td>29</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>42</td>
<td>30</td>
<td>39</td>
</tr>
<tr>
<td>Cumulative survival (mo)</td>
<td>615</td>
<td>243</td>
<td>247</td>
</tr>
<tr>
<td>Mean survival (mo)</td>
<td>21</td>
<td>14</td>
<td>62</td>
</tr>
</tbody>
</table>
| No. of deaths and/or
  graft failures (% of
deaths in each group) | 87/76 | 12/71 | 3/75 |
| Causes of deaths and/or
  graft failures (% of
deaths in each group) |       |       |       |
| Infection       | 8 (36) | 2 (17) | 2 (67) |
| Acute rejection  | 3 (14) | 3 (25) | 9 (40) |
| Chronic rejection| 9 (41) | 2 (17) | 1 (33) |
| Thromboembolism  | 2 (9)  | 1 (8)  | 0 (0)  |
| Malignancy*     | 0 (0)  | 1 (8)  | 0 (0)  |
| Other†          | 0 (0)  | 3 (25) | 0 (0)  |
| Total           | 22 (100)| 12 (100)| 3 (100)|
| Deaths associated
  with noncompliance (% of
deaths in each group) | 4 (18) | 4 (33) | 0 (0) |

IHD = ischemic heart disease; CM = cardiomyopathy; RHD = rheumatic heart disease.
*Gastric carcinoma.
†Deaths were due to suicide, acute hemorrhagic pancreatitis, and intravenous potassium arrest.

Survival defined. In patients with heterotopic transplants, graft failure from rejection may occur and yet the patient may survive and be retransplanted. If a second suitable donor does not become available, death may occur at some considerable interval of time after graft failure. In this analysis, “survival” was considered to end either with the death of the patient or with cessation of function of the graft from rejection. Survival of a patient with a nonfunctioning graft has been excluded. Retransplantation was considered as the beginning of a new and separate survival period.

RESULTS

In the combined groups several factors were found to correlate with improved survival: (1) recipient age of under 40 years at the time of operation, (2) recipient blood groups A and O (i.e., without B antigen), (3) donor race non-Caucasian, and to a lesser extent (4) history of previous blood transfusion in the recipient, and (5) compatibility of one or two human lymphocyte antigen (HLA) specificities between donor and recipient. These survival factors have been discussed fully elsewhere. Comparison of mean age, mean period of survival, number and causes of deaths occurring in patients from the IHD, CM, and RHD groups are shown in Table 1. CM patients were substantially younger than patients with either IHD or RHD, being on average 12 years younger than the IHD group (p < 0.01).

Survival. The mean period of survival of patients with RHD was significantly longer than that of patients with either CM (p < 0.02) or IHD (p < 0.05). The RHD patients survived, on average, over three times as long as patients from the combined IHD and CM groups (p < 0.01); the cumulative period of survival of the four RHD patients exceeded that of all 17 CM patients. Survival between the IHD and CM groups did not differ significantly.

Causes of death and/or graft failure. Infection and chronic rejection were the leading causes of death in both the IHD and RHD groups, whereas acute rejection and miscellaneous other causes were responsible for the majority in the CM group (Fig. 1). Fatal acute rejection was not seen in the four RHD patients, and the one patient dying of chronic rejection survived 12½ years; however, the overall number in this group is too small to draw any firm conclusions. There were considerably more deaths or graft failures from chronic rejection in the IHD patients (41%) than in CM patients (17%) (Table 1). Fatal infection was also twice as common in the IHD group (36%) as in the CM group (17%). None of these differences reached statistical significance.

Noncompliance with regard to advice and instructions given to the patient and to adherence to regular drug therapy has been a major contributory factor to mortality in both the IHD and, especially, CM groups, contributing to death or graft failure on four occasions in each group; one third of the deaths in the latter group were from this cause. By noncompliance we refer to any act or omission on the part of
the patient from whatever cause, excluding ignorance, which resulted in morbidity and mortality.

Major nonfatal complications. There were 12 nonfatal episodes of infection (2/100 cumulative months of patient survival or 0.41/patient) in the IHD group, which formed 75% of all such episodes (Table II). In contrast, there were only three (1.4/100 patient-months or 0.18/patient) in the CM group (19%), and one (0.40/100 patient-months or 0.25/patient) in RHD patients (6%). All four nonfatal episodes of pneumonia which occurred developed in those patients with IHD.

There was also a higher incidence of nonfatal major systemic and pulmonary emboli in IHD patients (0.82/100 patient-months) compared with CM patients (0.46/100 patient-months) or RHD patients (0.00/100 patient-months). None of these differences reached statistical significance.

One noncompliant patient in the cardiomyopathic group independently discontinued his immunosuppressive treatment; and rejection progressed to the extent that the transplanted heart fibrillated; massive immunosuppressive therapy and electrical defibrillation were successful in correcting the situation.

Factors influencing survival. A comparison of the favorable factors, outlined previously, in each group is shown in Table III. The majority of CM patients were under 40 years of age, and all had blood groups not containing B antigen. The IHD patients had better donor-recipient HLA compatibility, received slightly more non-Caucasian donor hearts, and had considerably more pretransplant blood transfusions. All RHD patients had positive histories of transfusion, all received non-Caucasian donor hearts, and all (one not known) matched at one or more HLA antigen specificities. The RHD patients would, therefore, appear to have rather more of the favorable factors.

An attempt has been made to assess the importance of each of the favorable factors influencing survival in relation to the underlying cardiac pathology and also to assess whether the underlying condition influences survival of the cardiac transplant recipient, per se, or whether the improved survival in patients with RHD is related solely to the higher incidence of other favorable factors in this group (Table IV). CM patients were on average over a decade younger than those in other groups, which should influence their survival favorably. However, if only patients under the age of 40 years are considered, the IHD patients survived over twice as long as CM patients (35 vs 15 months) and the RHD patients over five times as long (79 months). All of the patients in the CM group had the favorable blood groups A and O. When only those IHD patients with blood groups A or O were compared, the IHD patients survived nearly twice as long as the CM patients (24 vs 13 months) and the RHD patients over six times as long (80 months) (p < 0.02). Similarly, after correction for the other three favorable factors, there was improved survival in the RHD patients, with CM patients comparing very unfavorably. With the exception of survival related to favorable blood grouping, however, none of these differences quite reached statistical significance. An analysis of covariance, incorporating all five favorable survival factors, showed that the

Table II. Major nonfatal complications in heart transplant recipients in relation to underlying cardiac pathology

<table>
<thead>
<tr>
<th>Nonfatal complications</th>
<th>IHD</th>
<th>CM</th>
<th>RHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence/100 patient-months</td>
<td>Incidence/patient</td>
<td>Incidence/100 patient-months</td>
<td>Incidence/patient</td>
</tr>
<tr>
<td>Infectious episodes</td>
<td>12</td>
<td>2.00</td>
<td>0.41</td>
</tr>
<tr>
<td>Thromboembolic episodes</td>
<td>5</td>
<td>0.81</td>
<td>0.17</td>
</tr>
<tr>
<td>Malignancy*</td>
<td>1</td>
<td>0.16</td>
<td>0.05</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>3.00</td>
<td>0.61</td>
</tr>
<tr>
<td>Major complications associated with noncompliance (‘+’ of nonfatal complications in each group):</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Cerebellar meningioma treated with radiotherapy.
observed differences in survival between the IHD, CM, and RHD groups could, in fact, be ascribed to differences in clinical classification of disease, \( p < 0.05 \) for the RHD and IHD groups and \( p < 0.02 \) for the RHD and CM groups. There was no statistically detectable difference, however, between the IHD and CM groups.

**DISCUSSION**

**Underlying RHD as a favorable survival factor.** The statistically longer survival in patients with RHD may be related to an immunologic effect of RHD, as it does not appear to be related to a better combination of other survival factors in the patients in this series. One of these patients was, at 12 years, the longest surviving orthotopic cardiac transplant recipient in our series. Another, at over 7 years, is the longest surviving heterotopic recipient. Although there was a trend for IHD patients to survive longer than those with CM, this did not reach statistical significance. When correction was made for the individual survival factors, however, the difference in survival was nearly always twofold, the IHD group surviving longer, although again statistical significance was not achieved. This is of particular interest considering the CM patients were on average over a decade younger and on this basis would have been expected to fare better.

