La transplantation cardiaque

Deuxième symposium mondial

1969

Heart Transplantation

Second World Symposium

LES PRESSES DE L'UNIVERSITÉ LAVAL

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QUÉBEC 1970

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Avant-propos

Yves MORIN, M.D.

Tenu à Montréal en juin 1969 et fort habilement dirigé par notre ami Pierre Grondin, le deuxième Symposium mondial sur la transplantation cardiaque a fait, en réunissant toutes les compétences dans ce domaine, le point sur un épisode unique dans l'histoire de la médecine, celui des greffes cardiaques. Il est aujourd'hui curieux de constater qu'en si peu de temps, plus de quarante équipes chirurgicales à travers le monde se soient adonnées en même temps à une entreprise aussi complexe, aussi ardue et, il faut le dire, aussi hasardeuse, pour ensuite, en même temps, et de façon définitive, semble-t-il, cesser toute agression clinique dans ce domaine.

Il est trop tôt pour établir le bilan définitif de ces trois années mais il est indiscutable que plusieurs effets bénéfiques en ont résulté. Les greffes de cœur ont d'abord démontré de façon indiscutable l'assurance technique, les moyens matériels et dans plusieurs cas, la virtuosité des chirurgiens contemporains. Alors que la médecine se veut de plus en plus quantitative et automatisée, la chirurgie cardiaque est un des derniers châteaux forts de l'action individuelle qui ne peut être exactement reproduite. S'il en était besoin, ces transplantations ont encore une fois démontré que la médecine moderne est une médecine d'équipe réunissant des compétences diverses.

Sur le plan scientifique, ces greffes ont permis pour la première fois chez l'homme, l'observation de cœurs anatomiquement dénervés. Le syndrome clinique du rejet, avec ses trois phases successives, semble, sur les plans clinique, électrique et biologique, assez bien caractérisé. Personnellement, ce qui nous a le plus fasciné à cet égard est l'apparition inopinée, dans les cœurs greffés, d'une maladie coronarienne qui, chez les survivants du rejet précoce, a souvent (comme chez le docteur Blaiberg et le Père Boulogne) été la cause du décès de ces malades. Il faut au départ déterminer, sinon la similitude, du moins l'apparentage, entre ces coronaropathies et l'artériosclérose banale. Mais la réalité de ces insuffisances coronariennes se constituant en quelques mois pourrait possiblement aider à confirmer une hypothèse, chère à l'auteur de ces lignes et qui veut que la maladie coronarienne évolue par poussées relativement brèves.

La plupart des greffes ont donné sur l'écueil du rejet : ce revers de l'immunologie chez des malades par ailleurs fort bien contrôlés stimulera sûrement des recherches qui pourront en venir éventuellement à un « antibiotique » du rejet.

Malgré tout, surtout si l'on tient compte du nombre assez considérable (plus de 130) de cas étudiés, il est possible de penser que la masse des données objectives et des conclusions probables qui ont résulté de tout ceci est assez faible. Par nécessité, on n'a que rarement établi de protocole prospectif, ou procédé en cours de route à des réévaluations critiques et, à cet égard, il faut noter que les séries les plus considérables ne sont pas nécessairement celles qui présentent les survies les plus longues.

Il ne nous appartient pas de dire si chacune des 130 greffes était justifiée : on ne peut soustraire au médecin traitant, s'il tient compte à la fois du tableau clinique présenté par son malade et de l'état actuel des connaissances médicales, le droit de prendre de telles décisions et rien ne nous laisse croire, dans les cas qui nous intéressent, qu'elles étaient prises en dehors de ces normes.

Il faut, enfin, je pense, déplorer certains excès auxquels s'est livrée la presse populaire dans cette affaire. Dans plusieurs cas et malgré le désir de la famille, un exposé détaillé de l'état du donneur a été divulgué et comme le fait remarquer un des participants de ce Symposium, cette publicité de mauvais goût est responsable de la rareté actuelle des donneurs pour les greffes de reins.

Quoi qu'il en soit, le compte rendu de ce Symposium mérite d'être lu avec attention : son intérêt certain nous rend doublement heureux de publier ce qui est, pour une bonne part, l'oeuvre de collègues du Québec.

Introduction

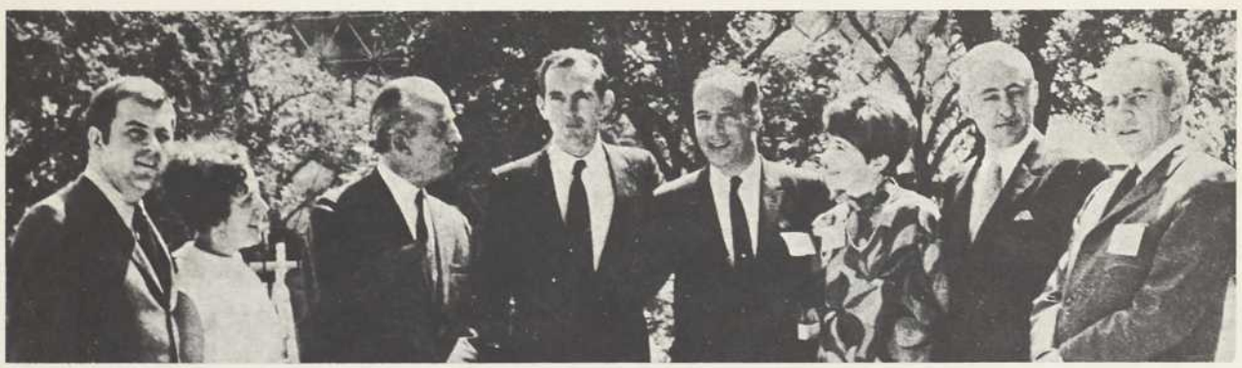
Pierre GRONDIN, S.M., M.D.

En raison de l'excellence des travaux et des discussions du Deuxième Symposium sur la transplantation cardiaque, tenu à Montréal les 6, 7 et 8 juin 1969, le Comité d'Organisation a réalisé l'importance de les colliger dans une publication. Cette réunion scientifique, nous l'espérons, a permis de faire le point sur les greffes cardiaques, et nous croyons que les informations échangées doivent être mises à la disposition de tous les Centres qui, de par le monde, s'intéressent à ces problèmes. De plus, l'annonce faite à Montréal qu'un Troisième Symposium serait tenu à Paris, en 1970, nous a stimulés à faire paraître ces travaux assez tôt pour qu'ils servent de référence à cette réunion et aux réunions futures.

L'organisation du Deuxième Symposium fut pour nous une expérience tout à fait enrichissante. L'idée première de tenir une telle réunion à Montréal revient à son Honneur le Maire

So outstanding were the papers and the discussions of the Second World Symposium on Heart Transplantation held in Montreal, June 6, 7 and 8, 1969, that the Organization Committee felt compelled to collect them in a monograph. This scientific gathering, we hope, has marked an important step in the history of cardiac allografts, and we believe that the informations released and the point of views expressed should be made available to all Centers in the World interested in this field. Moreover, the announcement made in Montreal that a Third Symposium would be held in Paris, in 1970, has stimulated us to publish these manuscripts early enough so that they can serve as references to this coming meeting.

The Organisation of the Second World Symposium was for us a most valuable experience. The first one to suggest that such a meeting be



Comité d'organisation. De gauche à droite : M. Jacques Lefebre, I.C.M., Mlle Alice Sanche, Montréal, Dr Charles Dubost, Paris, qui a accepté d'organiser un troisième Symposium, Dr Christiaan Barnard, Cape Town (Premier Symposium), Dr Pierre Grondin, Président, Mme Ghis P. Chouinard, I.C.M., Hon. Jean-Paul Cloutier, Ministre de la Santé au Québec, et Dr Napoléon Tremblay, Coordonnateur.

Jean Drapeau. Plusieurs participants ont, comme nous, regretté son absence physique aux réunions du Symposium. Monsieur le Maire était à cette époque en tournée européenne pour mousser la cause de la Cité de Montréal aux Jeux Olympiques. Monsieur Drapeau fut vraiment l'âme-clef dans la préparation de ce grand évènement. Son enthousiasme et son dévouement nous ont permis de former un Comité d'organisation que j'aimerais vous présenter :

En premier lieu, le Gouvernement du Québec sous l'instigation du Premier Ministre, le très honorable Jean-Jacques Bertrand, et du Ministre de la santé, l'honorable Jean-Paul Cloutier, a accepté d'être l'hôte de cette extraordinaire réunion. Ils ont délégué à notre Comité le docteur Napoléon Tremblay, coordinateur au Ministère de la santé. C'est grâce à l'expérience, au dévouement et à la sagesse de ce médecin, que le Symposium a pu être réalisé, et ce dans un décor vraiment merveilleux.

Quant à la Ville de Montréal, elle était représentée au sein du Comité par Monsieur Jean Dupire, hôte officiel de la Cité, assisté de Mademoiselle Alice Sanche, son adjointe. Si, comme le veut l'axiome « Le Québec sait faire », il est maintenant classique de dire « Montréal sait recevoir ». L'expérience acquise par ces deux personnalités lors de l'Expo 1967 s'est manifestée dans l'atmosphère d'hospitalité et de dignité qui a régné au cours de cette consultation internationale.

L'Institut de Cardiologie était représenté par monsieur Jacques Lefebvre, directeur des relations publiques, par madame Ghislaine P. Chouinard, secrétaire du Département de chirurgie, et par moi-même. A l'exemple des transplantations d'organes, l'organisation d'un Symposium est vraiment un travail d'équipe. Le succès remporté est dû en grande partie au travail constant de chacun. Je profite de l'occasion pour rendre un hommage tout à fait mérité à chacun de mes collaborateurs.

Les participants à cette réunion scientifique étant des universitaires de haute renommée, nul

held in Montreal was His Worship Mayor Jean Drapeau. Like many others, we have deplored his physical absence from the social activities of the Symposium. At that time, Mayor Drapeau was in Europe, promoting the cause of the City for the coming Olympic Games. Mayor Jean Drapeau was truly the life and soul in the preparation of this great event. His enthusiasm and his devotion have permitted to constitute an Organization Committee that I would like to introduce :

First, the Quebec Government, through his Prime Minister, the Honourable Jean-Jacques Bertrand, and his Minister of Health, the Honourable Jean-Paul Cloutier, has accepted to sponsor this extraordinary international scientific reunion. At the Organization Committee, the Quebec Government was represented by Doctor Napoleon Tremblay, Co-ordinator at the Ministry of Health. Because of his experience and his wisdom, the Symposium was held in a magnificent setting.

The City of Montreal has delegated to our Committee Mr. Jean Dupire, Official Greeter and Cultural Affairs Officer, and Miss Alice Sanche, his Assistant. Throughout the world, the hospitality of the City of Montreal is well known. The experience accumulated during Expo 67 by these two City officials was invaluable. It has widely contributed to the atmosphere of dignity that has prevailed throughout this international gathering.

The Montreal Heart Institute had three representatives on this Committee : Mr. Jacques Lefebvre, Director of Public Relations, Mrs. Ghislaine P. Chouinard, Executive Secretary in the Department of Surgery, and myself. The organization of the World Symposium requires a carefully planned team work much like an organ transplantation program. To achieve success, the constant offort of each member of the team is needed. I take this opportunity to pay a well deserved tribute to each of my collaborators in this venture.

ne pouvait mieux les recevoir que l'ont fait les Autorités de l'Université de Montréal. Tous se rappellent les commentaires élogieux à l'égard de notre Université canadienne française, entendus après la conférence de monsieur le professeur Roger Gaudry, Recteur, lors du déjeuner offert aux participants le samedi 7 juin.

Voulant assurer au Symposium un caractère vraiment international, nous avions invité tous les chefs d'équipes chirurgicales qui, au 1er janvier 1969, avaient effectué une transplantation cardiaque chez l'homme. Tous les Centres invités étaient représentés au Symposium, sans exception. A ces chefs de file, nous ajoutions leurs collaborateurs immédiats dans les disciplines connexes comme la cardiologie, l'immunologie, la pathologie, la bactériologie, la neurochirurgie, etc. En tout, 107 invités officiels participèrent à cette consultation internationale. Leurs connaissances et leur prestige ont, plus que tout autre facteur, contribué au succès de cette réunion.

En plus des invités d'honneur, 325 médecins se sont inscrits comme participants. Ils venaient de tous les coins du monde partager avec nos invités leur expérience tant au laboratoire qu'en clinique, sur les problèmes de la transplantation cardiaque. Ces derniers ont joué un rôle précieux dans les discussion et les conclusions de cette consultation scientifique.

The vast majority of our guest of honour at the Symposium were world renown University teachers. The Authorities of our University of Montreal have greeted them with a dignity second to none. We shall long remember the eloquent comments that have followed the conference of the Chancellor, Professor Roger Gaudry at the luncheon on Saturday.

To assure the world-wide character of the Symposium, we had invited each Director of the surgical teams who, as of January 1st, 1969, had performed one human heart transplant.

Discussion animée. Dr Jacques Gélinas, sous-ministre à la Santé, Dr Christiaan Barnard, Le Cap, Hon. Roch Boivin, ministre d'État, et Hon. Jean-Jacques Bertrand, Premier Ministre du Québec.

Animated discussion. From left to right : Dr. Jacques Gelinas, Deputy Minister Dept. of Health, Dr. Christiaan Barnard, Cape Town, Hon. Roch Boivin, State Minister and Hon. Jean-Jacques Bertrand, Prime Minister of Quebec.

Every Center invited was represented at the meeting.

With these surgeons, we had invited their immediate collaborators in related fields such as Cardiology, Immunology, Pathology, Bacteriology, Neurosurgery, etc.... A total of 107 official guests have actively participated to this international consultation. Their prestige and knowledge have, more than any other factor, contributed to the success of this meeting.

Besides these guest of honour, 325 medical specialists have registered as participants. They came from every corner of the world to share with our guest their experience both in the laboratory and in clinical situations. These have played an important role in the discussions and

C'est avec une légitime fierté que tout Québec se remémore ces trois jours de juin 1969, où il lui a été donné d'être l'hôte d'un nombre imposant d'illustres chercheurs. A tous, un grand merci.

in the conclusions of this scientific consultation.

It is with rightfull pride that the Province of Quebec remembers these three days of June 1969, when it was privileged to serve as host to such an illustrious group of researchers.

THE FIRST HEART TRANSPLANT IN MAN
Brief Review With Notes On A More Recent Case *

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Après une expérience de plus de 100 cas de transplantation cardiaque chez le chien, ainsi que des expériences additionnelles chez des veaux, des singes et des cadavres humains, la première transplantation cardiaque humaine fut effectuée le 22 janvier 1964. Le receveur fut un patient de 68 ans, hypertendu, atteint d'un infarctus du myocarde et en état de choc profond. À cette époque, le concept de mort cérébrale n'était pas accepté et il fallait attendre l'arrêt cardiaque complet avant d'arrêter la ventilation pour enlever un rein en vue d'une transplantation. Ainsi, on se rendit compte que le donneur humain sur lequel on comptait n'allait pas nécessairement mourir au moment même où le receveur entrait en phase terminale. La possibilité de greffer un cœur de primate fut retenue après la publication des premiers succès de Reemtsma et Creech dans la transplantation de reins de chimpanzé à l'homme. Dans ce premier cas de transplantation cardiaque humaine, le cœur d'un grand chimpanzé
(suite du résumé en page suivante)

We are met here to assess current horizons and progress in clinical heart transplantation. Virtually every group working in the field is represented, and this Congress will surely achieve significant progress. It was suggested that my opening remarks include a review of our 1964 case, as well as our more recent clinical experience.

In the Spring of 1963 our group concluded that animal studies, begun in 1956 and pursued continuously thereafter, justified a gradual approach to heart transplantation in man (3, 5, 6, 7, 10 and 11). Many potential recipients were screened throughout the ensuing months, but no appropriate and clearly terminal potential recipient presented until January 22, 1964. By this time our laboratory experience encompassed well over a hundred heart transplants in dogs, and additional experiments had been carried out in calves, monkeys and the human cadaver.

The 68-year-old chronically hypertensive patient had sustained myocardial infarction (and at eventual autopsy the pathologists estimated that approximately 90 per cent of his coronary arterial supply was occluded). When he lapsed into profound shock which could not be reversed, he was moved quickly to an operating room, anesthetized with minimal anesthesia through the tracheostomy tube, and placed on cardiopulmonary bypass through a median sternotomy incision. He had remained mentally obtunded since admission to the hospital, and permission for possible heart transplantation was obtained from the family (2). This document clearly stated that, while many heart transplants had been performed in animals by us and by others, any heart transplant would represent the first heart transplant in man. The proposed transplantation

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* Presented at the Second world symposium on heart transplantation, Montreal, Canada, June 6-8, 1969.

Dr. Carlos M. Chavez participated in both cases. Drs. Hilary H. Timmins, Akio Suzuki, Martin H. McMullan and Patricia C. Moynihan of the Department of Surgery and Drs. Patrick H. Lehan and Harper K. Hellems of the Department of Medicine collaborated in the second case.

mâle fut utilisé. Le débit cardiaque du cœur de primate était de 4,25 litres/minute et le débit du receveur avant l'état de choc, de 3,6 litres/minute. Durant l'intervention, le cœur du donneur fut perfusé par voie rétrograde dans le sinus coronarien et la défibrillation fut facile. La fréquence cardiaque fut maintenue à 100 par minute au moyen d'un *pacemaker* à fréquence fixe et après 30 minutes de circulation extracorporelle partielle le cœur put reprendre sa fonction seul. Une tension artérielle de 80 à 90 mm Hg fut obtenue durant une heure. À ce moment, on se rendit compte que l'état avancé de détérioration métabolique préopératoire du receveur ainsi que la taille modeste de l'organe transplanté rendaient aléatoire un succès à long terme. L'autopsie n'a révélé aucune preuve de rejet aigu. Cette première transplantation cardiaque chez l'homme établit d'abord la possibilité scientifique de transplanter un cœur et ensuite stimula de façon concrète la continuation d'études expérimentales en laboratoire.

La deuxième transplantation eut lieu le 6 janvier 1969 sur un receveur de 48 ans atteint d'une maladie coronarienne occlusive triple et ayant fait

was cleared with the administrative officials of the University of Mississippi Medical Center.

Although a patient with severe brain damage and "brain death" had represented a potential human donor, he remained alive supported by a ventilator. At that time the concept of brain death as we know it today was not accepted, and it was our policy to await complete cardiac arrest before stopping the ventilator and removing a kidney for transplantation. Accordingly, since we had realized that the potential human donor might not die at precisely the same time that the potential recipient went into terminal collapse, the availability and possible need of a lower primate heart for transplantation had been raised in discussions with the patient's family. Reemtsma and Creech had already reported early success with chimpanzee kidney transplants in man (9).

The heart of a large male chimpanzee was readily inserted as shown in Figure 1 (4). The cardiac output of the primate had been 4.25 L/min, and the pre-shock cardiac output of the human recipient had been 3.6 L/min. During transplantation the donor heart was well preserved by retrograde coronary sinus perfusion and it was easily defibrillated. The electrodes of a Chardack-Greatbatch fixed-rate pacemaker were applied to the left ventricle, and the heart rate was increased to 100 beats per minute. After 30 minutes of partial bypass support of the beating heart, as had been practiced in the laboratory, the bypass catheters were removed. The transplanted heart thereafter supported the blood pressure in the range of 60 to 100 mm Hg, usually 80 to 90, for approximately one hour (1).

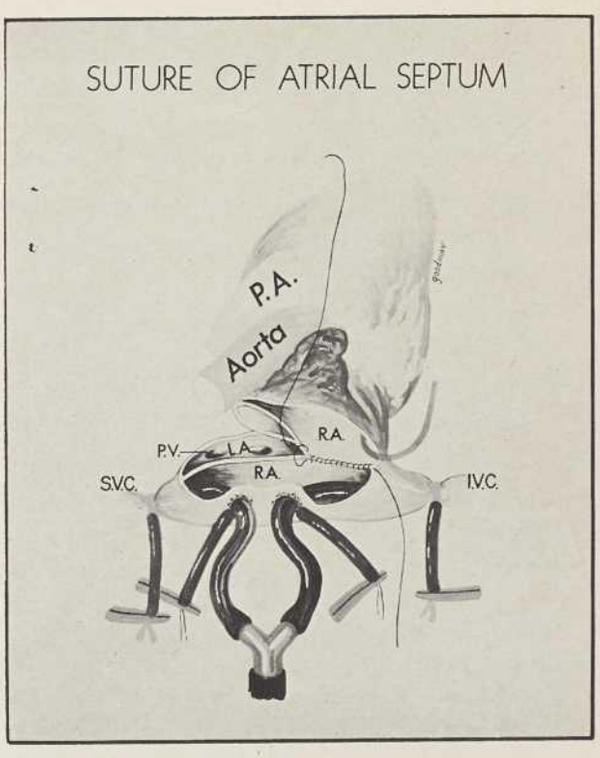

Figure 1 — Operative technique used for insertion of first heart transplant in man. (With permission from J.A.M.A.).

plusieurs infarctus. Le donneur mourut d'hémorragie intracrânienne à l'âge de 44 ans. Il était compatible au point de vue du groupe sanguin et présentait une histo-compatibilité du groupe C selon Terasaki. Après arrêt cardiaque, le cœur du donneur fut refroidi à 4° C, transporté chez le receveur et implanté. Après suture de l'oreillette gauche, l'aorte fut anastomosée et les autres anastomoses furent effectuées sous perfusion coronarienne. Le cœur reprit sa fonction facilement mais le patient mourut d'hémorragie intracrânienne le septième jour après l'opération. Une infiltration myocardique importante à cellules rondes fut notée malgré un traitement considéré optimal à l'Azathioprine, la Prednisone et la globuline antilymphocytaire.

En conclusion, si la transplantation cardiaque est réservée aux quelques patients manifestement en phase terminale et si d'autre part des critères stricts sont observés quant à la sélection du donneur et le choix du receveur il est possible de ne pas ébranler l'opinion publique de façon néfaste.

At this point it was concluded that the advanced state of preoperative metabolic deterioration of the recipient, plus the modest size of the transplanted organ, rendered long-term success unattainable in this instance. Microscopic studies of the transplant at autopsy revealed neither damage from the retrograde coronary sinus perfusion nor evidence of acute rejection (Figure 2).

On the basis of this initial clinical experience, the following conclusions were published at that time (4):

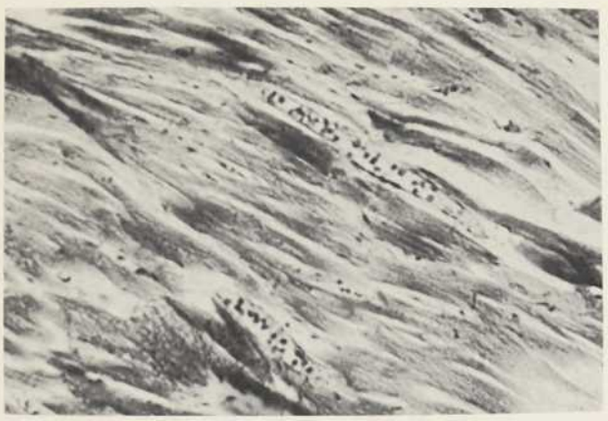

Figure 2 — Microphotograph from left ventricle heart transplant. Note absence of cellular evidence of acute rejection. In addition, the absence of red blood cell extravasation attests that the retrograde perfusion of the coronary sinus for preservation during transplantation did not result in excessively high intravascular pressures.

1. The first clinical heart transplant had been readily carried out with the methods which we had previously employed in a large number of laboratory experiments.

2. The suture techniques in use in many laboratories had been found satisfactory.

3. The heart had been well preserved by retrograde perfusion of the coronary sinus with oxygenated blood (5).

4. The heart had fibrillated throughout the anastomotic maneuvers, and it had been converted to a regular rhythm by a single weak shock of the pulse defibrillator.

5. The transplanted heart had reacted immediately to intravenously injected digoxin, as reflected in the development of partial heart block with pulsus bigeminus.

6. The cardiac pacemaker had readily broken through this arrhythmia when the amplitude of the current was increased.

7. The function of the transplant fully supported the scientific feasibility of heart transplantation in man, and the results rendered continuing laboratory studies more meaningful.

It was further stated that "with further refinements in physiology and drug therapy this operation may some day add years of life to many patients",

Perhaps most important of all, this first heart transplant in man assaulted the frontiers of human imagination and acceptance as no previous operation had done. It thus initiated vigorous and profound dialogue and debate which facilitated a more general acceptance of clinical heart transplantation later on. Actually, it was apparent that the technical aspects of heart transplantation were more easily carried out in the human being than in the dog, due to the larger size and toughness in man of the structures to be anastomosed.

A SECOND HEART TRANSPLANT

Our second clinical heart transplant was performed on January 6, 1969. The recipient was a 48-year-old white man who had severe, triple vessel coronary atherosclerotic occlusive disease, as demonstrated by coronary arteriograms. He had experienced multiple myocardial infarctions, had sustained one cardiac arrest, had a ventricular aneurysm of moderate size and had anginal pain at bed rest despite usual medication. The consulting members of the Department of Medicine, Cardiology Division, as well as members of the Committee on Human Investigation, concurred in the opinion that the patient represented an appropriate recipient for heart transplantation: he was immobilized by his disease, his life prognosis for more than a few months was poor, and heart transplantation could conceivably offer a rewarding prolongation of life.

The donor was a 44-year-old man who died from spontaneous intracranial hemorrhage. The donor and recipient were both ABO group O, and the histocompatibility matching was Terasaki Class C (with Class A the best match and Class D the worst). Following brain death, as certified by the neurological consultants, the donor was heparinized and cooled through the femoral vessels, using partial cardiopulmonary bypass with a disposable bag oxygenator. Appropriate written permission had of course been obtained from his family. The blood pressure had become unobtainable when the femoral vessels were exposed, but the heart was not removed until it had arrested in ventricular fibrillation. The organ was then cooled further in Ringer's lactate at 4°C, and was transported to an adjacent operating room, where it was inserted into the recipient using Barnard's modification of the basic Lower-Shumway technique. Although the chest of the recipient had been opened through a median sternotomy incision at the time the heart of the donor was exposed, the recipient was not placed on cardiopulmonary bypass and the heart excised until we were satisfied that a suitable donor heart was available. Thereafter the organ was readily sutured into place using double rows of 000 and 0000 mercilene sutures. After the left atrium and the aorta had been sutured, the left ventricle was vented and all air removed, and the aortic clamp was opened to perfuse the heart. The right atrial wall was then sutured, and last the pulmonary artery. The heart had exhibited total fibrillation soon after the aortic clamp had been released, followed by atrial beats with continued ventricular fibrillation. However, it resumed a normal rhythm spontaneously as the pulmonary artery anastomosis was being completed.

Postoperative course. The patient developed a marked systolic arterial hypertension in the postoperative period, at times in the range of 290/120 mm Hg. This was to be compared with his preoperative systolic pressure of 80–90 mm Hg. Unfortunately, perhaps the result of the severe hypertension, he suffered an intracranial hemorrhage and died on the seventh postoperative day. The autopsy confirmed the intracranial hemorrhage, and it also revealed a surprisingly extensive round cell infiltration of the myocardium (Figure 3), this despite what had been considered optimal treatment with azathioprine, prednisone and antilymphocyte globulin (ALG).

In retrospect, it might have been wise to have reduced the blood pressure with drugs. However, it was continuously expected that the circulation would adjust itself to the large new heart, and we were reluctant to complicate the postoperative course with a hypotensive drug.

Our present position regarding heart transplantation in man

Our attitude toward clinical heart transplantation remains unchanged. This is an experimental procedure, but we believe it is justified in the individual who has clearly pre-terminal heart disease and who has only a few weeks to live. My associates and I agree that the patient must understand the high risks involved, and that heart transplantation must not be employed where other and less drastic treatments are available to provide even a modest extension of reasonably comfortable life. The medical and the lay public may be expected to subject heart transplantation to an increasingly critical analysis. If we restrict heart transplantation to the few patients who are clearly terminal and who may benefit therefrom, we can preserve what we have gained, while extending knowledge and the potential for treatment with this method. If we do not rigidly enforce strict criteria for recipient and donor selection, much can be lost and progress can be seriously retarded.

SUMMARY

1. The first heart transplant performed in man has been reviewed. The organ was readily inserted, and it supported the recipient for one hour after cardiopulmonary bypass support had been discontinued. The organ failed because of the preoperative metabolic deterioration of the recipient and the relatively small size of the transplant. Microscopic sections demonstrated complete absence of myocardial injury from either the retrograde coronary sinus perfusion during transplantation or acute allograft rejection reaction.

2. This initial heart transplant demonstrated that the blood pressure could be thus supported in a human being, if for a limited period of time in this instance. The operation so assaulted the frontiers of human imagination and acceptance that world-wide dialogue was precipitated. This did much to prepare human minds for the cautious acceptance of heart transplantation which exists at the present time.

3. Our second clinical heart transplant is mentioned briefly. The insertion of a large and hypertrophied donor organ from a large subject into a slight recipient was followed by severe systemic hypertension in the recipient. Intracranial hemorrhage occurred, which proved fatal on the seventh postoperative day. Despite treatment with azathioprine, prednisone and ALG, an advanced stage of rejection was found at autopsy.

4. We believe that continued clinical heart transplantation is justified if rigid criteria for recipient selection are met. The patient must be clearly terminal, and all other modes of therapy must have been exhausted. In such instances, heart transplantation may prolong life and at the same time afford knowledge which alone will gradually improve results in the field.

Figure 3 — Microphotograph of left ventricle in human-to-human heart transplant, January 1969. Patient died on seventh postoperative day from effects of an intracranial hemorrhage. Note the remarkable round cell infiltration and evidence of myocardial necrosis, depiste treatment with azathioprine, prednisone, and antilymphocyte globulin (ALG). In contrast, the residual atrial segment of the recipient showed none of these changes.

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EXPERIENCE AT CAPE TOWN WITH HUMAN TO HUMAN HEART TRANSPLANTATION *

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L'auteur relate l'expérience de Cape Town avec cinq transplantations cardiaques.

Parmi ces cinq patients, deux souffraient de maladie cardiaque ischémique, un de myopathie cardiaque, et deux de fièvre rhumatismale.

Au cours de la transplantation, la technique était telle qu'elle évitait tout dommage au nœud sino-auriculaire, en ouvrant l'oreillette droite, par une incision partant de la veine cave inférieure vers l'appendice auriculaire droit.

Le diagnostic de rejet se basait d'abord sur l'évidence clinique d'une défaillance cardiaque et sur les changements électrocardiographiques. Parmi ceux-ci, c'est l'abaissement du voltage qui est le signe initial du rejet. L'étude des changements enzymatiques ne fut d'aucune aide dans le diagnostic. (suite du résumé en page suivante)

I have been requested to participate in this session by discussing our experience at Cape Town with human to human heart transplantation. The exploration of any new field in medicine brings forth many new problems and thoughts, but time will only allow my discussion to cover four aspects, namely:

a) The material selected;
b) Some aspects of the surgical technique;
c) The most important changes indicative of acute rejection; and
d) Our current views on the immunosuppressive regime.

To date we have performed five heart transplants. The first two patients suffered from ischæmic heart disease, the third from cardiac myopathy, and the last two from rheumatic carditis. We selected patients who had reached the end stage of their condition — patients who had run a progressively downhill course and in whom other medical and surgical forms of management had failed; patients in whom we anticipated death would occur within a very short time if they were not given this final, ultimate aid.

Four of the five patients are still alive. The first died 18 days after surgery. The remaining four patients are now alive eighteen months, nine months, two months and one month and a half respectively.

Before surgery all patients were fully investigated by means of right and left heart catheterization and angiography. The pertinent findings are given in Tables I, II, III, IV and V.

TABLE I

Catheter findings in the first patient (W.) six month before cardiac transplantation after extensive bed rest and medical treatment

Right atrium (mean)	10 mm Hg
Right ventricle	85/15-9 mm Hg
Right brachial	130/75 mm Hg
P.A. wedge pressure (mean)	35 mm Hg
Left ventricle	125/25-30 mm Hg
Cardiac index	2,43 l/min/m²
Pulm. vasc. resistance	11 units

* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6-8, 1969.

Le traitement immunosuppresseur après l'intervention, consiste en hautes doses de Prednisolone, 500 mg au cours des premiers jours, réduits à 100 mg au cours des jours suivants, l'Imuran, dont l'administration se fera ultérieurement en fonction de la formule leucocytaire, et les globulines antilymphocytaires données pendant trois mois à raison de 5 à 10 ml par voie intraveineuse deux fois par jour, au cours du premier mois. On donnera une seule dose par jour au cours du deuxième mois, et au cours du troisième mois on administrera la dose trois fois par semaine.

Pour éviter le rejet, on administrera une fois par semaine une dose élevée de stéroïdes. Ainsi, dans les cas de rejet, on note, après l'administration de Prednisone, une amélioration dans le voltage.

As already mentioned the first two patients suffered from coronary heart disease. This was verified by means of selective coronary angiography which showed that in both patients the three major coronary vessels were grossly diseased. Severe myocardial death was illustrated by the poor contractions of the left ventricles on the left ventriculograms. Total heart failure was shown in both patients with a rise in right atrial pressures, left atrial pressures, a rise in the end diastolic pressures of the left ventricles and low cardiac indices.

The highest pulmonary vascular resistance in this series was found in the first patient who had pulmonary vascular resistance one third of systemic.

Figures 1 and 2 show photographs of the hearts of the first two patients immediately after removal and the extensive myocardial death especially of the left ventricular muscle can be clearly seen.

The third patient suffered from cardiac myopathy. Haemodynamic studies again show both

TABLE II

Catheter findings in the second patient (B.) prior to heart transplantation

Right atrium (mean)	7 mm Hg
Right ventricle	50/3-7 mm Hg
Right brachial	150/90 mm Hg
P.A. wedge pressure (mean)	26 mm Hg
Left ventricle	125/25-30 mm Hg
Cardiac index	1,94 l/min/m²
Pulm. vasc. resistance	2,5 units

TABLE III

Catheter findings in the third patient (S.) three months before cardiac transplantation, after extensive medical treatment

Right atrium (mean)	15 mm Hg
Right ventricle	53/13-16 mm Hg
Right brachial	148/04 mm Hg
P.A. wedge pressure (mean)	34 mm Hg
Left ventricle	117/15-27 mm Hg
Cardiac index	1,66 l/min/m²
Pulm. vasc. resistance	4,2 units

TABLE IV

Catheter findings in the fourth patient (K.) after his condition failed to improve after aortic valve replacement

Right atrium (mean)	21 mm Hg
Right ventricle	75/12-32 mm Hg
Right brachial	110/60 mm Hg
P.A. wedge pressure (mean)	28 mm Hg
Left ventricle	120/20-12 mm Hg
Cardiac index	1,2 l/min/m²
Pulm. vasc. resistance	9,5 units

TABLE V

Catheter findings in the fifth patient (F.) after her condition failed to improve after mitral valve replacement

Right atrium (mean)	10,5 mm Hg
Right ventricle	32/9 mm Hg
Right brachial	100/72 mm Hg
P.A. wedge pressure (mean)	16,5 mm Hg
Left ventricle	116/9-11 mm Hg
Cardiac index	1,2 l/min/m²
Pulm. vasc. resistance	5,5 units

right and left heart failure, with a low cardiac index. His condition failed to improve on prolonged bed rest and medical treatment. The heart on removal (Figure 3) showed no coronary or valvular disease, but only the abnormally thickened pale muscle of the ventricular chambers.

The fourth patient presented with severe aortic incompetence and therefore was first submitted to an aortic valve replacement. Despite the haemodynamic correction he remained in total heart failure, as can be seen from the catheter findings three months before cardiac transplantation. It was thus concluded that the heart failure was now caused by disease of the heart muscle and this was verified on noting the extensive scarring of the muscle after the heart was removed (Figure 4).

The fifth patient also suffering from rheumatic heart disease presented with severe mitral incompetence. This was corrected with a xenograft replacement of the mitral valve (Figure 5) but, in spite of this, the patient remained in total heart failure with critical attacks of pulmonary œdema. Catheter findings a few weeks before surgery showed no valvular defect but total heart failure as a result of myocardial involvement.

Technique:

As changes in the electrocardiogram are early signs of the onset of acute rejection, a technique was evolved to ensure normal electrocardiogram after transplantation. The technique of Lower and Shumway (1) was modified in that the entire donor heart was removed after ligating the superior vena cava. The right atrium was opened for anastomosis by making an incision from the inferior vena caval orifice to the right atrial appendage (Figure 6), thus avoiding damage to the sino-auricular node.

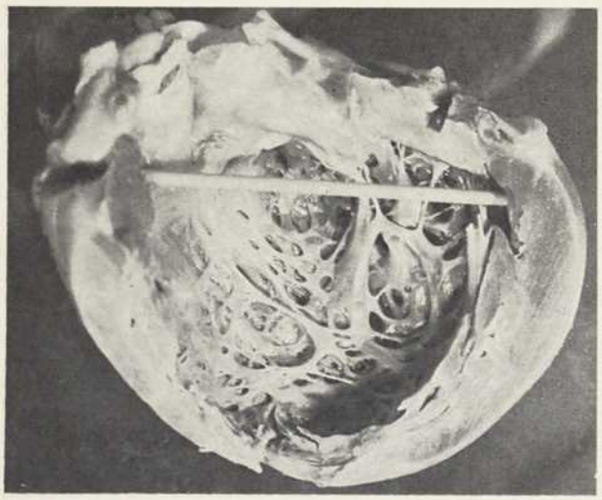

Figure 1 — The heart of the first patient after excision. Note extensive scarring in the left ventricle.

Figure 2 — The heart of the second patient. Note the dilatation of the left ventricle with extensive scarring and a localized aneurysm in the apex.

Figure 3 — Heart of the third patient after excision showing the pale, thickened left ventricular muscle.

The left atrium was opened by excising a square of myocardium between the entrances of the four pulmonary veins. The openings thus created are anastomosed to the remnants of the right and left atria of the patient. There is thus no danger to the sino-auricular or atrio-ventricular nodes and the transplanted heart will resume its beating in sinus rhythm with normal conduction.

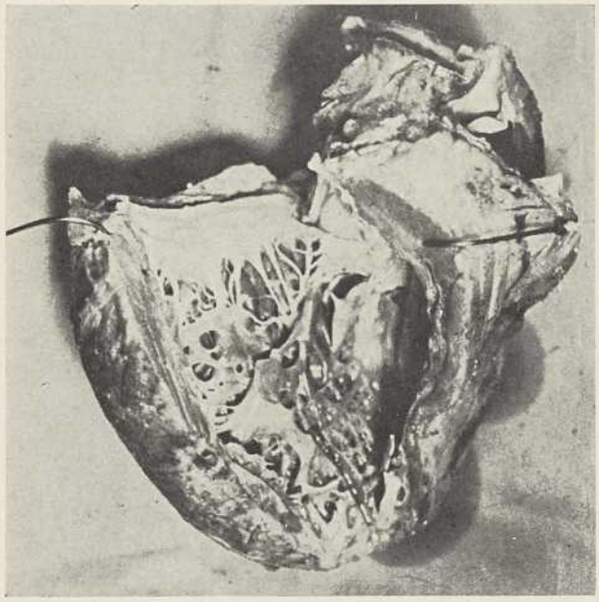

Figure 4 — Heart of the fourth patient after excision showing extensive muscular damage.

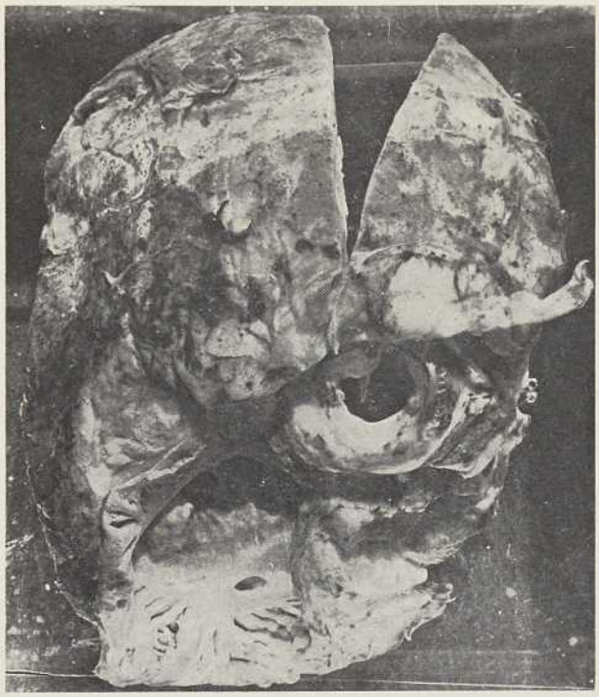

Figure 5 — Heart of the fifth patient after excision showing xenograft replacement of the mitral valve. The xenograft was in good condition.

Diagnosis of rejection:

In our experience the earliest changes indicative of rejection are:

Firstly, clinical evidence of cardiac failure. This can be noticed at the bedside by the onset of a rise in jugular venous pressure, ankle œdema and hepatomegaly. The patient may complain of shortness of breath. A gallop rhythm and a functional mitral systolic murmur indicative of cardiac dilatation can be heard on auscultation.

Secondly, changes in the electrocardiogram. Careful recording of electrocardiograph voltage is

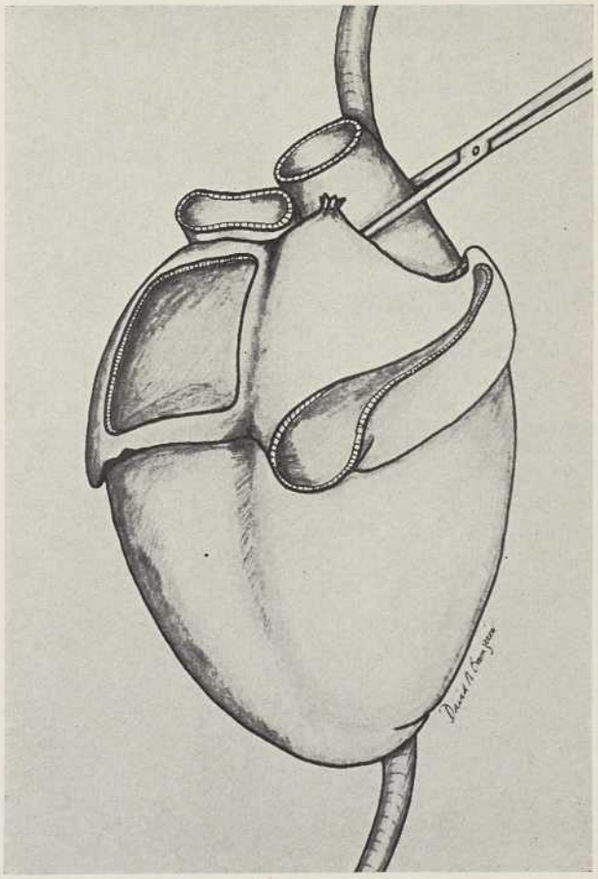

Figure 6 — Diagram illustrating opening of atrial chambers to avoid damage to conducting system.

of the greatest value in the early diagnosis of rejection. A progressive drop in the electrocardiograph voltage often occurs before any other symptoms or signs. An initial progressive drop in the electrocardiograph voltage following cardiac transplantation is probably due to œdema, surgical trauma and pericardial effusion, but the voltage rapidly recovers (Figure 7).

