Middle Articles

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.

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

FIG. 1.—Carrel and Guthrie's original method of cervical transplantation of an accessory heart (shaded).

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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 or transplantation 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 contractions. 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. Downie (1953) used the technique in an effort to elucidate the nature of the tachycardia found in hepatectomized 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 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.

Reemtsma *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 extra-corporeal 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.

Sinitzyn (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 parabolic 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 surrounding 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 intra-cardiac 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 Sinitsyn 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 Folts, 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 cine-angiography). 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, 1953, 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 system of respiration through the skin. (Sinitsyn (1963) subsequently made a study of transplantation of the turtle heart.)

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.

FIG. 2.—Demikhov's scheme 24 for the intrathoracic transplantation of a second heart (shaded), which functioned in accessory support of the host animal.

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 strophanthin 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.

Barrić *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, 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 aortae 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 25 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 tachypnoeic. 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 (Hurley *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 snares 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 snares 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 18 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 rewarming 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 post-operatively. 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 autografts, 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 Schuldts (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 lung 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. Cardiologist 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.

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