**Relationship of IHD and chronic rejection.** Of the 12 deaths due to chronic rejection, nine were in IHD patients. Histologically, the donor hearts of these patients were marked by occlusive intimal proliferation in the large and medium-sized coronary arteries bearing a close resemblance to atherosclerosis. Although the lesions have been thought to be the result of immunologic damage followed by reparative myointimal proliferation, Thomson and others attribute the lipid deposition in the thickened intima to a combination of immune-related damage and coexistent hypercholesterolemia. This latter conclusion is consistent with the finding of a higher incidence of hyperlipidemia in those of our patients who died or underwent graft failure from chronic rejection when compared with the other patients in this series. (0.21 vs 0.05).

**Noncompliance in CM patients.** The relatively high incidence of noncompliance was a major factor in mortality or graft failure in CM patients and may be related to their younger mean age; patients under 40 were found to have significantly more fatal compli-
cations related to noncompliance \( (p < 0.05) \). A difference in personality between patients with IHD and CM has also been observed and may be a factor.

**Influence of age on survival.** That the IHD patients were older may account, in part, for the significantly higher incidence of infection in this group, in which there were 20 fatal and nonfatal infectious episodes in contrast to only five in the CM patients \( (p < 0.01) \). There were significantly more deaths from infection over the age of 35 years in the overall series \( (p < 0.05) \), although this was not significant in any of the groups individually. Nine of all 11 fatal and nonfatal episodes of pneumonia occurred in patients in the IHD group; all developed in patients who were 35 years of age or older at the time of infection. These data correlate with those of Hassel et al. who found that in CM transplant patients there was a statistically lower incidence of infection in those under the age of 40 years. They also found fewer deaths from rejection in the younger group but no overall difference in survival rate.

**Risk of thromboembolism after cardiac transplantation.** One of the theoretic risks of heterotopic heart transplantation is the possibility of emboli arising from thrombus forming in the poorly contracting recipient ventricles. For this reason long-term anticoagulation is maintained in these patients. Nine major fatal and nonfatal systemic and pulmonary thromboembolic episodes occurred in five patients in this series, four of whom had undergone heterotopic and one orthotopic transplantation. Four episodes occurred in two noncomplaint patients, both with heterotopic grafts for CM, who failed to take anticoagulants regularly. If these patients are excluded, the remaining five episodes all occurred in three patients with IHD (two heterotopic and one orthotopic). The possibility arises, therefore, that such thromboembolic episodes may be related, at least in some cases, to the nature of the primary disease or possibly to the older ages of these patients, and not only to the retention of the recipient heart.

Conclusions. Our observations suggest that survival and morbidity of recipients of heart transplants might be influenced to some extent by the nature of the underlying primary cardiac condition, RHD being considered a favorable survival factor when compared with IHD, and CM being particularly unfavorable.

The authors thank the many members of the medical, nursing, and paramedical staff of Groote Schuur Hospital and the University of Cape Town Medical School who have contributed toward the care of these patients.

**REFERENCES**

NON-TRANSPLANT OPERATIVE PROCEDURES IN PATIENTS WITH HEART TRANSPLANTS: ANAESTHETIC AND SURGICAL CONSIDERATIONS.

BY

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INTRODUCTION

Cardiac transplantation is a recognised therapy for end-stage cardiac disease in selected patients for whom no other therapy is available (1). The number of patients undergoing heart transplantation each year worldwide is steadily increasing, and, with the improved results which have followed the introduction of cyclosporin A, this trend is likely to continue (2). Some of the successfully transplanted patients will develop other (non-cardiac) conditions which require surgical intervention. Increasingly, therefore, anaesthetists and surgeons may find themselves faced with the possibility of treating patients with well functioning heart transplants.

Such patients clearly present special management problems, which include, in particular, atypical responses to both stress and certain pharmacological agents, as the transplanted heart remains denervated, and increased susceptibility to infection.

We have reviewed our own patients with functioning cardiac transplants who have subsequently undergone other surgical procedures.

REVIEW OF CASES

Between December 1967 and December 1984, 73 human-to-human cardiac transplants were performed on 64 patients at our institution. Eight patients underwent second cardiac transplant procedures (3), and one of these subsequently received a third transplant. Fifteen of the 64 patients have undergone 39 surgical operations (excluding the retransplant procedures (3)) since the time of their original heart transplant (Table 1).
Two of these 15 patients also underwent further cardiac transplants (patients 10 and 11); one of these patients has undergone no fewer than 9 non-cardiac operations as well as the retransplantation procedure (patient 10).

The ages of the 15 patients at the time of their original heart transplant operations, performed between 1968 and 1984, ranged from 14 to 52 years with a mean of 38 years (Table 1). All but two (patients 2 and 15) were men. The underlying cardiac pathology was ischaemic in 7, myopathic in 5, rheumatic in 2, and endomyocardial fibrosis in one. Three of the patients had orthotopic transplants (patients 1, 2 and 15), the remainder heterotopic, where the donor heart is placed in parallel with the recipient heart and lies in the right pleural cavity (1). One of the retransplant procedures was orthotopic in the presence of an existing heterotopic allograft (3).

The non-transplant operations were performed between 2 weeks and almost 10 years after cardiac transplantation, though 15 were performed within the first year. Thirty four required a general anaesthetic; 5 were performed under local anaesthesia. The reasons for operation are listed in Table 1, as are the operative procedures and post-operative complications. Fourteen (36%) were for infective conditions, though this large proportion is influenced by the 2 patients who developed serious sternal wound infections after transplantation and who each required three operative procedures to eradicate this sepsis. Gastrointestinal procedures accounted for 9 operations (23%), vascular procedures (including amputation) were performed in 9 (23%), and 5 (13%) procedures were diagnostic, in 4 cases to identify tumours.
All except two of the operations were performed in patients immuno-suppressed at that time with a combination of azathioprine and methylprednisolone; the remaining two patients (patients 11 and 15) were receiving a combination of cyclosporin A, azathioprine and methylprednisolone.

Of the more major operations, a few are worthy of comment. Patient 1 underwent subtotal gastrectomy for what was believed to be a benign gastric ulcer, though histological examination later revealed it to be malignant. At the same procedure, a chronic dissection of the descending aorta was replaced by an aortic bifurcation graft. The patient rapidly developed widespread metastases and died within 3 months.

Patient 3 - only the second patient to undergo heterotopic heart transplantation at our institution - developed a *Staphylococcus aureus* endocarditis and thrombus sited on an indwelling aortic prosthetic valve in his own heart. At operation, the prosthesis was excised along with the free wall of the recipient left ventricle; the anterior leaflet of the recipient mitral valve was used to close off the root of the aorta, and the mitral valve orifice was itself closed by direct suture (4). The patient remains alive and well almost 9 years later.

The complications both of long-term immunosuppressive therapy and of the underlying pathological condition for which transplantation was performed, are illustrated by patient 10. Heart transplantation was performed for ischaemic disease; atheromatous disease of peripheral vessels has progressed and he has required 3 operations including right leg amputation. He awaits operation for the removal of corticosteroid-induced cataracts.
ANAESTHETIC CONSIDERATIONS

Standard general anaesthetic techniques were used in these patients. Induction was usually with intravenous thiopentone sodium, and maintenance of anaesthesia by inhalational agents such as nitrous oxide and halothane, with care being taken to maintain good oxygenation; narcotic agents such as morphine were used as necessary. Muscle relaxation, where necessary, was with pancuronium. The most common method of anaesthesia was the use of thiopentone sodium, nitrous oxide, morphine, and pancuronium.

When the surgical procedure was major, arterial and central venous pressures and electrocardiograms were monitored throughout the operation. No episode of intra-operative hypotension or arrhythmias occurred except in the patient with Clostridium welchii septicaemia, who was undergoing leg amputation and who died within 24 hours after operation.

Maintenance azathioprine (or cyclosporin A) and corticosteroid therapy was continued and, in addition, whenever a general anaesthetic was administered, hydrocortisone 100 mg intravenously was given immediately before the induction of anaesthesia, repeated once during the procedure if prolonged, and repeated at increasing intervals of 6, 8 and 12 hours for a maximum period of 48 hours, at which time it was discontinued.

MORTALITY

Nine of the 15 patients have subsequently died between one day and almost 4 years after the non-transplant procedure (Table 1). Only one of these deaths, that at 24 hours after operation (patient 5), was related
to the operative procedure; in this case death was from overwhelming Clostridium welchii septicaemia, for which the patient had undergone amputation of a leg affected by gas gangrene. Two of the other patients died from malignant tumours (patients 1 and 12), of which there is a high incidence in immunosuppressed patients (5,6), but at some time interval following the operative procedure. Five patients died from chronic rejection (accelerated graft arteriosclerosis) (patients 2,6-9); the final patient died from tuberculous meningitis for which he had undergone a lumbo-peritoneal shunt some 4 months previously (patient 13).