Our studies on the changes in the serum enzymes were of no value in the diagnosis of rejection.

Treatment of rejection:

As has been pointed out by other workers, the patient who receives a cardiac transplantation has probably a more active immunological system compared to that of a kidney transplant patient who has been chronically immunosuppressed by the chronic uraemic state. It therefore appears essential in the patient receiving a heart to initially use high doses of immunosuppression. We commence with 500 mg of Prednisolone on the day of operation, reducing it by 100 mg every day until the fifth day. For the following two weeks a dose of 100 mg per day is maintained and then it is slowly lowered to 50 mg per day. This dosage is continued for the first month, after which it is again gradually reduced to 30 mg daily and this dose is maintained for one year. Imuran is prescribed at the highest tolerated dose, observing carefully the white cell count and the liver function tests. Antilymphocyte globulin is given for three months in doses of 5 ml to 10 ml intravenously every 12 hours for the first month. This dosage is given once a day for the second month and for the third month is administered three months times a week.

Once rejection is well-established in the transplanted heart it may be difficult to reverse and as to date, an artificial heart has not been developed to take over cardiac function during this period, there is thus a great danger to the patient's life. Even if rejection is reversed it has been our experience that the patient never improves to the

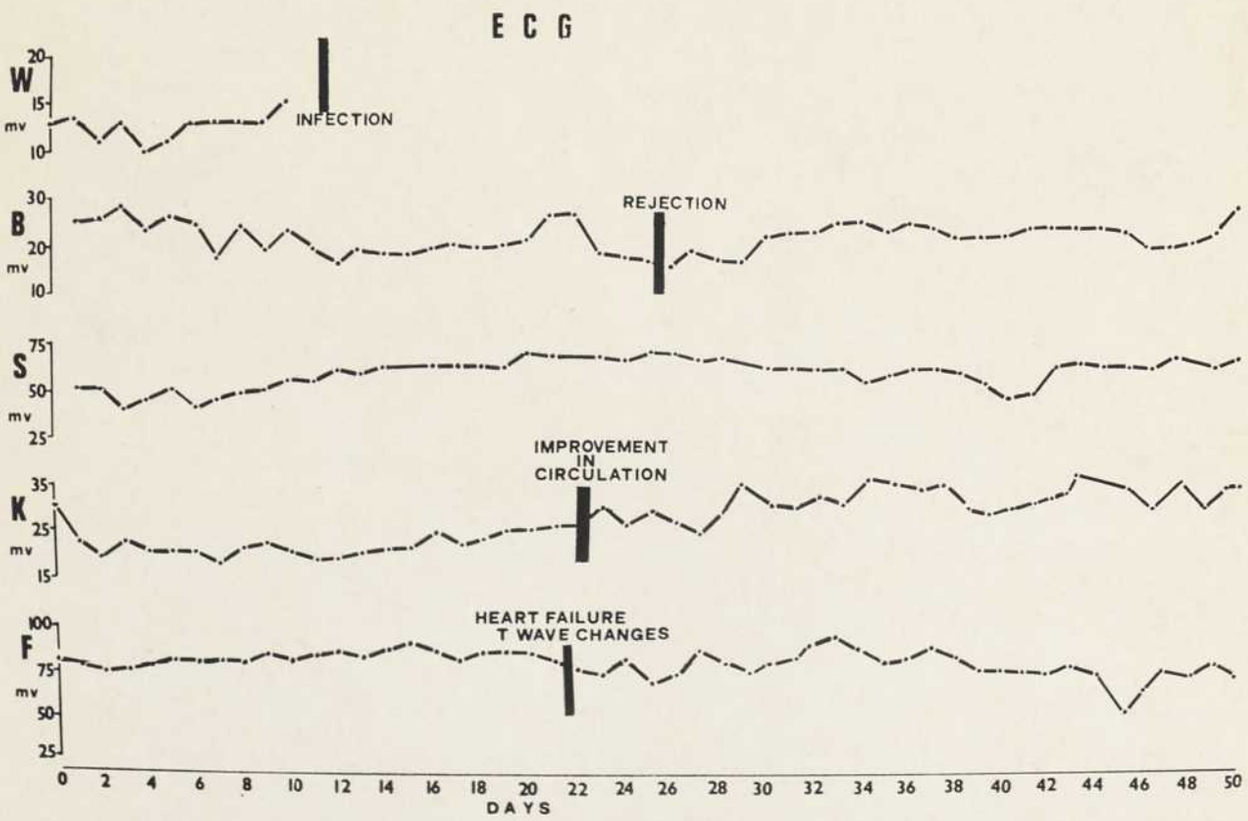

Figure 7 — Graph showing the initial drop in the electrocardiograph voltage, especially well show in patients one, three and five. In patients two and four prolonged diminution in voltage was associated with rejection.

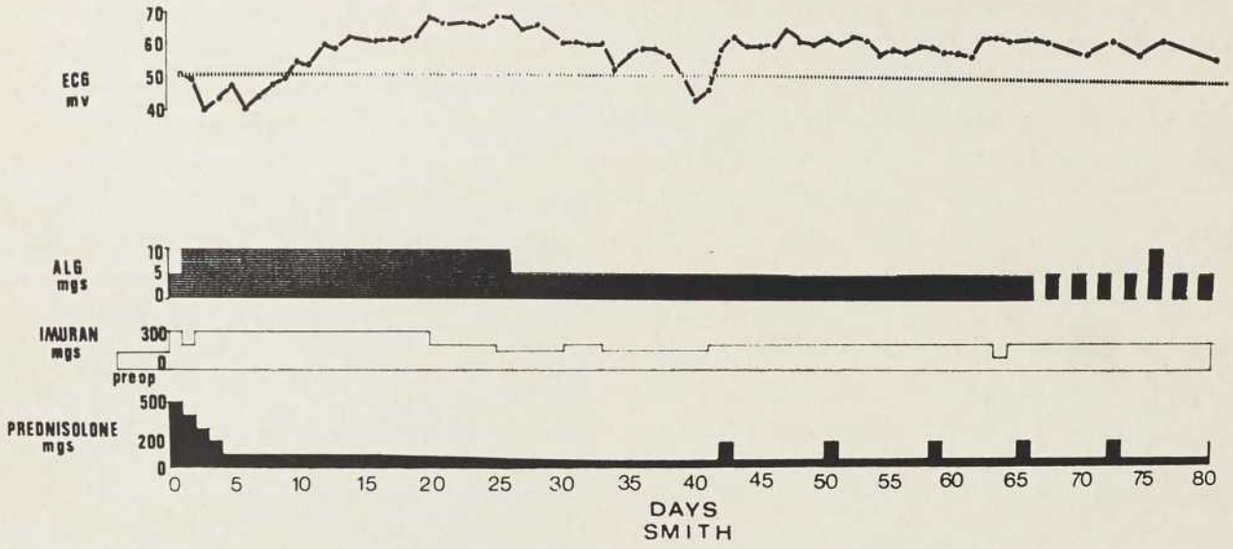

Figure 8 — A graph of the voltage and immunosuppressive regime in the third patient with the introduction if intermittent high doses of steroids. Note the improvement in the electrocardiograph voltage after the introduction of the intermittent steroids.

state he was in before the rejection episode. It therefore appears to be of utmost importance to prevent rejection, and we feel that this can be done by treating the patients weekly with a single high dose of steroids. In the third patient this regime was started on the fortieth day, once maintenance immunosuppression had been reached. The patient received 200 mg Prednisolone per week as a booster. It will be noticed from Figure 8 that when this was started there was an improvement in the electrocardiograph voltage andt he voltage never dropped below baseline again. So far there has been no clinical evidence of rejection. He has virtually normal activity and is able to play tennis.

In conclusion, we began with dying patients and in a year and a half have one man surviving eighteen months post-operatively. Another man is able to play tennis nine months after a heart transplantation. These achievements, in our opinion, make heart transplantation a worthwhile procedure, and although we realize that this is not curative surgery, there are many other forms of palliative surgery which are accepted, and this form of palliation should be continued too. We have many critics who say this is not worthwhile, but the persons really qualified to judge this are our patients. We are not treating our critics, we are treating our patients. When I asked Blaiberg: "Tell me, when was this operation worthwhile ?"... He replied: "Thirty minutes after it was performed, because then I could breathe again without having to struggle for my breath."

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EXPERIENCE WITH CARDIAC TRANSPLANTATION IN FOURTEEN PATIENTS *

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Avant la transplantation, onze patients étaient atteints d'une maladie coronarienne obstructive et trois d'une cardiomyopathie. Tous les patients subirent une exploration complète avant l'intervention sauf quatre dont l'état était trop précaire. En moyenne, l'index cardiaque était de 1,8 l/min/m². Une dysfonction hépatique fut notée chez dix patients et une dysfonction rénale chez sept. Tous étaient de classe IV au point de vue de l'incapacité fonctionnelle. Sur le plan immunologique, tous étaient compatibles au point de vue du système ABO et tous avaient un *cross-match* lymphocytaire négatif. Le cœur fut préservé par hypothermie externe. La modification de la technique chirurgicale concernant l'auriculotomie droite telle que décrite par Barnard fut adoptée.

Après l'opération, le myocarde fut supporté par *pacing* épicardique ventriculaire droit, Digoxin et Isoprotérénol. Quant au traitement immunosuppressif, huit patients reçurent de l'Imuran à la dose de 0,5 mg/kg/jour avant l'intervention pendant une à quatre semaines. Les corticostéroïdes furent prescrits au moment de l'intervention et la globuline antilymphocytaire immédiatement avant. En cas de rejet, un traitement à l'Actinomycine D et des doses massives de Méthylprednisone furent administrés. Chez
(suite du résumé en page suivante)

INTRODUCTION

Since January, 1968, 14 patients have undergone cardiac transplantation at Stanford University Medical Center. In this report we wish to present our general experience with patient selection, surgical technique, methods of postoperative study and management, and survival.

PATIENTS AND METHODS

The average age of these patients was 49 years, with a range of 33 to 58 years. Twelve were male and two female. In 11 patients the preoperative diagnosis was coronary artery disease and in three cardiomyopathy. Preoperative evaluation included, in addition to routine studies of pulmonary, hepatic, and renal function, complete hemodynamic assessment with left ventriculography in all but four patients whose preoperative conditions were too precarious to allow formal investigation. In those patients undergoing cardiac catheterization the average cardiac index at rest was 1.8 L/min/M² (range 1.2 to 3.2 L/min/M²). Pulmonary hypertension at rest was present in nine of ten patients, ranging from 33/13 to 78/33 mm Hg (mean 19 to 53 mm Hg), and elevation of the pulmonary vascular resistance to an average of 4.6 units (range 1.2 to 8.5 units). Preoperative evidence of hepatic dysfunction was present in ten patients and renal dysfunction in seven. All patients suffered Class IV disability.

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* Paper presented at the Second world symposium on heart transplantation, Montreal, Canada, June 6–8, 1969.

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la plupart des patients, l'héparinisation intraveineuse fut également employée au cours des épisodes de rejet. Le diagnostic de rejet est basé sur l'altération électrocardiographique. Des mesures de l'épaisseur de la paroi et du diamètre du cœur furent effectuées au moyen d'ultrasons. Dans les cas de rejet, une corrélation importante fut trouvée entre l'augmentation du diamètre du ventricule droit et l'augmentation totale de l'aire cardiaque. En plus, ces mesures permettent de déceler une diminution de l'amplitude du mouvement de la paroi postérieure au cours de la systole.

RÉSULTATS :

1. *En fonction de la greffe cardiaque.* Il n'y eut qu'un seul échec postopératoire immédiat. Ce cœur avait fait un arrêt anoxique avant l'opération et avait reçu une injection intracardiaque d'épinéphrine. Une nouvelle transplantation fut effectuée six heures après la première intervention.

Preoperative lymphocyte typing and crossmatch were performed in all cases and served as the basis for recipient selection in most. However, for the ten antigens typed (1) a compatible match was obtained in only three instances. In all cases the lymphocyte crossmatch was negative and ABO compatibility was present.

Surgical technique:

Surgical techniques have included routine cardiopulmonary bypass methods utilizing whole blood prime. A modified central cannulation technique was adopted after the first 5 cases because of a high incidence of postoperative complications related to the femoral cutdown site. At the present time arterial cannulation is performed via a stab wound in the ascending aorta; the inferior vena cava is cannulated through the superior caval — right atrial junction, and the superior vena cava via the right internal jugular vein approached through a separate incision. Since the institution of this technique for cannulation no further complications related to wound healing have occurred. Methods of excision and implantation of the donor heart have been previously described (4). In all cases hypothermia of the graft induced by surface cooling with saline at 2 to 6°C has been employed. Modification of the right atrial incision to avoid placing the sino-atrial node contiguous to the right atrial suture line has eliminated the intermittent nodal rhythm noted in two of the first five cases in which the donor right atrium was opened out by interconnection of the vena caval orifices posteriorly.

Postoperative management:

Three forms of myocardial support immediately following surgery have been used. These include cardiac pacemaking via a right ventricular epicardial wire to maintain the cardiac rate above 80 beats/minute, intravenous digoxin in standard doses, and isoproterenol. Each of these methods of postoperative support to the grafted heart has been shown in the laboratory to result in significant improvement in cardiac performance.

Respiratory assistance has been provided all patients for periods up to 24 hours postoperatively. Currently, nasotracheal intubation is avoided because of the danger of delayed pressure necrosis which occurred in two of the seven patients in which this route of intubation was employed.

Immunosuppressive therapy:

In all but the first two patients basic immunosuppressive agents have included azathioprine, corticosteroids, and antilymphocyte globulin (ALG). In eight patients pretreatment with azathioprine in a dose of 0.5 mg/kg/day has been given for periods of one to four weeks before surgery. A loading

Un infarctus hémorragique large fut observé dans la portion supérieure du septum musculaire et une injection intramurale d'épinéphrine fut mise en cause.

2. *Rejet.* Chez 11 des 14 patients un ou plusieurs épisodes de rejet furent diagnostiqués. Deux patients moururent de rejet aigu. Deux autres patients succombèrent au cours de la période postopératoire immédiate de complications chirurgicales. Aucun rejet ne fut trouvé à l'autopsie.

3. *Résultat global.* Actuellement, six des quatorze patients sont en vie, respectivement neuf mois et demi, huit mois, sept mois, trois mois et demi, deux mois et deux semaines après l'intervention. Onze autres patients complètement explorés et acceptés pour une transplantation moururent avant qu'un donneur compatible ne puisse être trouvé. Dans ce groupe, la survie maximale fut de 12 semaines et la survie moyenne de un mois.

dose of azathioprine, 4 to 5 mg/kg, is given immediately preoperatively. On the first postoperative day maintenance azathioprine in a dose of 2 to 3 mg/kg/day is begun. Corticosteroids are begun at the time of surgery with the intravenous administration of methylprednisolone, 3 to 4 mg/kg, during surgery. Maintenance therapy with prednisone, 60 mg/day, is begun on the first postoperative day, and gradually tapered after the first two postoperative weeks to 0.5 mg/kg/day by the end of the first postoperative month. ALG, initiated immediately preoperatively, has been administered postoperatively according to a regimen based on that originally described by Starzl (3).

Therapy for diagnosed rejection has consisted of a "pulse-type" regimen, employing actinomycin D and massive doses of methylprednisolone (500 to 1,000 mg) administered rapidly intravenously on a daily basis until initial reversal of the signs of rejection. In most patients intravenous heparinization has also been used during initial rejection episodes.

Diagnosis of rejection:

In our experience techniques directed at the documentation of the manifestations of local organ dysfunction have been the most effective in the early diagnosis of rejection. These include 12-lead electrocardiograms obtained twice daily during the first two postoperative weeks and daily thereafter until discharge, using marked skin sites for the precordial leads to insure uniform placement; ultrasound-cardiographic measurements (2) of posterior (left) wall thickness, right ventricular diameter, total heart size, and slope and amplitude of the posterior wall systolic movement; and auscultation and phonocardiographic documentation of abnormal sounds. Numerous other clinical and laboratory findings have been correlated with cardiac rejection, but none has proven, in our experience, to be as reliable in the early identification of advancing cardiac rejection. To date, however, no reliable as well as entirely specific index of cardiac rejection is available.

Electrocardiographic changes taken as indicative of rejection include progressive diminution in voltage, rightward deviation of the mean frontal plane axis, atrial arrhythmias, and ischemic ST depression. Significant correlation with rejection of various ultrasound-cardiographic measurements has included increase in posterior wall thickness, increase in right ventricular diameter, increase in total heart size, and decrease in the amplitude and rate of posterior wall movement during systole. The auscultatory finding most consistently associated with rejection and confirmed by phonocardiography has been an early diastolic gallop rhythm. Atrial gallop rhythms and pericardial friction rubs, though occasionally present, have been inconstant and have appeared transiently in some cases, unassociated

with other indication of rejection. In no patient in this series has rejection been diagnosed and treated without significant changes in these parameters.

RESULTS

Graft function:

In all cases but one immediate function of the graft has been satisfactory. In two patients with severe preoperative elevation of the pulmonary vascular resistance isoproterenol was employed in the immediate postoperative period because of moderate right ventricular failure. Intravenous digoxin was used in standard doses in eight other patients because of mild to moderate elevation of central venous pressure (to 14 to 20 cm water), and pacing was employed in five additional patients for periods of 24 to 60 hours postoperatively to maintain the heart rate above 80 beats/minute. By the third postoperative day clinically observed hemodynamic parameters have generally been normal, and digitalis has not been continued.

In one case immediate postoperative failure of the graft occurred, with hypotension and recurrent ventricular arrhythmias. In this instance anoxic cardiac arrest had developed in the donor preoperatively, requiring vigorous resuscitative measures including intracardiac administration of epinephrine. Retransplantation was performed six hours following the initial procedure. Examination of the excised first graft showed a large hemorrhagic infarction in the superior portion of the muscular ventricular septum, thought to be due to intramural injection of epinephrine. Following retransplantation myocardial function was satisfactory.

In long-term surviving patients normal activity has been well tolerated. Each patient has participated actively in a continuing physical therapy program. Measured work levels tolerated for ten minutes without undue symptoms by the four patients presently surviving beyond three postoperative months vary between 300 and 500 kilogram-meters, corresponding to 4 to 6-fold increases in oxygen consumption. Serial ballistocardiograms and exercise electrocardiograms in these patients have given normal results.

Rejection:

In 11 of the 14 patients acute rejection diagnosed by the parameters listed above were treated one or more times. Two patients died of uncontrolled rejection. In each of these the electrocardiographic, ultrasound-cardiographic, and auscultatory phenomena associated with rejection were progressive until death. In two additional patients succumbing to postsurgical complications in the early postoperative period rejection was not clinically identified. In only one long-term survivor have rejection phenomena been entirely absent. In the nine remaining patients 19 episodes of rejection were treated with reversal of the changes which prompted therapy. In none of the surviving patients has clinical evidence of residual myocardial damage been present.

One episode of possible acute rejection, diagnosed by electrocardiographic and ultrasound-cardiographic criteria, was treated and reversed at five months postoperatively. Although histopathological findings in other cases suggest a high incidence of obliterative arterial lesions, clinical evidence of chronic rejection sought by serial exercise electrocardiography and ballistocardiography has not been apparent.

Survival:

At the present time 6 of the 14 patients in this series are alive, at 9½, 8, 7, 3½, 2 months and 2 weeks postoperatively. All of these patients were treated at least once postoperatively for rejection. Additional causes of death include combined drug toxicity and rejection in two patients, a cerebrovascular accident at three weeks postoperatively in one patient, and fatal hepatitis at 4½ months postoperatively in one additional patient.

These survival data contrast with those characterizing non-transplanted cardiac recipients. Eleven patients in the latter group were fully evaluated and accepted for transplantation, but died before a

suitable donor could be obtained. In this group maximum survival was twelve weeks and the mean survival one month. Three additional patients await transplantation at the present time, all less than four weeks following acceptance into the program.

DISCUSSION

This experience demonstrates that successful cardiac transplantation can result in significant prolongation of useful life. The rapid rate of attrition in non-transplanted recipients, we believe, contributes to the justification of continued clinical investigation of this procedure.

Several factors combine to suggest an irreducible minimum mortality rate following cardiac transplantation, exceeding that achieved in the related field of cadaver renal transplantation. These include the generally greater age of cardiac recipients, the advanced effects of chronic congestive heart failure on secondary organs, the mechanical limitations imposed by persistent elevation of pulmonary vascular resistance in the recipient, the unavailability of effective temporizing support for graft failure, and difficulty in the diagnosis of chronic rejection. Improvements in understanding and management of these factors, as well as advances in donor-recipient matching and immunosuppressive therapy, imply improved results with extension of clinical experience.

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RESULTS OF CARDIAC TRANSPLANTATION *

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Ce travail rapporte vingt transplantations cardiaques chez dix-neuf patients. Dans cette série, on note une seconde transplantation six mois après le rejet du premier cœur, et une transplantation cœur-poumon chez un enfant. En plus, on rapporte un remplacement cardiaque total par une prothèse mécanique. Cette prothèse fut remplacée par une allogreffe 64 heures plus tard. La plupart des patients souffraient de maladie coronarienne occlusive. Un patient présentait en plus une maladie multivalvulaire. Deux cas de myocardiopathie furent également soumis à la transplantation cardiaque. La transplantation cardio-pulmonaire combinée fut effectuée pour un canal atrio-ventriculaire complet compliqué d'hypertension pulmonaire, de pneumonie et d'infarctus pulmonaire.

Au point de vue de l'histo-compatibilité tous les patients furent placés dans les groupes D et C de Terasaki.

Over the past year and a half the clinical feasibility of cardiac transplantation has been validated (1, 2, 4, 6 and 14). Salvage of patients dying of advanced incurable heart disease is now possible, but application of the procedure is limited and long-term results are hindered by a variety of unsolved problems which arise in selection of suitable recipients, timely acquisition of histocompatible donors, and management of effective immunosuppression without complications. At the Texas Heart Institute we have performed a total of 20 cardiac transplants in 19 patients. Included in this series are: a man who received a second allograft when his first was rejected after 6⅔ months, an infant who received a combined heart-lung transplant, and a man whose circulation was successfully supported by a total mechanical cardiac replacement until a suitable allograft was obtained 64 hours later.

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Indications:

All transplant recipients had irreversible end-stage heart disease refractory to other medical therapy. Coronary artery occlusive disease was the indication for operation in most patients. One patient had severe rheumatic multivalvular disease. A child with endocardial fibroelastosis and myocardiopathy and a man with myocardiopathy also received allografts. Subsequent experience suggests that myocardiopathy may be a contraindication, since the autoimmune factors which destroyed the original heart could react similarly to an allograft (11). The infant who received a combined cardiopulmonary transplant had complete atrioventricularis communis, pulmonary hypertension, pneumonia, atelectasis, and pulmonary infarctions (5). Irreversible cerebral damage (usually due to intracranial hemorrhage, trauma, or tumor) was confirmed in each donor by atonia, fixed and dilated pupils, arreflexia, apnea, and an isoelectric encephalogram (3).

Histocompatibility:

Through a collaborative study with Terasaki (15)

RÉSULTATS :

Cinq décès furent causés par infection. Cinq autres patients sont morts de rejet chronique entre le quatrième et le septième mois après l'opération. Le patient le plus âgé est mort d'une affection rénale et hépatique pré-existante. Le patient le plus jeune, celui qui reçut une transplantation cardio-pulmonaire mourut d'insuffisance pulmonaire après 14 heures. Le décès le plus tardif, du groupe C au point de vue de l'histo-compatibilité, est attribuable à une attaque de la maladie de Adams-Stokes au neuvième mois. Trois patients sont actuellement en vie respectivement à trois jours, trois mois et plus de six mois après la transplantation.

En conclusion, on note une plus longue survie lorsque l'histo-compatibilité est meilleure. La rareté en donneurs ainsi que le degré d'urgence dictés par une dégradation rapide du receveur furent à la base d'une médiocre compatibilité. Une façon d'éviter cet écueil pourrait être l'emploi d'une prothèse cardiaque totale maintenant la vie d'un patient moribond en attendant qu'une allogreffe histo-compatible soit obtenue.

donors and recipients were tissue typed and matched according to ABO red cell compatibility, lymphocyte cross match for preformed antibodies, and lymphocyte antigen match (Terasaki's grading scale: A-F). Our tissue match grades ranged from D to C plus, with a mean grade of C minus. No patients had A or B matches.

Immunosuppressive therapy:

Our immunosuppressive program is modeled after that employed by Starzl *et al.* (12) for renal allografts. Used in conjunction with azathioprine and corticosteroids, antilymphocytic globulin (ALG) has provided better control of rejection while allowing smaller dosages of other drugs.

Results:

Following implantation each cardiac allograft functioned satisfactorily resulting in improved cardiac output. Most patients were ambulatory in the first postoperative week. Three were able to leave the hospital, and two returned to work.

Infection caused five deaths (Table I). One patient died of gram negative bacterial pneumonia and sepsis 54 hours after operation. Sepsis and thrombocytopenia with gastrointestinal hemorrhage resulted in the early death of a woman who had blood cultures of Serratia marcescens, and one man died after nine weeks from a massive herpetic infection with secondary Pseudomonas pneumonia. Two other deaths were due to pneumonia. One of these occurred 32 hours after allografting in a man whose circulation had been maintained for 64 hours by an orthotopic cardiac prosthesis (7 and 8). The mechanical device was inserted when cardiac function failed during myocardial excision with ventriculoplasty. The prosthesis performed efficiently as a

TABLE I

Causes of death in cardiac transplant recipients

CAUSE OF DEATH	NUMBER OF PATIENTS
Infection	5
Chronic rejection	5
Acute rejection	3
Preexisting diseases	1
Pulmonary insufficiency	1
Probable arrhythmias	1
	16

total cardiac replacement until a suitable donor heart was obtained.

Chronic rejection accounted for five deaths (Table I) occurring from four months to nearly seven months after operation. One of these patients underwent re-transplantation when irreversible rejection of his first allograft set in after 6⅔ months. He died nearly three days later. Three patients died of acute rejection (Table I). Two of these had myocardiopathy, and all had grade D tissue matches. The oldest patient (62 years) died of pre-existing renal and hepatic failure. The youngest, a 2-month-old infant who received the heart-lung transplant, died of pulmonary insufficiency after 14 hours (7). The longest survivor, a grade C plus tissue match who had had no significant episodes of rejection, died of a probable Stokes-Adams attack after almost nine months. Three patients are alive today at three days, nearly three months, and more than six months after operation.

Hyperglycemia, cushinoid habitus, osteoporosis, leukopenia, thrombocytopenia, and reduced resistance to infection were among the complications of immunosuppressive therapy.

Discussion:

Reports of renal allografts have shown a strong relationship between histocompatibility and results (13). We have made a similar observation in this series of cardiac allografts, which have consistently displayed longer average survival with less incidence of rejection in higher grade tissue matches (10). Evidently, optimal histocompatibility is a basic requirement for improved results in cardiac transplants; but the emergency condition of potential recipients coupled with the scarcity of donors makes this a difficult requirement to meet. A hopeful solution to the dilemma is offered by the recent clinical success of an orthotopic cardiac prosthesis which sustained the life of a dying recipient until a suitable allograft was obtained (7 and 8).

The knowledge gained from the cardiac transplant program — that a cardiac allograft can support human circulation, and that the denervated allograft responds sluggishly to influences which increase or decrease cardiac output (9) — contributed heavily to the development of the orthotopic cardiac prosthesis as well as stimulating several other areas of research in immunology. These encouraging signs of progress give promise of enhancing the therapeutic value of cardiac transplantation by eventually solving its current problems.

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CLINICAL EXPERIENCE IN CARDIAC TRANSPLANTATION *

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Les auteurs discutent les points saillants de leur expérience avec quatre cas de transplantation cardiaque. Deux patients avaient une maladie coronarienne obstructive importante, les deux autres avaient des cardiomyopathies. Un de ces derniers patients présentait en outre une fibroélastose endocardique touchant principalement le cœur de gauche.

Les quatre patients subirent une exploration complète avant l'opération. L'intervention démontra que les niveaux d'hypertension pulmonaire pré-existants étaient compatibles avec de bons résultats. Il est évident qu'il est difficile d'évaluer le degré de résistance vasculaire pulmonaire fixée en présence d'un débit cardiaque aussi faible que celui qu'on observe chez ces patients. Le diagnostic de mort cérébrale fut établi suivant les critères discutés par le docteur Alksne au cours de ce symposium. Le pronostic après la transplantation cardiaque ne semble pas, dans cette série, en relation étroite avec les résultats de l'histo-compatibilité. Le premier patient, du groupe B, est mort de rejet aigu confirmé histologiquement.

Le second patient également du groupe B a fait trois épisodes de rejet. Chez le troisième patient, des anticorps préformés furent décelés et un rejet aigu l'emporta au quatrième jour. Ces faits démontrèrent qu'il

On the occasion of this Symposium, a review is presented of our experience with four patients undergoing cardiac homotransplantation between May and October of 1968. The details of the preoperative and postoperative findings in these four patients have been previously presented and published (1, 3) and this review will summarize those points which we consider of special importance.

The etiology of the heart disease in the first two patients was extensive three-vessel coronary atherosclerosis resulting in multiple episodes of infarction and fibrosis. The etiology in the latter two cases was unknown and classified as cardiomyopathy; the last patient had, in addition, gross endocardial fibroelastosis involving primarily the left atrium and ventricle. With respect to the significance of etiology in the selection of patients for transplantation and in the prognosis after transplantation, we must undoubtedly conclude that patients listed as "cardiomyopathy" represent a variety of entities which may well have widely varying prognoses after transplantation. Whether such conditions as autoimmune disease, myocarditis, antiheart antibodies resulting from myocardial infarction, rheumatic disease, hyperlipemia or other conditions predisposing to coronary atherosclerosis constitute special risks to the homografted heart remains to be determined as our experience increases.

The preoperative evaluation in all patients included complete cardiac catheterization (Table I), coronary arteriography and left ventriculography. The clinical and laboratory data revealed that each patient had refractory biventricular failure

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peut exister des antigènes d'histo-compatibilité importants ailleurs que sur le lymphocyte et qu'il faudrait employer d'autres cellules pour les dépister.

Le dernier patient, du groupe C, mourut au 18e jour après l'opération.

COMMENT DIAGNOSTIQUER LE REJET AIGU ?

La baisse de voltage du QRS est le signe diagnostique le plus important. L'accumulation du liquide médiastinal dans la phase post-opératoire immédiate peut cependant être responsable d'une baisse de voltage. Pour obvier à ce problème, l'implantation d'électrodes épicardiques est proposée.

Comme autres changements électrocardiographiques significatifs, on note une déviation de l'axe électrique à droite, avec des troubles de conduction atrio-ventriculaire ou intraventriculaire. Ces signes disparaissent après l'immunothérapie.

Le syndrome clinique de rejet cardiaque est d'abord caractérisé par une décompensation cardiaque droite. Un frottement péricardique peut être noté avec ultérieurement un souffle de régurgitation tricuspidienne ou mitrale. Le diamètre transverse du cœur augmente légèrement et les champs pulmonaires sont oligémiques. La congestion pulmonaire représente donc plutôt une infection ou une embolie. Afin de diagnostiquer rapidement un début de rejet, il est suggéré de supprimer la digitale et les diurétiques le plus rapidement possible après l'opération afin de ne pas masquer les premiers signes d'une insuffisance cardiaque droite. Quant aux enzymes, les faits suivants furent notés : LDH-1 présente une élévation assez constante en cas de rejet. L'élévation de LDH-5 peut être interprétée comme un premier signe de toxicité à l'Imuran.

with pulmonary hypertension and a low cardiac index. The last patient with a cardiac index of .99 L/min/M² was moribund when he underwent operation. Despite the severity of the cardiac failure, each patient made an initial good recovery from operation and showed marked improvement in cerebral, hepatic and renal function as well as cardiac performance in the early postoperative period. Thus it was clear that the levels of pulmonary hypertension existing in these cases were compatible with a potentially successful result. The operability or inoperability of patients with pulmonary hypertension should be related to the degree of fixed pulmonary vascular resistance — a measurement which is difficult to establish in the face of such low cardiac outputs. The question of how much increase in pulmonary resistance can be tolerated by the normal right ventricle of the transplant is reminiscent of the older discussions of the role of pulmonary hypertension in mortality

TABLE I

Cardiac transplantation preoperative cardiac catheterization

PATIENT	RA (M)	RV	PA	WEDGE	LV	CARDIAC INDEX
J.P.	1	45/0	50/15	15	120/0-23	1.18
L.R.	18	80/0-20	80/50	27	110/0-40	1.67
P.J.	20	75/0-20	80/44	29	99/0-27	1.62
D.S.	16	60/0-20	60/26	27	103/0-32	.995

following surgery from mitral stenosis. Only considerable experience can provide a valid answer.

In the case of each donor, the diagnosis of brain death was established by an independent team of neurologists and neurosurgeons according to criteria which are further discussed by Dr. Alksne in this Symposium. Although brain trauma and the prolonged intravenous administration of catecholamines have each been recognized as producing myocardial lesions in some patients, we doubt that these constitute a significant threat to the postoperative function of the usual donor heart. All efforts are pointed toward maintaining cardiovascular homeostasis in the donor prior to operation, and the prolonged administration of catecholamines or the occurrence of transient cardiac arrest are common occurrences and do not contraindicate use of the donor heart.

Histocompatibility typing (Table II) was performed prospectively in each case by the method of Terasaki. Our results and that of others suggest that "matching" as currently practiced probably does not bear a very close relationship to prognosis after cardiac transplantation. The first patient was a "B + match" and was the first in the world experience to die of fulminating rejection. The autopsy finding of massive infiltration of mononuclear cells, interstitial edema, hemorrhage and vascular necrosis dispelled any illusions we may have harbored that the human heart would be especially resistant to rejection.

The second patient, also a "B + match" with no detectable mismatch of major antigen groups, has weathered three rejection episodes. Nevertheless he has been completely rehabilitated and is at this moment the world's third longest surviving patient.

The third patient has provided some special insights into the problem of histocompatibility matching. While the preoperative lymphocyte-serum crossmatch revealed no evidence of preformed antibodies, subsequent analysis (3) of the preoperative serum by immune adherence revealed strong evidence that antibodies against cultured kidney cells of the heart donor existed in significant titer in the preoperative serum. The antibodies disappeared from the postoperative serum and were eluted in significant titer from the heart postmortem. The patient's clinical course strongly confirmed an element of previous sensitization, as fulminating rejection was manifested by the fourth day and inexorably progressed despite massive

TABLE II

Histocompatibility typing (lymphocytotoxicity)
MCV heart transplants

	8A	4A	4B	4C	7C	7D	LA1	LA3	MATCH
1. Recipient	—	+	+	+	—	—	—	—	
Donor	—	+	±	—	—	—	—	—	B +
				8% of sera mismatched					
2. Recipient	+	—	—	—	—	—	—	+	
Donor	—	±	—	—	—	—	—	—	B +
				6% of sera mitmatched					
3. Recipient	+	—	±	+	—	—	—	—	
Donor	±	±	±	+	—	—	—	—	
				9% of sera mismatched					
4. Recipient	±	±	±	+	±	+	+	—	
Donor	±	—	±	—	+	—	—	+	C
				9% of sera mismatched					

(Negative lymphocyte serum cross match in each case)

immunosuppressive therapy. Such findings indicate that some important histocompatibility antigens may not be expressed on the lymphocyte and require the use of other target cells for their detection if we are to achieve more precise matching and avoid some cases of prior sensitization.

The fourth patient, a "C match", made an initial good recovery from operation, had a rejection episode at the end of one week which was clinically reversed, but again showed evidence of severe rejection at the end of two weeks which became refractory to massive immunosuppressive therapy. The histology in this patient who died after eighteen days, was of particular interest in that there was extensive interstitial hemorrhage, edema and vascular damage typical of acute rejection, but almost complete absence of mononuclear cell infiltration in the myocardium. The leukocytes scattered through the myocardium were predominantly polys. Again antibody was eluted in significant titer from the myocardium postmortem.

The diagnosis of acute rejection:

Coincident with this unexpectedly high incidence of acute rejection we have formulated certain impressions with regard to the diagnosis of acute cardiac rejection. Many of these mirror the conclusions reached in the laboratory during the past ten years in the observation of more than 200 dogs which received orthotopic cardiac homografts (1, 4 and 5).

There seems little doubt that the electrocardiogram remains the most sensitive and specific examination for the detection of early threatened rejection, not only in the early postoperative weeks, but even months after transplantation. We conclude from our own observations and from those of others, as well as from the extensive animal work that a decrease in QRS voltage is an invariable accompaniment of acute myocardial rejection and is the most important single diagnostic criterion. Because of the possibility in the early postoperative weeks of non-specific fluid accumulations which might also depress voltage, diagnostic accuracy may be insured by the use of an implanted wire electrode to obtain tracings directly from the myocardium (6) or alternately, placing radio opaque markers on the epicardium to help in excluding pericardial effusion.

Other ECG changes which are of significant value in the diagnosis of rejection include an abrupt rightward shift in the mean frontal plane axis of the QRS, abnormal atrioventricular or intraventricular conduction and atrial arrhythmias. The decline in voltage and right axis shift occurred in each of seven rejection episodes observed in our patients. In the surviving patient, right bundle branch block was an additional finding in three rejection episodes at one week, three months and seven months. On each occasion the changes reverted to normal with immunosuppressive therapy. The vectocardiogram has also proven to be a sensitive and graphic means of demonstrating the changes in voltage during acute rejection.

The clinical syndrome of cardiac rejection is characterized initially by a predominance of right heart failure. The patient notes fatigue rather than dyspnea. There is incipient weight gain which may progress to clinically detectable edema. Venous pulsations become visible and ultimately the venous pressure elevated. Auscultation reveals, in addition to the frequent but somewhat nonspecific pericardial rub, the more important finding of a right ventricular diastolic gallop sound. Later in rejection the regurgitant murmur of slight tricuspid or mitral insufficiency can also be heard. Corresponding to predominant signs of right ventricular failure is the X-ray finding of a minimal increase in transverse cardiac diameter and lung fields which appear almost oligemic rather than congested. Thus when pulmonary infiltrates appear in the postoperative period there is a much greater likelihood that they represent infection or embolism rather than rejection. Fluoroscopy during acute rejection will generally show diminished amplitude and asynchrony of ventricular pulsations.

Because of the diagnostic importance of incipient right heart failure in the early detection of threatened rejection, it is strongly suggested that digitalis and diuretics be withdrawn from patients as

soon as practical after operation to minimize masking of this important early sign. When detected at a sufficiently early stage, threatened rejection should generally be reversible by appropriate increases in immunosuppressive therapy as demonstrated some years ago by prolonged survival in dogs with transplanted hearts (4) and confirmed more recently by repeated myocardial biopsies in dogs (7) and by the clinical observations of the many members of this Symposium.

Much attention has been given to evaluation of the serum enzymes in the diagnosis of early rejection — particularly the isozymes of LDH. I will only summarize our conclusions in this regard drawn from the clinical cases and from the animal studies. With a severe rejection crisis there is often a rise in LDH-I. However, typical ECG changes of rejection in such instances precede the enzymes changes. Moreover, one may see the typical ECG and clinical manifestations of acute rejection without any elevation in the serum enzymes. Elevations of the CPK or SGOT generally imply significant myocardial damage and may be considered an ominous sign in a severe rejection crisis. Serial determinations of the LDH isozymes may prove to be of further value in the detection of early imuran toxicity by a rise in Band 5.

The role of humoral antibodies in the acute rejection crisis is not yet clearly established. The hope that detection of serum antibodies might provide a useful means of monitoring the immunologic status of the graft has not yet materialized, undoubtedly because the graft too rapidly sponges up the antibodies. In the three patients dying of acute rejection in this series, antibody was eluted in significant titer from the heart muscle in each case postmortem, although antibody was not detectable in the postmortem serum or other tissues. Immunofluorescence of the myocardium revealed only a diffuse staining and failed to show localization of the antibody (3).

The most ominous threat to the prolonged survival and good function of the cardiac homograft is the development of the coronary arterial lesions of chronic rejection. These were reported (8) in long-surviving dogs with heart homografts before any clinical trials were undertaken and have been subsequently reported with alarming frequency in autopsies performed after human cardiac transplantation. The lesions appear to represent a manifestation of chronic endothelial injury resulting in platelet agglutination with subsequent formation and organization of thrombus. Such lesions result in mild to marked focal areas of narrowing of the vascular lumens ultimately resulting in significant areas of myocardial ischemia. It is hoped that this Symposium will address itself to the task of determining those factors which are associated with the high risk of chronic rejection and those associated with relative freedom from this condition. Insofar as acute rejection is concerned, the important aim is early detection and prompt treatment, whereas with chronic rejection it must be prevention.

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THREE CASES OF ALLOGENIC HEART GRAFTS IN HUMAN *

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TROIS CAS DE GREFFE CARDIAQUE

Les trois patients présentaient une insuffisance coronarienne sévère et avaient souffert de plusieurs épisodes d'infarctus myocardique. De multiples lésions athéromateuses furent mises en évidence par coronarographie sélective chez deux des trois patients. En plus, un cinéangiogramme gauche a démontré une stagnation du colorant dans le ventricule gauche, ainsi qu'une éjection systolique diminuée.

Histocompatibilité :

Quatorze antigènes leucocytaires furent testés chez les receveurs et les donneurs, suivant les techniques et la nomenclature décrites initialement par J. Dausset.

Dans le premier cas, huit antigènes furent trouvés identiques, trois compatibles et deux incompatibles.

Dans le second cas, on trouva également huit antigènes identiques, un compatible et deux incompatibles.