NON-FATAL POST-OPERATIVE COMPLICATIONS

Apart from the patient with gas gangrene, there were no per-operative or immediate post-operative complications related to any of these procedures, though one patient developed bronchitis 21 days after undergoing cholecystectomy. This was the only infective complication in this series; this low incidence may be related to the fact that (excluding the 6 operations on the 2 patients with infected sternal wounds) only 9 of the remaining 33 operations were performed during the first post-transplant year, when immunosuppression is at its heaviest (7).

DISCUSSION

This study suggests that surgical procedures in patients with cardiac transplants can be performed without undue anaesthetic or operative risk or complication. Stanford University has reported successful operations for hip replacement (as a complication of corticosteroid therapy) (8) and for abdominal aortic aneurysm (9) in patients with heart transplants; the anaesthetic management of such patients has also been reviewed (10,11).
Conditions for which Operation may be necessary in Patients with Cardiac Transplants (Table 2)

Patients with cardiac transplants may develop unrelated pathology requiring surgical intervention, as may any member of the population. They may also, of course, develop complications of the actual operation (e.g., incisional hernia, wound infection), or of subsequent diagnostic procedures (e.g., right ventricular perforation following endomyocardial biopsy). In addition, however, there are certain specific complications of long-term immunosuppressive therapy which may require surgical procedures. Immunosuppressed patients are more susceptible to infection (7,12) and to de novo malignant tumour formation (5,6); several procedures in the present series were for such complications. The immunosuppressive agents themselves may lead to complications which require surgical treatment (13); in particular, corticosteroid therapy may result in certain musculoskeletal disorders (including osteoporosis, vertebral compression fractures, pathological bone fractures, aseptic necrosis), gastrointestinal disorders (including peptic ulceration and pancreatitis), and ophthalmic disorders (including cataract, increased intraocular pressure, glaucoma, and exophthalmos), all of which may require operative procedures.

One of the disadvantages of heterotopic heart transplantation is that thrombus may form in the poorly functioning recipient's left ventricle, and embolisation may subsequently occur, either directly through the recipient's aortic valve or through the donor heart via an incompetent recipient mitral valve and the communicating left atria. Systemic emboli from left ventricular thrombus may also occur in patients with orthotopic transplants with poor cardiac function either during an acute
rejection episode or when chronic rejection is advanced. In view of
the risk of thromboemboli, all patients with heterotopic heart trans-
plants require long-term anticoagulation with warfarin sodium, and it
is general policy for patients with either type of transplant to receive
long-term anti-platelet agents; these patients may therefore be at a
risk from gastrointestinal haemorrhagic complications, particularly
when they are receiving high doses of corticosteroids as well.

When cardiac transplantation has been performed for ischaemic heart
disease, the atheromatous disease process may progress in peripheral
vessels and lead to ischaemic complications, particularly in the lower
limbs or brain.

Specific Problems of Anaesthesia and Surgical Procedures in Patients
with Cardiac Transplants

The special problems faced in managing patients with cardiac trans-
plants who require operative procedures include (i) the patient's
atypical response to stress and to certain drugs, as the transplanted
heart remains denervated, (ii) an increased susceptibility to infection,
(iii) an increased tendency to cardiac arrhythmias, particularly during
the first 3 months after transplantation or when acute or chronic
rejection is occurring, and (iv) an increased risk of complications re-
lated to drug therapy such as anticoagulation, corticosteroids, and
cyclosporin A.

With regard to drug-related complications, the increased risks of
managing a patient who has been on long-term anticoagulation are
obvious, and the need for increased corticosteroid therapy in patients
receiving these drugs over a long period of time, as their own adrenal
cortical response to stress is suppressed, is also well known. Cyclosporin A may result in impaired renal and/or hepatic function, which
may complicate the peri-operative period, and may also result in sys-
temic hypertension for which the patient may be receiving additional
hypotensive therapy.

The above problems can be overcome by certain prophylactic measures,
and by a full understanding of the physiology and pharmacology of the
denervated heart.

**Prophylactic Measures**

The general status of the patient should be assessed by physical exami-
nation. A full blood count and blood chemistry studies should be carried
out to detect the effects of current immunosuppressive therapy; elective
surgical procedures should be postponed if the white blood count is
particularly low (<2000 cells/cu mm).

Before any major surgical procedure is undertaken, it would seem wise
to check the status of the patient with regard to both acute and chronic
rejection. This may involve clinical examination for features of cardiac
failure or dysrhythmias, electrocardiography, endomyocardial biopsy
(14,15) or other technique such as radionuclide scanning (16) or echo-
cardiography (17) to detect acute rejection and even coronary
angiography or thallium scanning if significant chronic rejection is sus-
ppected (3).

To minimize the risk of post-operative infection, every care should be
taken by both the anaesthetist and surgeon to employ sterile techniques,
particularly with regard to the insertion of vascular monitoring and infusion lines and endotracheal intubation, should these be necessary. The patient should be extubated and all drains, vascular and urinary catheters removed as soon as possible after operation. As pulmonary infection is particularly common in immunosuppressed patients (7), they should receive physiotherapy to the chest until fully mobilized; chest radiographs should be taken frequently during the early post-operative days to monitor pulmonary status. Unless the operative procedure is being undertaken for an infective complication, e.g. the drainage of an abscess, and a specific antibiotic therefore indicated, our own policy has been to prescribe an anti-staphylococcal antibiotic as prophylaxis over the period of the operation; this should be administered initially approximately one hour before the surgical procedure begins, so that high blood levels are present at this time, and discontinued within 24 to 48 hours to minimize the risk of growth of resistant bacterial or, particularly, fungal organisms, which are not uncommon infecting agents in immunosuppressed patients (7). It is gratifying that in the present series only one possible infective complication occurred, and this was mild and easily treated.

As these patients are generally receiving long-term corticosteroid therapy, this should be supplemented to cover the operative procedure (as outlined earlier); it is not necessary to continue this extra therapy longer than 48 hours after operation, unless there is some specific indication.

Patients receiving long-term anticoagulation should have this therapy reduced for the period of operation. Our own policy has been to allow the prothrombin index to rise to 70% by discontinuing warfarin sodium
administration; this drug is begun again 48 hours after operation. Antiplatelet therapy such as dipyridamole or sulphipyrazone should be discontinued for the day of operation, but has not otherwise been found to lead to significant problems. (Cardiac transplant patients are rarely administered acetyl salicylic acid, as this drug increases the risk of gastrointestinal haemorrhage in patients receiving corticosteroids).

Early post-operative mobilization of the patient to minimize the risk of venous thrombosis and pulmonary emboli is equally important in these patients as in other patients undergoing operation.

**Physiology of the Denervated Heart**

The anaesthetist, in particular, needs to have a clear understanding of the atypical haemodynamic function of the denervated heart and of its response to surgical stress and to anaesthetic and other pharmacological agents. These have been fully reviewed elsewhere (18).

Cardiac transplantation results in complete denervation of the heart, which is permanent (19), thereby depriving the heart of the important neural regulating mechanisms. Following the abolition of the usually dominant inhibitory influences (by denervation), the resting rate of the transplanted heart may be higher than normal (19,20), (though not always), and does not reflexly alter in response to the Valsalva manoeuvre, carotid sinus massage, alterations in body position, or drugs such as phenylephrine, atropine, or amyl nitrate (19). Resting cardiac dynamics are essentially normal (21,22), though the cardiac index is in the low normal range.

During dynamic exercise, heart rate rises more gradually than in the
normal subject (19), due to a rise in the levels of circulating catecholamines. Cardiac output is increased initially mainly by the Frank-Starling mechanism (21,22,24), rather than by cardioacceleration (as in the normal subject), and even at peak exercise may remain lower than that of normally innervated control hearts (20,22). It has been suggested that this suboptimal cardiac function may be the result of sub-clinical rejection (26), as dogs with autotransplants are able to perform normally (27).

To avoid hypotension and maintain cardiac output during stress, therefore, an adequate preload must be available, in addition to the means to increase heart rate rapidly and also enhance contractile force, namely by inotropic agents.

**Arrhythmias**

Denervation does not protect the transplanted heart from arrhythmias. In one study, atrial arrhythmias occurred in 72% of patients and ventricular arrhythmias in 57% (28). Their incidence, however, decreases in long-term cardiac transplant survivors, both at rest and during exercise. Electrical conduction within the heart may be delayed, but the clinical significance of such findings is uncertain (29). Arrhythmias may be related to periods of acute rejection, and respond to increased immunosuppressive therapy. The increased sensitivity of the transplanted heart to catecholamines, and the lack of suppressant vagal tone, may be significant factors in the pathogenesis of those arrhythmias which are not related to acute rejection. Quinidine gluconate has been used successfully as an anti-arrhythmic agent in the transplanted heart, suggesting that innervation is not required for a cardiac response to such therapy (28). Most anti-arrhythmic agents are negatively inotropic
and should therefore be used with due caution.