Dans le troisième cas, neuf antigènes furent trouvés identiques, deux compatibles et deux incompatibles. (suite du résumé en page suivante)

Cardiac transplantation has entered its clinical phase since more than two years; the value of this procedure, as a palliation in curable heart diseases, was ascertained by several cases of long term survival (1, 2, 6, 7 and 8). However, this method, for the present, may not be considered, as discharged from the period of experimental and clinical research. Numerous problems remain to be solved concerning the physiology and the pathology of the grafted heart, as well as the early diagnosis of the rejection episodes, and more efficient and selective immunodepression.

CLINICAL DATA

The three patients who received a cardiac allograft were males aged 57, 48 and 56. All of them were suffering from a severe coronary insufficiency; all had several attacks of myocardial infarction and several episodes of left ventricular failure with pulmonary edema; two of them had rest and exertion angina, with a very high daily consumption of trinitrin, two of them had several episodes of congestive heart failure.

In three cases, ECG demonstrated definite sequelae of extended myocardial infarction, associated with ischemic chronic changes. X-rays of the chest showed in case 1 an almost normal heart size; on the other hand, a marked cardiomegaly was observed in cases 2 and 3; moreover, in the three cases fluoroscopic examination of the heart demonstrated a significant lowering of the ventricular kinetics.

Two of the patients were undergoing a selective coronarography; in both cases, coronarography was showing diffuse and severe atherosclerotic changes in the three main coronary arteries, but predominant in the left coronary tree. Left cineangiogram was also performed, and demonstrated a very

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Technique chirurgicale :

Au moyen d'un oxygénateur à bulles jetable, amorcé d'une solution de lactate Ringer, la température œsophagienne du cadavre est descendue à 20° C. Les veines caves sont alors clampées ainsi que l'aorte et la perfusion isolée du cœur amène ce dernier à 10° C. Le cœur est excisé suivant la technique de Barnard. Dans le premier cas, le cœur fut perfusé de sang froid durant toute la durée de la réimplantation. Dans les deux autres cas, aucune perfusion cardiaque ne fut pratiquée. Dans les trois cas, le cœur greffé reprit un rythme sinusal immédiatement après la ressuscitation.

Évolution postopératoire :

Les soins postopératoires furent prodigués dans un environnement rigoureusement aseptique, à cause du risque accru d'infections en présence de doses élevées d'immunosuppresseurs. De plus, on a rapporté certaines interférences entre les antigènes bactériens et les antigènes de transplantation. Toute inoculation septique pourrait donc stimuler les mécanismes de rejet.

Par ailleurs, un environnement stérile du patient permet la suppression d'une antibiothérapie non spécifique et prophylactique. De par cette suppression précoce d'antibiotiques, on évite la sélection d'une population

poor ejection stroke volume in the left ventricle and marked stagnation of the dye. Significant decrease of the cardiac output of the left systolic volume, systolic index, and left ventricle ejection time was appreciated in each case by the mean of cardio-green dilution curves.

In case 2, a scanning of the lungs was done, for dismissing the diagnosis of chronic pulmonary embolism.

In Table I are summarized the main clinical data concerning the recipients and the donors.

Several weeks before the procedure, the three patients were clearly informed of the risks incured by a still hazardous method; they came themselves to the operative decision.

HISTOCOMPATIBILITY

Fourteen leukocyte antigens have been tested in recipients and prospective donors according to the technics and nomenclature described initially by J. Dausset.

TABLE I

Histocompatibility matching in 3 cases of cardiac allogenic graft. Leukocyte antigents are determined in the Dausset's nomenclature. (? : identification of the antigents was questionnable).

	Da 1	Da 2	Da 3	Da 4	Da 5	Da 6	Da 7	Da 8	Da 10	Da 11	Da 12	Da 14	Da 16	Da 17
Recipient Bo.	—	+	+	+	—	—	+	—	—	—	+	+	+	?
Donor Ga.	—	—	+	+	+	+	—	—	—	—	+	+	—	?
Recipient Fo.	+	—	+	—	—	—	+	—	—	+	—	?	—	—
Donor Fr.	—	—	+	—	?	—	+	+	—	+	+	?	—	?
Recipient Ma.	—	—	—	—	—	—	+	—	—	+	—	?	—	—
Donor Du.	—	—	—	+	—	—	—	—	—	—	+	?	—	—

de germes endogènes résistants, ainsi que la prolifération d'agents mycotiques ou viraux.

Le régime immunosuppressif est ensuite décrit en détail.

Résultats :

Le troisième patient décéda environ 20 heures après l'intervention. Le décès semble être attribuable à une embolie gazeuse. À l'autopsie, aucun signe de rejet aigu ne fut noté.

Les deux autres patients sont en vie, respectivement 17 et 10 mois après l'opération.

Dans le cas du deuxième patient, par opposition au premier, aucun épisode de rejet aigu ne fut observé. Cependant, plusieurs données indiquent l'existence d'un processus de rejection chronique : il existe des troubles de la repolarisation, avec déplacement vers le bas du segment ST dans les dérivations précordiales, suggérant une ischémie myocardique chronique.

L'étude des enzymes sériques a démontré à plusieurs reprises des élévations des enzymes d'origine myocardique, à savoir la phosphocréatokinase.

Malgré un état clinique satisfaisant, la thérapie immunosuppressive fut augmentée à plusieurs reprises, afin de contrôler l'évolution chronique du phénomène de rejet.

The present properties of current immunodepressor means did not influence us in favour of slighting histocompatibility between recipient and donors. Therefore, no cardiac transplantation was deliberately planned outside a satisfactory leukocyte matching. Because of this delicate choice, some of the potential recipients on the waiting list for a cardiac allograft died, due to a lack of a suitable donor.

Table II summarize the leukocyte antigens tested in the three recipients and the three donors used; the nomenclature used is the Dausset (Da) system.

In case 1, identities were found in eight antigens (Da 1, Da 3, Da 4, Da 8, Da 10, Da 11, Da 12, Da 14); compatibilities in three antigens (Da 2, Da 7, Da 16); incompatibilities in two antigens (Da 5, Da 6). In case 2, identities were present in eight antigens (Da 2, Da 3, Da 4, Da 6, Da 7, Da 10, Da 11, Da 16); compatibilities in one (Da1); incompatibilities in two (Da 8, Da 12). In case 3, identities were present in nine antigens (Da 1, Da 2, Da 3, Da 5, Da 6, Da 8, Da 10, Da 16, Da 17); compatibilities in two (Da 7, Da 11); incompatibilities in two (Da 4, Da 12).

SURGICAL TECHNICS

The procedure of excising the cardiac transplant was the same in the three cases. Immediately after stopping the mechanical artificial breathing, the donor's body was perfused by the mean of a cardio-pulmonary bypass established between the right atrium and the ascending aorta. (Disposable plastic-bag Rygg oxygenator primed with Ringer-lactate solution). The esophagal temperature of the cadaver was lowered at 20° C; then the venæ cavæ and the aorta were clamped, and perfusion of the isolated heart was continued by the mean of the aortic cannula, until the myocardial temperature dropped around 10° C. Then the heart was excised following the technic described by Barnard (1).

In case 1, the transplant was perfused with cool blood through the aortic cannula during the whole re-implantation procedure.

In cases 2 and 3, no organ perfusion was done; the sole hypothermia was able to preserve the myocardium during the ischemic period.

The way of cutting the right atrium on the donor's heart was judged of great importance, in order to avoid the zone of the sinus node or the atrio-ventricular conduction pathways.

In this regard, incision was made vertically, from the I.V.C. to the S.V.C., parallel and close to the inter-atrial grove. The incision described by Cooley (7), from the I.V.C. to the apex of right appendage, was not used.

In the three cases, the grafted heart beated in sinus rhythm immediately after ressuscitation; in two instances, no electrical shock was necessary, and the heart resumed spontaneously his normal activity as soon as the clamps were released.

POST-OPERATIVE COURSE

Post-operative cares were managed in aseptic conditions, using a sterile unit especially built for this purpose and placed under continuous bacteriological supervision.

Such sterile conditions are probably a great benefit after cardiac transplantation. The reasons are:

1. The septic risk commonly observed after cardiac open surgery is substantially increased by the use of maximal doses of immunodepressor agents;

2. Interferences between bacterial antigens and transplantation antigens have been reported; any septic inoculation should stimulate the graft rejection mechanisms (9).

3. Sterile environment of the grafted patient allows the early suspension of non-specific antibiotic

TABLE II

Summarizing the main clinical data in recipients and donors in three cases of cardiac transplantation

CASES	DAYS OF TRANSPLANTATION	AGE	SEX	WEIGHT	ABO gr.	CLINICS
Recipient Bo.	5.12.1968	57	M.	63	A +	— Coronary insufficiency — Angina pectoris at rest — 3 myocardial infarctions — Atherosclerotic, obstructions in the 3 main coronary arteries on coronarography
Donor Ga.		38	M.	62	A +	— Massive spontaneous cerebral haemorrhage on the 5.7.1968
Recipient Fo.	24.11.1968	48	M.	55	A +	— Coronary insufficiency — Angina at rest — 2 myocardial infarctions — Cardiac failure — Triple atherosclerotic coronary disease
Donor Fr.		46	M.	65	A +	— Massive spontaneous cerebal haemorrhage
Recipient Ma.	26.11.1968	56	M.	96	B +	— Coronary insufficiency — 2 myocardial infarctions — Angina at rest — Cardiac failure
Donor Du.		33	M.	60	O +	— Cranio-cerebral trauma — Fracture of parietal bone with meningo-cerebral injury

prophylaxy; and thus, avoiding the selection of endogenous resistant population of germs, and the facilation of fungic or viral infestations.

Along the three first post-operative days, the patients received intravenously a solution of Isopropylnorepinephrine (Isuprel 1,2 mg in 250 ml of isotonic glucose). The immunosuppressive therapy involved:

1. Azathioprine (Imuran) 250 mg on the morning before the transplantation and then 3 mg/kg daily;
2. Prednisone: 200 to 300 mg intravenously on the first postoperative day, and then 1 mg/kg orally;
3. Antilymphocyte globulin (ALG).

An antilymphocyte IgG was prepared and purified by Choay Laboratories[1]. The treatment with ALG started on the day before the transplantation at the dose of 10 ml subcutaneously; the daily dose was then 20 ml during the first post-operative days. A horse ALG was used in the cases 1 and 3; in case 2, the patient became intolerant to the horse proteins, and developed fever and hypotension after injections of ALG; thus, a sheep ALG was prepared and used without any other intolerance symptoms.

Details concerning management of long terms survivors after cardiac allograft, the schedule of the controls, and the increasing detection of the early rejections signs are reported in another issue (3).

RESULTS

One patient (case 3) died on the first post-operative day, by a sudden cardiac arrest on the 20th hour after the procedure. The early post-operative cause had been uneventful: the patient was awake and soon conscious in the operating theater, E.C.G. was in sinus rhythm, arterial pressure and urinary flow were normal. However, routine recording of the E.E.G. had shown a transient and unilateral drop of the cerebral activity, during the heart's ressuscitation, which was interpreted as a gas embolism of slight degree; as a matter of fact, several clonic attacks occured on the 10th post-operative hour, resulting in the left brachial monoplegia.

Despite the spontaneous maintenance of consciensness and satisfactory A.P. levels, the patient was maintained under mechanical ventilatory assistance with tracheal intubation; suddenly, the E.C.G. tracing became isoelectric. The attempts for ressuscitation of the heart remained uneffective.

Microscopic examination of the grafted heart showed histological changes compatible with prolonged cardiac massage, but no patterns suggestive of an acute early rejection process.

Two other grafted patients are still alive respectively 17 and 10 months postoperatively[2]. In the two patients, cardiac sounds and peripheral arterial pressure are normal, pulsation rate is around 100 per mn, E.C.G. is almost normal, X-rays evaluation of the cardiac volume is normal, the apicogram and the systolic ejection time of the left ventricule measured from the indirect carotid pulse tracing are normal.

E.C.G. data concerning the two patients are reported in another paper (4). Several episodes of rejection have been early detected in patient n° 1; data about the rejection signs observed and the emergency treatment applicated to them are described in another publication (3).

In patient n° 2, no definite acute episode of rejection was so far encountered. However, several clues support the hypothesis of a latent chronic rejection process, *i.e.*:

- troubles in the electrical repolarization of the heart, with low shift of the ST segment in the precordial loads, suggestive of a chronic myocardial ischæmia;
- repeated peaks in seric enzymes levels of a myocardial origin, namely phosphocreatokinase (PCK) (5).

1. Laboratoires Choay, 48 ave. Th.-Gauthier, 75, Paris 16e, France.

2. Since this paper was submitted, one patient (case 1) died from a sudden cardiac arrest.

Despite a satisfactory clinical condition of the patient, in several instances the long term immunosuppressive therapy was increased, in an attempt to check the chronic evolutility of the rejection phenomenon.

SUMMARY AND CONCLUSIONS

Three cases of allogenic cardiac graft in humans are reported. The grafted patients were in the three instances males in the fifth or sixth decade of life; all of them were suffering from severe atherosclerotic coronary insufficiency, resulting in several attacks of myocardial infarction and in several episodes of cardiac failure.

Among the three patients operated upon, there was one operative death, from a sudden cardiac arrest, without any pattern suggestive of an acute rejection process.

Two patients are alive and in good clinical condition respectively 17 and 10 months after receiving the cardiac graft.

At least, two episodes of acute rejection have been observed in the first patient; early detection of the first signs of rejection and prompt and intensive immunosuppression have made the crisis reversible and healing without evidence of sequelae. In the second patient, no acute crisis of rejection was so far observed, but several symptoms consistant with a chronic evolutivity of rejection.

The satisfactory results observed in the two long-term survivors may be imputable to:

1. careful study of leukocytes compatibilities between donors and recipients, involving 14 antigens in the Dausset system;

2. adequate prophylaxy of the septic risk using a sterile post-operative unit under bacteriological control and thus avoiding the use of non specific antibiotics;

3. flexible immunodepression using an active heterologous ALG.

4. accurate and continuous detection of the early signs of rejection.

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EXPERIENCE OF THE MONTREAL HEART INSTITUTE *

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Les auteurs présentent neuf cas de transplantation cardiaque effectués du 31 mai au 29 novembre 1968. La méthode de protection du cœur greffé qu'ils décrivent est d'abord une perfusion hypothermique totale du donneur, ajoutant un refroidissement sélectif du cœur par irrigation au soluté salin froid. Après une période d'anoxie de l'ordre de 50 minutes, la racine de l'aorte est perfusée pendant 12 à 36 minutes, au cours de l'anastomose de l'artère pulmonaire, et l'anastomose de l'aorte est faite au cours d'une seconde période d'anoxie de 8 à 20 minutes. La protection myocardique ainsi obtenue permet une excellente fonction du cœur greffé sans l'aide d'aucun vasopresseur.

Les auteurs croient que deux antigènes incompatibles sont le maximum qui devrait être accepté, que l'électrocardiogramme quotidien est le meilleur indicateur du rejet, et que le traitement de la crise de rejet doit être précoce, vigoureux, mais préférablement de courte durée.

(suite du résumé en page suivante)

From May 31st to November 29, 1968, nine patients underwent total cardiac transplantation at the Montreal Heart Institute. The first patient died 42 hours after operation from low output failure which necessitated repeated and prolonged periods of assisted circulation with the pump-oxygenator and this resulted in an hemorrhagic diathesis with bleeding in the lungs and kidneys. He was conscious until shortly before his demise.

The transplanted heart was small and of poor quality, having suffered many anoxic episodes during the terminal illness of the donor, and it was unable to take over the full load of the circulation despite numerous cardiac drugs including calcium chloride, digitalis, isoproterenol and epinephrine. This heart was removed at normal temperature, immersed in cold saline for a few minutes and sutured in place, the longest period of anoxia being 38 minutes. After suture of the atria, the aortic root was perfused for nineteen minutes at normal temperature while the pulmonary artery suture line was completed, and a second period of anoxia of 16 minutes occurred during the aortic anastomosis.

Following this experience, our technique of protection of the donor heart was changed. All further patients received hearts that had been protected by hypothermic total body perfusion of the donor and cold saline irrigation of the heart until it felt cold to palpation, and had reached a temperature of approximately 18° C., a technique with which we were familiar, having used it in most of our open-heart cases for the previous three years. Again, after the atrial suture lines were completed, the heart was perfused through a canula inserted in the aortic stump (Figure 1) at a flow of 300 to 600 ml per minute at a temperature of 30° C for a period ranging from 12 to 36 minutes. The aortic suture was done during a further period of anoxia varying from 8 to 20 minutes. Upon release of the aortic clamp, vigorous ventricular fibrillation ensued in all patients, three of them reverting spontaneously to sinus rhythm, the others being easily defibrillated by electrical countershock. The bypass was discontinued while transfusing the patient from the pump to obtain a measured left atrial

* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6–8, 1969.

Ils hésitent à utiliser de fortes doses de Prednisone en même temps que la globuline antilymphocytaire, croyant que peut-être une corticothérapie poussée nuise à l'action de la G.A.L.

Ils croient que la radiothérapie cardiaque locale peut aider à prévenir le rejet précoce, qui survient de façon constante vers la fin de la première semaine.

Ils affirment enfin qu'il faudra trouver une façon pratique de doser l'activité immunosuppressive de la G.A.L., de façon à pouvoir diminuer rapidement l'administration des corticostéroïdes et ainsi diminuer leurs effets nocifs à long terme.

pressure of between 15 and 20 mm Hg. All those hearts maintained an excellent aortic pressure throughout the operation, at a heart rate varying between 60 and 120/minute. A single patient had a heart rate initially as fast as 140/minute. No episodes of hypotension occurred in any of the patients during the remainder of the operation, and no vasopressor drugs were needed either (Table I). The last patient received an isoproterenol drip to increase the heart rate which, by the end of the operation, had dropped to 55/minute. He was also intermittently stimulated by a pacemaker through a Teflon covered wire attached to the right ventricle at the time of operation. This wire has also been used to obtain an epicardial electrocardiogram which can be very useful in the diagnosis of early rejection, at a time when limb leads tracings are affected by the early local postoperative changes.

All of those patients recovered promptly and the early postoperative course was uneventful, with excellent cardiac performance. Indeed, one patient needed reoperation 36 hours after transplantation for a thrombosed aneurysm of the abdominal aorta

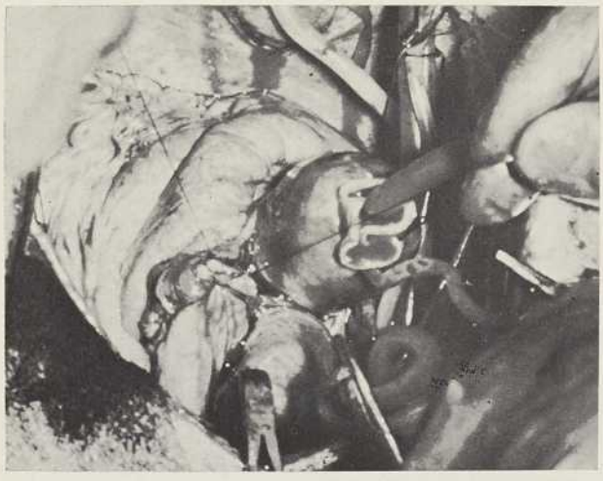

Figure 1 — Perfusion of aortic root during pulmonary artery anastomosis. View from head of table, showing the aortic root distended by blood perfusing the cononary arteries. The canula is connected to the arterial line through a standard coronary perfusion pump. Access to the pulmonary anastomosis, which here is almost completed, is not hindered by the perfusion. The clamp in foreground, is on the transsected aorta of the recipient.

TABLE I

Performance of transplanted heart, early postoperative. Blood pressure and heart rate at the end of surgery and during the first twenty-four hours. None of the last eight patients had to receive vasopressor drugs. The last patient received Isoproterenol for bradycardia, due to junctional rhythm.

RECIPIENTS - EARLY P.O. PERIOD

	B.P. + H.R. end of surgery		B.P. + H.R. first 24 hrs		Well oriented (hrs P.O.)	Extubation (hrs P.O.)	Ambulation (hrs P.O.)
I	90/60	100	intermitt. partial by-pass				
II	110/60	100	130 - 90/70	90	End of surgery	14 1/2	21
III	110/80	80	150 - 100/80 - 90	90	11 hrs	15 1/2	(aorto-iliac graft)
IV	130/80	90	130 - 90/60 - 80	85	7 1/2 hrs	12	32
V	115/70	90	115 - 100/60 - 70	75	End of surgery	7 3/4	30
VI	110/70	120	130 - 100/60 - 70	100	End of surgery	7 1/2	7 1/2
VII	120/90	110	140 - 100/70	70	18 hrs	5 1/2	24
VIII	110/60	95	130 - 90/60 - 80	90	End of surgery	6 3/4	6 3/4
IX	110/55	55	105 - 130/70 - 80	70	End of surgery	10 1/4	10 1/4

and both iliac arteries, which was treated by resection and bilateral bypass graft. During the five-hour operation (Figure 2), his new heart maintained a very stable blood pressure and the pulse rate did not vary more than 20 beats/minute from the usual steady rate of 80 to 90/minute. He recovered well from this second operation, but died on the eleventh day from a cerebrovascular accident. Severe acute rejection of the heart was found at postmortem examination. All seven other patients recovered very well and had a remarkably benign postoperative course.

Immunosuppressive therapy consisting of Azathioprine and Prednisone was used in all patients. All patients also received antilymphocyte globulin from the day of operation, except case 2, who was allergic to horse serum. Bovine A.L.G. was prepared for him and started during the seventh week. He received instead 500 roentgens of deep X-Ray therapy to the heart in five divided doses during the first six days. This patient was our longest survival. Azathioprine was given at approximately 6 mg per kg for the first 48 hours, and gradually lowered to 2 to 3 mg per kg in order to maintain a platelet count above 150 000 and a white blood count in the vicinity of 5 000 per cubic mm. Prednisone was given at a dose of 4 mg per kg initially and was gradually decreased so as to reach 2 mg per kg at two weeks and 0.5 mg per kg at one month in the early cases. In the last case, the plan was to reach 0.5 mg per kg at two months. However, these dosages had to be readjusted frequently according to the clinical status of the patient and the actual or suspected rejection episodes. Antilymphocyte globulin, prepared by the Institute of Microbiology of the Université de Montréal, was given at 500 mg daily for two weeks, 250 mg daily for a further two weeks, and then, the frequency of the injections was gradually decreased so that, by the third month, the patients were receiving 250 mg every three days. In the last patient, the decrease was less rapid and he was receiving 250 mg every other day by the third month. In addition, human gamma globulin was given periodically to all patients to try and prevent viral infections.

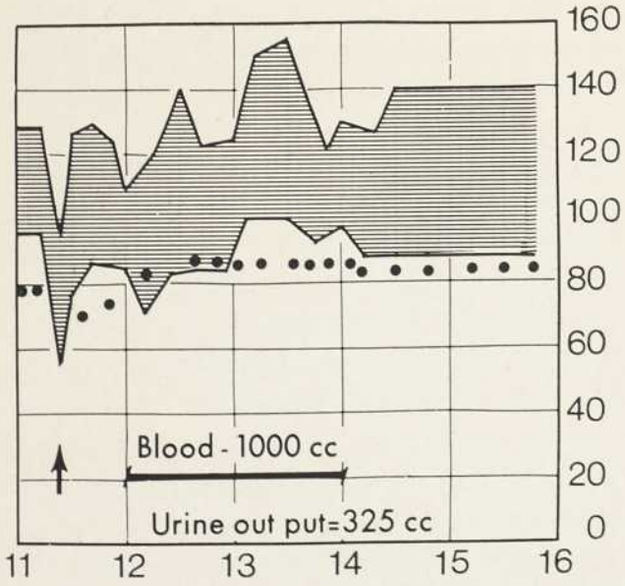

Figure 2 — Aneurysmectomy and aorto-femoral graft 33 hours post-transplantation. Pulse and blood pressure during resection of thrombosed aortic and iliac aneurysms, and bilateral aorto-femoral Dacron graft in patient No 3, 33 hours after heart transplantation. Blood pressure drop to 90 mm/Hg after tracheal intubation responded well to Vasoxyl, 2 mg i.v. (Arrow).

Despite the immunosuppressive therapy, six of the eight patients who survived operation experienced a mild rejection episode between the fifth and the seventh day, but it subsided without increasing the immunosuppressive therapy. Of the seven patients that survived more than one month, three died of acute rejection on the 38, 47 and 64th day, respectively, in spite of very intensive treatment including large doses of hydrocortisone, local X-Ray therapy, actinomycin and increased doses of A.L.G. and Prednisone. Three other patients died as a result of infection. One at 68 days from the consequences of a severe intercostal "herpes zoster" followed by herpes viremia, and two from pulmonary infection at 106 and 120 days. The longest survivor, the second patient, died from aspiration of vomitus 156 days postoperatively. Generalized osteoporosis and a compression fracture of the first lumbar vertebra resulting from corticosteroid therapy undoubtedly contributed to this episode. He also presented mild evidence of rejection in the last few days before his death.

Leucocyte typing had been done preoperatively on all patients by the "leuco-agglutination" method, and the length of survival was clearly related to the match (Figure 3). The four patients who had a B match, with only one or no incompatible antigens, had the longest survival, all over three months, the one exception being patient No. 8, where an antinuclear factor was demonstrated in the serum before transplantation. The three patients with C match, with two or three incompatible antigens, survived an average of two months, whereas the single patient with a D match, with more than three incompatible antigens, survived only eleven days and had acute and severe rejection at postmortem examination (Table II).

In conclusion, we believe that the method of protection of the donor heart that we have described, consisting of total body hypothermic perfusion of the donor, with added selective cooling of the heart with cold saline, and perfusion of the aortic root for a short period after approximately 50 minutes of anoxia, is a reasonable compromise and provides a good protection consistant with excellent cardiac performance in the critical period immediately post-transplantation.

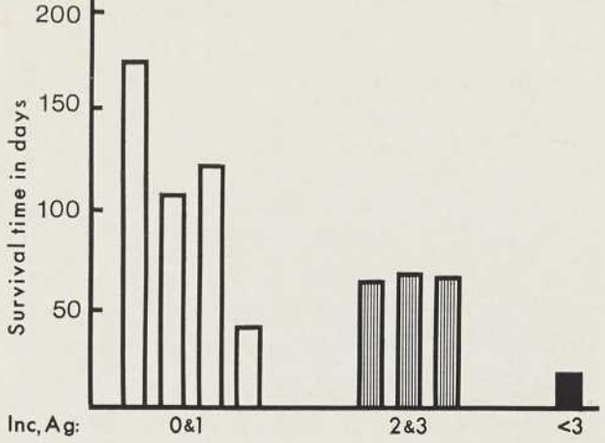

Figure 3 — Histocompatibility is clearly related to the number of incompatible antigens, and the resulting match beween donor and recipient.

TABLE II

Histocompatibility in nine cardiac transplantations. Incompatible antigens (Van Rood classification), in our nine cases. The darker columns indicate the so-called major antigens. A weakly positive direct cross-match does not appear to influence survival.

HEART TRANSPLANTATION - M.H.I.

Tissue Compatibility - Results

	X Match	HISTOCOMPATIBILITY													SURVIVAL - days -
		4A	4B	5B	5B'	6B	7A	7A'	7B	7C	8A	UNKNOWN			
1-A.M.	Neg.	+?	+?			+						+			+	2
2-G.P.	+ (weak)					+										156
3-E.Z.	Neg				+			+			*					10
4-E.D.	+ (¾)			+			+						+			67
5-R.B.**	Neg.						+									120
6-Y.T.	Neg.				+					+						47
7-L.S.	Neg.		+	+					+				+	+		64
8-A.M.**	Neg.				+											38
9-G.L.	Neg.															106

* Anti heart Antibody
** Antinuclear Factor

We believe that a good match between donor and recipient is a prerequisite, and that we should certainly not accept more than two incompatible antigens, and preferably less in the future. We also believe that the routine serial electrocardiogram is the best single indication of early rejection, and that the treatment of the rejection crisis, in order to be successful, should start early, be vigorous, but not necessarily prolonged.

We wonder if one should associate large doses of Prednisone with administration of A.L.G. since heavy doses of corticosteroids probably interfere with the action of A.L.G.

We also believe that early local cardiac X-Ray therapy might have a beneficial effect on the prevention of the early rejection episode, which is quite constant in our experience.

It is imperative that a test be found to ascertain the efficacy of the antilymphocyte globulin used so that, with good matching and Azathioprine, the corticosteroids might be decreased quite rapidly and drastically, because heavy steroid therapy seems to be the major cause of death next to rejection following cardiac transplantation.

EXPERIENCE ON THREE CASES OF HUMAN HEART TRANSPLANTATION *

E. J. ZERBINI and Luiz V. DÉCOURT,
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Le premier cas souffrant de cardiomyopathie chronique décéda d'embolie pulmonaire 28 jours après l'opération. Le second cas présentait de l'athérosclérose coronarienne, de la fibrose myocardique et une sténose aortique calcifiée. Ce patient est encore vivant neuf mois après la transplantation. Le troisième patient présentait également une maladie coronarienne obstructive avec fibrose myocardique et mourut 83 jours après l'opération par suite d'infection.

Les auteurs estiment qu'il est important de drainer le canal thoracique afin de diminuer le nombre de petits lymphocytes et le taux des gamma-globulines. Les effets de ce drainage se prolongent pendant environ 30 jours. Ces données sont basées sur notre expérience de 40 cas de transplantation rénale où le nombre des crises de rejet fut réduit après le drainage. Lorsqu'une crise de rejet survenait, elle arrivait plus tard dans le cours de l'évolution postopératoire et, en général, elle était de moindre importance. Ce drainage du canal thoracique fut effectué chez le deuxième patient. Dans les deux autres cas il fut impossible pour des raisons anatomiques. Les auteurs estiment également que la différence de poids
(suite du résumé en page suivante)

Three human heart transplants were performed in our institution between May 26th, 1968 and January 6th, 1969. The first patient had chronic cardiomyopathy; after transplantation, the immunosuppressive therapy was not efficient enough to control the rejection process. ALG was then not available for this case. Death occurred 28 days after surgery, when the rejection crisis seemed to be under control, and was caused by pulmonary embolism. The second case presented coronary atherosclerosis, myocardiofibrosis and calcific aortic stenosis and insufficiency; the postoperative course is excellent up till now, nine months after transplantation. The third patient, suffering from atherosclerosis, myocardiofibrosis and diabetes, had a satisfactory clinical course from the heart transplantation standpoint, expiring 83 days after surgery in consequence of infection.

In our opinion, previous drainage of the thoracic duct is a worthwhile preliminary step in heart transplantation, in order to deplet the small lymphocytes and gamma-globulins. These advantages are maintained for approximately 30 days after drainage. The experience on kidney transplantation performed in a series of 40 cases in our hospital (Prof. G. Campos Freire) demonstrated that patients with previous thoracic duct drainage presented fewer rejection crisis. When these crisis occurred, they appeared later in the postoperative course and were milder.

Thoracic duct drainage was possible in Case II, a survival of 9 months in good clinical condition.

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entre le donneur et le receveur et par conséquent entre les deux cœurs peut devenir un facteur important surtout dans la phase postopératoire immédiate où une fonction cardiaque optimale est désirable. Au point de vue de l'histo-compatibilité, les trois patients présentaient une incompatibilité majeure et une ou deux incompatibilités mineures.

Au point de vue clinique, la tachycardie était le premier signe de la décompensation cardiaque précédant le rejet. Ultérieurement on nota une diminution du pouls et une augmentation de la pression veineuse. À la radiographie, le cœur était augmenté de volume. La variation du nombre de leucocytes ne présentait pas de relation directe avec le rejet. Il en est de même pour le nombre de plaquettes. Par ailleurs dans les deuxième et troisième cas, le nombre de lymphocytes augmenta rapidement après que la baisse de voltage sur l'électrocardiogramme fut observée. À l'électrocardiogramme quatre variations furent mises en rapport avec le phénomène de rejet : *la diminution d'amplitude du complexe QRS ; une déviation de l'axe électrique à gauche ; des déplacements du segment ST surtout dans les dérivations précordiales gauches, ce qui suggère l'ischémie myocardique et le phénomène de rejet ; enfin, en phase finale de rejet, des dysrythmies telles que la fibrillation auriculaire.*

In the other two cases drainage failed for anatomical reasons.

Donor's body weight is another factor to be taken in account. In Case III, the donor weighed 25 per cent less than the recipient. In the first postoperative day difficulty to maintain adequate arterial pressure was observed. The transplanted heart probably became edematous and lost part of its contractile strength, not to mention the fact that this process developed in a denervated organ. When the donor and the recipient have approximately the same body weight, or when the donor is somewhat heavier, the transient decrease in the contractile capacity of the myocardium probably is more easily improved by inotropic agents, without great hemodynamic changes. When the donor is far lighter than the recipient, myocardial edema is likely to produce greater hemodynamic imbalance.

The histocompatibility tests in the present series were performed using 16 leukoagglutination sera provided by Doctor van Rood. These sera identify the following antigens, according to van Rood's classification : 4a, 4b, 6a, 6b, 7a, 7a′, 7b, 7c, 7d and 8a. Leukoagglutination and lymphocytotoxic sera obtained in our laboratory by Doctor Francisco Antonascio were also employed (Table I). In Case I

TABLE I

Preoperative donor and recipient erythrocyte and leukocyte typing in three heart transplant cases

CASE		BLOOD GROUP	LEUCOCYTE GROUP											
			IDENTIFIED SERA (SUPPLIED BY DR. VAN ROOD)										NON IDENTIFIED SERA INCOMPATIBILITIES	
			4a	4b	6a	6b	7a	7a′	7b	7c	7d	8a	MAJOR	MINOR
I	RECIPIENT	B	+	+	+	−	+	−	+	−	−	+	1 5 1	2
	DONOR	O	+	+	+	+	−	−	+	−	−	−		
II	RECIPIENT	B	+	+	+	−	−	−	+	−	−	+	3 2 -	2
	DONOR	O	+	+	+	−	−	−	+	−	+	+		
III	RECIPIENT	A	−	+	+	+	+	−	+	+	+	−	3 5 1	4
	DONOR	A	−	+	+	+	+	+	+	+	−	−		

there was one major specific incompatibility (6b) and two minor incompatibilities (7a and 8a). Among the non identified sera, one major and two minor incompatibilities were disclosed. In Case II, one major incompatibility (1d) and two minors among the unidentified sera were detected. In Case III the specific sera demonstrated one major (7a′) antigen incompatibility and one minor (7d) incompatibility. The 35 unspecific sera revealed one major and four minors incompatibilities.

In the postoperative course of our patients we strived to detect the earliest signs of rejection, in order to give the patient the best chances to profit from the increased immunosuppressive therapy. The analysis of our cases permits to set a hierarchy of useful signs for early detection of rejection.

General clinical data, such as asthenia, adynamia and anorexia, appear too late to be of value, usually when myocardial failure is present. The earliest clinical sign in our cases was tachycardia. Decreased pulse pressure and increased venous pressure, with or without jugular stasis and hepatomegaly, appeared only when myocardial contractility was impaired. They were usually accompanied by signs of myocardial insufficiency, such as hypophonetic first sound and a third sound in the mitral area.

Radiological evolution demonstrated that in every instance the transplanted heart increased in size, probably in consequence of edema and adaptation to new hemodynamic conditions. Later in the postoperative course the heart decreased in every case to approximate normal size. A new radiological enlargement of the heart was usually coincident with the appearance of clinical manifestations of myocardial insufficiency and of changes in the electrocardiographic tracings, suggesting rejection process.

Changes in leukocyte count presented no direct relationship to the rejection phenomena, but were

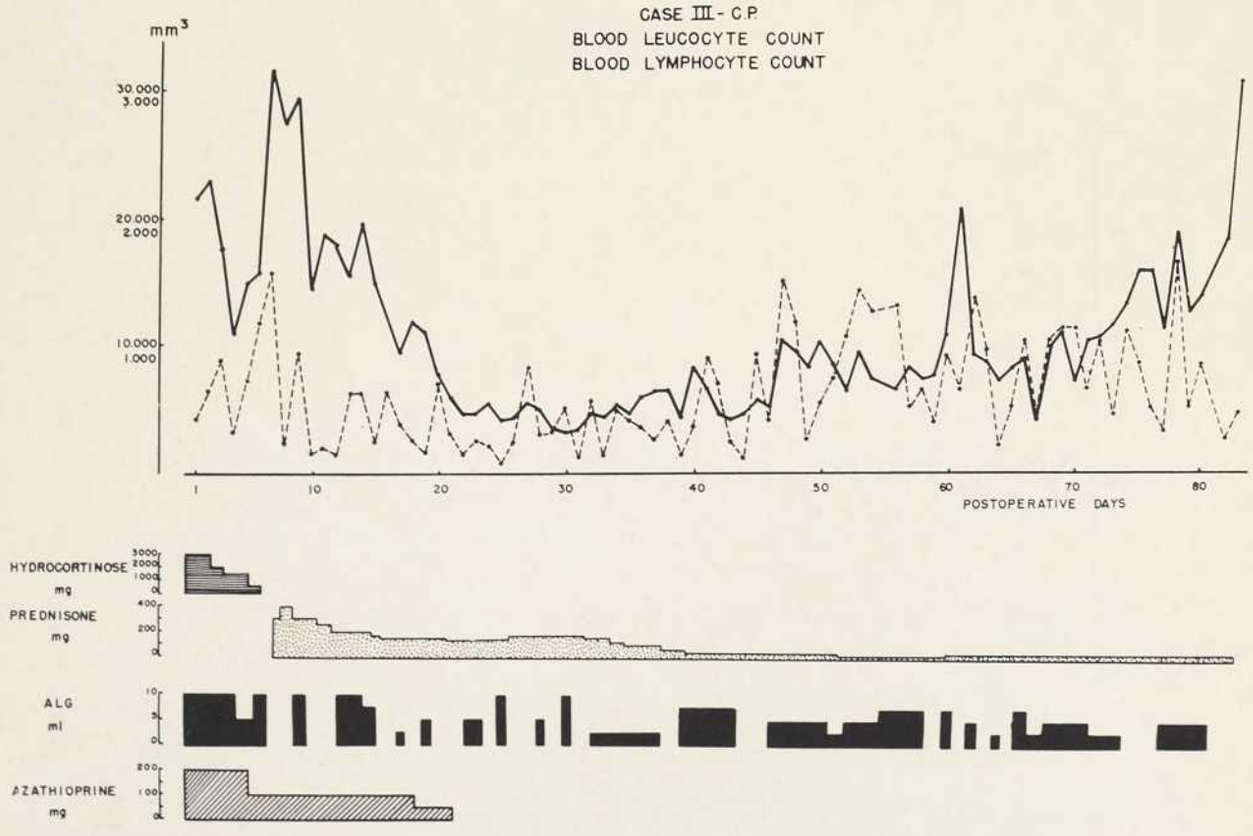

Figure 1 — Leukocyte and lymphocyte blood count in Case III. Leukocytes increased after 63rd and the 80th postoperative days due to infection.

influenced by the immunosuppressive drugs, increasing with corticosteroids and decreasing with azathioprine. Leukocytosis was present in one instance of mild infection (Case II) and in one case of severe infection (Case III), as seen in Figure 1.

Platelet count did not change in the rejection crisis. Individual sensibility was observed regarding immunosuppressive therapy and platelet count. Severe thrombocytopenia was observed only in Case III, in which azathioprine produced persistent platelet drop. In this very case, ALG determined immediate thrombocytopenia, with return to a normal count later on. The other two patients had their platelet count not influenced by the immunosuppressive agents.

Lymphocyte count was an useful index for the postoperative control. In cases II and III, peripheral lymphocytes increased in number soon after a decreased voltage of the electrocardiogram was observed. In Case I severe lymphocytosis was present early in the rejection crisis. ALG was an efficient agent to decrease the number of peripheral lymphocytes, specially in Case II (Figure 2), in which the count was kept under 500/mm³. However the number of circulating lymphocytes was not taken in account for the early diagnosis of rejection crisis.

A few enzymes were studied in our cases. Serum transaminases and dehydrogenases did increase only when cellular necrosis was present, in a late stage of the rejection process, as observed in Case I. These determinations, as well as the dehydrogenase isoenzymes studied in Case II, did not permit early diagnosis of the rejection crisis. The serum enzymes increased during the episodes of infection, as observed in cases II and III.

The electrocardiogram constituted the fundamental guide in the postoperative control of the transplanted heart. Four orders of considerations can be drawn from the electrocardiographic analysis.

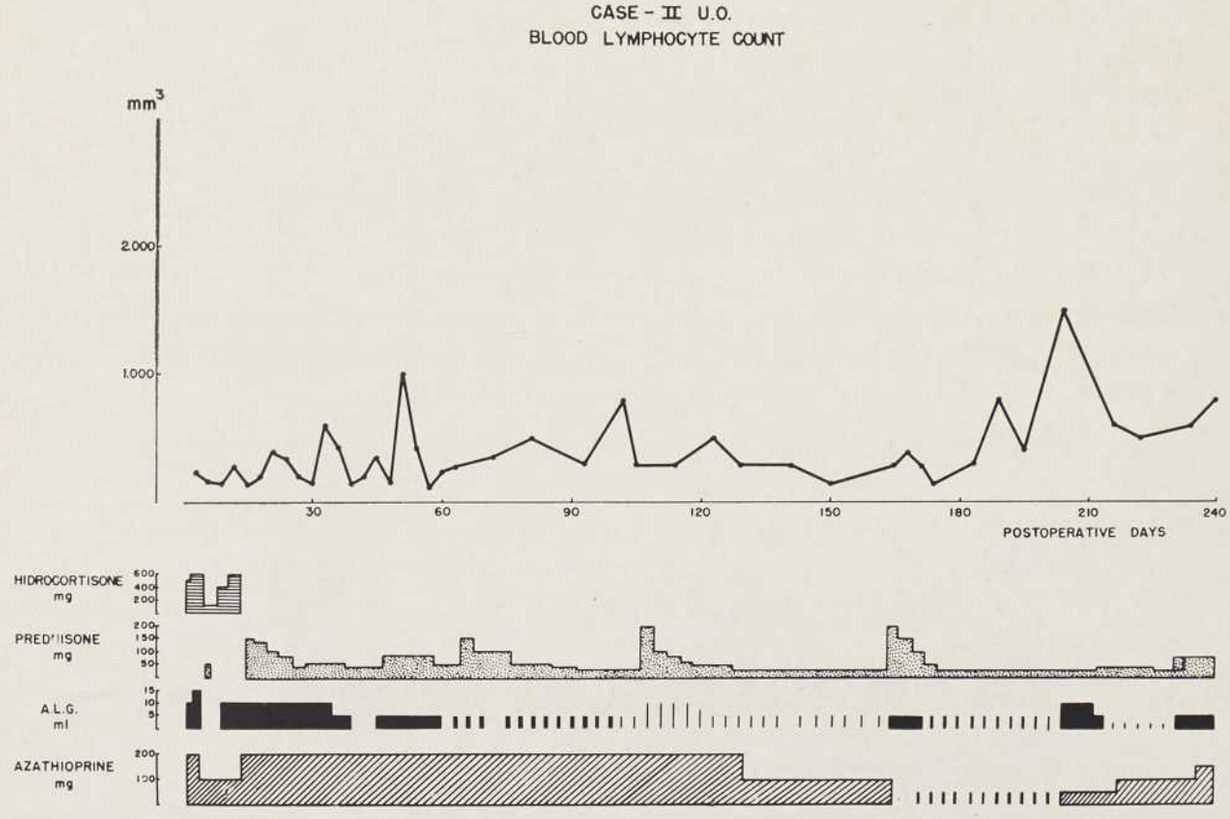

Figure 2 — Case II. Blood lymphocytes were under 500/mm³ during most of the postoperative course.