**Pharmacology of the Denervated Heart**

Data concerning the pharmacology of the transplanted heart are limited. A number of general principles, however, may be applied.

Firstly, drugs whose action on the heart is mediated by the autonomic nervous system (sympathetic and parasympathetic) will have no effect on the transplanted heart. As heart rate does not change following atropine administration, it would be expected also that no change would be seen with pancuronium, gallamine, neostigmine, or pyridostigmine. The effects of digoxin on sinus and atrioventricular node function and conduction are also largely mediated via the autonomic system, and are therefore diminished in the denervated heart (30).

Secondly, denervation of the heart may result in an enhanced inotropic and chronotropic response to certain drugs (e.g. beta receptor stimulants) (25). This sensitivity appears to be mediated by a change in the state of the beta receptors.

Thirdly, drugs which are negatively inotropic or which have an effect on peripheral vasculature may have a profound effect on cardiac performance, as the transplanted heart relies mainly on the Frank-Starling mechanism and on changes in contractility for its stress response. Beta blockers, in particular, by inhibiting one of the major compensatory mechanisms of the denervated heart, may have a marked effect in stress situations (23). Calcium antagonists all have direct binding sites on the myocardium, and therefore have an effect on the denervated heart. They are all negatively inotropic, and have an effect on
the peripheral vasculature; they may therefore produce exaggerated
haemodynamic changes in transplant recipients.
ACKNOWLEDGEMENTS

We wish to thank the many members of the medical, nursing and paramedical staff of Groote Schuur Hospital and the University of Cape Town Medical School who have contributed to the care of the patients reviewed in this series. We also thank the Chief Medical Superintendent, Johannesburg Hospital for permission to include data regarding one operation performed at that centre.
REFERENCES


11. KANTER, S.F., SAMUELS, S.I. Anesthesia for major operations on patients who have transplanted hearts, a review of 29 cases. *Anesthesiology*. 46, 65, 1977.


Table 1. Details of 15 patients with cardiac transplants who underwent subsequent non-transplant surgical procedures (O = orthotopic heart transplant; H = heterotopic heart transplant).

Table 2. Potential factors leading to the need for non-transplant operative procedures in patients with cardiac transplants.
<table>
<thead>
<tr>
<th>PATIENT</th>
<th>YEAR OF TRANSPLANT</th>
<th>AGE AT TRANSPLANT</th>
<th>TYPE OF TRANSPLANT</th>
<th>PERIOD SINCE ORIGINAL TRANSPLANT</th>
<th>DIAGNOSIS</th>
<th>OPERATION</th>
<th>OUTCOME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(PJS)</td>
<td>1968</td>
<td>52</td>
<td>O</td>
<td>1y 6m</td>
<td>Gastric carcinoma and abdominal aortic aneurysm</td>
<td>Subtotal gastrectomy, aneurysmectomy and aortic bifurcation graft (at same operation)</td>
<td>Died 2y 3m later from carcinomatosis</td>
</tr>
<tr>
<td>2(DF)</td>
<td>1969</td>
<td>38</td>
<td>O</td>
<td>9y</td>
<td>Perforated duodenal ulcer</td>
<td>Vagotomy and pyloroplasty</td>
<td>No recurrence of ulcer. Died 3 years later from chronic rejection</td>
</tr>
<tr>
<td>3(LG)</td>
<td>1974</td>
<td>47</td>
<td>H</td>
<td>1y 6m</td>
<td>Bacterial (staph aureus) endocarditis and thrombosis of prosthetic valve in recipient heart. Perianal abscess</td>
<td>Removal of prosthesis and recipient left ventricle.</td>
<td>No recurrence or septicaemia.</td>
</tr>
<tr>
<td>4(WF)</td>
<td>1975</td>
<td>32</td>
<td>H</td>
<td>&lt;$3m</td>
<td>Post-transplant mediastinitis and sternal osteomyelitis Cerebellar microglioma</td>
<td>3 operations to debride and drain mediastinal infection 2 diagnostic biopsies and one partial excision</td>
<td>Resolved; no further recurrence</td>
</tr>
<tr>
<td>5(HG)</td>
<td>1976</td>
<td>46</td>
<td>H</td>
<td>9m</td>
<td>Gas gangrene of right leg (clostridium welchii)</td>
<td>Amputation of right leg</td>
<td>Died 24 hours after operation from clostridium welchii septicaemia</td>
</tr>
<tr>
<td>6(SK)</td>
<td>1977</td>
<td>35</td>
<td>H</td>
<td>1y</td>
<td>Right femoral artery thromboembolus</td>
<td>Thromboembolectomy</td>
<td>No further thrombi. Died 1y 2m later from chronic rejection</td>
</tr>
<tr>
<td>7(LL)</td>
<td>1977</td>
<td>42</td>
<td>H</td>
<td>&lt;$3m</td>
<td>Post-transplant mediastinitis and sternal osteomyelitis</td>
<td>3 operations to debride and drain mediastinal infection</td>
<td>Resolved; no further recurrence. Died at 6y 2m from chronic rejection</td>
</tr>
</tbody>
</table>

Cont/....
<table>
<thead>
<tr>
<th>PATIENT</th>
<th>YEAR OF TRANSPLANT</th>
<th>AGE AT TRANSPLANT</th>
<th>TYPE OF TRANSPLANT</th>
<th>PERIOD SINCE ORIGINAL TRANSPLANT</th>
<th>DIAGNOSIS</th>
<th>OPERATION</th>
<th>OUTCOME</th>
</tr>
</thead>
<tbody>
<tr>
<td>8(MV)</td>
<td>1977</td>
<td>42</td>
<td>H</td>
<td>1y 5m</td>
<td>Cholelithiasis</td>
<td>Cholecystectomy</td>
<td>No further related problems but died 1y 4m after final operation from chronic rejection.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1y 8m</td>
<td>Right femoral artery embolus</td>
<td>Embolectomy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2y 4m</td>
<td>Right femoral artery thrombi with atheromatous narrowing</td>
<td>Thrombectomy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2y 5m</td>
<td></td>
<td>Thrombectomy and femoral artery bypass graft.</td>
<td></td>
</tr>
<tr>
<td>9(AA)</td>
<td>1979</td>
<td>45</td>
<td>H</td>
<td>4y 6m</td>
<td>Cholelithiasis</td>
<td>Cholecystectomy*</td>
<td>No further related problems, but died 1y 2m later from chronic rejection.</td>
</tr>
<tr>
<td>10(SVD)</td>
<td>1979 (RT 1981)</td>
<td>34</td>
<td>H</td>
<td>4m</td>
<td>Perianal fistula</td>
<td>Fistulectomy</td>
<td>Healed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36</td>
<td>H</td>
<td>10m</td>
<td>Abscess of back</td>
<td>Surgical drainage</td>
<td>Healed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2y 8m</td>
<td>Incisional epigastric hernia</td>
<td>Repaired</td>
<td>No recurrence</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3y 5m</td>
<td>Abscess of elbow</td>
<td>Surgical drainage</td>
<td>Healed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4y 3m</td>
<td>Perianal fistula</td>
<td>Fistulectomy</td>
<td>Healed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4y 4m</td>
<td>Rt femoral artery atheroma</td>
<td>Femoro-popliteal bypass graft</td>
<td>Healed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4y 7m</td>
<td>Ischaemic right leg</td>
<td>Amputation right leg</td>
<td>Still troubled by ischaemic disease of the left leg. Otherwise well 8m later.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4y 8m</td>
<td>Ischaemic left foot</td>
<td>Amputation toe of left foot.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5y</td>
<td>Cholecystitis</td>
<td>Cholecystectomy</td>
<td></td>
</tr>
<tr>
<td>11(PT)</td>
<td>1979</td>
<td>14</td>
<td>H</td>
<td>3y</td>
<td>Traumatic haematoma right leg</td>
<td>Drained</td>
<td>Bronchitis 21 days later; otherwise uneventful recovery.</td>
</tr>
<tr>
<td></td>
<td>(RT 1983)</td>
<td>18</td>
<td>O</td>
<td>3y 3m</td>
<td>Infected gunshot wound left hand</td>
<td>Drained (LA)</td>
<td>Healed</td>
</tr>
<tr>
<td>12(GW)</td>
<td>1980</td>
<td>51</td>
<td>H</td>
<td>4m</td>
<td>Soft tissue abscesses of legs: Kaposi's sarcoma</td>
<td>Surgical drainage</td>
<td>Died 7m later from disseminated Kaposi's sarcoma.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10m</td>
<td></td>
<td>Biopsy Kaposi's nodule of back (LA)</td>
<td>No recurrence</td>
</tr>
<tr>
<td>PATIENT</td>
<td>YEAR OF TRANSPLANT</td>
<td>AGE AT TRANSPLANT</td>
<td>TYPE OF TRANSPLANT</td>
<td>PERIOD SINCE ORIGINAL TRANSPLANT</td>
<td>DIAGNOSIS</td>
<td>OPERATION</td>
<td>OUTCOME</td>
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</tr>
<tr>
<td>13(RG)</td>
<td>1981</td>
<td>48</td>
<td>H</td>
<td>6m</td>
<td>Tuberculous meningitis</td>
<td>Burrhole+ventricular decompression</td>
<td>Died 4m later from tuberculosis meningitis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tuberculous meningitis</td>
<td>Lumbo-peritoneal shunt</td>
<td></td>
</tr>
<tr>
<td>14(QV)</td>
<td>1983</td>
<td>18</td>
<td>H</td>
<td>1m</td>
<td>Pulmonary tuberculosis</td>
<td>Thoracotomy and lung biopsy</td>
<td>Remains controlled on chemotherapy.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kaposi's sarcoma</td>
<td>inguinal lymph node biopsy (LA)</td>
<td>Regressed with reduced immunosuppression and cyclophosphamide therapy.</td>
</tr>
<tr>
<td>5(HVDM)</td>
<td>1984</td>
<td>20</td>
<td>O</td>
<td>2m</td>
<td>Perforated right ventricle at endomyocardial biopsy, producing cardiac tamponade</td>
<td>Emergency median sternotomy, evacuation of clot, and suture of right ventricular perforation.</td>
<td>Recovered without complication</td>
</tr>
</tbody>
</table>