1. The earliest change observed in ECG is the decreased amplitude of hte QRS complex (added voltage of QRS waves in $D_1 + D_2 + D_3$ leads). In our three cases this was the most reliable sign for the early diagnosis of rejection. In Case I (Figure 3), several days elapsed between the decrease of the QRS complex voltage and the onset of clinical manifestation. In Case III (Figure 4), the initial decrease of the QRS voltage, of great intensity, was followed by severe myocardial insufficiency. The ulterior increase in QRS voltage was followed by definite improvement of the clinical signs. In Case II (Figure 5), the initial decrease of the QRS voltage was followed by clinical manifestations a few days later. In the late postoperative course of this case, three other episodes of QRS voltage decrease were observed, each one without accompanying clinical signs and laboratorial changes (Figure 6).

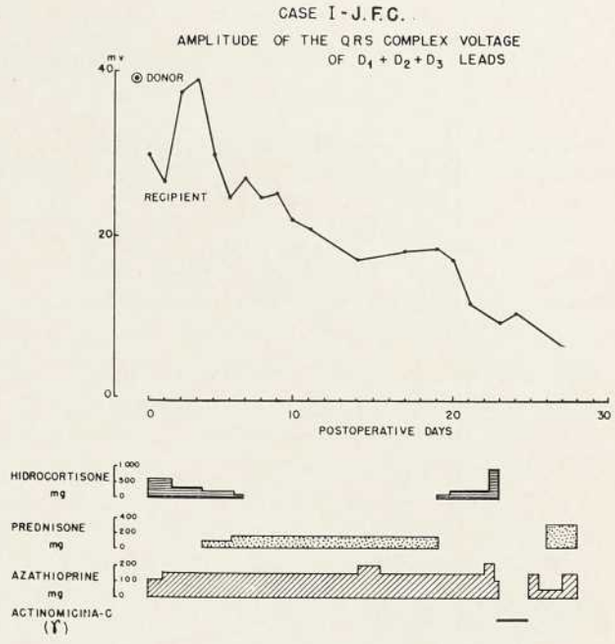

Figure 3 — Case I. Progressive fall in QRS voltage due to rejection.

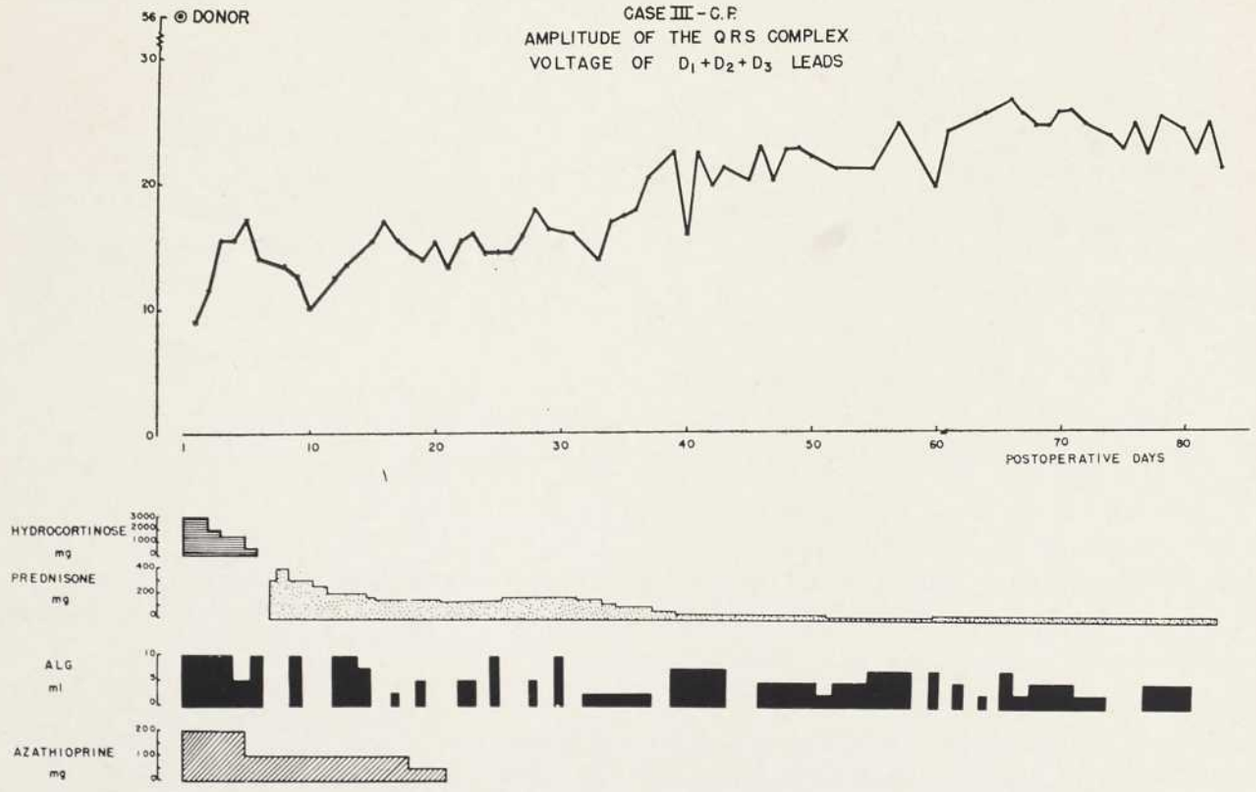

Figure 4 — Case III. The added QRS voltage in $D_1 + D_2 + D_3$ mv. was 9 after transplantation, but increased progressively during the postoperative period.

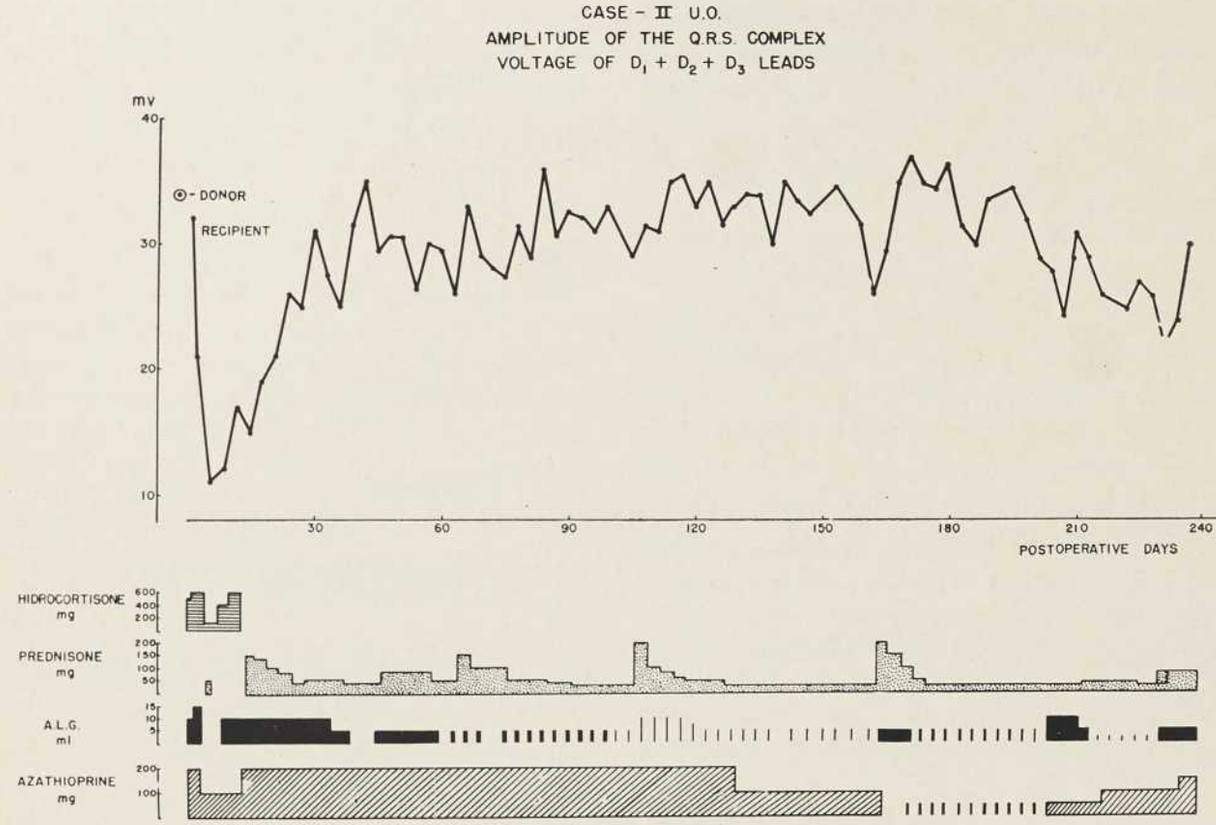

Figure 5 — Case II. Added QRS voltage in $D_1 + D_2 + D_3$.

Another fact was the observation made that, when the corticosteroid dosage was maintained, QRS voltage was influenced by different doses of ALG.

2. Electric axis deviation, specially to the right, was observed after the QRS voltage decrease, and was in every instance accompanied by increased venous pressure, suggesting right ventricular overload. Administration of high doses of prednisone in cases II and III restored the electric axis to the previous position.

3. Changes in ST-T segment, mainly in the left precordial leads were suggestive of myocardial ischemia and rejection crisis. In Case II, inverted T waves in the late postoperative course were never accompanied by clinical manifestations.

4. Dysrhythmias appeared in the late phases of the rejection process, when clinical changes were present; they constitued an ominous sign. This fact was observed in Case I, in which atrial fibrillation

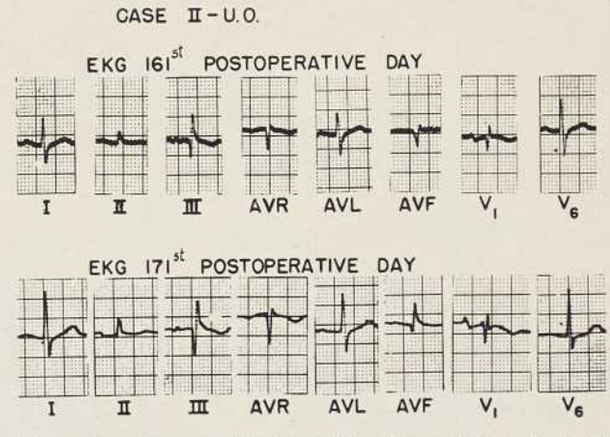

Figure 6 — Case II. Added QRS voltage ni $D_1 + D_2 + D_3$ increased from 26 mv. in the 161st postoperative day to 36 mv. in the 171st day.

was followed by ventricular fibrillation. In Case III paroxysmal atrial fibrillation was observed in the initial postoperative period; it did not revert with electrical countershock but disappeared after intravenous potassium administration.

IMMUNOLOGIC CONSIDERATIONS FOR FUTURE CARDIAC TRANSPLANTATION IN MAN *

William T. BUTLER, Roger D. ROSSEN, Evan M. HERSH, Arthur C. BEALL, Jr., Robert D. MORGEN, Edward B. DIETHRICH and Michel E. DEBAKEY

L'échec des programmes immunosuppressifs actuellement employés pour prolonger la survie des transplantations cardiaques entraîne l'évaluation de toute une série de méthodes possibles pour améliorer la longévité de ces patients. Les receveurs cardiaques potentiels sont immunologiquement parlant fortement réactifs. La formation d'anticorps humoraux n'est pas diminuée notablement avant la transplantation et même après la transplantation. D'importantes quantités d'anticorps sont formés contre l'immunoglobuline G de cheval même en présence d'un traitement immunosuppressif. Avant la transplantation les réactions d'hypersensitivité retardée et les réponses blastogéniques des lymphocytes en regard d'une stimulation antigénique *in vitro* sont normales. Les receveurs potentiels de reins montrent par ailleurs une réduction de leur réactivité immunologique lorsque ces paramètres sont mesurés de façon comparable. Ces faits font surgir plusieurs approches possibles au problème de l'amélioration les résultats de la transplantation cardiaque. Au point de vue immunologique, la sélection des receveurs potentiels devrait procéder par
(suite du résumé en page suivante)

THE DILEMNA OF REJECTION

Long term survival following human cardiac transplantation has occurred in only a small number of cases, and to date, no one has been able to successfully predict which patients will survive the longest. Based on retrospective histocompatibility testing, there appears to be a slight correlation of prolonged survival with close matching of donor and recipient lymphocyte antigens (6). Nevertheless, striking exceptions have occurred in that patients with at least two known major mismatches have survived for many months (10). This would imply that although close matching of donor and recipient tissues may be partially responsible for better survival, there undoubtedly are other factors which play an equally important role in determining prolonged survival. For instance, the presence of anti-heart antibodies may be detrimental to graft survival. Patients who have had repeated episodes of myocardial cell damage may also have triggered an autoimmune response to myocardial tissue manifested by the production of anti-myocardial antibodies (7). If these antibodies behave in a manner analogous to those seen in kidney disease (9), they may be difficult to detect in the circulation, especially if they have strong avidity for myocardial tissue. More needs to be known about the frequency and localization of immunoglobulin and anti-heart antibodies bound to myocardial tissue in end stage

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étapes. La première étape serait une sélection basée sur la présence d'une maladie cardiaque terminale dont l'étiologie n'entraîne pas de contre-indication à la transplantation. En second lieu, les patients présentant des antigènes rares seraient exclus étant donné l'improbabilité de trouver un donneur compatible. Dans une troisième étape, l'évaluation de la réactivité relative tant humorale que cellulaire serait effectuée. Les patients présentant un haut degré de réactivité seraient exclus. Finalement, les candidats recevraient des injections d'immunoglobuline G purifiée, de cheval, dans le double but d'abord de déterminer si une tolérance à des protéines purifiées peut être facilement obtenue et en second lieu afin d'être complètement préparés pour une transplantation ultérieure. Au moment de l'intervention, la globuline anti-lymphocytaire pourrait être administrée immédiatement sans risque de développement d'anticorps contre cette dernière. Il est à espérer qu'une sélection minutieuse de receveurs améliorera les chances de succès des transplantations cardiaques futures en même temps que d'autres méthodes immunosuppressives prometteuses soient développées tel que le drainage des lymphocytes par le canal lymphatique thoracique.

heart disease and about the possible role of these antibodies in pathogenesis of disease in a transplanted heart.

There is little doubt that the most difficult management problems of the postoperative period are those concerned with rejection and infection and that patients live in a perilous balance between the two. The transplanted heart cannot be maintained artifically as can the transplanted kidney during periods of failure of function. The lack of this "rejection reserve" in the cardiac patient means that treatment by necessity includes higher doses of immunosuppressives and that drugs, including corticosteroids, most often need to be given indefinitely. Despite heavy immunosuppression, 13 (50 per cent) of the 26 deaths in the Houston patients have been caused by rejection, and eight (31 per cent) by infection.

We find ourselves, therefore, in a disconcerting position. From the technical standpoint there is no contraindication to pushing forward with cardiac transplantation. Moreover, the improved circulatory dynamics that follow the operation cause patients to feel better, thereby winning their overwhelming acceptance of the procedure. On the other hand, we are hesitant to push forward without restraint because of the realization that the ultimate goal, production of tolerance to an allograft in an outbred species such as man, has not yet been achieved, and until this goal is realized, it is unlikely that survival of cardiac grafts will reach the proportion that now follows transplantation of the human kidney. In the latter situation, the genetic barrier is minimized by the use of well-matched, living-related donors, a situation obviously impossible in a cardiac transplant program. What, then, can be done at this stage of cardiac transplantation to improve the present survival rate?

In an attempt to find a rational approach to the resolution of this dilemma, we have begun by proposing two basic questions:

1) Can we predict in advance the degree of immunologic response a potential cardiac recipient will manifest against his graft? and,
2) Can we manipulate the immunosuppressive methods already available to make them more efficient and efficacious?

IMMUNOLOGIC REACTIVITY OF POTENTIAL RECIPIENTS

Initially, the basis for selection of patients for cardiac transplantation was entirely non-immunologic (3 and 4). Irreversible end stage heart disease

was the major consideration. Subsequently, histocompatibility matching has been taken into account, and patients have generally not been transplanted if more than one major mismatch exists between the recipient and the potential donor. More recently, we have studied the immunologic reactivity of patients prior to institution of immunosuppressive treatment by measuring the reactivity of cellular and humoral immune mechanisms. Initial results have been compared with those from patients awaiting kidney transplantation. In general, potential cardiac recipients as a group may be distinguished from kidney recipients in that the cardiac patients appear to have immunologic reactivity which is more like that of normal persons.

We first became aware of the immunological capabilities of heart transplant recipients when we undertook to study the plasma survival of anti-lymphocytic globulin (ALG) in these patients. Since anti-lymphocytic serum has reportedly resulted in improved survival and function of transplanted kidneys (13), we have given anti-lymphocytic serum prepared in horses against human thymus cells to all cardiac transplant patients in the hope that its use would lessen the need for steroid and antimetabolite immunosuppressives. Since treatment of anaphylaxis is quite difficult and dangerous in a patient with a denervated heart, considerable pains were taken to make the ALG which was given to the patients hypoallergenic by highly purifying the raw anti-lymphocytic serum to yield only the IgG fraction (11). We were gratified when it was found that the purified horse IgG could be given repeatedly intravenously or intramuscularly with no reaction whatsoever on the part of the patient. However, when a portion of the ALG was labeled with radioactive iodine and injected into the patients to study the plasma survival of the administered ALG, it was found that in most patients the plasma half-life was extremely short (Figure 1). Subsequent work showed that this was because the patients had developed specific antibodies to the ALG. Although these antibodies did not cause precipitation of horse gamma globulin *in vitro* or anaphylaxis *in vivo*, they were able to interact with the administered horse ALG molecules to cause their rapid removal from the plasma (2). Patients differed considerably, however, in their response to the horse IgG. For instance, patient 37 who received a total of 2,498 mg of horse IgG over a 22 day period (average dose = 114 mg/day) and patient 28 who received only 333 mg over 58 days (average dose = 6 mg/day) developed the same degree of rapid immune clearance. Patient 18 who received an average dose of 7 mg/day for 31 days did not develop rapid immune clearance. All three patients received azathioprine and corticosteroids along with ALG throughout the study. The fact that two of the patients developed significant amounts of antibody against the horse IgG indicates that the ALG is highly antigenic in man despite simultaneous administration of large doses of chemical immunosuppressive agents.

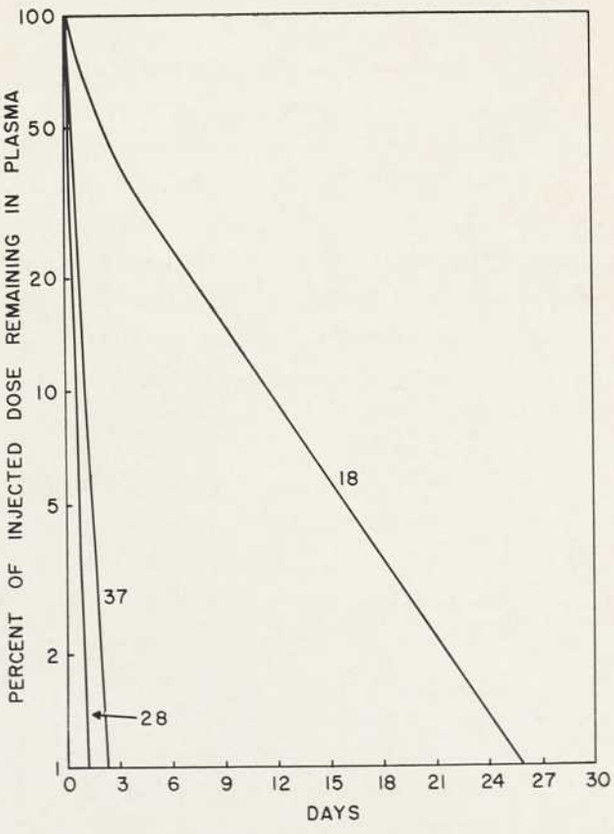

Figure 1 — Plasma survival of 125 I-labeled ALG in 3 recipients of heart allografts who at the time of the tracer study had received 333, 2498, and 217 mg of ALG (no's 28, 37 and 18, respectively).

As mentioned above, the heart recipients as a group were more reactive immunologically than the kidney recipients. An example of the difference is illustrated by the difference in response to injection of keyhole limpet hemocyanin (KLH), a primary antigen in man. Hemagglutinating antibody to KLH has developed within two weeks in all potential heart recipients studied to date, with titers ranging from 1:4 to 1:64. In contrast, none of the potential kidney recipients have developed circulating antibody within two weeks. It is evident, therefore, that primary antibody formation is not significantly impaired in the potential cardiac recipient, as it is in the potential recipient of a kidney allograft.

Cellular immune reactivity was also found to be greater in the heart recipients, an example of which is summarized in Table I. Although the frequency of the presence of established delayed hypersensitivity to streptokinase-streptodornase was approximately the same in kidney and heart recipient groups, forty per cent of the potential heart recipients had skin test reactivity greater than seen in any of the potential kidney recipients. These findings are not surprising in view of studies of other investigators in which humoral and cellular immune mechanisms have been shown to be suppressed in the presence of uremia (14).

A more quantitative assessment of cellular immunity would have obvious advantages in an attempt to evaluate patients preoperatively. The blastogenic responses of the lymphocytes of waiting recipients to phytohemagglutinin, a non-specific stimulator of the transformation of small lymphocytes to lymphoblasts, and to antigens which provoke blast transformation of previously sensitized memory cells provide such a quantitative measure of lymphocyte function (8). Table II shows that lymphocytes from about one-half of potential heart recipients react more vigorously to streptolysin-O added to the cultures than do those from kidney recipients. Our data at this stage is not sufficiently complete to attempt correlation with the subsequent course of the patients. Preliminary analysis of the data, however, indicates that this technique of measurement of lymphocyte reactivity is quite helpful in assessing the effect of the immunosuppressive treatment postoperatively.

TABLE I

Delayed skin test reactivity to SK-SD *

Recipient group	Number of patients			
	Millimeters of induration at 48 hours			
	< 5.0 (negative)	5.0 – 9.9	10.0 – 19.9	20.0 – 29.9
Kidney	3	2	0	0
Heart	7	2	4	2

* Streptokinase and Streptodornase (Veridase, Lederle) ; test dose is 0.1 cc containing 10 units SK-SD given intradermally.

TABLE II

Blastogenic response of lymphocytes to antigen in vitro

Recipient group	Number of patients				
	Counts/minute/10^6 lymphocytes *				
	0 – 4 999	5 000 – 9 999	10 000 – 14 999	15 000 – 19 999	20 000 +
Kidney	8	1	0	0	0
Heart	7	4	1	1	2

* Measured as incorporation of C^{14} labelled thymidine in cultures containing 10^6 lymphocytes after stimulation with streptolysin-O.

INCREASING THE EFFECTIVENESS OF CURRENTLY AVAILABLE IMMUNOSUPPRESSIVE TREATMENT PROGRAMS

We wish to mention briefly two methods which can be applied to cardiac transplantation in an effort to increase the effectiveness of immunosuppression. The first provides a method to increase the effectiveness of ALG, and the second a method to reduce the total mass of reactive lymphoid tissue.

There is much to be said on a theoretical basis for the use of anti-lymphocytic globulin. It is highly specific for lymphoid tissue and in this respect it has considerable advantage over chemical immunosuppressive agents. The latter agents also have profound effects on other vital metabolic processes, often leading to increased susceptibility to infection, osteoporosis, gastrointestinal bleeding, and so forth. It was discouraging, therefore, when we found that the plasma survival of ALG was shortened by antibody formation against it in the majority of patients who were treated with ALG. In addition, the return of established delayed hypersensitivity correlated with the appearance of rapid immune elimination of the horse IgG in a kidney patient who was treated with ALG alone for three weeks prior to transplantation. This observation led us to conclude that ALG loses considerable effectiveness after the appearance of rapid immune elimination. In an attempt to circumvent this problem, a model of induction of low-zone tolerance proposed by Dresser (5) was applied to five potential allograft recipients. The patients were treated intravenously with aggregate-free normal horse IgG for variable periods prior to ALG administration in an attempt to produce tolerance to the horse protein. Results have been reported in detail elsewhere (2). In brief, only one of the five patients developed evidence of rapid immune elimination of horse IgG. Interestingly, three of the patients also received additional immunosuppressive agents concurrently. In these patients, the added immunosuppression may have been a decisive factor in preventing the development of rapid immune elimination of the horse IgG. We believe that these results are encouraging and further experiments are in progress to determine their full meaning.

Another method of depleting lymphoid tissue is that of thoracic duct drainage. This method has been successfully used recently in pre-treating potential kidney recipients (12). Although this method has not yet been applied to potential cardiac recipients, we recently used chronic thoracic duct drainage as a means of immunosuppression in a potential lung recipient (1). Within two months of instituting drainage it was shown that the patient's peripheral lymphocyte count had stabilized near 300 per cubic mm; he no longer responded to skin test antigens which had previously provoked typical delayed hypersensitivity reactions; the blastogenic response of his lymphocytes to antigenic stimulation *in vitro* decreased by over 90 per cent; and he responded with a much delayed primary antibody response to KLH, which despite a booster injection of the antigen failed to persist. Moreover, when given horse IgG over a period of two months, he did not develop rapid immune elimination of the horse protein. Taken together, these findings suggest that chronic drainage of thoraic duct lymphocytes profoundly suppressed cell mediated immunologic reactivity and reactivity to new antigens in this patient.

SUMMARY AND PROPOSED CRITERIA FOR SELECTION OF RECIPIENTS

The failure of currently used immunosuppressive programs to cause prolonged survival of heart transplant patients comparable to that seen following kidney transplantation has led to an evaluation of possible methods to improve chances for long-term survival.

It has been found that potential cardiac recipents are highly reactive immunologically. Humoral antibody formation is not significantly impaired prior to transplantation and even after transplantation when steroids and antimetabolites are given along with anti-lymphocytic globulin substantial amounts of antibody are formed against

horse IgG. Prior to transplantation, delayed hypersensitivity reactions and the blastogenic responses of lymphocytes to antigenic stimulation *in vitro* are normal. By comparison, potential kidney recipients show reduction of immunologic reactivity when the above parameters are measured in a comparable manner.

These findings raise for consideration several possible approaches to improving results of cardiac transplantation. Evidence can now be marshalled to suggest that from an immunological point of view selection of potential recipients should proceed in stages. The first stage would be the careful selection of patients based on the presence of end stage heart disease of an etiology suitable for transplantation. In the second stage, patients with few or rare histocompatibility antigens would be excluded due to the improbability of finding a suitable donor. The third stage would be an evaluation of the relative reactivity of both humoral and cellular immune mechanisms. Patients with high levels of reactivity would be excluded. In the final stage, candidates would be given injections of aggregate-free horse IgG for the dual purpose of first determining whether tolerance to purified proteins can easily be achieved and secondly to be fully prepared for subsequent transplantation at which time ALG could immediately be started without the hazard of developing antibody against it. Hopefully such careful selection of suitable recipients along with addition of newer and promising immunosuppressive methods such as depletion of lymphocytes by thoracic duct drainage will improve the chances of successful cardiac transplantation until that time when methods are worked out to produce specific tolerance to the transplanted allograft.

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HEART TRANSPLANT EXPERIENCE *

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En Angleterre, la publicité autour des cas de transplantation cardiaque a ému le public au point d'affecter la collection de donneurs cardiaques potentiels. Les craintes légitimes du public devant le problème de la transplantation cardiaque ne peuvent pas être ignorées. Un programme d'éducation est nécessaire et il est souhaitable qu'une organisation telle que celle-ci puisse rapporter ses conclusions au public. Devant la difficulté d'obtenir des cœurs de donneurs, nous avons développé les méthodes de transport et de préservation des cœurs. Dans ce domaine le docteur Proctor a réussi à réanimer des cœurs de chien après trois jours de préservation.

La première transplantation cardiaque eut un bon résultat clinique immédiat. Le patient mourut cependant six semaines plus tard d'embolies multiples et finalement d'embolie pulmonaire massive. À l'autopsie, on a pensé que ces embolies pourraient provenir de l'appendice auriculaire droit. L'excision des appendices auriculaires est donc préconisée et en plus on pense qu'il faut employer des anticoagulants durant la première période postopératoire, période durant laquelle les oreillettes du receveur sont encore actives. (suite du résumé en page suivante)

The development of heart transplantation in England has been characterized by a small number of clinical cases punctuated by frenzied emotional outbursts from the daily newspapers. The net effect has been to spread suspicion and distrust widely through the country and this has reflected badly on the supply of donors both to myself and to my colleagues working in related fields of liver and kidney transplantation.

These outbursts have been largely of a destructively critical nature with a demand for full details of the surgery and all domestic details of donor and recipient together with their names. The distressed donor relatives particularly have been subjected to harassment and probing for intimate details.

Although the subject of press relations in transplant surgery is not strictly part of my brief it has an important bearing on the further development of the field of cardiac transplantation certainly in England and I feel it should be aired.

While I am in favour of a medically educated and informed public we are not used to having to carry out our everyday surgery which commonly involves decisions of life and death in the full glare of publicity. I believe the individual patient has a right to expect to maintain a private and confidential relationship with his doctor and only the techniques used should be publicly reported.

The newspapers would have a much stronger case to justify their sensational headlines if they showed the same public-spirited watchdog qualities in relation to the almost daily cadaveric kidney transplants and also to the liver transplants — all of which involve the death of a donor. In the case of the liver the conditions of urgency are just as great. This seems to be the crux of the matter and clearly the public must be weaned from the idea that heart donors fall in a special category.

* Paper presented at the Second world symposium on heart transplantation, Montreal, Canada, June 6-8, 1969.

Une seconde tentative fut faite chez un jeune patient atteint d'une hypertrophie musculaire sous-aortique dont le cœur ne put être réanimé après l'intervention. Un cœur de porc prélevé immédiatement fut greffé en série avec le cœur du patient afin d'agir comme pompe auxiliaire. Cette xénogreffe a présenté des contractions vigoureuses pendant quatre minutes pour ensuite s'altérer et s'arrêter en systole en présentant une couleur rouge brique.

Le second receveur cardiaque était en décompensation et en coma urémique au moment où un donneur fut trouvé. Le cœur du donneur s'était arrêté environ deux heures avant la transplantation et la circulation avait été maintenue au moyen d'une circulation extracorporelle. Le receveur a vécu environ 48 heures mais resta inconscient. Par après, six receveurs potentiels sont morts à l'hôpital en attendant une possibilité de transplantation.

Le dernier cas est un patient atteint d'une cardiomyopathie de cause inconnue et le donneur une jeune fille de 23 ans décédée par suite d'un traumatisme cérébral. L'histo-compatibilité est du groupe B +. Ce dernier patient mourut après la présentation de ce travail, le 31 août 1969.

Furthermore intelligent medical correspondents know quite well that the heart action is not the determining factor in the death of the donor but the emotive term 'beating heart' is still widely splashed across the headlines.

I am sure we cannot ignore the need to allay the legitimate fears of the public. This can only be done through a programme of education and I think it would be appropriate for an organization such as ours to take the lead in this respect, possibly by compiling a report of our views for lay readers. I am sure we would all welcome a code of conduct for transplanting surgeons and a clarification of the law. At the same time I am equally sure there should be a similar code of conduct for the news media if we are to get on with the job.

As in most things it is an ill wind that blows nobody any good and our difficulty in getting donors and the embargo or their movement between hospitals has meant we have concentrated on organ transport and storage methods.

As far as transport is concerned we have a simple perfusion apparatus which we believe will maintain myocardial function for 2-3 hours. More recently we have favoured a simple aortic flush method with cold Ringer's solution to induce hypothermia for transplant purposes. In the organ storage field my colleague Doctor Proctor has achieved successful resuscitation of dogs' hearts after three days storage but he will be reporting this work in another session.

As far as our clinical experience is concerned there have been three transplants only. Two of these were about a year ago and one three weeks ago. There was one additional attempt to use a pig xenograft as an assisting device for a failing circulation.

Our first case was a satisfactory tissue match (B) and there was a good early clinical result. However he died after six weeks from multiple and finally massive pulmonary emboli. Autopsy showed these to have arisen in the recipient's right atrial appendage and I am sure there is general agreement that the atria must be widely excised together with the atrial appendages. In addition we now feel there is a case for anticoagulants during the early postoperative stages while the recipient's atria are still active or arrhythmic.

This patient was on small initial doses of immunosuppressives and no ALG and he had a definite and severe rejection episode after six days. A further finding at autopsy was a fairly advanced concentric coronary artery narrowing presumably as a result of platelet deposition.

Although death was undoubtedly due to repeated pulmonary emboli, pyocyaneus infection was present throughout both lungs in the areas of infarction and terminally there was a florid septicaemia indicating that immunosuppressives also played their part in his death.

Our second attempt was in a young patient with hypertrophic sub-aortic stenosis who would not come off the pump after surgery. In this case we had a freshly killed pig heart which we connected in series with the patient's heart, with the idea of its acting as an auxillary pump till the patient's own heart took over.

The xenograft beat strongly for about four minutes then its colour changed to bright brick red, and it went into systolic arrest and exuded a lymph-like fluid. Obviously we are still some way from using non-primate hearts and simple perfusion of a further pig's heart with human blood produced an identical pathological picture.

The second true recipient was in congestive failure and uremic coma by the time we were able to get a donor. Also the donor heart had been arrested for about two hours prior to transplant and the circulation was maintained on a femoral bypass. Although a technically successful transplant was achieved the recipient lived for only 48 hours and never regained consciousness.

Subsequently six potential recipients died in hospital while awaiting a transplant due to lack of available donors and lends support to the fact that we have reserved this type of therapy to terminally ill patients.

Our most recent case is one with longstanding cardiomyopathy of unknown cause and the donor was a girl of 23 who suffered a fatal brain injury. The tissue match is classified as a (B+).

This patient had been given Imuran (50 mg a day) for the month previous to transplant and we have used heavy immunosuppression with Prednisolone, Imuran and ALG according to Barnard's current programme. In addition he had both right and left atrial appendages removed, anticoagulants are being used and coronary perfusion was used to maintain the viability of the donor heart with only about eight minutes of cardiac ischaemia.

Gross fluid retention was present by the 12th day and this has responded to cutting back the steroids. The ALG (Brendel) has been given intravenously (20 ml per day) without untoward effect, apart from a slight rigor with the first injection.

Sinus rhythm has been present throughout and the recipient atrial pacing waves are still visible but have shown transient atrial flutter and we believe this justifies continuing the anticoagulants meanwhile.

Wound healing has been defective and the sternum had to be resutured on the 18th postoperative day but there is no evidence of infection.

There has been no clinical or objective evidence of rejection and provided there are no further complications it is proposed to allow the patient out of hospital as soon as sound wound healing is achieved [1].

It is our intention to continue with a programme of experimental and clinical cardiac transplantation with the experimental emphasis on donor organ transport and storage.

1. This last patient died after this paper was written on August 31st 1969.

TRANSPLANTATION OF THE HUMAN HEART *

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and Herbert SLOAN, M.D.

Trois transplantations cardiaques furent pratiquées au Centre médical de l'Université du Michigan et ces trois patients diffèrent de la plupart des autres cas rapportés ailleurs par le fait que tous les trois avaient une maladie myocardique idiopathique plutôt qu'une maladie coronarienne obstructive. Ces patients étaient en phase terminale et ne répondaient plus au traitement médical. Ils étaient tous les trois au grade IV au point de vue de l'incapacité fonctionnelle et leur ventriculogramme présentait dans les trois cas une contraction ventriculaire gauche faible. L'histocompatibilité fut déterminée par le docteur Terasaki et les trois patients furent classés dans le groupe C, présentant une incompatibilité majeure, respectivement HL-A2, HL-A8 et HL-A5.

Le décès du donneur fut confirmé dans chaque cas par deux tracés électroencéphalographiques iso-électriques espacés de 24 heures. La modification de Barnard de la technique de Lower-Shumway fut adoptée

At the University of Michigan Medical Center the first heart transplant operation was carried out in September, 1968. Two more patients have since been operated upon, one in December, 1968 and the other in March, 1969. The patients were men, aged 50, 39, and 43, respectively, and at present all are alive and well.

These three patients differed from most of those reported elsewhere in that all represented idiopathic myocardial disease rather than disease involving the coronary circulation. This was not a matter of selection, however; it was only by chance that such similar candidates for heart transplantation appeared within a relatively short period. Otherwise the indications for operation were much the same as in other centers: In all patients the disease was in the end stages, no longer responding to medical management, and no other cardiac surgical procedure was possible. All patients were so ill that they could not safely be discharged from the hospital even for two or three month periods.

Cardiac catheterization in all three patients confirmed the clinical evidence of severe congestive heart failure, with significantly increased pressures in all the heart chambers (Figure 1). Left ventricular cineangiocardiograms showed a generalized

D.K. 10-29-68

Catheter Position	Pressure (mmHg)	Oxygen Saturation (%)
Right Atrium	25/10 (mean 20)	
Right Ventricle	70/20	
Common Pulmonary Artery	70/32 (mean 41)	22
Right Pulmonary Artery Wedge	21/16 (mean 19)	
Left Ventricle	100/29	
Ascending Aorta	100/80 (mean 88)	
Brachial Artery	100/75 (mean 88)	89

Figure 1 — Preoperative catheterization findings in the second heart transplant.

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dans les trois cas. Aucune difficulté de réanimation cardiaque ne fut rencontrée. Dans les trois cas, le thymus fut enlevé en fin d'intervention. Dans la phase postopératoire immédiate, l'isoprotérénol fut employé comme soutien myocardique dans un cas et prophylactiquement pour 48 heures dans les deux autres cas. Dans les trois cas on observa que la congestion périphérique par décompensation droite avait provoqué une hypervolémie considérable : 2 000, 3 500 et 5 000 ml de sang ne furent pas retournés aux patients après la restauration d'une action hémodynamique satisfaisante par la transplantation cardiaque. Une disparition très rapide de l'hépatomégalie extrême fut notée après la transplantation. Un patient fit une infection à pseudomonas au lobe pulmonaire inférieur droit. Un traitement par drainage avec Colistin et immuno-globuline anti-pseudomonas fut administré. La thérapie immunosuppressive ne fut pas interrompue et l'infection disparut après trois mois. Il existe certaines preuves que l'anticoagulothérapie peut diminuer la fréquence du rétrécissement des petits vaisseaux coronariens en cas de rejet chronique. En conséquence, de l'héparine fut administrée à partir du quatrième jour après la transplantation et ce durant trois semaines aux trois patients. À ce moment, l'héparine fut remplacée par le Coumadin.

Durant le premier mois après l'opération, les leucocytes s'élevèrent en moyenne entre 15 000 et 20 000 probablement à cause des fortes doses de Prednisone. Par après, ce taux diminua à 6 000 ou 8 000. Les lymphocytes n'étaient que de 0 à 5 pour cent et après réduction de la Prednisone à la fin du premier mois, ils s'élevèrent à 10 pour cent.

poorly contracting left ventricle. Physical limitations in all three patients were such that they could not walk more than a few steps. The first patient experienced four or five episodes of cardiac shock during his period of waiting until the operation became possible.

Histocompatibility:

Histocompatibility studies were performed by Doctor Paul I. Terasaki. Tests were performed for ABO red cell compatibility, lymphocyte crossmatching for preformed antibodies and lymphocyte antigen matching, with the use of the Terasaki microdroplet lymphocyte cytotoxicity testing technique and grading scale (4). In all three patients the tissue was classified as type C; each patient showed one major incompatibility, at the HLA_2 locus in the first case, HLA_8 in the second, and HLA_5 in the third. The blood type was A in the first and third patients, both of whom received hearts from type O donors; in the second patient the blood type was B for both patient and donor.

Surgical procedure:

In each instance, the clinical evidence of the donor's death was confirmed by two isoelectric encephalograms, the second being recorded after an interval of 24 hours during which respiration and blood pressure were artifically maintained in the donor's body. Only after this proof had been obtained were the recipient and donor taken to the operating room. The donors also had dilated fixed pupils and absent reflexes before the first electroencephalograms were obtained.

At operation the first step was to institute cardiopulmonary bypass for the recipient. The donor heart was then removed and immediately connected to the pump oxygenator for perfusion through a Bardick tube placed in the aorta, where a temperature of 32° C. and pressures of 70 to 80 mm Hg

were obtained. The recipient heart was excised, leaving an atrial cuff but removing the atrial appendages. Installation of the donor heart began with left atrial anastomosis, followed by the interatrial septum and the right atrium. In keeping with Barnard's modification (1) of the Lower-Shumway (2) technique the superior vena cava was tied off, the right atrium was split open toward the atrial appendage, and the left and right atrial walls of the donor heart were sutured to the interatrial septum of the recipient. Thus the sino-atrial node was not injured and no sutures were placed in the interatrial septum of the donor heart. The pulmonary artery was anastomosed after the atrial anastomoses were complete. The donor heart was then disconnected from the pump oxygenator and aortic anastomosis in the transplanted heart was completed.

The establishment of normal sinus rhythm posed no difficulty. In the first patient a normal sinus rhythm developed spontaneously, in the second patient defibrillation occurred with the first electric shock; and in the third a normal rhythm was maintained throughout the entire operative procedure.

As a possible aid in the management of immune responses, the operation included removal of the thymus in every case. The blood pressure, pulse, and cardiac output were normal in all three patients at the end of the operation.