* Operation performed at Johannesburg Hospital
<table>
<thead>
<tr>
<th>CAUSATIVE FACTORS LEADING TO OPERATION</th>
<th>RESULTING CONDITIONS REQUIRING OPERATION IN THE PRESENT STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. General - unrelated to the cardiac transplant procedure</td>
<td>1. Carcinoma of stomach (not related to long-term immunosuppressive therapy (6)).</td>
</tr>
<tr>
<td></td>
<td>Gunshot wound.</td>
</tr>
<tr>
<td></td>
<td>Cholecystectomy. (?)</td>
</tr>
<tr>
<td>2. Complications of the cardiac transplantation operation.</td>
<td>Traumatic haematoma (?)</td>
</tr>
<tr>
<td>3. Complications of subsequent diagnostic procedures.</td>
<td>2. Wound infection (mediastinitis).</td>
</tr>
<tr>
<td>(a) Infection</td>
<td></td>
</tr>
<tr>
<td>(b) De novo malignant tumour formation.</td>
<td></td>
</tr>
<tr>
<td>(c) Corticosteroid-induced (skeletal, gastro-intestinal, ophthalmic, etc.)</td>
<td>4. (a) Wound infection (mediastinitis), leg abscesses, perianal abscess, gas gangrene of leg, tuberculous meningitis, pulmonary tuberculosis.</td>
</tr>
<tr>
<td></td>
<td>(b) Cerebellar microglioma, Kaposi's sarcoma.</td>
</tr>
<tr>
<td>5. Thromboembolism</td>
<td>(c) Duodenal ulcer</td>
</tr>
<tr>
<td>6. Complications of long-term anticoagulation</td>
<td>5. Thromboembolectomy</td>
</tr>
<tr>
<td></td>
<td>6. Traumatic haematoma (?)</td>
</tr>
<tr>
<td>7. Progression of underlying pathology (atheroma)</td>
<td>7. Dissecting abdominal aortic aneurysm</td>
</tr>
<tr>
<td></td>
<td>Peripheral vascular disease</td>
</tr>
<tr>
<td></td>
<td>Cholecystectomy (?) (hyperlipidaemia)</td>
</tr>
</tbody>
</table>
Transplantation of the Heart and Both Lungs

INTRODUCTION

Transplantation of the heart and both lungs as a form of therapy is clearly still in its infancy. Most groups which have performed the operation have not yet overcome the considerable technical difficulties involved. For the foreseeable future, the number of patients submitted to this procedure will remain small, largely as a result of the strict selection requirements for potential donors. In recent years, however, an improved understanding of the blood supply of the trachea and bronchi has resulted in a better prospect of satisfactory healing at the site of the airway anastomosis. The introduction of cyclosporin A has allowed control of the acute rejection process without the need for corticosteroid therapy during the first 2–3 weeks. These recent advances, largely the work of the Stanford group, will almost certainly lead to an improved outlook for selected patients with pulmonary vascular disease, various advanced pulmonary diseases, and complex developmental abnormalities of the heart and lungs.

EXPERIMENTAL BACKGROUND

The early experimental work in this field has been reviewed fully elsewhere. Although attempts to transplant the heart and lungs into the neck were made as early as 1907, and into the abdomen in 1951, it was Demikhov who made the first attempt to carry out orthotopic transplantation of these organs in the late 1940s and early 1950s. Demikhov achieved survival for up to 6 days, death resulting mainly from thrombosis at the sites of blood vessel anastomosis, or from bronchopneumonia in the lower lobes.

With the advent of supportive techniques, such as hypothermia and cardiopulmonary bypass, further attempts were made in 1953 by Neptune et al. and
in 1957 by Webb and Howard\textsuperscript{7}, though in all cases the bronchi were the site of airway anastomosis. Lower \textit{et al.} (1961)\textsuperscript{8} and Longmore and his colleagues (1968)\textsuperscript{9} introduced simplified techniques in which the trachea was the site of anastomosis, which have been the basis for the recent successful clinical attempts of transplantation of the heart and both lungs.

The results in the dog were poor, and there is evidence that this animal requires the presence of afferent pathways from the lungs to maintain spontaneous breathing.\textsuperscript{10} Primates, however, appear to tolerate total denervation of the lungs and do not require the Herring–Breuer reflex as the dog does\textsuperscript{11}: spontaneous respiration, controlled by the midbrain, is preserved.

\section*{EARLY CLINICAL EXPERIENCE}

\textbf{Single lung transplantation}

The results of transplantation of a single lung, with anastomoses of pulmonary artery, left atrial cuff, and bronchus, have been extremely disappointing\textsuperscript{11}. Hardy was the first to carry out this operation clinically in 1963\textsuperscript{12}. By 1980, 38 such transplants had been performed, with a mean survival of only 8.5 days; two patients survived for 6 and 10 months respectively\textsuperscript{13}. Most of the deaths occurred from complications of the bronchial anastomosis, with necrosis of the bronchial stump and dehiscence of the suture line\textsuperscript{13–15}. The second major complication was pneumonia. The differentiation between acute rejection, reimplantation response\textsuperscript{16}, and infection was, and remains, difficult, and may, on occasion, be impossible\textsuperscript{14}.

Strong evidence that a major limiting factor preventing the success of single lung transplantation is an inadequate blood supply to the bronchial stump was put forward by Haglin \textit{et al.}\textsuperscript{17}. Their patient underwent bilateral lung transplantation. A bronchial artery from the donor aorta was preserved for the left lung and reimplanted in the recipient's aorta. Due to technical difficulties, the right bronchus was not revascularized in this way. Eleven days later the patient died from sepsicaemia and pulmonary insufficiency. The postmortem findings showed the right bronchus to have necrotic changes, while the left bronchus was entirely normal. This problem of maintaining an adequate blood supply to the bronchus is minimized when the heart and both lungs are transplanted in a single unit as a block, preserving the carina and both bronchi intact. The blood supply to the airway from small branches of the coronary arteries in the region of the bare area of the heart is retained\textsuperscript{18}.

A second major advantage of this technique is that acute rejection of the lung and myocardium frequently occur together\textsuperscript{19}, and therefore pulmonary rejection can be strongly suspected by the findings of endomyocardial biopsy\textsuperscript{20}. In all the earlier patients undergoing single lung transplantation conventional immunosuppression was used. The introduction of cyclosporin A and a...
concomitant reduction in the use of corticosteroids has improved the chances of bronchial healing. In single lung transplantation, however, bronchial ischaemia remains a major factor in the failure of the operation.