Postoperative problems:

Our experience with laboratory animals, both dogs and calves, indicated that isoproterenol was needed after cardiac transplantation, and it appears that the drug should be given prophylactically in any case of human heart transplantation. This was not done in our first patient and it became necessary to reopen the incision when sinus arrest was followed by a brief period of inadequate cardiac output two hours after the operation. This patient showed an excellent cardiac output just after the cardiopulmonary bypass was removed; oxygenation was adequate, blood volume was normal, and no arrhythmia was apparent. After two hours, however, the patient's blood pressure dropped to about 20 mm Hg and the emergency administration of isoproterenol was necessary to restore heart beat and cardiac output. In the subsequent patients, the drug was given prophylactically for 48 hours, and these patients did not experience similar postoperative difficulty.

The severe preoperative congestive heart failure and hypervolemia in all three patients was found to have considerable bearing on calculations of blood loss and blood replacement immediately after the operation. It was noted that the pump oxygenator contained a large amount of the patient's blood following the bypass procedure. Since this large blood volume was no longer needed when myocardial function was restored, the postoperative volume was lower by a considerable amount — 2,000 ml in the first patient, 3,500 in the second and 5,000 in the third. The third patient was particularly dramatic. Before the operation this patient's liver had been noted to extend below the right iliac crest; afterward it was at the rigth costal margin even before the patient was moved to the recovery room. Apparently such patients have a great deal of excess blood pooled in their hepatic system, and any attempt to restore the preoperative blood volume after adequate cardiac function has been achieved may create a typical picture of hypervolemic heart failure.

Our single instance of major postoperative infection occurred after the chest was reopened under nonsterile conditions in the first patient in order to correct sinus arrest. As a result, a pseudomonas infection developed in the right pleura, mediastinum, and lower lobe of the right lung. Treatment involved tube drainage of the chest and the administration of colistin, on the basis of sensitivity studies, as well as pseudomonas-immune globulin. Immunosuppressive therapy was not interrupted, and the infection cleared during the patient's three month period of convalescence in the hospital. The other two patients had uncomplicated postoperative courses and were discharged about six weeks after operation.

Immunosuppression:

Thymectomy was carried out at the time of cardiac transplantation. Our experience with animal studies, as well as that reported by other workers (3), had shown that survival time was greatly increased in adult animals when thymectomy was added to other types of immunosuppressive therapy.

Treatment with azathioprine and prednisone was started 12 hours before the operation at a daily dosage level of 200 mg and 150 mg respectively, and continued for three weeks after the operation. The prednisone dosage was then lowered to 75 mg per day between the third and fourth week and gradually decreased during the next three months to a maintenance level of 30 mg per day. Azathioprine was decreased to 150–175 mg per day after two months and maintained at that level.

Although there is as yet little information regarding the possible value of anticogulant therapy following heart transplantation, there is some evidence that it may decrease the incidence of small-vessel involvement and help to maintain a more normal coronary blood flow. Accordingly, heparin administration was started on the fourth postoperative day and continued for three weeks. At that time coumadin replaced heparin for maintenance therapy. In two patients studied with radioactive cesium^{131} immediately after operation and again two to eight months after operation, coronary blood flows have been shown to be relatively unchanged from the immediate postoperative values.

Rejection episodes:

The early detection of rejection phenomena has been based on examination of LDH, with isozyme 1, as well as electrocardiograms, chest X-rays to reveal heart size, changes in the leukocyte count, and the general condition of the patient from the clinical viewpoint. In none of our patients have we seen any abnormalities that would suggest an acute rejection episode. The LDH value, initially high after the operation, returned to normal and has remained so. Heart size has not changed, and the patients have remained clinically well. The third patient has had major psychiatric problems.

Electrocardiogram:

There have been some variations in the electrocardiograms, the significance of which we are uncertain. The only arrhythmia occurred in the first patient who developed atrial flutter in the donor heart on the thirty-eighth postoperative day. He had a mediastinal infection at the time. He returned to sinus rhythm twenty days later on digitalis therapy.

The sum of the QRS voltage in leads, I, II, and III dropped markedly in the first patient on the third postoperative day and even more on the ninth postoperative day. It did not increase appreciably until the thirty-ninth postoperative day. He was not treated for rejection. The second patient did not show a drop in voltage, but rather, a progressive rise since the time of his transplantation. The third patient showed no significant change in his voltage.

All three patients developed marked terminal inversion of the T waves in V_4 - V_6 between the third and thirteenth postoperative day. T wave inversion occurred somewhat later in the limb leads. These changes fluctuated but they had largely disappeared by the fiftieth postoperative day in two of the three patients.

Effect on lymphocytes:

Immediately after the operation, all three patients showed a normal leukocyte count with a normal proportion of lymphocytes — 25 to 30 per cent. During the first month after the operation, the leukocyte count in all patients rose to 15,000–20,000, which was judged to represent the effect of the large doses of prednisone. Subsequently the leukocyte count dropped to the range of 6,000–8,000 and remained at that level. Meanwhile the percentage of lymphocytes in the total leukocyte volume dropped precipitously, so that during the first month after the operation the level varied between 0 and 5 per cent. After the prednisone dosage was

reduced at the end of the first month, the lymphocyte content rose to about 10 per cent and has remained at about this level.

Pathologic features:

The first patient clearly had a family history suggesting a familial type of cardiomyopathy. His father and his father's brother died of a similar type of heart disease before their fiftieth birthday. The patient has no brothers; he does have younger sisters who have not shown any cardiac disorder so far. Our other two patients had no familial history, and their personal histories disclosed no etiologic factor such as alcoholism or myocarditis.

The heart ventricles were greatly enlarged in all three patients, the hearts weighed 533, 476, and 522 g respectively. Microscopic findings in the first and third patients were similar, showing extreme hypertrophy of the muscle fibers and some focal vacuolar changes in the myocardial fibers. There was endocardial scarring secondary to the chronic heart failure. The large coronary arteries showed mild atherosclerosis, but no artery was narrowed more than 50 per cent. In the small arteries, however, the hearts in these two patients showed marked muscular hypertrophy which had resulted in narrowing of these vessels. There was no significant myocardial fibrosis. In the second patient there was not only muscle hypertrophy but also diffuse micro-infarction throughout the heart. The small arterioles, however, were normal, in contrast to those in the other two patients. There was some coronary arteriosclerosis in this patient also, with narrowing to 30 per cent of the lumen of the right coronary artery, but this was not considered a significant degree of obstruction to explain his diffuse myocardial fibrosis.

Hemodynamic features:

Cardiac output in all three patients, which had amounted to less than a liter before the operation, increased afterward to five or six liters per minute. One patient has been studied by graded exercise tests at three months and six months after the operation. On both occasions his cardiac output was over six liters per minute at rest, with a heart rate of 100, and with exercise the output rose to over nine liters and the heart rate to 130 (Figure 2). His heart rate did not change with carotid sinus pressure or 0.6 mg atropine indicating that the heart has not, as yet, become reinervated. The other two patients have not yet undergone exercise tests; their cardiac outputs at rest range from five to six liters.

SUMMARY

Three patients with end stage myocardial disease have undergone heart transplantation with excellent results in terms of hemodynamic improvement, general physiologic well-being, and continued absence of rejection phenomena. Immunosuppression was achieved by thymectomy and chemotherapy involving azathioprine, prednisone, and anticoagulant agents. With this program the number of circulating lymphocytes was lowered to under 5 per cent for the first month and under 10 per cent for eight months. In one patient a major infection by pseudomonas organisms, introduced during an emergency operation, was successfully treated without interruption of the immunosuppressive treatment.

CARDIAC TRANSPLANTATION

D.K.	Cardiac Output (liters/minute)	Stroke Volume (ml)	Heart Rate
Preoperative	< 1		
Post-transplant			
24 hours	5.6	62	
48 hours	6.1	72	
72 hours	6.9	85	
3 mos. (resting)	6.8	67	100
3 mos. (exercise)	9.4	72	130

Figure 2 — Postoperative cardiac outputs from the same patient whose properative catheterization data is seen in Figure 1. Studies at six months also show a resting cardiac output ovec six liters per minute and an exercise output of over nine liters per minute.

There were no other complications in these three patients.

All three patients differed at one HLA locus from their donor, making them a type C tissue match. Hemodynamic studies show that these patients have a normal resting cardiac output postoperatively and that the cardiac output in one patient increased with exercise with both an increase in heart rate and an increase in stroke volume.

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HEART TRANSPLANTATION. University of Toronto *

W. G. BIGELOW, M.D.[1], **D. R. WILSON**, M.D.[2] and **C. B. BAKER**, M.D.[3].

Trois hôpitaux différents ont participé à ce travail sous l'égide d'un service central de typage tissulaire, le tout basé sur une recherche fondamentale en immunologie unifiée au Toronto General Hospital. Cinq transplantations furent effectuées dont quatre pour des patients atteints d'une maladie coronarienne obstructive terminale et une pour un patient atteint d'une cardiomyopathie de Chagas. Des cinq donneurs, trois avaient subi des traumatismes cérébraux et deux des hémorragies intracrâniennes.

ÉVOLUTION CLINIQUE : Le deuxième cas mourut une semaine après l'intervention de complications résultant de la prolongation d'une circulation extracorporelle d'assistance.

This is a report of Toronto's experience with heart transplantation. In one respect Toronto has the largest "series" to be reported here today ! It may be unique to have a "series" of three hospitals with the same University affiliation engaged in this work.

It has been a co-operative effort in the sense that there is: (*a*) free communication and co-operation between surgical teams, (*b*) one central tissue typing service at the Toronto Western Hospital, and (*c*) one fundamental immunological research study at the Toronto General Hospital using human material from all three hospitals.

Each hospital has independently: (*a*) made it's own decision to enter the heart transplant field, (*b*) selected it's own recipients and donors, and (*c*) decided upon the surgical technique to be used.

The record with five heart transplants may be summarized in Table I. Figure I demonstrates this experience graphically. Three patients were operated at the Toronto Western Hospital, one at St. Michael's Hospital and one at the Toronto General Hospital.

Four of the five recipients were terminal coronary heart disease in chronic cardiac failure. Case 2 was diagnosed preoperatively as a cardiomyopathy and pathological diagnosis confirmed this as Chaga's disease.

Of the five donors, three were head injuries and two cerebral haemorrhage, age 20 to 40 years. Four were male donor to male recipient and one a female to a male.

TABLE I

Cardiac transplantation — Human
(May 1969)

OPS.	OP. DEATH	POSTOP.	LATE DEATH	A & W
5	0	2	2	1

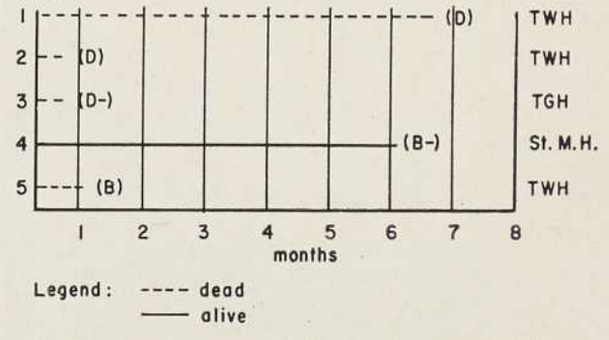

Figure 1 — Summary of five heart transplantations,

* Paper presented at the Second world symposium on heart transplantation, Montreal, Canada, June 6–8, 1969.

1. Toronto General Hospital.
2. Toronto Western Hospital.
3. St. Michael's Hospital.

Le troisième cas fit une embolie cérébrale six heures après l'intervention et mourut une semaine plus tard.

Le premier cas, du groupe D au point de vue immunologique, mourut d'infection pulmonaire virale et mycotique après sept mois. Peu de signes de rejet furent trouvés dans ce cas.

Le quatrième cas, du groupe B est actuellement en excellent état.

Le cinquième cas, également du groupe B, avait un voltage abaissé et mourut sept semaines après la transplantation. Devant l'excellent résultat du cas 4, ce fut une surprise de trouver un rejet sévère dans le cas 5, également du groupe B. Ce dernier cas fut le seul à ne pas recevoir de sérum antilymphocytaire et le seul à recevoir un cœur de sexe opposé. Ce dernier cas est une exception à la règle démontrant qu'en présence d'un *matching* d'histo-compatibilité optimal une évolution fatale peut survenir. Il faut en conclure que les tests actuels n'englobent en fait qu'une fraction du profil antigénique.

Technique:

Hypothermic perfusion through the innominate artery was used in the first two cases (Figure 1). Transplantation was followed by prolonged periods of low cardiac output.

Anoxic arrest was used in the last three cases. Suture time was 33 to 45 minutes and total period of donor heart anoxia was under 54 minutes in each case.

In the first two cases of anoxic arrest (Cases 3 and 4) the donor was maintained at normal body temperature and normothermic bypass perfusion was used with no vasopressor. Both hearts took over with one shock and an immediate blood pressure of 130 mm Hg. In Case 5 the donor was hypothermic, vasopressor was used for 12 hours and bypass was at 31°C. The heart was slow to develop expulsive beats.

Tissue typing was by Doctor S. Sekiguchi using Terasaki testing technique with 11 groups.

Clinical course:

Case 2 died one week postoperatively of complications resulting from a prolonged period of bypass due to technical surgical problems. There was moderately severe rejection pathologically.

Case 3, with a good cardiovascular response, experienced a cerebral embolus six hours after surgery from which he did not recover, dying one week later. There was mild rejection pathologically.

The late deaths provide more information (Table II). Case 1, a D match, was diagnosed and treated aggressively as a continued rejection but died

TABLE II

Cardiac transplant — Relation of tissue type and immunosuppressive therapy

Cases	Tissue Type	A.L.G.	Average daily dose of Prednisone		
			1st month	2nd month	3–6 month
1	D	5 months	170	75	156
4	B–	4 months	75	40	20
5	B	0	82	49	

of virus and fungus pneumonia at seven months. There was a surprising finding of only slight evidence of rejection pathologically.

Case 4, a B match, has had a smooth and completely uneventful course with minimal therapy. He is very active, cheerful and intelligent. He walks and jogs about a mile a day.

Conversely Case 5, a B match, did not show laboratory evidence of rejection. There was, however, a low QRS voltage and high end diastolic pressure in the right ventricle which was present from the time of surgery. He was treated with modest immunosuppressive therapy with no ALG and died suddenly seven weeks later. The pathological picture of severe rejection was not expected.

Cases 1 and 5 point up the need for a more objective index of rejection. With such an excellent result in Case 4, the first B match, it was a surprise to find severe rejection in Case 5, also a B match. This latter case was the only one that did not receive ALS and it was the only instance in which the sex barrier was crossed.

What have we learned:

1. The technique of normothermic anoxic arrest is very satisfactory and minimizes the bypass period.

2. To use normothermic anoxic arrest the donor should be maintained at near normal body temperature with normal blood gases and avoidance of vasopressor if possible.

3. ALG requires standardization but it may be an important suppressive agent.

4. A well trained psychiatric social service worker for screening and mental preparation is an important adjunct.

FUNDAMENTAL RESEARCH

Although histocompatibility typing appears to have some value in predicting long term survivors, there have been exceptions to the rule that good matches survive for a longer time than poor ones. One can speculate as to the reasons for this. We are probably only testing a fraction of the antigenic profile. The essential problem, however, is that the actual cause and the mechanism of rejection is not yet known.

Although it is commonly accepted that cellular immunity is the critical factor in rejection, one is forced to ask if circulating anti-heart antibodies develop in cardiac transplant recipients and, if so, when, and what role do these humoral antibodies play in rejection. Does treatment with ALS have any effect on this response?

These questions are being explored by an interdisciplinary research team from the Toronto General Hospital. Doctor B. S. Goldman, Doctor K. H. Shumak and Doctor M. D. Silver, from Surgery, Medicine and Pathology, are the principle investigators, with Doctor John Crookston directing the immunological studies. The experimental surgical technique involves the use of mongrel dogs with heterotopic cardiac allotransplants into the abdomen. This is the Abbott modification of the Mann-Markowitz dog used by Doctor Gergli of London, Ontario, and others. This it is a simple technique with a low mortality rate and it eliminates the unsatisfactory features of cardiac bypass. With this heterotopic model the classic pathologic picture of rejection will develop. With the beating heart in the abdomen it allows serial biopsy. The antigen-antibody response to removing the transplanted heart may be studied as well as the reaction to a second heart transplant into the abdomen. This is a satisfactory method of studying modification of rejection by immunosuppressive agents. Doctor Goldman will present a preliminary report of some interesting results in the "Rejection" study group tomorrow. There is evidence that tumoral antibodies do play an important part in rejection and there appears to be hope for an objective test for rejection.

GENERAL OBSERVATIONS

1. Heart transplantation has reached the stage where it is the responsibility of each surgical team

to become involved in a fundamental immunological research program to further our knowledge of rejection.

2. The clinical work of heart transplantation should be continued in a careful honest and informed manner. This symposium organized by Doctors Grondin, Lepage, David and their associates is a fine positive step forward.

INDICATIONS FOR CARDIAC TRANSPLANTATION *

Charles K. FRIEDBERG, M.D. †

Devant les résultats actuels de la transplantation cardiaque, on se demande s'il y a lieu de continuer cette expérience clinique. Parmi 130 receveurs connus en fin d'avril 1969, 35 sont vivants au 6 mai 1969. Des 95 sujets décédés, 17 ont survécu plus de trois mois, et des 35 encore en vie, 24 ont déjà survécu trois mois et 18 pour cent plus de six mois. En excluant les décès chirurgicaux, 55 pour cent ont survécu trois mois et 30 pour cent plus de six mois. Quelles sont les raisons de continuer la transplantation cardiaque ?

1. Par analogie à l'expérience des transplantations rénales, on peut anticiper une extension de la longévité consécutive à la transplantation.
2. La survie de plus d'un an chez deux patients suggère que cet objectif pourra à l'avenir être atteint pour un grand nombre de receveurs.
3. L'amélioration prévue des techniques de typage et de « matching » immunologiques sera à la base d'une survie prolongée.

The poor overall long-term results of cardiac transplantation have raised serious doubts as to whether there is any indication for continued human cardiac transplantation at the present state of the art. Those who would call a halt to human cardiac transplantation maintain that the unconquered problems of rejection and the need for immunosuppressant drugs and their consequent complications should be resolved by animal experimentation rather than by continued cardiac transplantation in the human.

An evaluation of cardiac transplantation and possible indications for the operation are based in large measure on the results, especially the rate of survival obtained up to the present time (Table I). As of May 6, 1969, there were 130 recipients of a cardiac transplant, and two of them had received two transplants. Seventy-four had been performed in the United States and 58 in other countries around the world. Of the 74 in the U.S.A., 44 had been performed by three surgeons, and 12 of the other 21 surgeons had performed only a single cardiac transplant. Of the 58 cardiac transplants outside of the U.S.A., 22 had been performed by four surgeons, nine of them here in Montreal by Doctor Grondin and his team. Of the 130 recipients, 95 were dead and 35 alive as of May 6, 1969, *i.e.* as of one month ago.

TABLE I

Recipients of cardiac transplants (May 6, 1969)

TOTAL	130	
U.S.A.		72
Others		58
LIVING	35	
U.S.A.		19
Others		16
DEAD	95	

* Paper presented at the Second world symposium on heart transplantation, Montreal, Canada, June 6–8, 1969.

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4. Malgré que l'expérimentation animale devrait prédominer dans les études de transplantation cardiaque, il est souhaitable de continuer l'expérience de transplantation cardiaque chez l'homme du fait que les observations animales ne sont pas toujours applicables à l'homme.

5. L'expérience chirurgicale de transplantation cardiaque a démontré que le cœur greffé, dans la plupart des cas, est capable d'assurer une circulation satisfaisante.

Les indications générales pour la transplantation cardiaque sont actuellement :

1. Un receveur en période terminale d'une maladie cardiaque, rebelle à toute médication ;

2. Une incapacité fonctionnelle et une symptomatologie extrêmes ;

3. De plus, il faudrait ajouter l'improbabilité d'une survie de plus de trois mois.

Les principales contre-indications sont : une maladie avancée d'autres organes, un cancer incurable, une maladie pulmonaire irréversible, une maladie rénale, hépatique ou pulmonaire associée, ainsi que l'association d'autres maladies du système nerveux central, le diabète, une maladie psychiatrique évidente et, finalement, l'histo-incompatibilité.

The outlook in terms of survival after the operation is indicated by the following data (Table II) : Of the 95 recipients who succumbed as of May 6, 1969, 17 had survived for more than three months, and of the 35 patients still alive, 24 had already survived for more than three months. Excluding eleven living patients who were operated less than three months previously, there were 41 of the remaining 119 recipients, or 35 per cent, who had survived for more than three months. There were three survivors for more than six months among the 95 recipients who had died by May 6, and 17 of the 35 living patients had also survived for more than six months. Thus, excluding 18 living patients who were operated too recently to have survived six months, there were 20 of the remaining 112 recipients, or 18 per cent, who had survived for more than six months.

TABLE II

Cardiac transplantation. Survival of recipients (May 6, 1969)

	LIVING	DEAD	SURVIVAL RATE
Total	35	95	
Longer than 3 months	24	17	35% *
Longer than 6 months	16	3	18% **

* Excluding 11 living patients operated less than 3 months previously.

** Excluding 18 living patients operated less than 6 months previously.

These results must be interpreted also in terms of an unexpected high early mortality for a new surgical procedure which has been performed only once by 35 different surgeons, *i.e.* in 27 per cent of the 130 recipients. If one excludes the 45 deaths occurring within the first week after operation as essentially surgical deaths, then 41 of the remaining 74 recipients, or 55 per cent, survived more than three months, and 20 of 67, or 30 per cent, survived more than six months. Only one recipient had survived for more than a year as of May 6, but I presume there are two survivors as of this day.

These data, indicating only one chance in three of surviving for more than three months after the operation, and only one chance in five of surviving more than six months, are indeed cause for pessimism, but not for despair and surrender. Despite the relatively poor outlook for prolonged survival at the present time, there are cogent reasons for continuing cardiac transplantation in the human:

1. By analogy with the experience in renal transplantation, one may anticipate, with increased experience, continued improvement in the rate and duration of survival. At the present time, the majority of cadaveric renal grafts are functioning for more than two years.

2. A postoperative duration of more than a year in two patients and of more than eight months in perhaps another ten of the surviving patients suggests that survival for more than a year is an attainable objective for many recipients in the near future.

3. It is probable that the high incidence of early graft rejection and death of recipient were due to poor matching, even though there are occasional examples of relatively favorable survival despite relatively poor histocompatibility. There is reason therefore to hope that with better sera for typing and with improved and standardized technics, and especially with greater knowledge of the transplantation antigens which are important in rejection, there will be improved matching of recipient and donor cardiac graft and a reduced incidence of early rejection. This will be aided also by national or international registries containing the types of potential recipients and by greater insistence on donor-recipient histocompatibility before cardiac transplantation is performed.

4. Although animal experimentation should predominate in any further studies of cardiac transplantation and the immunology of rejection, some human cardiac transplantation must also be performed, since it is doubtful that the observations from animal experimentation can be consistently applied to the human, or that the necessary information for controlling rejection can be obtained from animal experimentation alone. The knowledge already obtained from human cardiac transplantation supports this viewpoint.

5. Human cardiac transplantation has been demonstrated to be a feasible surgical procedure with an acceptably low surgical mortality, considering the early stage of its development. Furthermore, the grafted cadaveric heart has proven to be consistently capable of maintaining a satisfactory circulation. It must be regarded as an available therapeutic procedure for those patients, whose degree of suffering, disability and very poor prognosis with other available treatment justifies the risks and promise of cardiac transplantation.

The real issue is not whether cardiac transplantation should be halted or continued as a clinical procedure, but whether the physician can make the wise decision as to the indication of the operation in a particular patient. Since transplantation of the human heart is a surgical procedure, its recommendation or not, depends on the general principles which govern the performance of any serious surgical operation. These include an assessment of (*a*) the relative risk of the operation versus that of continued medical treatment; (*b*) the severity of symptomatic distress and functional disability; (*c*) the probability of clinical improvement and its duration after the operation; (*d*) postoperative complications and problems in management; (*e*) the relative risks of delaying surgery versus the advantages of delay, in the hope of improvements in medical and surgical treatment. The patient's desire to receive a cardiac graft also must be rationally motivated and must be based on the physician's fair evaluation of the patient's outlook without a graft, in addition to his honest presentation of the risks and uncertain prognosis of the transplantation procedure.

The *general indications* for cardiac transplantation at the present time are:

1. The end-stage of intractable cardiac disease and
2. Intolerable symptomatic distress of functional disability and
3. Absence of response to medical and surgical therapy.

At the present time, end-stage would mean, in addition to intolerable symptoms, great improbability that the patient would survive for more than three months.

Contraindications include:

1. Generalized advanced disease of other organs;
2. Associated incurable malignant disease;
3. Heart disease, secondary to advanced, irreversible pulmonary disease;
4. Hematologic neoplastic disease or incurable hemorrhagic disease;
5. Associated intrinsic renal, hepatic or pulmonary disease;
6. Advanced disease of the central nervous system;
7. Diabetes mellitus;
8. Obvious severe psychiatric disease;
9. Histocompatibility.

The *specific indications* for cardiac transplantation may be considered in terms of the particular cardiac disease, but more cogently in terms of clinical syndromes. Thus regardless of the etiologic cardiac disease, end-stage intractable heart failure is the most common indication. Cardiogenic shock, as in myocardial infarction, may become an important indication if an assist device is available until a suitable donor is obtained. A temporary assist device or artificial heart may also be used until a donor heart is available when there is inability to restore an effective heart beat off the pump, following open-heart surgery. Recurrent cardiac arrest due to refractory ventricular tachycardia-fibrillation may become a compelling indication.

Of the specific cardiac diseases, coronary heart disease is the most common indication, chiefly because it is the most common cardiac disease. The myocardiopathies may be a common indication, because of their consistent progression to intractable heart failure and serious arrhythmias. Rheumatic cardio-valvular disease is amenable to other surgical as well as medical therapy, but it may require cardiac transplantation when valvular surgery is ineffective. Cardiac transplantation may also be indicated for a number of complex congenital cardiac lesions which cannot be treated surgically or have not responded to surgical palliation or a partial prosthesis. Other diseases representing indications for cardiac transplantation may include occasional cases of extensive, uncorrectable cardiac trauma and primary localized sarcoma of the right atrium. All of these indications, if strictly considered, should result in no more than 75 to 85 cardiac transplants throughout the world in the coming year.

THE DONOR HEART : BRAIN DEATH AND PATHOLOGICAL CHANGES IN THE HEART *

H. Alexander HEGGTVEIT, M.D. †

Cette étude concerne l'apparition fréquente de dommage myocardique à la suite d'une lésion intracrânienne aiguë. Les cœurs de 100 sujets morts subitement après un traumatisme cérébral ont été étudiés. Les cas de choc, de traumatisme thoracique ou de maladie coronarienne sont

Most human hearts transplanted to date have been obtained from persons dying of craniocerebral trauma or spontaneous intracranial hemorrhage. Such cases would appear to be the major source of donor hearts in the forseeable future. Apparently, little attention has been paid to the fact that these "normal" hearts are frequently the site of significant structural alterations. This report calls attention to the common occurrence of myocardial damage following acute intracranial lesions.

MATERIAL

Hearts from 100 patients with cerebral hemorrhage or head injury, who died suddenly or at varying intervals after the acute event, were examined grossly and microscopically. The material was seen on the autopsy service of the Ottawa General Hospital over a five year period. Cases with significant thoracic injury, shock or coronary disease were excluded.

OBSERVATIONS

One half of the hearts exhibited gross subendocardial hemorrhages situated usually over the left side of the interventricular septum and less commonly on the papillary muscles of the left ventricle. These hemorrhages varied from several mm to 7 cm in diameter. Histologically, interstitial hemorrhages involved the underlying myocardium to varying depths and often were interspersed with ramifications of the left bundle branch. The subjacent heart muscle invariably showed severe edema, fragmentation of myocardial cells, dehiscence of intercalated discs and a variety of degenerative changes including segmental contraction bands, hyalinization and fuchsinophilia. Twenty-five per cent of cases manifested more severe lesions in the form of multifocal myocardial necroses, discrete areas of myocytolysis and interstitial cellular infiltrates which included lymphocytes, histiocytes and neutrophils. Well established lesions were most often found in persons who survived the acute episode and succumbed later to the effects of cerebral swelling. Two patients, both young women with no coronary disease, had large necrotic zones which, in reality, could be termed myocardial infarcts. Cases without grossly visible subendocardial hemorrhage all contained marked interstitial edema with fragmentation and dehiscence of myofibers.

COMMENT

In 1954, Burch and co-workers (2) correlated specific electrocardiographic changes simulating myocardial ischemia with the occurrence of intracranial hemorrhage. The same year, Smith and Tomlinson (11) noted the association of subendocardial hemorrhage with intracranial lesions. The

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exclus. Des hémorragies sous-endocardiques du ventricule gauche et du septum furent trouvés dans 50 pour cent des cas. En outre, le myocarde présentait invariablement de l'œdème, une certaine altération cellulaire, et des anomalies ultramicroscopiques au niveau des disques intercalés. Dans 25 pour cent des cas les lésions étaient plus sévères et il existait des nécroses multifocales. La cause de ces lésions est probablement en relation avec la décharge de cathécholamines au cours des traumatismes crâniens. La noradrénaline peut induire un état d'anoxie relative par suite de l'augmentation de la consommation d'oxygène par le myocarde.

En conclusion il est possible que l'échec de certaines transplantations cardiaques puisse être imputé « à des lésions cardiaques neurogéniques ».

electrocardiographic pattern of cerebrovascular disorders has attracted wide interest (2, 7, 10 and 12) but an anatomic basis for such changes has been disputed. Only a few investigators have documented myocardial damage in the human heart secondary to intracranial lesions (4, 5 and 11), although the conditions have been reproduced experimentally by different techniques (3, 6, 8 and 9).

The cardiac alterations are most likely the result of a pressor sympathetic effect mediated by a neurohormonal mechanism (3 and 9). Catecholamine release, primarily noradrenaline from the terminal nerve endings in the mycardium, may induce a state of relative anoxia due to augmented myocardial oxygen consumption (6). Altered hemodynamic patterns (1), mechanical factors (6) or excessive production of adrenal corticosteroids (3) may also contribute to the development of the lesions.

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 obscure or complicate the histological manifestations of rejection in others.

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BRAIN DEATH AS SEEN BY THE NEUROSURGEON *

John F. ALKSNE, M.D. †

Il est de l'intérêt du neurochirurgien d'éviter des efforts extraordinaires de réanimation lorsqu'il se trouve en présence d'une mort cérébrale certaine. Les critères de mort cérébrale furent déterminés par le Comité Harvard : absence de réponse aux stimulus externes, absence de mouvement spontané ou de respiration, absence de réflexes et absence d'activité électroencéphalographique chez un patient qui n'est pas sous l'influence des sédatifs ni en état d'hypothermie. Lorsqu'il existe un doute quant à la sédation, on conseille de refaire un électroencéphalogramme 24 heures plus tard. L'hypotension et l'anoxie peuvent produire une

As a neurosurgeon I am happy to witness the increasing interest and acceptance of the concept of brain death which has been brought about by the development of transplantation techniques, particularly cardiac. Even before the era of transplantation the need for brain death criteria existed; however, because the availability of artificial respiratory support equipment created the possibility of sustaining the circulatory system in an individual with irreversible coma. Neurosurgeons had to make the decision to discontinue therapy in such cases. From the standpoint of the survivors, the protracted dying of irreversibly comatose patients is agonizing. From the standpoint of society, the use of medical resources to prolong death is wasteful. The responsibility of the physician to patients families and to the community demands that brain death be recognized and that useless support measures be appropriately discontinued whether or not transplantation is contemplated. The potential utilization of viable organs from the deceased has served to focus the attention of the entire medical and lay community on this important aspect of medical practice. Although the majority of this discussion will be focused on brain death as it relates to organ transplantation it should be clear that the establishment of brain death is equally important when transplantation is not at issue.

The guidelines set forth by the Harvard Committee (1) have served a very useful function in assisting the physicians and the public in understanding and accepting the concept of brain death as well as in guiding neurologists and neurosurgeons in establishing the diagnosis. Such guidelines are essential. Nevertheless, one must remember that the actual pronouncement of death remains the responsibility of the physician. Each case must be evaluated carefully and judged individually. As more information becomes available the guidelines may need modification.

The criteria for brain death as outlined by the Harvard Committee are: no response to external stimuli, no spontaneous movement or respiration, no reflexes, and no electroencephalographic activity in a patient who is not sedated or hypothermic. It is recommended that these findings should be observed on two successive examinations performed 24 hours apart. In general, these criteria have proved satisfactory in neurosurgical practice but there are some aspects which deserve discussion.

One of the most important aspects of the Harvard Criteria is the preface that the patient must not be under the influence of sedative medications.

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dysfonction cérébrale et ces états doivent être corrigés avant d'évaluer la qualité de l'électrogenèse cérébrale. L'areflexie peut ne pas être totale, dans ce sens que certains réflexes tendineux profonds peuvent persister en présence de mort cérébrale. L'absence de réflexes tendineux profonds signale une nécrose médullaire et non cérébrale. Le contrôle de la mort cérébrale 24 heures après un premier électroencéphalogramme peut s'avérer un soucis excessif lorsque l'on pense à une transplantation cardiaque. En cas de lésion cérébrale extensive et évidente, une importante décharge sympathique peut provoquer des changements nécrotiques au niveau du myocarde. Dans ces cas, afin de décider plus rapidement de la mort cérébrale, on peut recourir à une artériographie cérébrale. En cas de destruction cérébrale, la substance de contraste ne pénètre pas dans le cerveau après une injection intracarotidienne. Enfin, l'urgence d'une transplantation cardiaque ne doit pas influencer les obligations du médecin envers la famille d'un donneur éventuel.

A recent report at the American Academy of Neurology meeting (5) indicated that the only documented instances where a "flat" electroencephalogram had been followed by clinical recovery were cases of sedative overdose. The emergency room physician must remember that patients with obvious head injuries may have taken sedatives as well, and he must remember that alcohol itself is a sedative. If there is any uncertainty about the patient's clinical history, blood alcohol, barbiturate, etc., levels should be assayed and the patient observed for 48–72 hours. Similarly, hypotension or anoxia can produce cerebral dysfunction and must be corrected immediately in the emergency room in order for a meaningful neurological examination to be performed.

One facet of the neurological examination merits further consideration. It has been widely assumed that the criterion — "areflexia" — applies to spinal reflexes as well as cerebral reflexes, although the Harvard report is evasive on this point. Becker and Robert (2), however, have shown that patients who meet all the other criteria for brain death may maintain deep tendon reflexes until cardiac arrest occurs. Since it is brain death, not spinal cord death, which is at issue, it seems reasonable that a clear distinction should be made between cerebral and spinal reflexes. Pupillary reaction, eye movements to ice water labyrinthine stimulation, gag, and decerebrate posturing are cerebral reflexes; whereas deep tendon reflexes are of spinal origin and should not be considered an essential part of the brain death criteria.

Another point which deserves discussion is the period of time which the patient must be observed before a diagnosis of brain death is established. The recommended 24 hours does insure the reliability of the criteria but when the use of viable organs from the deceased neurosurgical patient is contemplated it may be excessive. Many patients suffering from intracranial hemorrhage or penetrating gun shot wounds of the brain cannot be maintained for 24 hours because of cardiovascular collapse. Although the mechanism responsible for this phenomena is not well understood the fact that brain dysfunction may adversely effect the heart has been clearly demonstrated. EKG changes have been reported clinically in association with subarachnoid hemorrhage, intracranial infections, and cerebral mass lesions. Recently, Greenhoot and Reichenbach (4) have reported that EKG changes can be produced in animals by midbrain stimulation and that chronic stimulation produces myocardial necrosis. On the basis of their experiments they suggested that excessive sympathetic discharge may be etiologically responsible. In clinical practice hypotension or pulmonary edema followed by cardiac arrest may occur in spite of extensive therapy.

Therefore, it seems reasonable to leave to the discretion of the responsible physician the determination of the length of time a patient with obvious extensive brain damage must be observed before brain death is established.

One method of establishing brain death without a waiting period is to perform cerebral arteriography. Crafoord (3) reports that in 50 cases when no angiographic contrast media entered the head after intracarotid injection the brain was always necrotic at autopsy. He recommends repeating the X-rays after a 10–15 minute period. For rapidly deteriorating neurosurgical cases this may be the best means of making a definitive decision.

In addition to adhering to the established brain death criteria the neurosurgeon has definite responsibilities regarding his relationship with the relatives of dying patients and his relationship with the hospital staff caring for these patients. The pressures of transplantation must not be allowed to interfere with these responsibilities. It is impossible for a family to discuss organ donation while they are hoping for the recovery of their loved one. Therefore, the family must be advised of the brain death prior to being introduced to the physician who will request the use of viable organs. In addition, it is unjust for the family to bear any expenses whatsoever after brain death has been established whether or not they agree to organ donation.

Communication is also essential between the physicians and the hospital personnel. Hospital morale is severely damaged when personnel must care for hopeless cases, particularly when they have had no opportunity to discuss the reasons or to witness the eventual beneficial results. The nurses and aides must understand the irreversibility of the patient's condition and they must know that everything possible has been done to improve the situation. All hospital personnel who have patient contact should have the opportunity to participate in discussions about the concept of brain death and the value of transplantation. If permission for organ transplantation is given by the family the donor should not be kept in the Neurosurgical Intensive Care Unit and the time of death should be recorded on the death certificate before the respirator is disconnected.

With the passage of time and the accumulation of increased experience, continued sophistication about the criteria for brain death will be required. It is essential, therefore, that we not become fixed in our concepts or rigidly adherrent to the printed word, and that we, as physicians, not relinquish our responsibility for the pronouncement of death. Also, we must remain sensitive to the effects of our actions on families and nurses, as well as on public opinions, and we should always attempt to be clear in our own minds whether our therapeutic endeavors are prolonging life or protracting death.

In summary, I would recommend:

1. Extreme caution be taken to insure that a potential donor not be sedated or anoxic.
2. Loss of spinal reflexes not be included as part of the brain death criteria.
3. Flexibility be allowed in the length of time a patient must be observed before brain death is established.
4. Cerebral arteriography be used to assist the physician in establishing brain death when a long period of observation is impractical.
5. The urgencies of transplantation not be allowed to interfere with the physicians obligations to the potential donor's family.
6. Nursing morale be supported by frequent discussions of the concept of brain death and the values of transplantation.

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L'ÉLECTROENCÉPHALOGRAMME COMME CRITÈRE DE LA MORT CÉRÉBRALE *

Fernand POIRIER, M.D.

L'électroencéphalographie permet de déceler l'activité ou l'absence d'électrogenèse cérébrale et par le fait même de distinguer entre la vie et la mort de l'individu. Un électroencéphalogramme dit « plat » ou « iso-électrique » peut être obtenu dans d'autres états que la mort cérébrale, par exemple, en cas d'anxiété marquée ou d'une tumeur cérébrale. En fait le cerveau comporte une multitude de points iso-électriques. Le silence électro-cérébral est une expression plus conforme à la réalité. La mort cérébrale sera soupçonnée en l'absence d'électrogenèse cérébrale et démontrée si ce silence électro-cérébral se prolonge après certaines stimulations par agents chimiques, comme le Métrazol, après une
(suite du résumé en page suivante)

C'est pour moi un grand honneur que d'avoir l'occasion de vous entretenir d'un sujet aussi grave que celui de la mort cérébrale. Ce fut également pour moi, au cours de l'année 1968, une lourde responsabilité que d'avoir à décider de celle-ci dans le contexte des transplantations cardiaques pratiquées par l'équipe de l'Institut de cardiologie de Montréal.

J'entends aujourd'hui faire le procès de cette technique paraclinique qu'est l'électroencéphalographie et désire me poser devant vous quatre questions fondamentales:

1. L'électroencéphalogramme est-il capable de déceler la mort cérébrale?
2. Un électroencéphalogramme plat signe-t-il à coup sûr une mort cérébrale?
3. Quelles sont les conditions requises pour que cet électroencéphalogramme soit fiable?
4. Pour ce qui intéresse surtout les participants à ce symposium, est-ce que l'électroencéphalogramme est nécessaire, utile ou superflu dans le cas de transplantations cardiaques imminentes?

Premièrement, à la question, à savoir si l'électroencéphalogramme est capable de déceler la mort cérébrale, je répondrai oui, puisque la vie n'est pas une structure ou même un état mais une fonction, et que la vie humaine, pour sa part, est par excellence fonction cérébrale.

Des fonction cérébrales, la plus fondamentale et la plus permanente est sûrement l'activité électrique spontanée du cerveau que nous appelons l'électrogenèse cérébrale. Et l'électroencéphalographe est justement un appareil inventé pour détecter celle-ci.

Deuxièmement, un électroencéphalogramme plat signe-t-il à coup sûr une mort cérébrale? Je dois dire: hélas! pas encore.

Signalons tout d'abord que le terme « plat » n'est pas très heureux dans ce contexte et que celui de « isoélectrique » ne l'est guère davantage puisqu'une anxiété marquée peut donner lieu à un tracé plat tout autant qu'un coma, sinon davantage, et qu'une tumeur cérébrale peut se manifester par la présence d'une ligne isoélectrique. De plus, le cerveau comporte une multitude de points ''isoélectriques'', ce qui veut dire que l'activité électrique ou le voltage sur chacun de ces points est égal, pas plus.

Un silence électrocérébral, expression beaucoup plus conforme à la réalité, se vérifie soit:

1° spontanément, et l'on peut dire alors que l'écran de télévision n'est pas allumée;

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application d'électro-chocs, ou encore après une stimulation sensorielle tactile, douloureuse ou visuelle. L'hypothermie et l'intoxication médicamenteuse donnent des E.E.G. pratiquement plats. Par ailleurs, dans les cas de décès cérébral où la circulation est maintenue, celle-ci peut être responsable d'une certaine activité électrique cérébrale. Les complexes QRS notés sur l'E.E.G., en phase terminale représentent probablement un électrocardiogramme que l'on ne reconnaissait pas sur le tracé ordinaire puisque ces complexes sont camouflés par l'activité électrique cérébrale régulière.

2° par la réponse à certaines stimulations, que celles-ci soient :

a) des agents chimiques comme le métrazol,

b) des moyens physiques comme l'application d'électro-choc,

c) des stimulations sensorielles, tactiles, douloureuses ou visuelles.

On dit alors que non seulement l'écran de télévision n'est pas allumée, mais aussi qu'elle ne transmet pas les images. Ainsi, non seulement la télévision n'est plus bonne, mais elle a perdu sa fonction principale, spécifique.

Puisque, cependant, les réactions cérébrales à certaines stimulations peuvent être très faibles dans le cas qui nous concerne, le recours à des amplifications plus poussées et à l'usage d'ordinateurs afin de les mieux mettre en évidence par un processus de sommation et nous mieux permettre de les détecter s'avèrent fort utiles dans ce cas-ci.

Laissez-moi vous signaler deux situations qui vous sont bien connues comme susceptibles de donner des tracés E.E.G. facticement plats, sans que l'issue pour le sujet soit nécessairement fatale, à savoir :

a) l'hypothermie, avec une température de moins de 90°F, par exemple ;

b) l'intoxication médicamenteuse, notamment par les barbituriques.

Remarquons que ces états représentent des exemples non pas de fonctions cérébrales, ou vitales, perdues mais seulement suspendues qui, d'ailleurs, peuvent être utilisées dans certains contextes thérapeutiques.

Ce qui est beaucoup plus sérieux et urgent, à mon sens, c'est de découvrir un moyen de reconnaître à coup sûr, facilement et instantanément, ou encore de faire un diagnostic différentiel irrécusable entre le tracé plat d'une intoxication médicamenteuse et le tracé nul d'une mort cérébrale.

Pour ma part, je ne vois pas comment ces tracés pourraient être identiques ; seule la grossièreté de nos appareils de mesure et même de notre interprétation peut être responsable de cette confusion.

D'autre part, je ne crois pas que le tracé puisse être plat ou silencieux lorsque le cœur continue à battre et à envoyer des ondes pulsatiles au cerveau dont l'activité de base ne m'apparaît, à moi du moins, que comme une forme étalée, redondante, et je dirais « transducée » de celles-ci.

Je crois même que les complexes QRS que l'on observe mieux sur l'EEG à la phase terminale, lorsque celui-ci est plat, surtout dans les régions postérieures et plus particulièrement à gauche, ne sont probablement pas dus à une tranmission plus facile sur la surface cutanée de ces potentiels mais représentent possiblement une forme d'électroencéphalogramme qu'on ne reconnaissait pas sur le tracé ordinaire, puisque ces complexes étaient camouflés par l'enveloppe de l'EEG. Ceci reste évidemment à vérifier.

Troisièmement, quelles sont les conditions requises pour que l'électroencéphalogramme soit assez faible pour nous permettre d'attester avec quelque assurance d'une mort cérébrale ?

Celles-ci sont connues, et puisqu'elles présentent un aspect technique avec lequel la plupart des participants à ce symposium ne sauraient être

familiers, je me permets de vous les signaler brièvement et de fournir quelques commentaires appropriés.

Je m'en réfère surtout au rapport du Comité *ad hoc* de l'université de Harvard publié au mois d'août 1968.

1° L'appareil doit bien fonctionner, et c'est évident; la calibration du tracé doit en faire foi;

2° Les électrodes doivent être bien placées. Pour ma part, je ne verrais pas d'objection à insérer des aiguilles au travers du cuir chevelu d'un malade profondément inconscient ou même de les implanter au travers de la table crânienne jusqu'au niveau de la dure-mère ou encore jusqu'à l'intérieur du parenchyme cérébral;

3° L'amplitude doit être au moins double de celle qui est utilisée habituellement; il faut cependant se méfier de ne pas porter l'appareil à son amplification maximale parce qu'on augmente alors l'importance des artéfacts qui nécessairement sont très nombreux, et pour ainsi dire aux aguets, dans des salles de soins intensifs;

4° On dit que l'enregistrement doit durer au moins dix minutes; pour ma part, je vois plutôt qu'un tracé de dix secondes soit amplement suffisant dans certaines circonstances pathologiques, tandis que dans d'autres un tracé de dix heures ou de dix jours serait loin d'être rassurant.

Dans le même contexte, on suggère de répéter l'électroencéphalogramme 24 et 72 heures après, mais ceci est loin de me satisfaire; les chirurgiens cardiaques responsables de la transplantation non plus, à cause du délai encouru.

Il est donc d'ores et déjà évident que la mort survenant à un moment précis et non pas à un moment quelconque puisqu'il s'agit d'un phénomène absolu et sans nuance, négatif d'ailleurs (autrement dit, on ne peut pas être un peu moins ou plus mort), il nous incombe de trouver un moyen sûr d'en arriver à un diagnostic formel, final et irrévocable.

Quatrièmement, pour ce qui est de l'utilité de l'électroencéphalogramme dans le contexte des transplantations cardiaques à pratiquer, je dirai que dans l'état actuel de nos connaissances, en ce mois de juin 1969, malgré les embûches que comporte cette technique paraclinique, les chirurgiens cardiaques doivent se soucier de solliciter cet examen et de s'assurer qu'il y a vraiment silence électrocérébral chez le malade qu'on considère comme donneur imminent.

En effet, certains états cliniques peuvent s'accompagner d'un coma extrêmement dense et laisser percer une activité électrique cérébrale qui est sûrement beaucoup plus subtile, précise, et spécifique de vie humaine, que tous les autres critères connus actuellement.

D'autre part, il est évident que l'EEG ne constitue pas l'unique critère de mort dont on dispose et qu'il doit être placé dans le contexte clinico-pathologique particulier à chaque cas: traumatisme crânien, absence de respiration spontanée, etc.

En terminant, je forme le souhait que l'analyse plus poussée et précise des derniers instants de la vie d'un homme nous aidera à mieux planifier et préparer avec assurance le sursis de vie qu'on veut offrir à un autre être humain et surtout à mieux comprendre le principe fondamental de cette même vie.

Espérons, en somme, qu'en apprenant comment l'on ne vit plus, on comprendra mieux comment l'on vit.

ANESTHETIC MANAGEMENT IN NINE HEART TRANSPLANTATIONS *

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Ce travail décrit la conduite de l'anesthésie au cours de neuf transplantations cardiaques et rapportent les observations faites chez les donneurs et les receveurs de cette série.

Les donneurs. Les donneurs, apnéiques par définition, doivent être ventilés artificiellement, ce qui ne pose habituellement pas de problèmes, à moins qu'il n'y ait eu un traumatisme thoracique. Les donneurs n'ont pas présenté de difficultés en ce domaine.

L'usage de vasopresseurs a été nécessaire pour maintenir une pression artérielle satisfaisante chez tous les donneurs, sauf dans un cas.

Human heart transplantation is a new endeavour for anesthesiologists (1 and 3). The purpose of the present paper is to review the problems encountered in the transport and maintenance of donors, and to report our experience in the anesthetic management of nine human heart transplantations performed at Montreal Heart Institute in 1968.

I. DONORS

The responsibility for the safe transport of donors from their respective hospitals to the Institute was given to the anesthesiologists.

All our donors had extensive brain damage and behaved like patients under total spinal anesthesia:

1. They were apneic and had to be ventilated.
2. They developed hypotension even in the presence of normovolemia and required vasopressors to maintain an adequate circulation.
3. Finally they were quite sensitive to changes of position and had to be moved with care.

Equipment:

The material used for the transport was simple (Figure 1). Ventilation was performed manually with an Ambu bag connected to an oxygen

Figure 1 — Equipment used during the transport of donors.

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* Presented at the Second world symposium on heart transplantation held in Montreal, June 6, 7 and 8th, 1969.

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Des hypovolhémies ainsi que des déséquilibres acido-basiques et électrolytiques ont été fréquemment observés et, en autant que possible, nous nous sommes efforcés de les corriger avant le prélèvement du myocarde.

Les receveurs. Une anesthésie légère est de mise dans ces cas, comme chez tous les grands malades. Nous avons opté pour le mélange protoxyde d'azote-oxygène, avec de petites doses intraveineuses de curare et de narcotiques.

Une attention particulière a été apportée par les chirurgiens à la protection des myocardes transplantés : prélèvement sous circulation extracorporelle avec hypothermie, dans les huit derniers cas, refroidissement par du soluté physiologique froid, et perfusion coronarienne durant une partie de la greffe. Chez huit de ces receveurs, une excellente circulation a été maintenue par l'organe greffé dès l'interruption de la circulation extracorporelle.

Des suites opératoires immédiates relativement bénignes ont été observées chez sept des neuf receveurs.

cylinder. We took along a portable suction apparatus and an assortment of the drugs used in the treatment of cardiac arrest. A portable battery-operated cardioscope has been an excellent addition. For long trips a battery-powered defibrillator was also carried.

Vasopressors:

When we took charge of these cases at the time of transfer, all of them but one were already receiving a vasopressor (Table I). The selected agents were of all types. We usually continued to use the same agent during transportation to the Institute, and afterwards if needed before surgery.

TABLE I

Vasopressors received by the donors

DONOR N°.	BEFORE TRANSFER	DURING TRANSPORT	AT M.H.I. BEFORE TRANSPLANT
1	Epinephrine	Epinephrine	Epinephrine
2	Metaraminol	Metaraminol	Metaraminol
3	—	—	—
4	Isoproterenol	—	—
5	Methoxamine	Methoxamine	—
6	Methoxamine	Methoxamine	—
7	Metaraminol	Metaraminol	Metaraminol
8	Phenylephrine	Phenylephrine	Phenylephrine
9	Epinephrine	Isoproterenol	Isoproterenol

Transfusion:

Many of the donors were hypovolemic and needed transfusions (Table II) to restore an adequate blood volume.

Electrolytes:

Electrolytes (potassium range from 1.7 to 6.5 mEq/L) and acid base disturbances were found in most cases and appropriate therapy was applied to keep "the donor organ in the best possible state before the transplant team is able to take action (3).

II. RECIPIENTS

All our recipients were males (ages from 35 to 59 years, average 50) with advanced coronary disease, multiple infarctions, severe incapacity and progressive heart failure despite active treatment.

Anesthetic management:

Figure 2 summarizes our anesthetic management before the bypass.

1. Five of these cases had signs of severe heart failure at the time of surgery.

2. Four of them were orthopneic and anesthesia was induced in a sitting position.

3. A very light plane of anesthesia was maintained, as usually is the case in cardiac surgery.

4. We experienced only three short episodes of moderate hypotension (Figure 2) in recipients before the bypass.

ECG, central venous pressure, intra-arterial pressure and œsophageal temperature were monitored.

Perfusion:

Perfusions were made with a Sarns pump and disposable (Travenol) oxygenators primed with dextrose 5 per cent in half strength saline (1,200 to 1,400 ml), with added bicarbonate and potassium chloride. During the bypass, blood or dextrose solution were added to the pump if needed. Moderate hypothermia (29 to 30° C) was used.

Except for case No. 1, all donors' hearts were removed under hypothermic extracorporal circulation and further cooled with cold saline. The myocardium was also perfused with a coronary pump during the suture of the pulmonary arteries (4).

Observations after the bypass (Figure 3):

1. Case No. 1 presented a low cardiac output with bradycardia. This small, poor-quality heart could not carry the full load of the circulation (4).

2. In all other cases the heart performance after the bypass was good. Hemodiluted pump blood or fresh blood was given as needed to maintain the left atrial pressure around 15 mm Hg (2).

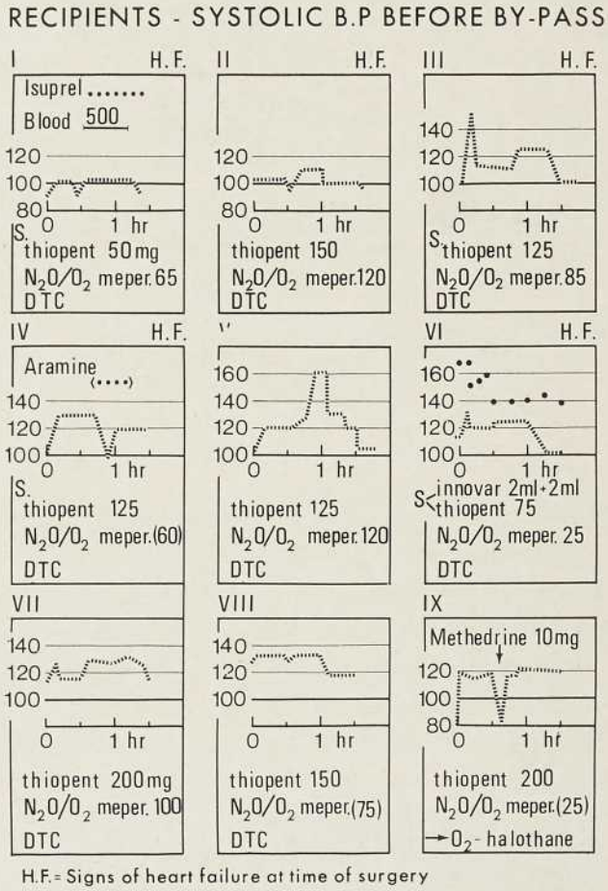

Figure 2 — Anesthesia before the by-pass. Following pre-medication with Scopolamine or Atropine and Pentobarbital, light plane of anesthesia was maintained with Nitrous oxide, oxygen, curare and small doses of narcotics.

TABLE II

Amount of blood received by the donors [1]

DONOR N°	1	2	3	4	5	6	7	8	9
In hospital before transfer	—	2 000	—	1 000	—	2 000	6 000	500	2 500
During transfer ..	—	500	—	—	1 000	—	—	—	—
At M.HI. before transplant	—	1 500	—	1 000	—	500	—	—	—

1. Many of these patients were actively bleeding or hypovolemic and required blood to restore an adequate circulation.

3. We did not experience the repeated episodes of hypotension shortly after bypass as reported by Keats *et al.* in Doctor Cooley's series (7). We feel that the hypothermic perfusion of our eight donors before their hearts were removed and again after approximately 45 minutes of anoxia contributed to the better immediate performance after transplantation.

4. An excellent diuresis (average 690 ml) followed bypass.

5. All recipients were digitalized shortly after surgery or immediately after the pump.

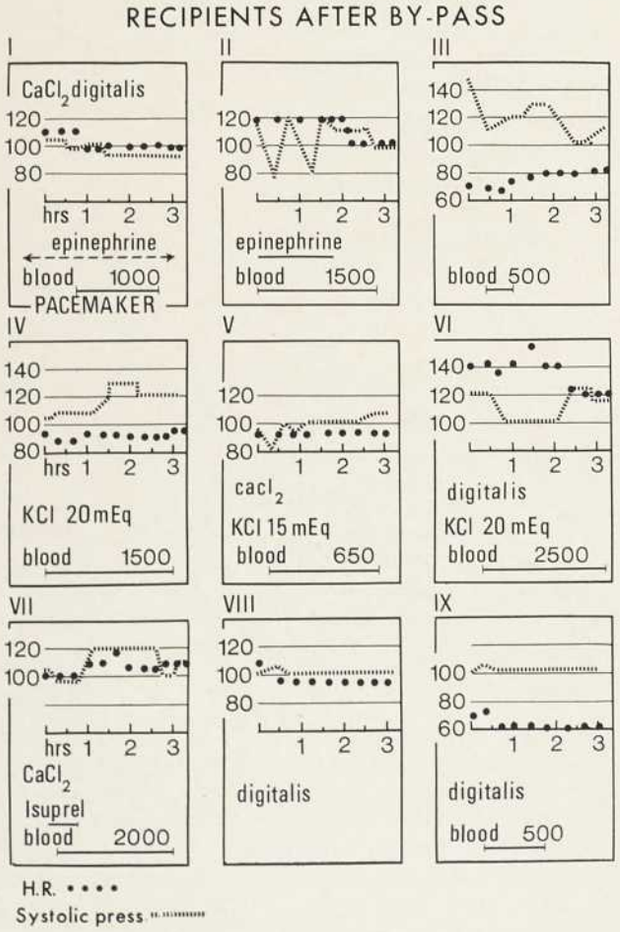

Figure 3 — In case No 1, right after the by-pass, hypotension and bradycardia were experienced. Tachycardia and arrhythmia followed Isoproterenol administration. A good response was obtained with Epinephrine. This small heart could not carry the full load of circulation. Heart action after the pump was excellent in all other cases. One could observe the steady stable heart rates of denervated hearts. Digitalis was started shortly after surgery in the first few cases and right after the pump in the last ones.

6. Nitrous oxide and oxygen were the anesthetic agents used after bypass.

Early postoperative period (Table III):

1. Six recipients were awake and well-oriented at the end of surgery. One patient was still curarised and cold: he recovered slowly over the next 18 hours.

2. After surgery our patients were moved to an adjoining sterile operating room which was used as an intensive care unit for the next 24 to 48 hours.

3. In case No. 1 the circulation had to be supported by intermittent partial bypass till the death of the patient 46 hours later.

4. Our recipient No. 3 presented an ischemic right leg shortly after the surgery and was taken back to the operating room 33 hours later. He tolerated very well (4) a five-hour intervention (resection of an abdominal aortic aneurysm with bilateral aorto-femoral graft).

5. All other patients maintained an excellent circulation during the postoperative period. The steady, stable rate of denervated hearts could be observed in all cases.

6. Recipient No. 9 had junctional bradycardia which responded well to Isoproterenol.

7. Extubation was performed 7 to 15 hours after surgery and

8. As confidence increased, the last ones were extubated, sat in their bed, encouraged to cough, then helped to their feet on a stool. They made a few standing steps while the bed sheets were being changed. This was well tolerated.

SUMMARY AND CONCLUSIONS

Observations made during nine human cardiac allografts have been reported with the anesthetic management of the recipients.

What struck us most was the benign early postoperative course of most of these cases: they compared favorably to ordinary transventricular commissurotomy cases.

The excellent circulation observed after the bypass is thought to be due to the protection of the

TABLE III

Recipients — Early P.O. period [1]

		B.P. + H.R. END OF SURGERY A = AWAKE AT END			WELL ORIENTED (hrs P.O.)	EXTUBATION (hrs P.O.)	AMBULATION (hrs P.O.)
			(adrenalin)				
I	intermitt. partial by-pass	A	90	100			
			—	—			
			60	min			
	130 – 90 90	A	110	100			
II	——— —		—	—	End of surgery		
	70 min		60	min		14½	21
	150 – 100 90		110	80			
III	———		—		11 hrs		
	80 – 90		80			15½	(aorto-iliac graft)
	130 – 90 85		130	90			
IV	———		—		7½ hrs		
	60 – 80		80			12	32
	115 – 100 75	A	115	90			
V	———		—		End of surgery		
	60 – 70		70			7¾	30
	130 – 100 100	A	110	120			
VI	———		—		End of surgery		
	60 – 70		70			7½	7½
	140 – 100 70		120	110			
VII	———		—		18 hrs		
	70		90			5½ (acc.)	24
	130 – 90 90	A	110	95			
VIII	———		—		End of surgery		
	60 – 80		60			6¾	6¾
	105 – 130 70	A	110	55			
IX	———		—		End of surgery		
	70 – 80 (on Isuprel)		55			10¼	10¼

1. Despite active treatment, case N° 1 developed progressive hypotension. His sternum was reopened and circulation was supported by intermittent partial hy-pass during the next 43 hours. Case N° 3 presented an ischemic right leg after surgery and had an aorto-iliac graft 2 days after the transplant. All other cases had bening early post-operative course.

donors's hearts by hypothermia and coronary perfusion during the operation.

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OPERATIVE TECHNIQUE FOR CARDIAC TRANSPLANTATION *

Grady L. HALLMAN, M.D., and **Denton A. COOLEY,** M.D.

Au moyen d'une circulation extracorporelle avec oxygénateur à bulles amorcé avec un soluté de glucose à cinq pour cent, le cœur du receveur est enlevé en prenant soin de laisser en place les parois postérieures des oreillettes, les veines caves et les veines pulmonaires. Le cœur du donneur est complètement excisé. Une ligature est placée sur la portion cardiaque de la veine cave supérieure. L'oreillette droite est incisée à partir de la veine cave inférieure vers l'appendice auriculaire droit. De cette façon le nœud sino-auriculaire et les voies des conductions vers le nœud auriculo-ventriculaire sont préservés. L'allogreffe cardiaque n'est pas perfusée ni refroidie. L'anastomose du nouveau cœur commence sur le versant externe de la paroi auriculaire gauche au moyen d'un surjet. En second lieu les septums sont anastomosés et l'anastomose de l'oreillette droite complète la fixation. L'artère pulmonaire est taillée à une longueur convenable et anastomosée au moyen d'un surjet simple. Enfin, l'anastomose aortique est pratiquée de la même façon. Après l'évacuation de l'air des cavités cardiaques, le cœur est défibrillé. Dans certains cas, l'action cardiaque fut stimulée par de la digitale ou de l'Isoprotérénol.

(suite du résumé en page suivante)

Cardiac transplantation is scheduled when brain death of the donor is confirmed. Excision of donor and recipient hearts is carried out simultaneously in adjacent operating rooms. Using a disposable bubble oxygenator primed with 5 per cent dextrose in water, temporary cardiopulmonary bypass is instituted in the recipient (2 and 3), and the heart is removed by transecting the right atrium, ascending aorta, pulmonary artery, and left atrium. The posterior walls of the atria with attached venae cava and pulmonary veins remain to permit attachment of the donor heart (4) (Figure 1).

After the donor is given heparin the donor heart is excised and prepared by opening the posterior left atrium between the ostia of the pulmonary veins, ligating the superior vena cava, and incising

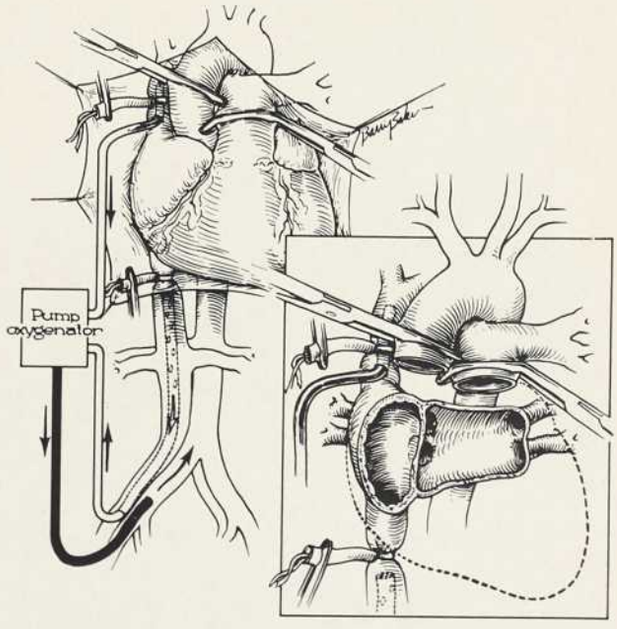

Figure 1 — Technique of cannulation for cardiac transplantation. Inferior caval catheter may be inserted through right atrium. (Inset) Portions of atria and great vessels remaining after excision of heart. Reprinted by permission from Cardiac transplantation for advanced acquired heart disease, Cooley, D.A., Bloodwell, R.D., and Hallman, G.L., *J. Cardiov. Surg.* (Blalock Issue), 9 : 403-413, (Sept-Oct) 1968.

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* Paper presented at the Second world symposium on heart transplantation, Montreal, Canada, June 6–8, 1969.

From the Cora and Webb Mading Department of Surgery, Baylor College of Medicine, and the Texas Heart Institute of St. Luke's and Texas Children's Hospitals, Houston, Texas.

Supported in part by U.S. Public Health Grants (HE-03137) (HE-05387) (HE-05435).

Un des patients, un bébé de deux mois présentant un canal atrio-ventriculaire commun complet avec complication pulmonaire sévère, reçut une transplantation cardio-pulmonaire combinée à partir d'un donneur anencéphalique. Le cœur et les poumons du donneur furent excisés en bloc en divisant la tranchée, l'aorte ascendante et les deux veines caves. Entre-temps le cœur et les poumons du receveur furent excisés en laissant ouvert la trachée et l'aorte. Des rebords auriculaires furent préservés au niveau de chaque veine cave. Les nerfs phréniques furent également préservés sur des ponts de péricarde. Les poumons de l'allogreffe furent introduits dans chaque cavité pleurale à travers les orifices du péricarde postérieur. Les anastomoses trachéales, aortiques et les veines caves furent effectuées au moyen de surjets. Ces deux techniques opératoires sont illustrées de huit schémas.

the right atrium from the inferior vena cava toward the right auricular appendage (Figure 2). This technique preserved the sino-auricular node and most of the internodal conduction pathways to the atrioventricular node (6, 7, 10 and 11). Neither perfusion nor hypothermia are used for the allograft (1 and 9).

The donor heart is inserted by continuous suture anastomoses beginning at the lateral margins of the left atria (Figure 3) and progressing to the right atria. Adjacent septal margins of both atria are attached to the recipient's atrial septum (Figure 4). The main pulmonary arteries and then the aortae are tailored and anastomosed, completing insertion (Figures 5 and 6). Following aspiration of intracardiac air with a needle and syringe, the aortic clamp is removed, and coronary flow restored. Cardiac action returns in either sinus rhythm or ventricular fibrillation (8). In case of the latter, conversion is accomplished with a direct current countershock. At termination of cardiopulmonary

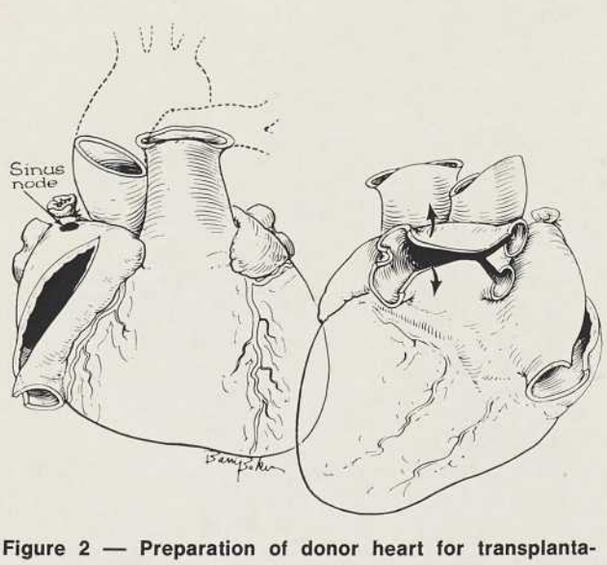

Figure 2 — Preparation of donor heart for transplantation. Incision in right atrium extends from inferior vena cava into appendage, avoiding S-A node. Left atrium opened posteriorly with incision connecting pulmonary veins. Reprinted by permission from Cardiac transplantation for advanced acquired heart disease, Cooley, D.A., Bloodwell, R.D., and Hallman, G.L., *J. Cardiov. Surg.* (Blalock Issue), 9 : 403-413, (Sept-Oct) 1968.

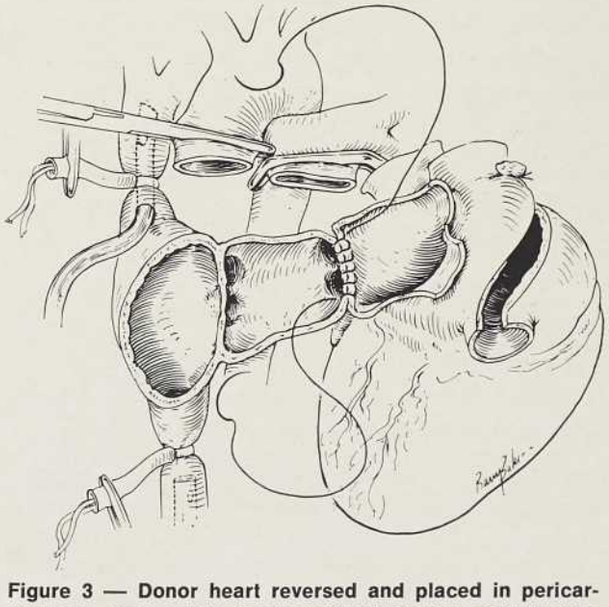

Figure 3 — Donor heart reversed and placed in pericardial sac. Suture line begins at left border of left atria and is completed at atrial septum of recipient. Reprinted by permission from Cardiac transplantation for advanced acquied heart disease, Cooley, D.A., Bloodwell, R.D., and Hallman, G.L., *J. Cardiov. Surg.* (Blalock Issue), 9 : 403-413, (Sept-Oct) 1968.

bypass cardiac output and blood pressure are usually normal, although digitalis, and sometimes isoproterenol, are occasionally necessary. After operation an isolated recovery room receives the patient whose physiologic functions are then monitored continuously for 24–36 hours.

One patient in our series, a 2-month-old infant with complete atrioventricularis communis and severe pulmonary complications, received a combined cardiopulmonary transplant from an anencephalic donor (15). The heart and lungs of the donor were excised as a unit by dividing the trachea, ascending aorta, and both venae cava. Meanwhile, the recipient's heart and lungs were excised leaving cut ends of the trachea, aorta, and cuffs of atrium at each vena caval orifice (Figure 7). (The phrenic nerves were preserved on pedicles of pericardium). Metal caval catheters provided venous return to the pump oxygenator and arterial perfusion was through the ascending aorta. The lungs of the allograft were introduced into each pleural cavity through the openings in the posterior pericardium. Continuous suture technique was used for tracheal, aortic, and venae caval anastomoses respectively (Figure 8). Blood flow was restored upon release of the aortic clamp.

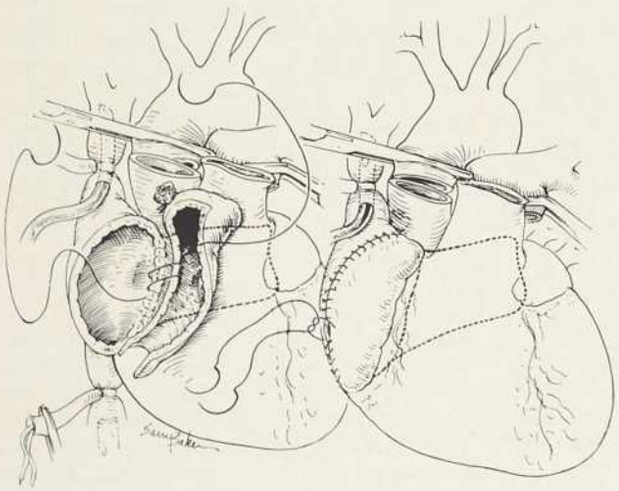

Figure 4 — Anastomosis of right atria begins at atrial septum of recipient and is completed laterally. Reprinted by permission from Cardiac transplantation as palliation of advanced heart disease, Cooley, D.A., Hallman, G.L., Bloodwell, R.D., Nora, J.J., and Leachman R.D., *Arch. Surg.*, 98 : 619-625, (May) 1969.

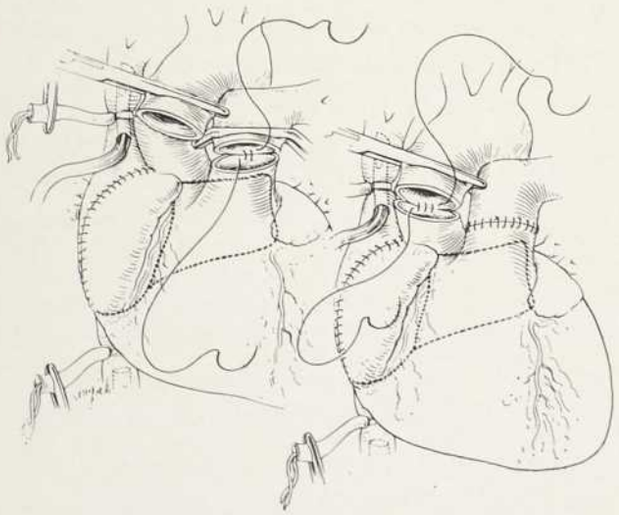

Figure 5 — Pulmonary artery and aorta anastomosed with continuous suture. Reprinted by permission from Cardiac transplantation as palliation of advanced heart disease, Cooley, D.A., Hallman, G.L., Bloodwell, R.D., Nora, J.J., and Leachman, R.D. : *Arch. Surg.*, 98 : 619-625, (May) 1969.

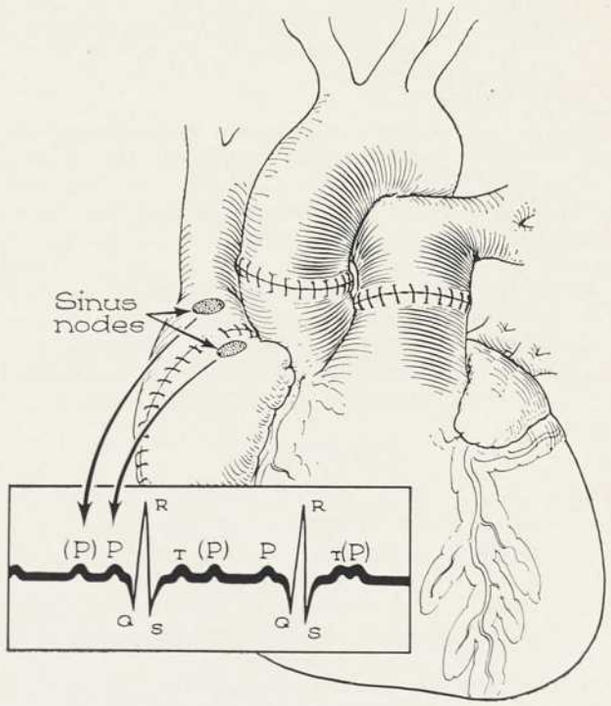

Figure 6 — Anastomoses completed and cannulae and clamps removed. Patient has two S-A nodes and each produces a P wave in the ECG. Only the P wave from the S-A node in the donor heart is followed by a ventricular contraction. Reprinted by permission from Transplantation of the heart, Cooley, D.A., Hallman, G.L., and Bloodwell, R.D., Chapter in Craft of Surgery, *Little, Brown and Company*, (Ed. Philip Cooper, M.D.), Boston, In press.

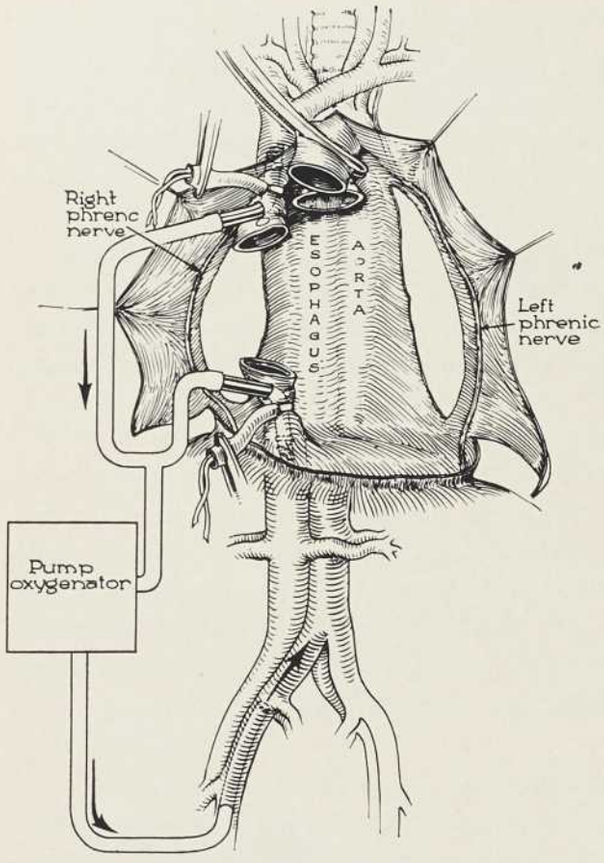

Figure 7 — Diagram of operative technique for cardiopulmonary transplantation. Appearance after removal of recipient heart and lungs. Cut ends of trachea, aorta, and cuffs of atrium at each vena caval orifice. Venous return to pump-oxygenator through metal cannulae in cavae : arterial perfusion into ascending aorta. Phrenic nerves preserved on pedicles of pericardium. Reprinted by permission from Organ transplantation for advanced cardiopulmonary disease, Cooley, D.A., Bloodwell, R.D., Nora, J.J., McNamara, D.G. Leachman, R.D., and Hallman, G.L., *Ann. Thorac. Surg.*, 8 : 30, 1969.

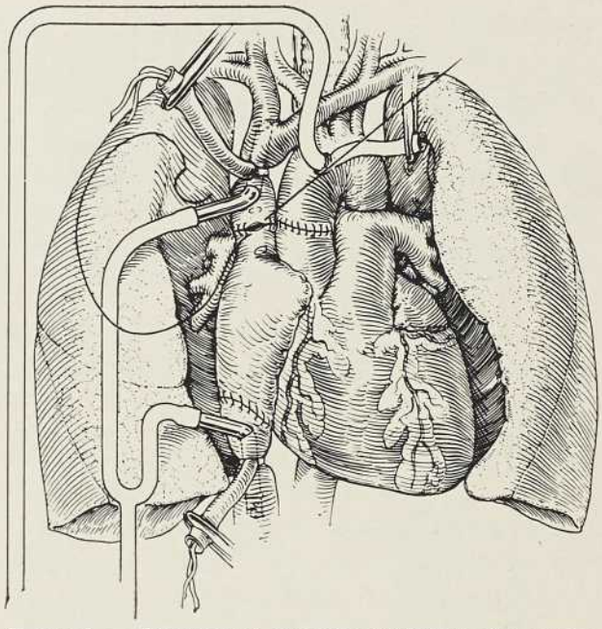

Figure 8 — Diagram of operative technique for cardiopulmonary transplantation. After completion of tracheal and aortic anastomoses, venae cava approximated. Blood flow restored to donor heart when aortic clamp removed. Reprinted by permission from Organ transplantation for advanced cardiopulmonary disease, Cooley, D.A., Bloodwell, R.D., Nora, J.J., McNamara, D.G., Leachman, R.D., and Hallman, G.L., *Ann. Thorac. Surg.*, 8 : 30, 1969.

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MYOCARDIAL HYPOTHERMIA FOR CARDIAC TRANSPLANTATION *

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William W. ANGELL, M.D. and **Norman E. SHUMWAY,** M.D. †

Quinze cents patients furent opérés par les auteurs pour remplacement valvulaire. Dans la moitié des cas, la seule méthode de préservation myocardique fut l'hypothermie locale externe. L'hypothermie s'est avérée efficace et un retour de la fonction cardiaque fut observé dans tous les cas même après deux heures et demie d'arrêt anoxique.

Expérimentalement, cette méthode fut vérifiée lors de transplantations cardiaques. Le cœur excisé est immergé dans un soluté salin à 4°C pendant 10 minutes. Après l'anastomose des oreillettes, le cœur est de nouveau immergé au moyen d'un cathéter intraventriculaire gauche passé par l'auricule gauche. Grâce à cette méthode, la survie opératoire est de 90 pour cent. Le temps d'anoxie myocardique était d'environ 55 minutes. Des études élaborées, basées sur des mesures biochimiques et mécaniques démontrèrent qu'un cœur normothermique reste viable en autant que la période d'anoxie ne dépasse pas 30 à 40 minutes. A 24°C la période d'anoxie peut atteindre 90 à 140 minutes et à 15°C le cœur peut reprendre sa fonction après un arrêt anoxique qui se prolonge pendant 180 à 280 minutes. (suite du résumé en page suivante)

INTRODUCTION

It is apparent that various methods may be used satisfactorily for preservation of myocardial viability during clinical cardiac transplantation. These include coronary artery perfusion *via* the aortic arch (2), localized myocardial hypothermia (7), and simple exposure of the graft to room temperature with rapid reestablishment of coronary circulation (4). For each method advantages and disadvantages have been proposed. In the absence of controlling data, we wish in this report simply to present our technique and results of myocardial hypothermia in cardiac transplantation.

That local myocardial hypothermia induced by surface cooling with isotonic saline is effective for short-term preservation of myocardial viability is attested by its successful clinical use in approximately 1,500 patients undergoing valve replacement or ventriculotomy at Stanford University Medical Center. In approximately half these cases hypothermia of the heart produced by surface cooling constituted the sole method of preservation; in the other half this was combined with selective low pressure perfusion of the left coronary artery. Periods of myocardial protection with hypothermia alone have ranged up to 2.5 hours with full return of function. In no case in this clinical experience has myocardial dysfunction related to inadequate protection during anoxia been identified.

Laboratory experience:

Myocardial protection obtained by hypothermia has contributed significantly to the success of

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L'hypothermie locale externe fut employée comme méthode de préservation du myocarde au cours de 15 transplantations cardiaques chez 14 patients. Dix minutes de refroidissement par immersion furent pratiquées et après l'anastomose auriculaire gauche, le cœur fut de nouveau irrigué au moyen de soluté salin froid.

L'ischémie myocardique dans les 15 cas fut en moyenne de 59 minutes. Dans tous les cas, un rythme sinusal fut obtenu lors de la réanimation. La fonction myocardique fut satisfaisante dans tous les cas sauf un. Dans ce cas particulier, on constate une hypotension et des arythmies ventriculaires récidivantes après l'implantation d'un cœur qui avait présenté un arrêt cardiaque pré-opératoire.

Quoique cette technique soit suffisante pour le transfert d'un cœur du donneur au receveur, des modifications additionnelles seront à l'avenir nécessaires pour prolonger la conservation afin de pouvoir utiliser de façon efficace tous les organes possibles en vue d'une transplantation.

cardiac transplantation in the laboratory. Current techniques involve surface cooling of the heparinized donor heart immediately upon excision by immersion in saline at 4–6°C. After ten minutes an intramyocardial temperature of 6–10° is attained. The heart is then removed from saline and implantation in the recipient accomplished by methods previously described (5). Following completion of the atrial anastomoses, the heart is again briefly cooled by irrigation of the left ventricle through an indwelling polyethylene tube placed through the left atrial appendage. Coronary circulation is reestablished following completion of the aortic and pulmonary artery anastomoses. In the past fifty consecutive cardiac homograft and autograft procedures performed in this laboratory, operative survival has been 90 per cent. The average myocardial anoxia time has been 55 minutes. Neither digitalis nor vasopressors have been employed; atrial pacing to maintain a heart rate greater than 120/beats/min. has been used, if necessary. In all subjects surviving operation cardiac output has been mildly to moderately depressed immediately following surgery, rising to normal levels within 48 to 72 hours postoperatively.

In an attempt to define the relationships between preservation of organ viability, duration of anoxia, and mycoardial temperature during the anoxic interval, the following experiments were performed (1). Ten donor hearts were stored 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 carotid artery of a second dog. Viability of the graft perfused in this manner by an intermediate host was assessed by observation of the cardiac rate, rhythm, and appearance, measurement of the strength of left ventricular contraction with the use of an intraventricular balloon, serial weights, measurement of serum lactic acid dehydrogenase, glutamic oxylacetic transaminase, and creatine phosphokinase levels in coronary sinus return, light microscopy, and electron microscopy. Orthotopic transplantation of five hearts from each of the subgroups served as the ultimate test of viability and provided confirmation of the validity of the other parameters listed as indices of viability.