Transplantation of the heart and both lungs

This operation was first performed clinically by Cooley on 31 August 1968. The patient was a 2-month-old infant with a complete atrioventricular canal defect, pulmonary hypertension and pneumonia. The patient required re-opening for bleeding and died 14 hours after the initial transplant operation. In December 1969, Lillehei performed the second such operation on a 43-year-old patient with emphysema and pulmonary hypertension; the patient survived 8 days, dying from pneumonia.

The third operation was performed at our own institution in Cape Town in July 1971. Details of this patient have been reported elsewhere and only a brief summary will be presented here. The patient was a 49-year-old man with chronic respiratory failure from longstanding airways disease and bronchiectasis. By 1970 he had become bedridden, requiring oxygen therapy, and anti-failure therapy for cor pulmonale. The donor heart was intermittently perfused with blood from the recipient’s cardiopulmonary bypass machine throughout the surgical procedure (Figure 21.1). Anastomoses were performed, in turn, between the donor and recipient bronchi, right atria, and aortae. The bronchi, rather than the trachea, were chosen as the site of anastomosis of the air passages, as it was believed at that time that this would preserve both the blood supply to the carina and the cough reflex at the carinal area more satisfactorily.

The patient recovered satisfactorily from the operation and was extubated within 20 hours. Immunosuppression was with azathioprine and corticosteroids. On the 8th postoperative day a right pneumothorax developed and, at right thoracotomy, a bronchopleural fistula on the posterior aspect of the bronchial anastomosis was repaired and covered with an intercostal flap; the air leak ceased. By the 18th day, however, the patient showed features of a tracheobronchitis and the pneumothorax recurred. A second thoracotomy was performed, at which complete necrosis of the mucosa of the donor right bronchus was found, necessitating pneumonectomy. The patient tolerated this procedure moderately well, but developed a septicaemia and died on the 23rd day. Autopsy showed bronchopneumonia of the left lung, pericarditis, mediastinitis and meningitis, but no significant rejection of the transplanted organs. The cause of death was considered to be a klebsiella pneumonia and septicaemia.

It was not until another 10 years had elapsed that a fourth transplant of heart and lungs was performed, on this occasion at Stanford University. The availability of an improved immunosuppressive regimen, including cyclosporin A, the better understanding of the reimplantation syndrome, the
availability of endomyocardial biopsy to diagnose acute rejection\textsuperscript{20,28}, and an improved understanding of the blood supply of the trachea and bronchi\textsuperscript{29}, resulted in the first long-term survival of such a patient.

INDICATIONS FOR HEART–LUNG TRANSPLANTATION

Though patients with a variety of pulmonary and cardiac disorders might be considered for heart and lung transplantation (Table 21.1), at the present time the operation is being restricted mainly to those with severe irreversible pulmonary vascular disease, either primary idiopathic or secondary to congenital heart disease (Eisenmenger’s syndrome).

Table 21.1 Major indications for transplantation of the heart and both lungs

<table>
<thead>
<tr>
<th>Cardiac:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiogenic pulmonary hypertension:</td>
</tr>
<tr>
<td>(a) Congenital – Eisenmenger’s syndrome</td>
</tr>
<tr>
<td>(b) Acquired – high fixed pulmonary vascular resistance with right ventricular failure</td>
</tr>
<tr>
<td>Pulmonary:</td>
</tr>
<tr>
<td>Primary (idiopathic) pulmonary hypertension</td>
</tr>
<tr>
<td>End-stage chronic obstructive airways disease (emphysema)</td>
</tr>
<tr>
<td>Interstitial pulmonary fibrosis (fibrosing alveolitis)</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>Toxic pneumonitis (e.g. Paraquat poisoning)</td>
</tr>
</tbody>
</table>

SELECTION OF THE RECIPIENT

The potential recipient must clearly have a severely restricted exercise tolerance and a life expectancy of less than 1 year\textsuperscript{28}. He or she must otherwise fulfil the requirements for any patient being considered for cardiac transplantation, with the exception that a recent pulmonary infarction is not a contraindication for heart–lung transplantation. Patients requiring this operation are frequently within their third or fourth decades of life. They often have marked nutritional derangement, resulting from longstanding cardiac failure and hypoxaemia, and systemic hypoxaemia with an elevated haemoglobin and haematocrit. The onset of syncopal episodes or oxygen dependence indicate an extremely poor prognosis.

SELECTION OF THE DONOR

Selection criteria of the donor for heart and lung transplantation are much more strict than for heart transplantation alone. Probably only 25\% of brain-dead donors can be used for donation of the heart and lungs\textsuperscript{25}, the remainder
being excluded largely because of pulmonary problems. Potential donors have frequently undergone prolonged ventilation, and may have developed atelectasis or pulmonary infection. It is essential to exclude respiratory infection in such potential donors. The chest radiograph should be clear, with no evidence of pulmonary contusion or infection. Bronchial lavage is performed and microscopic examination carried out to exclude the presence of micro-organisms and pus cells; the presence of epithelial cells is considered normal.

The potential lungs should show good function. Simple tests, such as estimating the arterial blood gases with the donor ventilated temporarily on 100% oxygen, are carried out: the arterial $P_{O_2}$ should rise above 350 mmHg under such conditions. Lung compliance should be shown to be normal for a normal tidal volume.

There must also be a close anatomical match between the sizes of the chest cavities of the potential recipient and donor. An excessively large lung placed

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**Figure 21.1** The donor organs as used in the Cape Town case (1971). Note transection of both bronchi. The donor aorta was perfused from the aortic line of the recipient cardiopulmonary bypass machine via a perfusion cannula inserted into the donor ascending aorta. RA = right atrium; RV = right ventricle; PA = pulmonary artery; PV = pulmonary vein; LA = left atrium; AO = aorta
in a small pleural cavity will be compressed, resulting in atelectasis and arteriovenous shunting; there would be a high risk of infection in such atelectatic regions in an immunocompromised patient.

DONOR LUNG PRESERVATION

Methods of successfully preserving viable lung tissue during periods of ischaemia are still in their infancy. Experimental work has to date been disappointing. Flushing with a modified Collins' solution before storage by immersion in 4°C ice slush has not been shown to preserve lung integrity and function satisfactorily even after such a short period as 3 hours. Maintaining lung inflation, flushing with and subsequently storing at 4°C in modified Sacks' solution has resulted in adequate short-term function of single canine lungs after periods of ischaemic storage ranging from 7 to 21 hours. Flush cooling with cold blood followed by hypothermic storage has resulted in adequate storage for approximately 20 hours; these lungs showed minimal injury on light and electron microscopy, with normal ventilatory parameters and blood oxygenation during the first 24 hours after transplantation.

SURGICAL TECHNIQUE OF TRANSPLANTATION OF THE HEART AND BOTH LUNGS

As the preservation of lung tissue is unsatisfactory at the present time, the donor and recipient operations are performed simultaneously in adjacent theatres. Our own personal experience of this procedure is based on extensive experimental work in baboons (Novitzky et al., unpublished).

Anatomical considerations

The blood supply of the trachea is shared with that of the oesophagus and adjacent mediastinal tissues. It is derived from three major sources, between which there are poor collateral circulations. As a result, dissection of only 1-2 cm of trachea circumferentially may lead to ischaemic necrosis.

The upper portion of the trachea is supplied predominantly by branches of the inferior thyroid artery (Figure 21.2). The mid-portion and carina derive blood directly from the bronchial arteries, which originate from the aorta. The bronchial collateral circulation around the hilum of the lung is fairly good, and permits segmental resection of the bronchus. The carina and both bronchi also receive small but important arteries which arise from the major coronary arteries, predominantly the left; small vessels penetrate the mediastinum in the region of the 'bare' area of the posterior aspect of the left atrium. (These arteries can be an important source of collateral blood supply to the
heart when both main coronary arteries are occluded at their origin\textsuperscript{16,33,34,}.

In their normal state the diameter of these vessels varies between 80 and 100 µm. Though in the normal subject they play but a small role in the blood supply of the trachea, once the donor trachea has been transected for heart and lung transplantation they become essential. They are crucial for adequate healing at the tracheal suture line. The Stanford team has shown by angiography that after transplantation these arteries dilate and form a collateral anastomotic network with branches from the descending bronchial arteries of the recipient\textsuperscript{26}.