These experiments served to roughly define the allowable anoxic intervals for maintenance of organ viability at the given temperatures. Hearts stored at 37° remained viable for greater than 30 but less than 40 minutes, those at 24° remained viable for greater than 90 but less than 140 minutes, and those at 15° remained viable for greater than 180 but less than 280 minutes. The relationship of maximum duration of allowable cardiac anoxia to temperature is best expressed as a linear function of

the logarithm of the temperature of storage. Two points derived from this relationship deserve emphasis. First, hearts remaining anoxic at greater than 37°C suffer rapid irreversible damage, and under the usual circumstances of organ procurement, cannot be transplanted successfully. Secondly, even moderate degrees of organ hypothermia significantly extend allowable anoxia time.

Clinical experience:

In the transplantation of 15 hearts in 14 patients local myocardial hypothermia has been employed as the sole method for organ protection. After full heparinization of the donor, the heart is excised and immediately immersed in saline at 4–6°C. Following a 10-minute period of initial cooling, during which time the atria and great vessels are prepared for anastomosis, the heart is removed from saline and the left atrial anastomosis begun. After completion of the left atrial anastomosis, the recipient pericardial well is irrigated continuously with saline at the same temperature to provide continued cooling of the dependent portions of the ventricles. Cooling is discontinued upon release of the aortic cross-clamp to reestablish coronary circulation. With this technique the myocardial ischemia time in 15 cases has averaged 59 minutes (48 to 85 minutes).

In all cases resuscitation of the graft and establishment of normal sinus rhythm was easily accomplished. In all but one instance immediate postoperative function was satisfactory. In patients with severe, long-standing pulmonary hypertension preoperatively, however, central venous pressure remained mildly to moderately elevated for 48 to 60 hours postoperatively, and myocardial support with digitalis and occasionally isoproterenol was employed. Signs of low cardiac output have been absent.

In the one exception, immediate graft failure, including hypotension and recurrent ventricular arrhythmias, developed immediately following transplantation of the heart from a donor in whom cardiac arrest had occurred preoperatively. Vigorous resuscitative measures, including intracardiac administration of epinephrine, were required. In this case successful retransplantation was performed six hours following the initial procedure. Examination of the excised first graft showed a large hemorrhagic infarction in the upper portion of the muscular ventricular septum, thought to be caused by intramural injection of epinephrine.

DISCUSSION

The laboratory and clinical experience described here demonstrates that local myocardial hypothermia alone is satisfactory for short-term preservation of the heart. The limits of this technique are not well-defined, but have been extended to seven hours of hypothermic, anoxic cardiac arrest followed by successful orthotopic transplantation by Lower (6).

The significant extension of allowable anoxia time by even moderate degrees of hypothermia is an important consideration in view of the usual circumstances surrounding transplantation. In our experience body temperature of organ donors has been maintained at 35–36°C to facilitate stable cardiovascular control. Myocardial temperature is further lowered by thoracotomy with exposure to room atmosphere and by ventilation with inspired gases at ambient temperatures. Simultaneous measurements of rectal and intramyocardial temperatures under open chest conditions in both dog and man have consistently demonstrated a temperature gradient producing myocardial temperatures 1–4°C less than general body temperature. Following excision of the donor heart, simple exposure to room atmosphere has resulted in an additional lowering of myocardial temperatures by approximately 3° over 30 minutes. These factors combine to produce a significant degree of moderate hypothermia in the heart which is not actively maintained at 37°C. The resulting extension of allowable anoxia time with maintenance of viability, as extrapolated from laboratory data, is approximately one hour (from 30–40 minutes to 80–100 minutes).

Although this technique can easily accommodate the requirements for myocardial preservation in the current clinical situation of transfer of graft from

a neurologically dead donor immediately to the recipient, additional modifications must be induced for prolonged storage because of persistent metabolic needs at organ temperatures above freezing (3). In the future such systems will become necessary for the efficient utilization of all available organ grafts.

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STORAGE OF THE ISOLATED HEART *

E. PROCTOR †

En parvenant à entreposer un cœur isolé, on gagne du temps pour le transport du cœur, pour pratiquer une opération élective, pour choisir le receveur immunologiquement le plus compétent, pour obtenir l'anonymat du cœur et ainsi éviter une publicité indésirable. De plus, il est possible que par cette méthode l'on puisse ranimer un cœur endommagé par une anoxie *ante mortem*.

Si le temps prévu pour le transport n'excède pas une heure et demie, le cœur peut être immergé, refroidi et transporté dans un sac de plastique contenant de la glace.

Pour la préservation à long terme, jusqu'à 72 heures, une réanimation satisfaisante a été obtenue par une perfusion avec un soluté de Krebs modifié. La faible pression oncotique fut corrigée par l'addition de Dextran à 0,6 pour cent et par une basse pression de perfusion (20 cm d'eau). Le système complet est placé dans un réfrigérateur à 5°C. Les cœurs ainsi traités furent vérifiés quant à leur performance mécanique, leur activité électrique et au point de vue microscopie électronique. Ces cœurs

(suite du résumé en page suivante)

The essential gain from storing the isolated heart is time.

1. Time to transport the heart from donor to recipient.

2. Time to do an elective operation; this is even more important with the heart than the kidney due to the complexities of cardiopulmonary bypass and the larger number of staff involved.

3. Time to tissue-type the heart and find the best recipient, instead of having a typed terminal recipient and hoping that the heart is suitable.

4. Time to gain anonymity of the heart and thus reduce much of the undesirable publicity that clings to this field.

5. Time, one hopes, to resuscitate a heart too damaged by ante-mortem changes to transplant immediately .

Realistic methods of preserving the heart (1) have included 1) metabolic inhibitors; 2) hypothermia; 3) hyperbaria; 4) 'supercooling'; 5) perfusion — either normothermic or with elements of 1–4. The chosen method of storage depends upon the requirements — if all that is need is to transport a heart from one hospital to another within a city, with a travelling range of up to an hour and a half, then flush-cooling the heart with a water-based physiological solution at 5° C. followed by packing it in a plastic bag in ice for transport, should be adequate; we have satisfied ourselves of the reliability of this procedure by experimental orthotopic transplantation. On the other hand, if a period of storage of 24 hours or more is required, then, for all practical purposes hypothermic perfusion offers the most consistent results. 'Supercooling' with glycerol or dimethyl sulproxide — so successful with blood and semen — could be the method of the distant future, but currently the results with organs are disappointing.

Our own method of storing hearts in a viable condition for up to 72 hours (2), uses a modified Krebs' solution (containing one quarter of calcium concentration; insulin 15 units/litre; hydrocortisone

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furent comparés à des cœurs de contrôle isolés et non préservés. La résistance vasculaire coronarienne semble être l'indice le plus sûr quant à la possibilité de la performance future. Une augmentation de 60 pour cent de la résistance coronarienne après 10 minutes de perfusion par rapport à la résistance coronarienne initiale dénote une diminution de la viabilité.

Cette méthode permet d'envisager l'établissement d'une banque cardiaque avec des réfrigérateurs de grande capacité dans lesquels seraient placés des modules isolés de perfusion.

Un tel module est capable de préserver un cœur pendant dix heures sous sa forme portative, après quoi, en plaçant le module dans un grand réfrigérateur et en le branchant au réseau d'électricité, il devient ainsi une unité de la banque. La viabilité de ces cœurs reste connue à tout instant par la mesure de la résistance coronarienne.

40 mg/litre; and procaine HCl 0.013 per cent) and on-line filtration with eight micron pore size cellulose filters. The lack of oncotic pressure is offset by the addition of 0.6 per cent dextran (70,000 m.w.), and a low (20 cm H_2O) perfusing pressure. Normal pH, pCO_2, and pO_2 are maintained with a 97/3 per cent oxygen/carbon dioxide gas mixture. The whole apparatus is placed within a refrigerator at 5°. Figure 1 shows three such systems photographed through the inner transparent door of a large refrigerator. The pots act as reservoirs, filters, and oxygenators, thus considerably simplifying the apparatus — an essential feature if a number of hearts are to be stored at the same time, as in a 'heart-bank'.

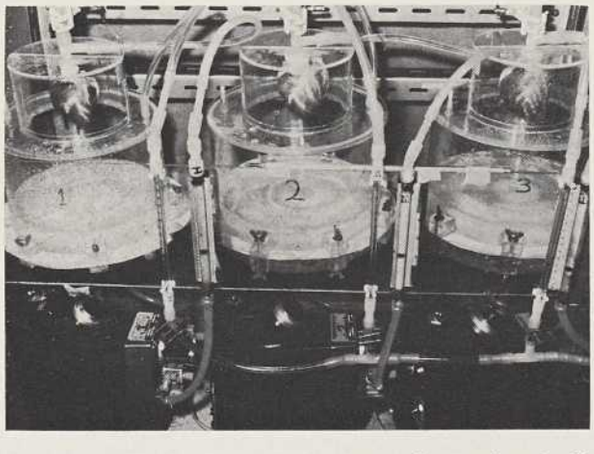

Figure 1 — Shows three small hearts being perfused with separate perfusion systems within a large refrigerator. The 8 micron cellulose filters are bolted to the bottom of the reservoir pots. Flowmeters measure gas and perfusate flow.

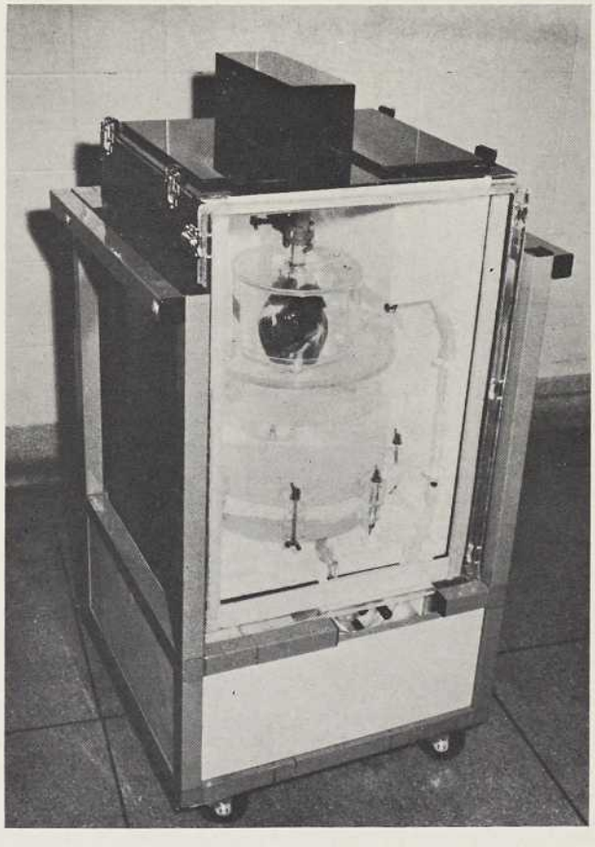

Figure 2 — Insulated portable container for transport of hearts (10 hour travelling capacity). Contains 350 g. greyhound heart. Cooling blocks of 10% potassium chloride maintain temperature of 5°C, and battery drives perfusion pump. Gas cylinder at rear. Proposed basic module for « heart-bank»" system (see text).

Due to using several hearts at a time, we have, for convenience, tested the majority of them on the arterio-venous circulation of larger dogs. They are assessed for viability by mechanical performance; electrical activity; electron microscopy; and by comparison with control isolated hearts that have not been stored.

Viability during storage is difficult to assess, but, empirically, we find that the coronary vascular resistance (CR) is the most reliable guide to future performance. Values vary with different perfusates, but, with the above solution the final CR should not be more than 60 per cent greater than the initial CR after ten minutes of perfusion; higher values reflect decreasing viability. Additionally, when conditions are standardised, we find that the initial CR becomes a measure of pre-existing damage. This may become a useful parameter when techniques are developed for resuscitating hearts too damaged by ante-mortem changes to transplant immediately. If one could resuscitate a basically good heart that had been arrested for 30–40 minutes in an unheparinised body, not only would the number of hearts available for transplantation be increased, but it would avoid the ethical problem of whether a patient was dead or not on the basis of the EEG, since few would dispute cerebral death after this period of unheparinised circulatory arrest.

'Heart bank':

Based on the above long term storage system one can envisage a 'heart-bank' as consisting of a large refrigerator containing a number of self-contained perfusion modules of the type shown in Figure 2. This battery powered system is capable, in the first instance, of supporting a heart for ten hours in the portable mode, after which, by insertion into a large refrigerator and switching to main electricity it becomes a unit of the 'bank'. Tissue-typing is done during this period, and when the correct recipient is located and prepared, the module becomes portable again and is transferred to the theatre, its viability known at all times from the CR.

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EVALUATION OF THE CONDITION OF THE ISOLATED HEART DURING PRESERVATION *

S. PITZELE, S. SZE, N. LAUZON, A.R.C. DOBELL and L.D. MACLEAN

L'énergie myocardique est dérivée de la transformation d'ATP en ADP. Grâce à des processus oxydatifs, cette réaction est suivie de la resynthèse d'ATP. Une capacité contractile élevée sera en rapport avec un taux élevé d'ATP, provenant d'une déplétion progressive du liquide extracellulaire en phosphate inorganique. A l'inverse, un faible niveau de contractilité ira de pair avec une élévation des phosphates inorganiques. Cette hypothèse de travail fut vérifiée chez le chien et une relation inverse fut démontrée entre la concentration en phosphates inorganiques dans le liquide de perfusion et la capacité contractile du cœur. Expérimentalement, après 20 heures de perfusion au moyen de plasma, le phosphate inorganique total montre une élévation importante et irréversible et ce malgré l'administration de calcium. L'électrocardiogramme est normal, le cœur n'est pas dilaté et cependant ce cœur est incapable d'effectuer du travail mécanique. Ces dosages de phosphate sont faciles et rapides à effectuer et peuvent présenter un intérêt pour l'évaluation du cœur lui-même et pour l'évaluation des liquides de perfusion expérimentaux.

Successful heart storage demands the delivery of a heart that will immediately and totally support the recipient's circulation.

The experience gained during prolonged storage experiments using hypothermic perfusion techniques (1 and 2) have shown that the mechanical function of the ventricular myocardium can be altered or even disappear completely even though the general appearance of the heart, the regularity of its rhythm and the ECG tracing remain satisfactory.

These observations underline the need for a test directly related to the mechanical function of the myocardium that can be monitored during the storage period.

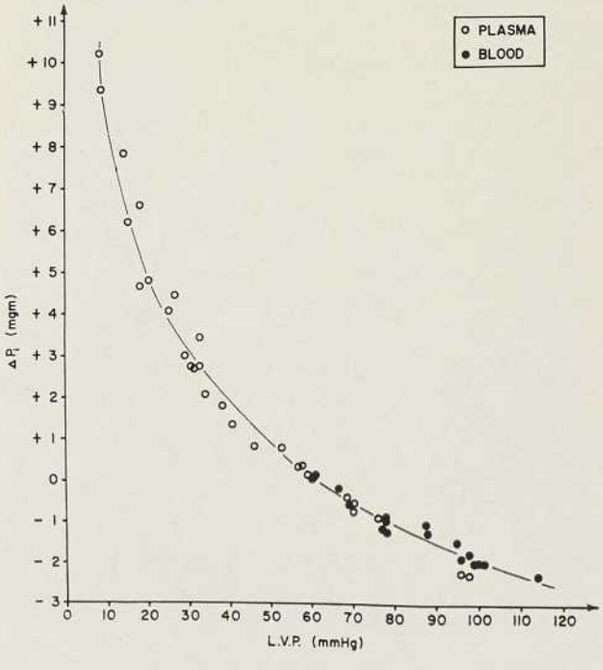

Figure 1 — Relationship between total inorganic phosphorus (Pi) and left ventricular pressure in 5 perfused hearts. Pi values on the ordinate are expressed in terms of positive or negative differences in relation to the initial value at the start of perfusion.

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* Presented at the Second world symposium on heart transplantation, Montreal, Canada, June 6–8, 1969.

From the Department of Experimental Surgery, McGill University.

Supported by the Medical Research Council of Canada, the Canadian Heart Foundation and the John A. Hartford Foundation.

Theoretical basis:

The mechanical energy of the heart muscle is derived from uncoupling of high energy phosphate bonds from adenosine triphosphate (ATP) yielding ADP and inorganic phosphate followed by resynthesis of ATP as a result of oxidative processes. Every level of contractility corresponds to a given turnover rate of inorganic phosphorus in the cycle. A higher level of contractility will draw an additional amount of inorganic phosphorus (Pi) into the high energy pool and thus decrease the total amount of inorganic phosphorus in the extracellular fluid and perfusate. A lower level of contractility will have the opposite effect.

Experimental technique and results:

Five hearts from dogs weighing 50 to 65 lbs. were removed extrapericardially and perfused at 26°–28° C. through the aortic arch, first with heparinized blood, then with pooled filtered plasma. A left intraventricular balloon was inserted for measurement of left ventricular pressures (LVP). Variable levels of contractile activity were achieved either by stimulating the myocardium with calcium, or depressing it with pronestyl, potassium chloride or anoxia. Total circulating inorganic phosphorus was measured during steady state periods by the method of Taussky and Shore (3).

The results illustrated graphically in Figure 1

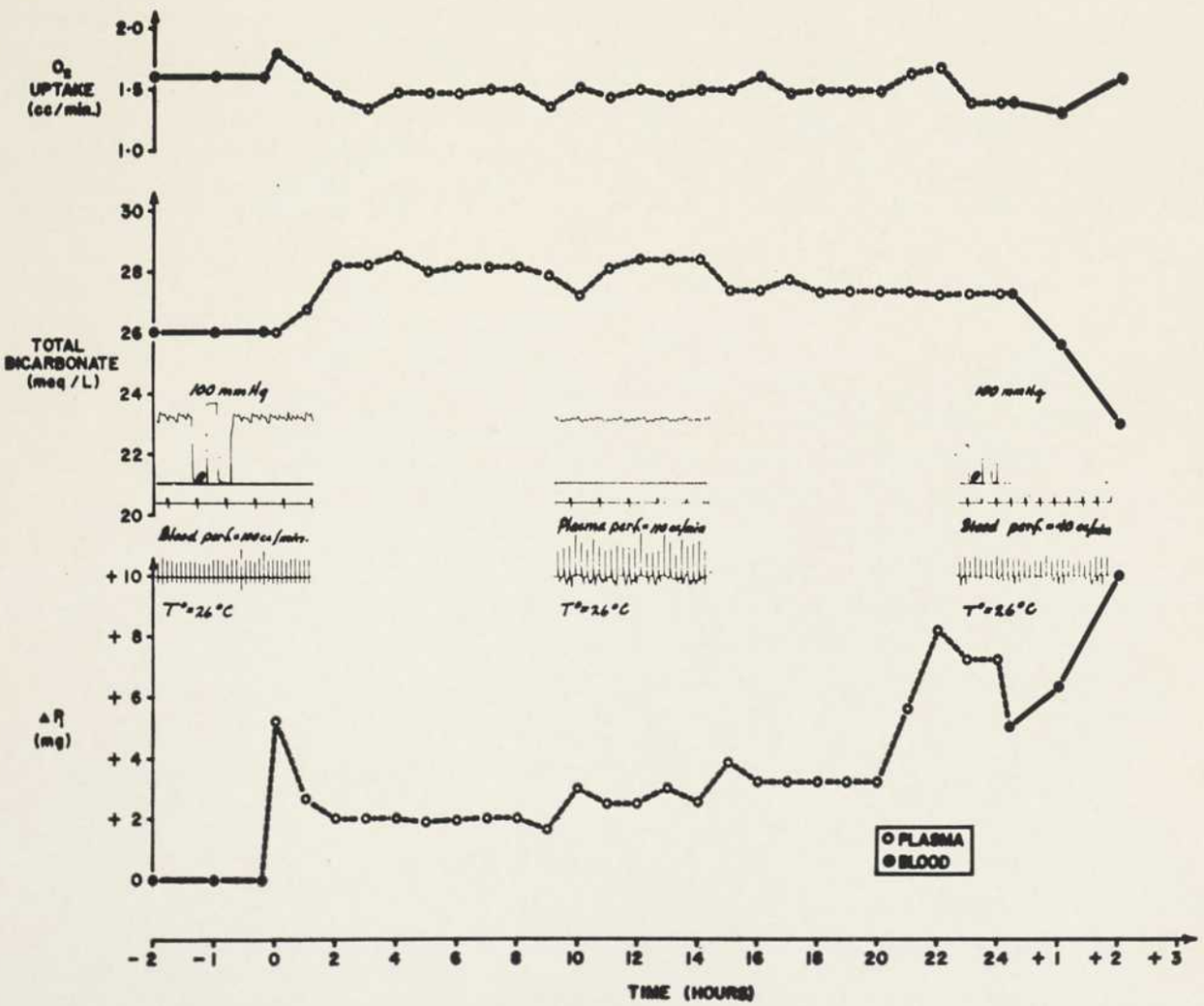

Figure 2 — Variation of total Pi level, total bicarbonate concentration and O_2 uptake during preservation of a heart from a 57-lb dog.

verify the existence of an inverse relationship between total inorganic phosphorus in the perfusate and the contractile ability of the heart. An increase in total Pi is associated with a decrease and, in extreme cases, with an almost complete disappearance of the left ventricular ability to develop pressure. The results also show that the same hearts will develop a higher pressure and keep a better contractile function with blood perfusion rather than with plasma. Two of the five hearts tested behaved as well with plasma as with blood but it took comparatively larger doses of calcium to achieve this level of performance.

Figure 2 shows the changes in circulating inorganic phosphorus during a long-term perfusion with blood and plasma. After the twentieth hour of plasma perfusion, total Pi showed a significant rise that became even more pronounced despite administration of calcium. Towards the end of the perfusion, the heart was not visibly enlarged and the electrocardiogram was regular, yet it was incapable of performing mechanical work.

SUMMARY

Evidence has been presented that the variations in inorganic phosphorus in the circulating perfusate are related to the mechanical capacity of a perfused heart. The measurement is rapid and straigthforward and may be of value in evaluating the heart itself and the adequacy of various experimental perfusates.

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PERFUSION OF THE GRAFT DURING HEART TRANSPLANTATION *

C. CABROL, A. CABROL, G. GUIRAUDON, A. ZAFY, P. LE PICARD, S. MATTEI and J. LUCIANI †

La transplantation cardiaque ne semble plus poser de problèmes quant à la préservation de la viabilité du cœur du donneur. Cependant, dans l'expérimentation animale, on a observé une mortalité assez élevée. En vue de réduire la mortalité et d'obtenir beaucoup plus d'animaux pour l'étude du rejet et de son traitement, plusieurs techniques de préservation myocardique ont été utilisées.

Trois méthodes furent employées :

1. Aucune protection myocardique ;
2. Après excision, immersion du cœur pendant 10 minutes dans du soluté salin à 4° C ;
3. Après excision, perfusion du cœur avec du sang oxygéné.

Résultats

Avec la première méthode, les résultats étaient très médiocres. La cause principale de la mort était la défaillance myocardique aiguë. Les contractions du cœur greffé restaient faibles, irrégulières, malgré l'utilisation d'une pompe de soutien, l'utilisation de cardiotoniques et de vasopresseurs. (suite du résumé en page suivante)

Preservation of the donor heart viability during clinical transplantation seems to be no longer a problem. Successful cases have been reported in which various methods were used, including no myocardial protection at all. However, in animal experimentation this problem remains important and in our experience high immediate mortality following orthotopic heart homotransplantation in dogs was mainly due to acute myocardial failure in relation with poor preservation of the graft.

In order to reduce the mortality and to obtain more animals for the study of early recognition and adequate treatment of rejection which is now the major concern in clinical heart transplantation, several technics of myocardial preservation were investigated.

Material:

Orthotopic heart homotransplantation was performed on 84 mongrel dogs weighing between 18 and 24 kilograms using an equal number of heart donors with the same characteristics.

In the recipient animal the heart was exposed through a right lateral thoracotomy in the fourth intercostal space. Cardiopulmonary bypass was instituted between the two venæ cavæ (cannulated by way of the right femoral and external jugular veins) and the right femoral artery. General hypothermia (30° C) was induced. The ventricles were excised leaving the entire atrial cavities and the proximal aorta and pulmonary artery.

In the donor, the heart was exposed through the same incision, fibrillated and excised without any special preparation, by division of the venæ cavæ and the pulmonary veins at their respective atrial junctions and by division of the pulmonary artery at the level of its bifurcation and the aorta after the innominate artery. A left ventricular vent was

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* Paper presented at the Second world symposium on heart transplantation, June 6-8, 1969.

† Division de chirurgie thoracique, Service N° 8, Hôpital de la Pitié, boulevard de l'Hôpital, Paris XIII^e^, France.

Avec le refroidissement local, la fonction myocardique n'apparaît guère meilleure, bien que les complications (hémorragie, perfusion médiocre et insuffisance respiratoire) aient été moins fréquentes que lorsqu'aucune protection n'était assurée.

Dans les cas où l'on utilisa une perfusion, l'activité cardiaque, après retrait de la pince aortique, était certainement meilleure. Le cœur restait rose, et non dilaté. La fibrillation ventriculaire active était transformée en rythme sinusal, à l'aide d'un seul choc électrique, et les contractions cardiaques étaient nettement vigoureuses et régulières. Dans certains cas cependant, on observa une réponse myocardique médiocre, en relation avec une perfusion insuffisante de la greffe. Cette complication fut éliminée en maintenant un bon remplissage de l'aorte ascendante. En vue d'éviter une pression excessive dans le lit vasculaire coronarien, on utilisa une ligne de perfusion reliée à la pompe. La pression dans la ligne n'excède jamais les 100 cm d'eau. Le cathéter de perfusion introduit dans l'artère innominée doit être suffisamment gros pour assurer un flot capable de fermer la valve aortique, qui peut être occasionnellement ouverte durant les manipulations du cœur.

En résumé, on peut dire que dans l'homo-transplantation cardiaque orthotopique chez les chiens, les meilleurs résultats sont obtenus lorsqu'on perfuse le cœur. Le cœur du chien est très sensible à l'anoxie.

inserted through the left appendage. Anastomoses were carried out in the following sequence: left atrium, interatrial septum, right atrium, pulmonary artery, aorta. After removing of the aortic clamp, body temperature was raised to 35° C and ventricular fibrillation was reverted to normal rhythm with a direct current countershock. Two pacemaker wires were sewn on the right ventricule and cardiopulmonary bypass discontinued as soon as the heart appeared able to maintain arterial and venous pressures at normal levels. The catheters were removed and the chest closed with bilateral underwater tube drainage.

Concerning the management of the graft during implantation three methods were employed.

In 14 dogs no cardiac protection was used and the anastomoses were done on an anoxic heart.

In 20 dogs immediately after excision the heart was immersed in cold saline at 4° C for ten minutes then sutured. In some cases after completion of the left atrial anastomosis the left ventricular vent was used for cold saline irrigation of the left heart.

In 50 animals we started after its excision a perfusion of the graft with oxygenated blood by the way of the innominate artery. Perfusion was stopped during the aortic anastomosis (Figures 1, 2 and 3).

Results:

When no protection of the graft was used the results were very poor (Figure 1). Except various other complications (hemorrhage, inadequate perfusion, defective lung function) the main cause of death (50 per cent) was acute myocardial failure. Contractions of the grafted heart remained weak and irregular in spite of further pump assistance

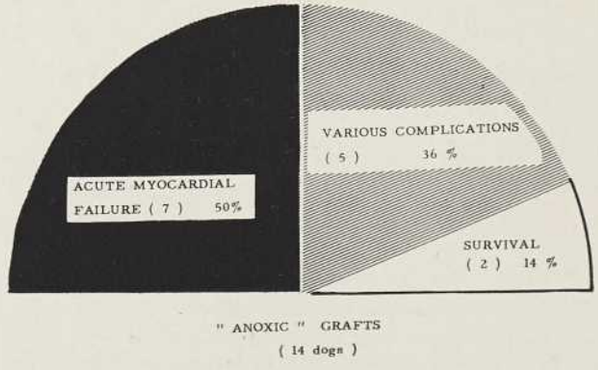

Figure 1 — Results with "anoxic" grafts (14 dogs).

and the use of cardiotonic and vasopressive drugs (Figure 4).

With local cooling (Figure 2) myocardial function of the transplant did not appear much better (Figure 5) although other complication were less frequent certainly due to increased technical experience.

In cases where we employed perfusion of the graft (Figure 3), cardiac activity after removal of the aortic clamp was definitively better. The heart remained pink and not dilated. Active ventricular fibrillation readily reverted to sinus rhythm with a single counter shock and cardiac contraction were immediately vigorous and regular. Although the heart was still in bypass systolic peaks were often seen on the arterial pressure tracing. The by-pass could be easily discontinued with prompt restoration of a normal circulatory function without any drug support (Figure 6). In some cases (13 per cent) however we did observe the same poor myo-

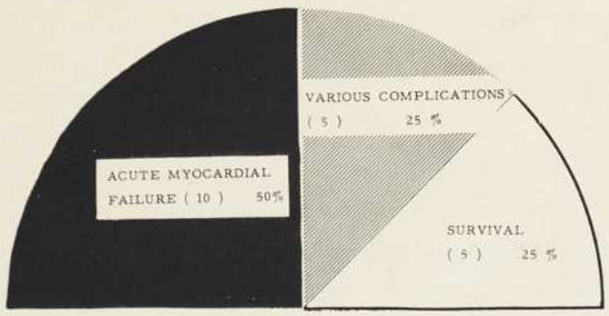

Figure 2 — Results with cooling of the heart (20 dogs).

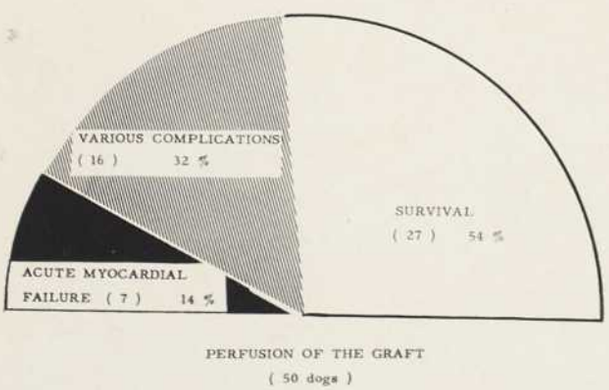

Figure 3 — Results with perfusion of the heart (50 dogs).

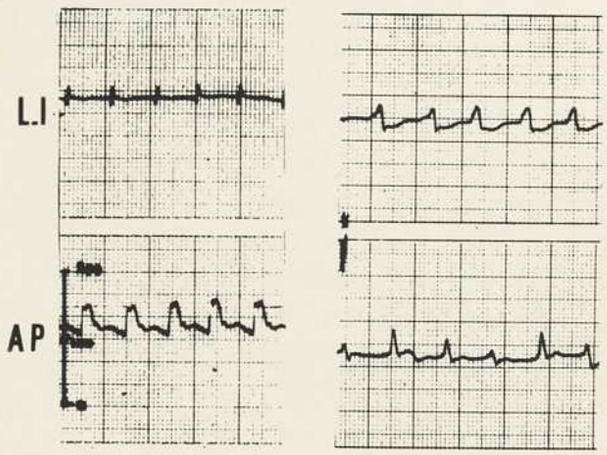

Figure 4 — L 1 : Lead one of the electrocardiogram. — A P : Arterial pressure (femoral artery) before and after homotransplantation. No protection of the graft.

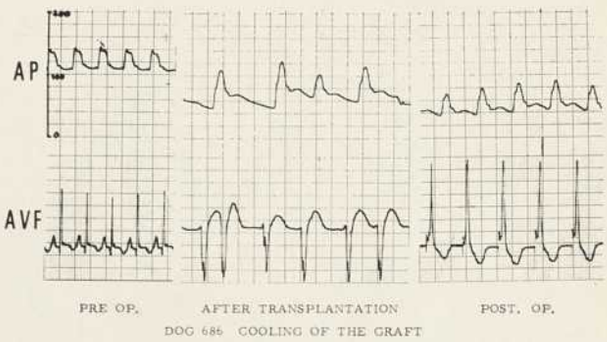

Figure 5 — A P : Arterial pressure (femoral artery) and electrocardiogram (AVF) before, immediately after and six hours after homotransplantation. Protection of the graft by local hypothermia.

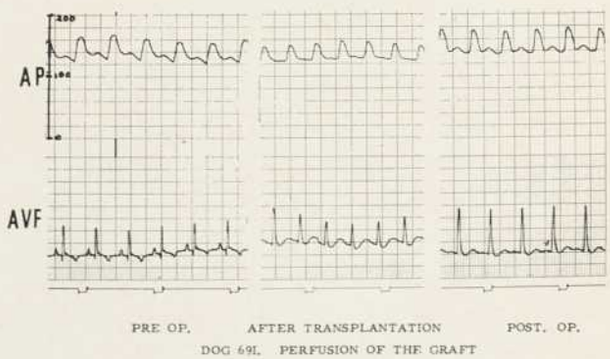

Figure 6 — A P : Arterial pressure (femoral artery) and electrocardiogram (AVF) before, immediately after and six hours after homotransplantation. Protection of the graft by perfusion.

cardial response we have seen in the two previously described methods. This was almost invariably due to inadequate graft perfusion. To obviate this complication it is necessary to maintain a good filling of the ascending aorta. That can be easily appreciated by the palpating finger. But on the other hand we must avoid excessive pressure in the coronary vascular bed. For this purpose we used the same method we have always employed in our clinical coronary perfusion (Figure 7). The perfusion line is branched directly on the arterial line after the pump. The pressure which never exceed 100 cm of water is easily monitored with the help of a screw clamp and a simple manometric tube. Moreover the perfusion catheter inserted in the innominate artery must be large enough to insure a high flow capable to close promptly the aortic valve which can be occasionally opened during cardiac handling.

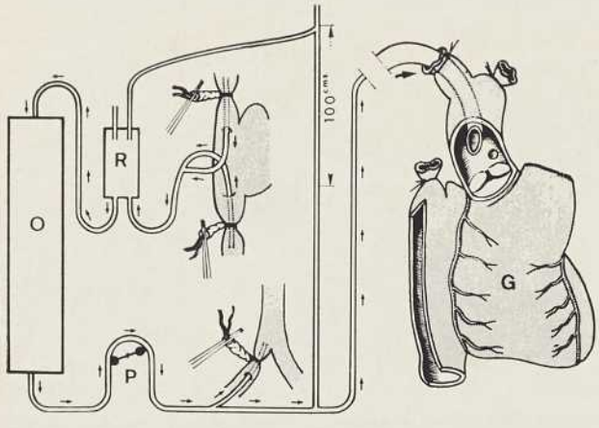

Figure 7 — Extra-corporeal circuit and manometric tube used in heart homotransplantation.
G : heart transplant
O : oxygenator
R : reservoir
P : pump

Discussion:

The present study convinced us that the perfusion of the heart is the best way to prevent myocardial damage during cardiac transplantation. This is specially important in dogs because of the peculiar sensibility of the canine heart to anoxia.

This is also true in human transplantation although the human heart can tolerate longer ischemic periods. Judging also from our clinical experience with operations on the aortic valve, perfusion of the myocardium appears to be a more physiologic and safer approach to the problem of cardiac preservation. It gives the best chance to provide the recipient patient with a functionally sound organ and relieves the transplant team of the necessity for extreme speed in connecting the graft to the recipient's blood vessels.

SUMMARY

In order to obtain acceptable survival rate after orthotopic heart homotransplantation in dogs several methods of graft protection during implantation were investigated on 84 animals.

Continuous perfusion of the transplant although it represents a more complicated method than simple anoxia or local hypothermia is certainly the safest technique at the present time.

A PROPOS DE LA CONSERVATION DES ORGANES *

Jean-Paul BINET †

L'auteur rapporte 25 expériences pour mettre au point une technique d'autoperfusion de l'ensemble cœur-poumons prélevé chez le chien. Le cœur, les poumons et la trachée sont prélevés en bloc avant la mort de l'animal, donc sans ischémie, et raccordés d'une part à un réservoir contenant du sang et, d'autre part, par la trachée, à un appareil « respirateur » à pression positive. La plus longue conservation de cette autoperfusion atteint 18 heures. L'œdème pulmonaire qui survient en général entre la septième et la dixième heure empêche la poursuite de l'expérience. De nombreux paramètres hémodynamiques et humoraux ont été étudiés au cours de ces perfusions. Ce travail doit permettre d'étudier les constantes de base de la conservation de l'ensemble cœur-poumons, par une technique relativement simple.

À propos de la discussion en cours, je viens rapporter l'expérimentation faite à l'hôpital Marie-Lannelongue (129, rue de Tolbiac, Paris XIII[e]) par mes collaborateurs, M. Weiss, A. Brunet, A. M. De Groote, A. Pellet et C. Planche.

Cette expérimentation a pu être faite avec le concours de l'INSERM et de la DGRST.

Il s'agit de la conservation « à court terme » du bloc cœur-poumons par autoperfusion.

Vingt-cinq expériences ont été faites pour mettre au point une technique d'autoperfusion de l'ensemble cœur-poumons prélevé chez le chien. Le bloc cœur-poumons-trachée est prélevé en bloc avant la mort de l'animal (donc, sans ischémie) et raccordé, d'une part, à un réservoir contenant 500 ml de sang homologue, d'autre part, par la trachée à un appareil « respirateur » à pression positive. Les poumons sont régulièrement insufflés, la température de l'ensemble étant abaissée à 29° C.

De nombreux paramètres ont été suivis de façon permanente pendant la durée de l'expérimentation :

a) *Les paramètres hémodynamiques:* pression dans les diverses cavités cardiaques et les gros vaisseaux, volume sanguin, débit ;

b) *Les paramètres humoraux:* équilibre acido-basique, glycémie, lactate, potassium.

Dernièrement, la possibilité de régler un débit d'apport à l'oreillette droite a permis d'étudier les performances de la fonction cardiaque à différents débits. Toutes ces études ont été faites avant d'essayer la transplantation.

La plus longue conservation avec bonne fonction a atteint dix-huit heures. Cependant, mises à part les premières expérimentations du débit, entachées d'erreurs techniques, elles ne semblent pas, en moyenne, pouvoir dépasser douze heures. C'est l'œdème pulmonaire qui survient en général entre la septième et la douzième heures qui empêche la poursuite de l'expérimentation. Ce travail doit permettre d'étudier les constantes de base de la conservation de l'ensemble cœur-poumons par une technique relativement simple. Les efforts devront être poursuivis concernant les variables, les températures de débit, la pression, la composition du sang, ainsi que les modifications de la pression de la pompe. Mais, en fait, seules la réimplantation orthotopique du poumon, hétéro ou orthotopique du cœur, permettront de tester la viabilité des organes conservés.

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* Communication au Deuxième symposium mondial sur la transplantation cardiaque, Montréal, 6–8 juin, 1969.

† 3, rue Christophe-Colomb, Paris VIII[e].

ABOUT PERFUSIONAL METHOD OF ISOLATED HEART PRESERVATION BEFORE ITS TRANSPLANTATION *

Alexandr A. VISHNEVSKY and Vladimir F. PORTNOY,
Moscow, USSR.

Les auteurs décrivent une méthode réduisant au minimum la période d'ischémie myocardique du donneur avant que sa circulation ne soit rétablie.

Après l'exposition du cœur, des canules sont introduites dans l'aorte ascendante, l'oreillette droite et le ventricule gauche. Après quoi, on commence la perfusion des coronaires. À ce moment, le cœur est excisé. Le cœur est prélevé, toutes ses cavités étant fermées. La perfusion d'un tel cœur humain pendant une période de sept heures, fut observée à l'aide d'une circulation extracorporelle, tout en maintenant en activité cardiaque électrique et une pression partielle d'oxygène avec un maximum de 200 mm Hg. Avec cette technique on peut préserver un coeur avant sa transplantation.

In this report will be discussed one particular moment of the heart transplantation problem, which we think is of some interest.

This is how to bring to a minimum the period of myocardial ischemia of a donor's heart before its circulation is finally restored in the recipient's organism.

The perfusion techniques of the isolated heart during implantation, as is generally known, has its advocates: professor Hardy (1964), professor Bernard (1967) and some others of our colleagues. The first transplantation of the heart from one dog to another was performed in USSR in 1940 by V. Demichov, his experiments being well known in the world.

We have devised in experiment and used in the clinical observation the technique of organ perfusion in order to preserve the heart during the period of its excision, transportation to recipient and some waiting before the recipient is prepared.

After exposure of the donor's heart, catheters were introduced into ascending aorta, right atria and left ventricle. Then the coronary perfusion began. The heart is excised under the continuing perfusion (Figure 1).

In the clinical observation we took the heart out with closed cavities; the vessels were ligated before excision. In experiment the same was done without ligation of vessels.

Then, the isolated heart, placed into the cuvette, was well supplied with blood (Figure 2).

We used a special reduced model of an apparatus

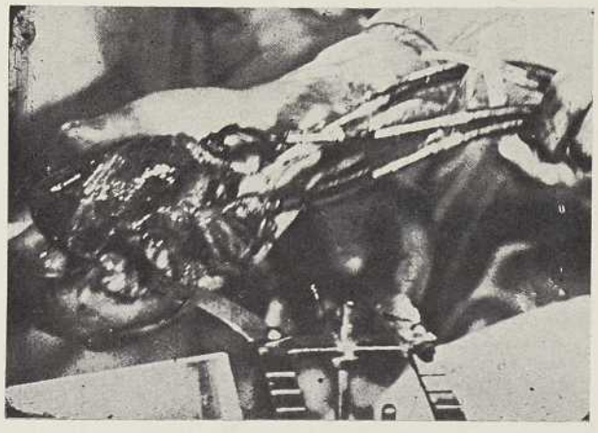

Figure 1 — Excision of the heart under continuing perfusion

* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6–8, 1969.

for artificial circulation (Figure 3). Our preliminary experiments have shown that it is possible to avoid myocardial damage for two to three hours, if we keep the blood pressure at a level of about 60 mm Hg; the temperature at 36° C; the pO_2 of arterial blood at no more than 200 mm Hg.

Naturally, it is desirable that the period of isolated perfusion of the heart, out of the organism, must not be too prolonged.