In patients with severe pulmonary hypertension, a further possible source of blood supply to the bronchus is by retrograde flow from the pulmonary artery, via a collateral circulation which develops with the bronchial arteries. After transplantation, however, this pulmonary hypertension no longer exists and any retrograde flow from the pulmonary artery will be negligible. The

![Figure 21.2](image-url)  
**Figure 21.2** Posterior view of heart and trachea, showing blood supply to the trachea, carina and bronchi. \( \text{LV} = \) left ventricle
absence of this retrograde supply may possibly contribute to a relative ischaemia and necrosis of the mucosa of the bronchus if the anastomosis is performed at this level.

![Diagram of blood supply and anastomosis](image)

**Figure 21.3** Donor trachea and bronchi, with transection above the carina, showing disruption of the blood supply to the carina and bronchi from the bronchial arteries, and retention of the blood supply from the coronary arteries.

To preserve the blood supply of the carina and bronchi from the coronary arteries, it is essential to avoid dissection in the areolar fat tissue which is interposed between the donor trachea and left atrium, through which these vessels run. Retention of the carina in the donor heart-lung block would appear to be advantageous as this blood supply from the coronary arteries is therefore retained (Figure 21.3). The Stanford group has not reported significant air leaks or dehiscence at the tracheal anastomosis in its series, suggesting that there has been no significant ischaemia of the trachea following the transplant operation. If the bronchi are used for the sites of anastomosis, as in our own case in 1971 (Figure 21.4), the blood supply of the distal bronchus, derived from both bronchial and coronary arteries, is divided, with a resulting high risk of ischaemic necrosis at the suture line. The high incidence of this complication following single lung transplants is almost certainly for the same reason.
**Donor operation**

Through a midline sternotomy the heart and ascending aorta are exposed. The superior vena cava is doubly ligated and divided proximal to the azygos vein, and the inferior vena cava clamped at the diaphragm and divided cephalad, thus decompressing the right side of the heart. The tip of the left atrial appendage is transected to decompress the left side of the heart. The aorta is then immediately cross-clamped at the level of the brachiocephalic artery, and cardioplegic solution infused into the root of the aorta. Cold saline is applied over the heart. Whilst the cardioplegic solution is still being infused, the pleuropericardium is incised on each side from the sternum to the hilum of the lung. The phrenic nerve is divided and the hilum dissected free. This dissection is extended posteriorly to mobilize the posterior aspect of the left atrium. The aorta is then transected at the level of the cross-clamp. The trachea is mobilized and transected superior to the carina. No attempt is made to mobilize the carina, thus preserving the blood supply from the coronary arteries. The donor heart and lungs can then be removed as a single block of tissue.

![Diagram of donor trachea and bronchi](image)

**Figure 21.4** Donor trachea and bronchi, with transection at the level of the bronchi, showing disruption of both bronchial and coronary arterial supplies to the distal bronchi.

These organs are placed in a bowl of cold saline, care being taken not to allow saline to run into the bronchial tree. An incision is made in the right atrium, beginning in the posterolateral aspect of the inferior vena caval orifice.
and extended up into the base of the right atrial appendage (as for orthotopic heart transplantation). The trachea is trimmed to within one or two cartilages of the carina. The heart and lungs are then transferred to the adjacent theatre for insertion into the recipient.

**Recipient operation**

*Excision of the recipient organs*

Through a midline sternotomy, cardiopulmonary bypass is initiated through an arterial cannula inserted into the aorta (at the origin of the brachiocephalic artery) and venous cannulae introduced through the lateral wall of the right atrium into both superior and inferior venae cavae. Snare is placed around both cavae, converting cardiopulmonary bypass to total bypass. Systemic cooling to reduce the body temperature to 20 °C is begun.

The aorta is cross-clamped proximal to the arterial cannula, and cardiectomy carried out as for orthotopic heart transplantation. Right and left pleural cavities are then entered through incisions in the pleuroperticardium made parallel and posterior to the phrenic nerves, great care being taken to avoid any damage to these structures (Figure 21.5). Each lung can then be withdrawn through its corresponding pleuroperticardial incision into the pericardial cavity. The right and left pulmonary arteries are divided, a cuff of pulmonary artery tissue being left in situ under the arch of the aorta to prevent

![Figure 21.5 View of recipient pericardial cavity after resection of the recipient heart and lungs, showing the remnants of the right (RA) and left (LA) atria, aorta and pulmonary artery. The incisions in the pleuroperticardium posterior to the right and left phrenic nerves are indicated](image-url)
possible damage to the left recurrent laryngeal nerve. The pulmonary veins are transected close to the posterior wall of the left atrium. Great care is taken to obtain haemostasis in this region as it will be extremely difficult to examine at the end of the operation. The trachea is transected above the carina, a minimum of mobilization having been carried out (Figure 21.3). The two lungs can then be removed from the chest cavity. A further check is made to ensure that adequate haemostasis has been obtained.

The posterior pericardium is left intact where possible to avoid excessive dissection of the posterior mediastinum, and thus further reduce the risk of subsequent haemorrhage. In the dissection and division of the trachea, care must be exerted to prevent damage to the vagus nerve plexus which surrounds the oesophagus, or gastric dilatation and impaired pyloric function may result.

**Implantation of the donor organs**

The donor heart and lungs are then introduced into the pericardial cavity, each lung being passed through the respective opening in the pleuropericardium, posterior to the phrenic nerve (Figure 21.6). The right lung is passed posterior to the remnant of the recipient right atrium. The recipient aorta and trachea are trimmed to the appropriate lengths. The recipient and donor tracheae are anastomosed using a continuous suture of 4/0 polypropylene. The lungs are gently inflated; cold saline is applied around the tracheal suture line to inspect for air leaks. If there is no leak, a few interrupted sutures are placed through the surrounding areolar fat tissue to further cover the anastomosis. The donor and recipient right atria are anastomosed using a continuous suture of 5/0 polypropylene, as in the operation of orthotopic heart transplantation (Figure 21.7). Finally, the two aortae are anastomosed end-to-end, using a continuous suture of 4/0 polypropylene. Myocardial protection is maintained throughout operation by the continuous or intermittent topical application of cold (4°C) saline.

A vent is introduced into the left ventricle, either through the left atrial appendage, which is to be preferred, or through the ventricular apex. The caval snare is released and a needle introduced into the ascending aorta and pulmonary artery to evacuate air. The venous pressure is raised to facilitate the expulsion of air from the heart and lungs. The aortic cross-clamp is removed, thus reintroducing coronary artery perfusion to the donor heart. It is usually necessary to defibrillate the heart. The patient is rewarmed and, if haemodynamically stable, cardiopulmonary bypass is discontinued, and the various cannulae removed (Figure 21.8). Drains are introduced into the pericardial and pleural cavities, and the chest is closed.

In our experience in the experimental animal, the cardiopulmonary bypass time varies between 1 hour 30 minutes and 1 hour 50 minutes, the total ischaemic time of the graft being ± 45 minutes.
IMMEDIATE POSTOPERATIVE CARE AND MAINTENANCE IMMUNOSUPPRESSION

The immediate postoperative care is very similar to that of a patient who has undergone heart transplantation alone. The patient is nursed under isolation conditions, and is initially ventilated with a volume ventilator. The central venous pressure is kept low in an effort to prevent reimplantation response (see below). If the venous pressure should rise, diuresis is encouraged by drug therapy. Chest physiotherapy is extremely important to prevent atelectasis and clear secretions from the tracheobronchial tree. Suction of the tracheobronchial tree is, however, carried out with extreme care to prevent trauma to the tracheal suture line, and also to minimize the risk of introducing infection. In the Stanford experience it is usually possible to extubate the patient within the first 24-48 hours. Chest radiographs are carried out twice daily during the

![Donor heart and lungs](image)

**Figure 21.6** Insertion of donor heart and lungs into the recipient thorax. The remnants of the recipient right (RA) and left (LA) atria can be seen. The arrows indicate the route of insertion of the lungs into their respective pleural cavities. Note that both lungs pass posterior to the phrenic nerves and that, in addition, the right lung passes posterior to the recipient right atrium.
first few days to detect any possible pneumothorax resulting from a tracheal leak. If no pneumothorax (or haemothorax) is present, it is usually possible to remove the pleural and pericardial drains on the second postoperative day, after which time the patient is actively mobilized.

A major potential complication of the operation is a failure of the tracheal anastomosis to heal. It is therefore important not to depress fibroplastic activity during the first 2-3 weeks. and for this reason, corticosteroids are avoided unless a definite acute rejection episode has been diagnosed. The major components of the immunosuppressive regimen during this early period therefore consist of cyclosporin A and azathioprine. Methylprednisolone is administered as a single intravenous dose of 500 mg immediately after operation, followed by three further doses of 125 mg during the next 24 h period.25 No further corticosteroids are administered during the following 2-3-week period, by which time satisfactory healing of the tracheal suture line should have occurred. To compensate partially for the lack of corticosteroid therapy, antithymocyte globulin may be given during the first 2-3 days.26

Cyclosporin A is begun at 18 mg/kg per day, but reduced with the aim of maintaining a blood trough level of approximately 400 ng/ml, as for patients undergoing heart transplantation alone. Azathioprine dosage is adjusted to
maintain a total white blood cell count of 3–5000 cells/mm$^3$, and is usually of the order of 1.5 mg kg per day. Unless there is evidence suggesting that the tracheal anastomosis has not healed satisfactorily, during the second or third week azathioprine is discontinued and methylprednisolone introduced in a dosage of 0.2–0.3 mg kg per day. If there is doubt with regard to the healing of the tracheal suture line, it is occasionally necessary to inspect this area by flexible bronchoscopy.

Figure 21.8 The completed operation. The three anastomoses (tracheal, right atrial, and aortic) are shown. The trachea and bronchi, which lie posterior to the other structures and which therefore cannot be seen from the anterior view, have been shaded.

COMPLICATIONS

The complications of this operation clearly include those possible following open heart surgery, together with those relating to transplantation of the heart alone. Delayed or imperfect healing of the tracheal suture line has already been discussed. Any air leak with resultant pneumothorax should be treated with intercostal tube drainage; if this fails to correct the situation, further operation is indicated.

Technical

In the initial Stanford series, three of the first six patients required exploration for postoperative bleeding. Haemorrhage may occur from posterior
mediastinal dissection in the recipient during excision of the heart and lungs, but also from bleeding points on the donor organs which have been implanted. Retention of the posterior wall of the left atrium has reduced the dissection necessary in the recipient.

Other technical complications in this first Stanford series included three patients who suffered injury to nerves in the mediastinum (Figure 21.9). One required a subsequent pyloroplasty to allow satisfactory gastric emptying following injury to the vagus. A second sustained transient paralysis of the left phrenic nerve, and a third sustained loss of function of the left recurrent laryngeal nerve, requiring Teflon injection into the vocal chord. Subsequent modifications to the surgical technique (incorporated in the section on surgical technique above) have reduced the excessive dissection which was originally carried out and which resulted in these nerve injuries. In particular, retention of the pericardium, the posterior wall of the left atrium, and a small cuff of pulmonary artery attached to the arch of aorta, have reduced the possibility of damaging these mediastinal nerves.

Figure 21.9 Drawing to illustrate the proximity of major thoracic nerves to the trachea, bronchi and aorta. The potential sites of damage of these nerves during the operation of transplantation of the heart and lungs are obvious.

**Reimplantation response**

This complication, which is still poorly understood, has occurred in a large number of the lung transplants performed to date, and may occur whether the
transplant involves the heart and both lungs or a single lung. Pulmonary function usually recovers promptly after operation, enabling the patient to be extubated within 24-48 hours. Chest radiographs are normal. During the next few days, however, the radiological appearances change markedly, showing features of a diffuse pulmonary infiltrate. The patient becomes febrile and tachypnoeic, and shows signs of respiratory failure. These are related to a loss of pulmonary compliance, elevation of the pulmonary vascular resistance, a marked fall in arterial $PO_2$ and an elevation of $PCO_2$, and pulmonary 'shunting' with ventilation-perfusion imbalance. This syndrome has occurred most commonly between the 4th postoperative day and the end of the 3rd week, after which time it has not been reported.

This clinical picture is difficult to differentiate from an acute rejection episode or from infection. The Stanford group has shown evidence that acute rejection in the lungs occurs concomitantly with acute rejection of the myocardium, and endomyocardial biopsy may therefore be helpful in differentiating an acute rejection episode from this 'reimplantation oedema'. If the endomyocardial biopsy shows no features of acute rejection, it is unlikely that rejection is occurring in the lungs. Aggressive investigation for a possible pulmonary infection must be carried out; this may involve fiberoptic bronchoscopy. Microscopic examination of fluid collected following bronchial lavage is of little value in differentiating the reimplantation response from acute rejection. It may be helpful, however, in differentiating infection, as micro-organisms may be seen. Microscopic examination of lung affected by this complication shows perivascular cuffs of mononuclear cells, associated with alveolar exudates containing a large quantity of fibrin, pneumocytes and mononuclear cells. If infection is also excluded, then a diagnosis of reimplantation response, as it has become known, can be made with some certainty.

It is likely that many patients with single lung transplants were treated for acute rejection episodes with increased immunosuppression, when in reality they were suffering from this phenomenon of reimplantation oedema. Without the facility for endomyocardial biopsy to distinguish acute rejection in these cases, the diagnosis is particularly difficult. This is clearly one of the great advantages of transplantation of the heart and both lungs over transplantation of a single lung alone.

The aetiology of the reimplantation response is uncertain, but it appears likely that it is related to the ischaemia which the lung has experienced during removal from the donor and reimplantation in the recipient. Though in most reported cases the ischaemic time has ranged between 1 hour 30 minutes and 1 hour 50 minutes, this period is thought to be long enough for the lung to sustain damage; hypothermia alone does not appear to protect the lung completely. Several other factors, however, have been considered as possible aetiological factors in the reimplantation response, though these are probably of less importance than ischaemia. These include the effect of denervation of
the lungs, disruption of the lymphatic circulation, and pulmonary trauma from manipulation during the operative procedure.

To a certain extent this complication can be prevented by maintaining a low venous pressure and inducing a diuresis during the early days following transplantation. Should the complication develop, then diuresis is essential, and oxygen should be administered by mask or nasal cannulae. If the pulmonary compliance continues to deteriorate and the arterial PO$_2$ cannot be maintained above 60 mmHg (8.0 kPa), then continuous positive airway pressure must be introduced. If this should prove inadequate, or if the patient becomes distressed by his respiratory effort, endotracheal intubation and ventilation may be necessary for several days until lung function recovers. Other therapeutic measures which have been found helpful include the infusion of a colloid osmotic agent such as albumin, which helps to draw fluid from the interstitial spaces of the lungs into the circulation.

DIAGNOSIS AND MANAGEMENT OF ACUTE REJECTION

The extreme difficulty of diagnosing acute rejection in the lungs has been discussed above. Fortunately, it would appear that when rejection is occurring in the lungs it is also occurring in the myocardium, and therefore methods of diagnosing acute rejection episodes in the heart can be employed. It is therefore important to perform endomyocardial biopsy at frequent intervals, or to employ other methods of detecting rejection, such as radionuclide scanning (Chapter 11).

Acute rejection of the lungs is associated with an infiltration of mononuclear cells, small lymphocytes, and polymorphonuclear leukocytes; interstitial and intra-alveolar fibrin and erythrocytes may also be present. During infection similar changes can be observed, but significant micro-organisms can also be identified. Bronchial lavage does not usually provide information helpful in making the diagnosis but, on some occasions, can be of value.

The treatment of an acute rejection episode is identical to that occurring in a patient with a heart transplant alone. Intravenous boluses of methylprednisolone are administered, together with antithymocyte globulin in severe cases. If a prolonged course of antithymocyte globulin is required, it is essential to regulate the dosage by monitoring the number of circulating T cells.

Though the Stanford experience is that rejection of the heart and lungs occurs simultaneously, our own studies in the Chacma baboon receiving cyclosporin A have shown that the lung may undergo a severe rejection episode whilst the heart shows minimal changes only. It is difficult to obtain satisfactory immunosuppression in the baboon with cyclosporin A, even when administered intravenously twice daily, and this may be a factor in accounting for this observed difference. Our findings would at least suggest,
however, that in an inadequately immunsuppressed patient, rejection may occur more rapidly in the lung than in the heart.

RESULTS

The Stanford group has the major experience of patients undergoing transplantation of the heart and both lungs. Of 19 such transplants performed since March 1981, actuarial survival has been 71%, 62%, and 62% at 1, 2, and 3 years respectively. The first long-term survivor following this procedure was a 45-year-old woman with primary pulmonary hypertension, operated on at Stanford Medical Center on 9 March 1981. She suffered two documented episodes of acute cardiac allograft rejection at 10 and 25 days respectively, which were reversed using methylprednisolone. Early deaths were from haemorrhage and impaired renal function.

In all, approximately 50 heart and lung transplants have been performed by various groups, including those at Stanford, the University of Pittsburgh, the University of Texas Medical Center at Houston, and a small number of European centres. Early results were disappointing and showed a high mortality, but subsequent results have been encouraging.

References