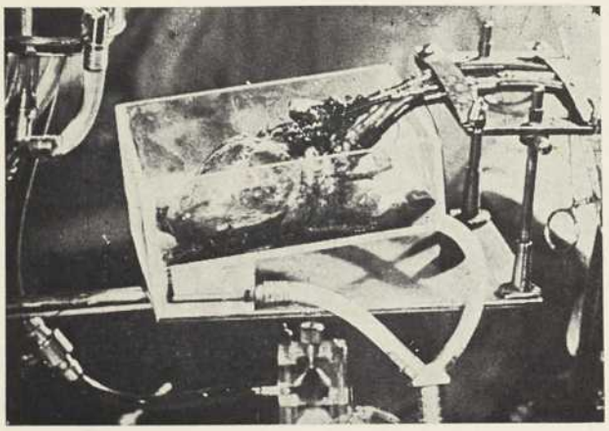

Figure 2 — Perfusion of the isolated heart

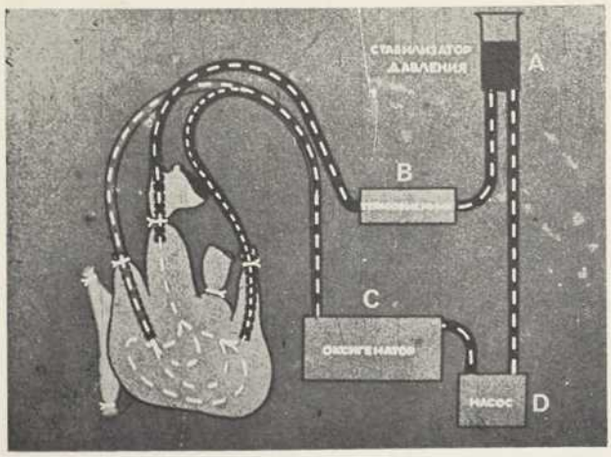

Figure 3 — Reduced model of an apparatus for artificial circulation. A : pressure stabiliser ; B : heat exchanger ; C : oxygenator ; D : circulation pump.

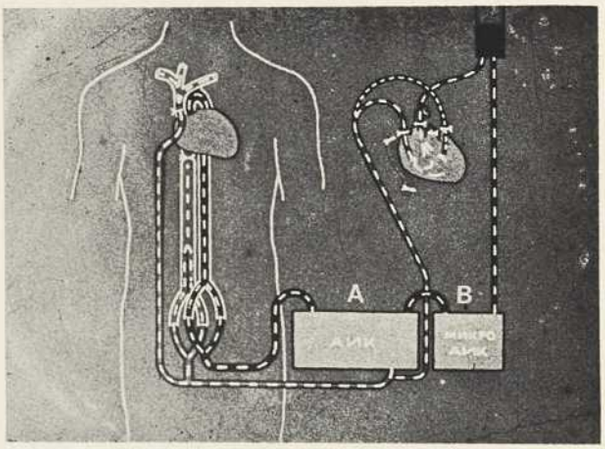

Figure 4 — Connection of the small apparatus for isolated organ perfusion (B) to the apparatus for extracorporal circulation (A).

After the apparatus for extracorporal circulation of the recipient was on operation the small apparatus for isolated organ perfusion was connected with it (Figure 4).

In these conditions professor Shumway's method of heart transplantation worked well.

We have an interesting observation concerning a 7-hours perfusion of animated human heart without its transplantation. In that experiment a steady coronary circulation was maintained as well as a certain level of O_2 consumption and electrical activity of the heart.

With this technique we performed the transplantation of the heart from one woman to another in November 1968. The patient lived during 33 hours and died from right ventricle insufficiency.

We think that with good reason we can consider perfusional technique for myocardial preservation before transplantation is a highly perspective method for practical purposes.

GENETICS OF THE HL-A SYSTEM *

J. DAUSSET and J. COLOMBANI

Durant les dernières années, on opéra un progrès rapide dans la connaissance et la compréhension de la génétique du système HL-A. Deux séries d'antigènes furent décrites et les antigènes de chaque série se comportent — au moins dans une population causasienne — comme allèles mutuellement exclusifs, situés sur deux sub-loci rapprochés. Dans ce travail, de nouvelles données concernant les antigènes Da6 et Da9 (6b) sont présentées avec l'hypothèse que ceux-ci appartiennent également au deuxième sub-locus. Cette étude fut menée chez 83 familles ayant 246 enfants. Les conclusions qui en découlent amènent l'hypothèse que les deux antigènes Da6 et Da9 (6b) appartiennent ou sont extrêmement apparentés au deuxième sub-locus du système HL-A.

L'étude des phénotypes de 312 individus de race caucasienne montre que lorsque l'antigène HL-A5 est présent, l'antigène Da6 est également présent. De même lorsque l'antigène HL-A7 est présent l'antigène Da9

The HL-A system is in man analogous to the H2 system in mouse and to all the other main histocompatibility systems which have been described in many species. Its importance in human histocompatibility is now well documented between relatives. The relationship between HL-A groups and graft survivals in non related pairs is now also established, but for a long time it was not obvious because of the extreme complexity of the HL-A system and of the fact that many of the antigens of this system were unknown. However during the late years rapid progress was made in the knowledge and the understanding of the genetics of this system (2, 4, 8, 10, 12 and 14). Two series of antigens were described and the antigens of each series were shown to behave, at least in the Caucasian population, as mutually exclusive alleles at two very closely linked sub-loci (6, 8 and 10).

In this paper new data concerning the antigens Da6 and Da9 (6^b) are presented with the hypothesis that they also belong to the second sub-locus. New family studies have been made which make it possible to increase the number of genotypes and haplotypes genetically deducted.

MATERIAL AND METHODS

Eighty-three families with 246 children have been studied. The antigenic determination was carried out with the help of monospecific or practically monospecific sera used in microlymphocytotoxicity and complement fixation on platelet techniques. The method for the deduction of genotypes and haplotypes has been described elsewhere (7). The allelism of the antigens was studied by double backcross method.

RESULTS

The antigens of the first sub-locus:

Six well defined antigens behave as mutually exclusive alleles at this first sub-locus. They are antigens HL-A1, HL-A2 (Mac), HL-A3, Da15, Da16

* This work was carried out in the "Institut de recherches sur les maladies du sang, Hôpital Saint-Louis, Paris X^e (France)". It was supported by Grant CA 5573 of the National Cancer Institute, Bethesda, Md, U.S.A., and contract PH 43 65 986 of the National Institute of Allergy and Infectious Diseases, Bethesda, Md, U.S.A. and "Unité de recherche n° 93" of the "Institut national de la santé et de la recherche médicale", Paris, France.

(6b) est toujours présent. Ceci est appelé phénomène d'inclusion. En plus si on assume que ces deux associations constituent deux entités, on n'a trouvé aucun individu ne possédant plus que deux des antigènes ou entités Da4, HL-A8, Da6, Da9 (6b), « HL-A5-Da6 » « HL-A7-Da9 », il est fort probable que d'autres sub-loci du système HL-A seront postulés. Par exemple, il y a lieu de supposer que les antigènes de Waldford Lc-17, Lc-20 sont des allèles au troisième sub-locus. De tout ceci il apparaît que le système HL-A est complexe et consiste en plusieurs sub-loci dont chacun d'eux possède plusieurs allèles. Il est intéressant de noter que ces sub-loci sont dépendants l'un de l'autre. Ivanyi et Dausset ont postulé que chacun des antigènes connus — ainsi que ceux qui sont encore inconnus dans le système HL-A — est lui-même probablement formé par de nombreux facteurs antigéniques. Pareille hypothèse est basée sur l'analogie de ce qui est connu pour l'antigène D du système Rhésus qui lui-même consiste en plusieurs facteurs RhA, RhB, RhC et RhD. Finalement, l'indépendance du système HL-A, du système ABO, Rh, MNS, Kell, P. JR, et d'autres a été établie.

La plupart des antigènes connus du système HL-A ont trouvé place dans l'un ou l'autre des deux premiers sub-loci. Il semble probable que le comportement d'un greffon cardiaque puisse être déterminé pour une grande part, par des incompatibilités de ces antigènes.

(Lc-11) and very probably Da17 (10). The existence of at least one and very likely several other alleles must be postulated at this first sub-locus.

The mutually exclusive relationship between these antigens has been proved by the double back-cross method using informative families. It must be underlined that a cross-reaction has been observed between two antigens of this series, the antigens HL-A2 and Da15 (5). This observation has been confirmed by Svejgaard and Kissmeyer-Nielsen (13).

The antigens of the second sub-locus:

Four antigens behave like alternative alleles at the second sub-locus. They are antigens HL-A5, HL-A7, HL-A8, and Da4 (8). As for the first sub-locus, the existence of at least one or probably several other alleles must be postulated at this sub-locus (6, 8 and 10).

Informative families by the double back-cross method gave evidence, at least in the Caucasian population, of the allelism of these antigens.

Looking at two other antigens not yet classified at any sub-locus, the following facts are observed: the haplotypes which govern antigen Da6 (4) never govern antigens Da4, HL-A8, HL-A7. On the other hand, there are haplotypes which govern both antigens Da6 and HL-A5; the haplotypes which govern antigen Da9 (8) [Van Rood's antigen 6^b (15)] never govern antigens Da4, HL-A5, or HL-A8. On the other hand, there are haplotypes which govern both antigens Da9 (6^b) and HL-A7. The 189 haplotypes observed are given in Table I.

The study of the phenotypes of 312 Caucasian individuals shows that when antigen HL-A5 is present, antigen Da6 is also present (8). Likewise when antigen HL-A7 is present, antigen Da9 (6^b) is also present (4 and 15), this phenomenon being the so-called "inclusion" phenomenon. Furthermore, if we assume that the association HL-A5 – Da6, and HL-A7 – Da9 (6^b) constitutes two entities, no individual has been found possessing more than two of the antigens or entities Da4, HL-A8, Da6, Da9 (6^b), "HL-A5 – Da6", "HL-A7 – Da9".

These facts lead us to propose the hypothesis that both antigens Da6 and Da9 (6^b) would belong or would be extremely close to the second sub-locus of of the HL-A system.

Among the possible interpretations, two possibilities are to be considered first: *a*) the possibility

of a reaction of the anti-Da6 antibodies, simultaneously against antigen Da6 and HL-A5, and of a reaction of anti-Da9 (6^b) antibodies against both antigens Da9 (6^b) and HL-A7. The existence of antigenic community between HL-A allelic products (HL-A2 and Da15) was already observed (5 and 13); *b*) the possibility of the existence of a third sub-locus extremely close to the second one, between which there would be a strong linkage desequilibrium, the antigens Da6 and Da9 (6^b)

TABLE I

88 genotypes (80) phenotypes observed in 148 individuals (Genotypes limited to the antigens at present known at the first and second sub-loci)

		NUMBER OBSERVED			NUMBER OBSERVED
HL-A1	/ unknown	3	HL-A2, 5	/ Da16	2
HL-A1	/ HL-A5	1	HL-A2, 5	/ Da16, 4	1
HL-A1	/ Da4	1	HL-A2, Da4	/ unknown	1
HL-A1	/ HL-A3	4	HL-A2, Da4	/ HL-A3	2
HL-A1	/ Da16	3	HL-A2, Da4	/ Da15	2
HL-A1	/ Da17	3	HL-A2, Da4	/ Da16	2
HL-A1	/ Da17, HL-A7	1	HL-A2, Da4	/ Da17	1
HL-A1, Da4	/ HL-A5	1	HL-A2, Da4	/ Da17, HL-A7	1
HL-A1, Da4	/ Da16	1	HL-A2, 7	/ HL-A3	1
HL-A1, Da4	/ Da17	1	HL-A2, 7	/ HL-A3, 5	1
HL-A1, 7	/ HL-A1, 8	1	HL-A2, 8	/ HL-A3	1
HL-A1, 7	/ HL-A2	2	HL-A3	/ HL-A3	1
HL-A1, 1	/ Da16	1	HL-A3	/ HL-A3	3
HL-A1, 7	/ Da16, HL-A5	1	HL-A3	/ HL-A3	3
HL-A1, 7	/ Da16, HL-A8	8	HL-A3	/ HL-A3, 7	1
HL-A1, 8	/ unknown	2	HL-A3	/ Da15	2
HL-A1, 8	/ HL-A5	1	HL-A3	/ Da16	2
HL-A1, 8	/ HL-A2	3	HL-A3	/ Da16, 4	2
HL-A1, 8	/ HL-A2, 5	1	HL-A3	/ Da16, HL-A7	1
HL-A1, 8	/ HL-A2, Da4	1	HL-A3	/ Da17	17
HL-A1, 8	/ HL-A3	3	HL-A3, 5	/ unknown	1
HL-A1, 8	/ HL-A3, 7	1	HL-A3, 5	/ Da17	1
HL-A1, 8	/ Da16	1	HL-A3, Da4	/ Da4	1
HL-A1, 8	/ Da16, 4	2	HL-A3, 7	/ Da15	1
HL-A1, 8	/ Da17	1	HL-A3, 7	/ Da16	2
HL-A2	/ unknown	2	HL-A3, 7	/ Da17, HL-A7	1
HL-A2	/ HL-A5	1	Da15	/ unknown	1
HL-A2	/ HL-A2	5	Da15	/ Da4	1
HL-A2	/ HL-A2, Da4	1	Da15	/ HL-A7	1
HL-A2	/ HL-A2, 5	1	Da15	/ Da16	2
HL-A2	/ HL-A2, 7	1	Da15	/ Da17	1
HL-A2	/ HL-A3	13	Da16	/ unknown	3
HL-A2	/ HL-A3, Da4	1	Da16	/ Da16	1
HL-A2	/ HL-A3, 7	2	Da16	/ Da17	1
HL-A2	/ Da15	2	Da16	/ Da17	1
HL-A2	/ Da16, 4	1	Da16, HL-A5	/ unknown	1
HL-A2	/ Da16	8	Da16, HL-A5	/ HL-A5	1
HL-A2	/ Da16, HL-A5	1	Da16, HL-A5	/ Da17	1
HL-A2	/ Da16, 4	1	Da16, 4	/ unknown	1
HL-A2	/ Da17	2	Da16, 4	/ Da17, HL-A7	1
HL-A2	/ Da17, 4	1	Da17	/ unknown	1
HL-A2	/ Da17, HL-A7	1	Da17, HL-A5	/ Da4	1
HL-A2, 5	/ HL-A7	1	Da17, 4	/ unknown	1
HL-A2, 5	/ HL-A3	2	Da4	/ unknown	1
HL-A2, 5	/ HL-A3, 7	1	Unknown	/ unknown	1

being very frequently associated, at least in the Caucasian population, on the same haplotype, respectively with antigens HL-A5 and HL-A7, or with one of still unknown antigens of the second sub-locus. Serological and genetic studies are still necessary to decide between the various possibilities.

Very likely other sub-locus in the HL-A region must be postulated. For instance, there are grounds for supposing that Waldford's antigens Lc-17 and Lc-20 are alternative alleles at a third sub-locus (10).

From all this above it appears that the HL-A system is a complex system which consists of several sub-loci, each of them being multi-allelic. It is worth noting that these sub-loci are dependent upon one another. It is said that there is a "linkage desequilibrium" between them which is shown by the positive association that take place between some antigens at the first sub-locus and some others at the second sub-locus. For instance antigens HL-A1 – HL-A8 or HL-A3 – HL-A7 are frequently governed by the same chromosome.

Thanks to family studies the genotypes of 148 persons have been deducted. It can be seen on Table II that the frequency of each phenotype even limited to the antigens of the two first sub-loci is very low.

The HL-A system is certainly even more complex. Ivanyi and Dausset (11) have postulated that each of the known and still unknown antigens was itself probably formed of numerous antigenic factors. Such an hypothesis is based on the analogy with what is known of antigen D of the Rhesus system which itself consists of several factors Rh^A, Rh^B, Rh^C and Rh^D.

Finally the independence of the HL-A system

TABLE II

189 haplotypes of the HL-A system observed in 378 individuals

HL-A1,	Da4	=	1	0.5	Da15,	Da4	=	0	0
HL-A1,	HL-A5, Da6	=	0	0	Da15,	HL-A5, Da6	=	0	0
HL-A1,	Da6	=	1	0.5	Da15,	Da6	=	1	0.5
HL-A1,	HL-A7, Da9	=	4	2	Da15,	HL-A7, Da9	=	0	0
HL-A1,	HL-A8	=	9	4.5	Da15,	HL-A8	=	0	0
HL-A1,	Da9	=	1	0.5	Da15,	Da9	=	0	0
HL-A1,	unknown	=	4	2	Da15,	unknown	=	5	2.6
HL-A2,	Da4	=	5	2.5	Da16,	Da4	=	3	1.5
HL-A2,	HL-A5, Da6	=	5	2.5	Da16,	HL-A5, Da6	=	4	2
HL-A2,	Da6	=	8	4	Da16,	Da6	=	1	0.5
HL-A2,	HL-A7, Da9	=	3	1.5	Da16,	HL-A7, Da9	=	2	1
HL-A2,	HL-A8	=	2	1	Da16,	HL-A8	=	0	0
HL-A2,	Da9	=	4	2	Da16,	Da9	=	3	1.5
HL-A2,	unknown	=	20	10.5	Da16,	unknown	=	12	6.3
HL-A3,	Da4	=	2	1	Da17,	Da4	=	1	0.5
HL-A3,	HL-A5, Da6	=	2	1	Da17,	HL-A5, Da6	=	1	0.5
HL-A3,	Da6	=	4	2	Da17,	Da6	=	2	1
HL-A3,	HL-A7, Da9	=	3	1.5	Da17,	HL-A7, Da9	=	1	0.5
HL-A3,	HL-A8	=	0	0	Da17,	HL-A8	=	0	0
HL-A3,	Da9	=	2	2	Da17,	Da9	=	1	0.5
HL-A3,	unknown	=	16	8.4	Da17,	unknown	=	7	3.6

Unknown,	Da4	=	7	3.5
Unknown,	HL-A5, Da6	=	4	2
Unknown,	Da6	=	4	2
Unknown,	HL-A7, Da9	=	2	1
Unknown,	HL-A8	=	2	1
Unknown,	Da9	=	2	1
Unknown,	unknown	=	28	14.7

from the ABO, Rh, MNSs, Kell, P, Jk, Duffy, Secretor systems, from the Ko platelet system, from the Gm, Gc, Lp, Ag and haptoglobin serum systems has been established.

The consequences in transplantation of a better knowledge of the genetics of the HL-A system are obvious. Most of the known antigens have found their place in one or the other of the first two sub-loci. It seems probable that the behaviour of a graft is to a large extent determined by the incompatibilities with these antigens. Skin grafts made in collaboration with F.T. Rapaport (9 and 10) between children and fathers have shown that the identity (or the absence of incompatibility) for the two sub-loci leads to a long survival of skin grafts. This is a statistical truth, but not always an individual one. Some skin grafts are rejected which have no incompatibility at the two sub-loci, and, on the contrary, others survive a long time in spite of some incompatibilities. It is known that the complete neutralization of the HL-A system, which is obtained in grafts exchanged between sibs who received from their parents the same two HL-A haplotypes (HL-A identical sibs) is followed by a survival of the skin grafts of 22 to 25 days (1 and 3). Likewise the neutralization of the two sub-loci in parent-child combinations leads to a survival time of 17 to 25 days (9 and 13).

In order to obtain such a neutralization in non-related pairs, the choice should be made among a large number of individuals. It has been calculated that a recipient that would be compatible for the AB antigens, for seven antigens of the first sub-locus (one postulated) and for seven antigens of the second sub-locus (Da6 and Da9 (6^b) included, and one postulated) could be found for a given donor, among a waiting list of 373 individuals, with a 5 per cent risk of not finding such recipient.

The size of the waiting list will probably even increase when new antigens will be discovered. However we now assume that the identity (or impossibility of incompatibilities) for the antigens of the first two sub-loci of the HL-A is essential for a long survival of transplanted organs, and should be the first aim to be reached before transplantation.

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REPORT OF HISTOCOMPATIBILITY IN 70 HEART TRANSPLANTS *

E. F. POTWOROWSKI, Ph.D. †

Dix-neuf centres de transplantation cardiaque — totalisant 70 cas — ont répondu à un questionnaire. Dans plusieurs cas, les informations obtenues furent incomplètes et aucune corrélation définitive ne fut possible. Treize centres sur 16 connaissaient le résultat de l'histo-compatibilité avant la transplantation. Le nombre d'antigènes leucocytaires (HL-A et autres) variait fortement d'un centre à l'autre. Dans neuf centres, dix antigènes ou plus sont détectés. Une classification de l'histo-compatibilité fut attribuée à chaque transplantation cardiaque suivant le système de Montréal :

Groupe B : 0 ou une incompatibilité ;
Groupe C : 2 incompatibilités ;
Groupe D : 3 incompatibilités ou plus.

Ainsi groupées les différentes transplantations cardiaques furent comparées quant à la fréquence, l'importance et le délai postopératoire des crises de rejet. Aucune différence statistiquement significative ne put être établie entre ces trois groupes. Quarante et un cas seulement étaient suffisamment détaillés pour pouvoir être pris en considération dans une étude de survie à long terme. (suite du résumé en page suivante)

A survey[1] dealing with histocompatibility in heart transplantation has been conducted in an attempt to obtain, on as large a number of heart transplants as possible, pertinent informations as to the role of histocompatibility in transplant survival.

The survey was aimed at establishing the policy of each center and at studying how the results of the histocompatibility tests, performed according to this policy, could be related to the outcome of the transplant.

A total of 19 centers answered the questionnaires and reported on seventy cases. In several instances, information obtained was not complete so that correlations could not be made. It appears that in 13 centers out of 16, the outcome of the compatibility is known before the transplant operation. Thirteen centers use the lymphocytoxicity technic, while five centers use leuko-agglutination, one center complement fixation on platelets and one center mixed leukocyte culture.

Out of seventeen centers, fifteen take into account leukocyte cross-match, seventeen consider ABO compatibility, eight consider Rh compatibility while four consider other red cell antigens such as Kell, Duffy and P.

Only six centers out of sixteen test for pre-existing anti-heart antibodies, while four centers out of sixteen preclude intersexual donor-recipient combinations.

All the leukocyte antigens are considered as equal in strength by six out of fifteen centers.

The number of leukocyte antigens (HLA and others) determined by the various centers vary greatly. However nine centers detect ten or more,

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1. This survey was made possible through the cooperation of the following transplantation centers : Ann Arbor, Bordeaux, Buenos Aires, Cape Town, Chicago, Dallas, Houston (De Bakey), Jackson, Milwaukee, Montreal, New York, Paris, Pittsburgh, Richmond, Sao Paulo, Sapporo, Toronto, Valparaiso.

La survie moyenne des patients décédés était de 14 semaines pour les groupes B et C et de 5 semaines pour le groupe D. Le pourcentage de survie était de 56,5 pour cent dans le groupe B, de 40 pour cent pour le groupe C et de 14,3 pour cent pour le groupe D.

Une des raisons pour laquelle aucune corrélation ne put être établie avec les crises de rejet est probablement la difficulté actuelle d'avoir des critères du phénomène de rejet cardiaque qui soient universellement acceptés. En conclusion, un registre mondial de transplantations cardiaques serait des plus utiles pour obtenir des données standardisées portant sur un grand nombre de cas.

three centers less than ten and two centers detect none (one of the latter uses mixed leukocyte culture).

The main source of typing sera seems to be the NIH serum bank (used by nine centers) while eight centers use sera of local source and eight use sera from one of the well-established histocompatibility centers.

A uniform compatibility grading was assigned to each transplant according to the Montreal system, *i.e.*

B : 0 or 1 incompatibility;
C : 2 incompatibilities;
D : 3 or more incompatibilities.

These transplant patients, thus grouped, were compared with respect to the frequency, strength and time of rejection crises: no statistically significant difference could be established between these three groups.

Only forty one cases had enough data to be used in a survival study. These were again grouped according to their match and each group compared as to the duration of survival and percentage of living patients (Figure 1).

For the deceased patients, the average survival time was 14 weeks for both B and C matches, and 5 weeks for D matches. The percentage of survival is 56.5 per cent for the B group, 40 per cent for the C group and 14.3 per cent for the D group. In spite of the great heterogeneity of the crude data, there appears to be a good correlation between the histocompatibility match and the survival of the transplants.

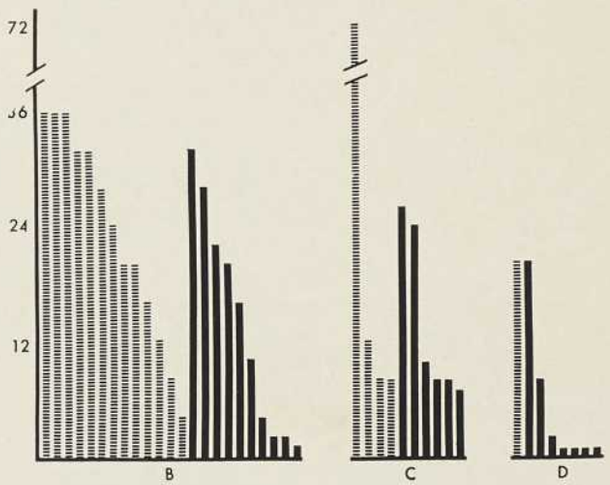

Figure 1 — Surviving (striped) and deceased (black) heart transplants grouped according to histocompatibility match (letters) and weeks of survival (numbers).

One of the reasons why no correlation was established with rejection crises could well be the present difficulty of having objective and universally accepted criteria for heart rejection.

A world wide heart transplant registry would indeed be of great assistance in obtaining standardized data on a large number of cases.

RED CELL ANTIGEN COMPATIBILITY IN HEART TRANSPLANTATION *

Shin SEKIGUCHI, M.D. †

Il existe peu d'information concernant la comptabilité antigénique du globule rouge dans la transplantation cardiaque. Il existe deux approches à ce problème.

1. Des expériences de greffes cutanées sont effectuées dans le système antigénique des globules rouges A et B, sur les groupes compatibles et incompatibles afin de déterminer la différence en survie des greffes dans les deux groupes.

La plupart des expériences sont faites en prenant la peau d'individus A, B ou AB et en greffant cette peau sur un receveur O. Une présensibilisation avec des globules rouges A ou B ou avec de la substance antigénique soluble A ou B est effectuée avant de pratiquer les greffes cutanées sur le receveur O. Une réjection accélérée survient chez le receveur lorsque le donneur de globules rouges et de peau était identique ou de même groupe ABO.

L'induction d'un accroissement de possibilités immunologiques est démontrée lorsqu'une quantité donnée de substance antigénique (1 mg) est injectée chez des individus de groupe O avant que les greffes cutanées de A ou de B sont placées sur le receveur O. Comme résultat, on note une prolongation dans la survie des greffons cutanés plutôt qu'une

(suite du résumé en page suivante)

There is not enough information about red cell antigen compatibility in heart transplantation (1 and 11) for an extensive discussion of the subject therefore, it would be worthwhile to review briefly what we know about red cell antigens particularly of A and B, in relation to organ transplantation in general.

Basically, there have been two types of approach to this problem:

1) The first being a series of skin graft experiments conducted in red cell antigen systems A and B (ABO system thereon), compatible and incompatible groups, in order to ascertain the difference in survival time of the grafts in the two groups.

Most of the experiments were done by taking skin from A, B, or AB individuals and grafting it on an O recipient (12, 13 and 14). Presensitization with A or B red cells (2 and 15) or soluble A or B antigen substance was done previous to the skin grafts on the O recipient, resulting in an accelerated rejection of the grafts in the recipient if the donor of the red cells and the skin happened to be the same individual or had the same ABO group (2 and 15).

Induction of immunological enhancement has also been reported in the experiment where a given amount of antigenic substance (1 mg) was injected to the group O individuals before the incompatible skin grafts from A or B were placed on the O recipient (2)

This resulted in the prolongation of skin grafts rather than the acceleration of the rejection of the grafts. Also, an *in vitro* coating of A1 to O skin grafts by Anti-A antibodies has induced a significant increase in graft survivals (2).

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accélération du rejet des greffons. De même, le revêtement *in vitro* de greffes cutanées A par des anticorps anti-A induit une augmentation significative de survie du greffon.

Des données cliniques détaillées de transplantations rénales démontrent des rejets immédiats en présence d'incompatibilité du système ABO.

2. La seconde approche au problème est l'identification des antigènes des globules rouges dans les tissus humains. Grâce au développement du test d'agglutination mixte ceci est accompli par plusieurs investigateurs. Les antigènes A et B sont retrouvés dans les globules blancs, les plaquettes, les cellules épidermiques, le rein fœtal, le foie, la rate, les poumons, le cœur et la peau.

Les autres antigènes de globules rouges, mis à part A et B et H, n'ont pas été identifiés positivement dans les cellules du tissu humain excepté dans les leucocytes où l'on a trouvé les antigènes MN, et Tja (ou P). L'antigène Rh n'a jamais été trouvé dans les cellules humaines autres que les globules rouges. D'où on a pu conclure que cet antigène n'a pas une importance significative dans la transplantation clinique. Il devient évident qu'une investigation plus poussée est nécessaire dans le domaine des antigènes des globules rouges afin d'exclure la possibilité de négliger d'autres antigènes de globules rouges qui pourraient être des antigènes importants dans la transplantation cardiaque.

Here, it is intriguing to note that the incompatibility within the subgroups of A, that is A1, A2 and others makes some difference in the host when they are used to sensitize the O recipient and also in the survival of the skin graft from the subgroups of A (2). For instance, skin from A1 would react violently with O recipient whereas skin from A2 individual would not be rejected as the second set reaction which is analogous to the situation in hemolytic diseases of the newborn due to AB incompatibility where only A1 or B children are affected while A2 children always escape the disease (2 and 17).

There have been well-controlled clinical data of renal transplantation in which immediate graft rejections were encountered in ABO incompatible pairs (6 and 17).

Besides A and B antigens, P antigen has been attracting attention because of its possible role as a transplantation antigen. Shorter than normal survival time of the skin grafts has been reported in P incompatible donor-recipient pairs (3) and supportive evidence to the effect has been presented in human renal transplantation (6). Skin grafts' experiments by others however, show the results to be otherwise. It has also been shown that ABO and HLA compatible control groups did not differ from each other as far as survival is concerned, suggesting that the other red cell antigens are not strong transplant antigens (15).

No other important contribution regarding red cell antigen system in relation to organ transplantation has been available except one of Doctor Dossetor's cases in his series of renal transplantation in which red cell antibodies might have been responsible for the failure of the kidney.

2) The second approach to the problem is the identification of the red cell antigens in human tissue cells. With the development of Mixed Agglutination Test, this has been accomplished by many investigators. A and B antigens have been found to be present in blood leukocytes (7), platelets (4), epidermal cells (5), fetal kidney, liver, spleen, lung, heart and skin (7).

Immunofluorescent technique has contributed some information regarding the distribution of the

TABLE I

Red cell antibody screening (with pool of O cells)

C,D,E,	c,d,e,	C^{w}—,	V—,	M,S,N,s,	PI,	Lu (a—),	Le^{a},	k,	Kp^{b},	Fy^{ab},	Jk^{ab},
Di (a—),	Vel,	Wr (a—),	Mi (a—),	Lu^{b},	Le^{b},	KK,	Js (a—),				
JK^{a},	Ge,	V^{w}—,	VS—,	(P2,	Jk^{b},	Xg^{a})					

A and B antigens on endothelium of vessels and sinusoids in spleen. These findings are in agreement with experimental works on skin grafts and with clinical results of renal transplantation.

The other red cell antigens, besides A, B, and H have not been positively identified in human tissue cells except in leukocytes where MN and Tj^{a} (or P) antigens have been identified (7). Among other things, this is due to such technical difficulties as host red cell contamination and loss of antigenic sites during the process of culturing (8, 10, 16 and 19). Rh antigen has never been found to be present in human cells other than in red cells (7 and 8) and this is a part of the theory that the said antigen is not considered to be significant in clinical transplantation.

Thus it becomes obvious that more investigation is needed in the field of red cell antigens in order to exclude the possibility of missing out on some other red cell antigens besides A, B antigens which are significant transplantation antigens.

We have been doing genotypes of Rh system and red cell antibody screening test in order to avoid any obvious incompatibility (Table I). So far none of the cases in our institution (University of Toronto Transplantation Team) has developed red cell antibodies.

A complete profile of red cell antigens including the subgroups of A antigen (7) as well as HLA system are needed in all cases of organ transplantation in the future, placing more efforts on identification of red cell antigens on tissue cells, in order to accumulate data for better understanding of the relationship between red cell antigens and organ transplantation.

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PRE-EXISTING ANTIBODIES : RELEVANCE TO HEART TRANSPLANTATION *

J. B. DOSSETOR †

À quels antigènes correspondent les anticorps pré-existants ? Il faut considérer cinq groupes d'antigènes :

1. Le globule rouge et les systèmes ABO, Rhésus et autres ;
2. Le système HL-A ;
3. Les antigènes anti-cœur spécifiques ;
4. D'autres antigènes à l'exclusion des premiers, trouvés en dehors du cœur, à savoir la membrane cellulaire, la membrane nucléolaire, le DNA, le RNA, les plaquettes et les granulocytes ;
5. Les antigènes streptococciques et d'autres micro-organismes.

GROUPE I : Les antigènes du globule rouge. Les antigènes A et B doivent être considérés comme des antigènes de transplantation. Ils apparaissent dans le cœur, et sont importants dans les greffes cutanées de jumeaux identiques dans le sytème HL-A. Une présensibilisation cause un rejet accéléré. Quoique les antigènes Rhésus ne semblent pas importants dans la transplantation, il est prudent de ne pas pratiquer de greffe tissulaire chez un receveur Rh négatif sensibilisé si les autres antigènes du globule rouge sont négligés dans la transplantation parce que considérés comme immunologiquement faibles. Dans ces cas la présensibilisation
(suite du résumé en page suivante)

Pre-existing antibodies to which antigens? That is the first question. There are five groups of antigens that have to be considered: 1) red cell: ABH, Rhesus and others; 2) HL-A; 3) organ specific heart antigens; 4) other antigens (not red cell, not HL-A) but found in tissues other than heart: cell membrane, nucleolar, DNA, RNA, platelet and granulocyte; and 5) streptococcal and other micro-organisms. Although many of these are not of the histocompatibility type, it is the latter, or HL-A, that are the most important and crucial to success. The importance of the antigens of group 3 is unknown. For others, such as the less common red cell antigens, tissue distribution is not known for sure. Many of these are weakly immunogenic but may still be of significance when pre-immunisation happens to be present prior to transplantation.

The rôle played by these non HL-A antigens may be determined by studies to detect their presence prior to transplantation, and to follow changes in antibody titers with transplantation and with subsequent rejection episodes.

Group 1: Red cell antigens. Pre-existing antibodies exist 'naturally' to antigens A and B, and these two must be considered to be transplantation antigens. They occur in the heart (7, 15 and 16) they are important in skin grafts between HL-A identical siblings (1) and pre-sensitisation causes accelerated rejection (13). Early reports of success with renal allografts despite ABO incompatibility (3 and 14) were not borne out by later results (5 and 6). Although Rhesus antigens do not seem to be important in transplantation (5), it would

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peut bien devenir un risque de transplantation. Par ailleurs, il est possible que le système antigénique P puisse être important dans la transplantation.

GROUPE II : Antigènes HL-A. Des anticorps pré-existants contre les antigènes HL-A peuvent être décelés par un *cross-match* entre le sérum du receveur et les lymphocytes du donneur. La positivité du test ira de pair avec un rejet immédiat de la greffe. Cependant, des tests faussement négatifs sont signalés par les auteurs. Ce principe peut être important dans la transplantation cardiaque chez une femme multipare qui peut avoir eu et perdu ultérieurement des anticorps lymphocytotoxiques contre les antigènes incompatibles de l'époux. Si le donneur cardiaque a des antigènes en commun avec l'époux, des anticorps lymphocytotoxiques peuvent rapidement être reformés par un clone latent et ainsi causer une réjection cardiaque rapide.

GROUPE III : Anticorps contre des antigènes cardiaques (spécifiques d'organes). Kaplan donne une revue de l'apparition d'anticorps contre le

still be considered inadvisable to implant tissues into a sensitised Rh negative recipient as studies on tissue distribution of these antigens cannot be accepted as having been exhaustive. Most believe that pre-existing antibodies to rarer red cell antigens would not be important. We have evidence to the contrary, with pre-sensitisation to Duffy antigen (Fy^a) where renal implantation was associated with disappearance of antibody, and excision of the failed transplant was associated with immediate reappearance this antibody (2). If other red cell antigens are being disregarded in transplantation not because of poor immunogens, then pre-sensitisation may well be a transplantation hazard. There is evidence that P may be important in transplantation (5) though this was not confirmed by others (11 and 12).

Group 2: HL-A antigens. The evidence is now so strong that pre-existing antibodies to HL-A antigens, detected by a positive recipient serum/donor lymphocyte crossmatch, will cause immediate graft failure (4, 9 and 10), that it would be wrong to carry out any allograft without a negative crossmatch of this type. Recent work in our laboratory has shown that there may be occasions when this crossmatch test is *falsely negative* (8). Thus, one of our patients with renal failure, whose lymphocytes carry Terasaki antigens (Te 2, 3, 5, 6 and 8) became sensitised by transfusions and hemodialysis to antigens (Te 4, 7, 9 and 11). After unsuccessful renal transplantation, during which time he was given two months of intensive immunosuppression, lymphocytotoxic antibodies were found to have disappeared completely from his serum, including after excision of the graft. He was then dialysed with buffy coat free red cells for a five month period. Lymphocytotoxic antibodies were absent throughout this period. On exposure to his mother's blood antibodies returned against Te 4, the only incompatible antigen she carried. During subsequent hemodialyses with whole blood, containing white cells, the other antibodies previously present, against Te 7, 9 and 11, reappeared. It was fortunate, indeed, that the mother's kidney was not transplanted into this patient during the five month period when the direct crossmatch was *falsely negative.* This principle might be important in heart transplants into multiparous women who might have had, but later lost lymphocytotoxic antibodies against their husbands, incompatible antigens. If the donor of the heart had antigens in common with the husband, lymphocytotoxic antibodies might rapidly be reformed by a latent clone and cause a rapid heart rejection *because of latent sensitisation.*

Group 3: Antibodies to organ-specific heart antigens. Kaplan (8) has written a recent succinct

sarcolème, le sarcoplasme, les éléments intermyofibrillaires. Par ailleurs, des anticorps contre les antigènes cardiaques peuvent apparaître dans différentes maladies incluant la période consécutive à la chirurgie cardiaque. L'importance de ces anticorps dans la transplantation cardiaque est actuellement inconnue mais il est certain qu'ils peuvent être mesurés avant et après la transplantation cardiaque, et au cours des crises de rejet.

GROUPE IV : Anticorps contre les antigènes cellulaires ne faisant pas partie des trois groupes précédents. Ces anticorps pourraient devenir d'une grande importance si, à l'avenir, on faisait des transplantations cardiaques pour des maladies du collagène avec phénomène vasculaire aigu ou, encore, en phase aiguë de fièvre rhumatismale ou d'autres formes de myocardite.

GROUPE V : Anticorps contre le streptococque et d'autres organismes. Ces anticorps furent décrits par Rappaport et leur importance peut être plus grande qu'on ne le croit actuellement.

review of the incidence of antibodies to sarcolemmal, sarcoplasmic, intermyofibrillary structures, using fluorescence microscopy and to various heart antigen extracts, using *in vitro* techniques. Antibodies to heart antigens may occur in different disease states, including after cardiac surgery. Their pathogenic importance in heart transplantation is quite unknown and may not be great; but they should certainly be measured before and after heart transplantation and in rejection crises.

Group 4: Antibodies to cellular, non RBC, non HL-A, non heart-specific antigens. These antibodies would be of great importance if the time ever came when heart transplantation was done for acute collagen vascular diseases, acute rheumatic fever or other forms of acute myocarditis. At present this is not the case.

Group 5: Streptococcal and other organisms. Preformed antibodies of this type, and their significance are reported elsewhere in these proceedings by Rapaport. Their importance may be greater than is presently realized.

Analysis of rôle of preformed antibody in heart transplants, to date:

Of 19 centres contributing data on 64 heart transplants, all were compatible with respect to ABO; 4 were incompatible for Rhesus and in 26 Rhesus compatibility does not seem to have been determined. The less common red cell antigens were not studied in 16 centres nor did any reporting centre record whether antibodies against Rhesus or other red cell antigens pre-existed in their patient's plasmas. Four centres did not do the recipient serum/donor-lymphocyte crossmatch but the large majority who use this test included all the larger centres. One pre-transplant test in Montreal was positive by micro-leucoagglutination and it may be presumed this compares, prognostically, with the lymphocytotoxicity crossmatch. Acute rejection coincided with development of cytotoxic antibody against donor cells around the 7th day in one New York patient; these antibodies had not been present prior to transplant raising the possibility of a false negative pre-transplant crossmatch.

Sixteen of 19 centres do not seem to have looked for any organ specific heart antibodies — a fact which is both surprising and regrettable. We have no information of changes in organ specific antibodies after transplantation, or with rejection.

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COMPTE RENDU DE LA PREMIÈRE RÉUNION PLÉNIÈRE — PROCEEDINGS OF THE FIRST GENERAL SESSION :

— Problems related to the choice of both donor and recipient ;
— Surgical technique and organ preservation ;
— Histocompatibility in heart transplantation.

Chairman : Christiaan N. BARNARD, Cape Town.
Reporters : Wilfred G. BIGELOW, Toronto, and Gilles LEPAGE, Montréal ;
Denton A. COOLEY, Houston, and Yves CASTONGUAY, Montréal ;
Jacques COLOMBANI, Paris, and E. F. POTWOROWSKI, Montréal.
Transcript Editor : Gilles LEPAGE.

Doctor Christiaan Barnard:

Ladies and Gentlemen. This afternoon in this session, we are going to discuss three aspects of heart transplantation. This first is the problems related to the choice of both donor and recipient. I have no doubt in my mind, in the light of the experience we have had in the last year and a half, we have to change certain of our criteria, which we accepted a year ago, in both the choice of the donor, and the choice of the recipient.

Next is the surgical technique and organ preservation, which have been pretty uniform. But there are a few new points which, I think, should be mentioned.

And lastly, the very important aspect of histocompatibility in heart transplantation.

We will now first hear from Doctor Bigelow and Doctor Lepage.

Doctor W.G. Bigelow:

We have had a stimulating and interesting session and I noticed that the audience were principally medical men so that we had to be rather careful to manage the discussion properly.

The severest critic of surgery would have to admit that heart transplantation at least stimulates interest in a number of fields that would perhaps never be explored. This was the case in point this afternoon. The first speaker, Doctor Freidberg, gave some very interesting statistics. We are all statistically oriented and we like to behave like exact scientists, and we feel better if our statistics are proving our point, although this is very difficult in the biological sciences. However he has taken 130 heart transplants that have been operated upon and he finds that there are 35 living. That, in itself, does not sound very happy. However, in analysing this, 35 per cent have lived over three months and 18 per cent over six months. Those are very interesting statistics. Then he does a very interesting bit of paper work: he excludes all those that died in the first week and finds that 41 of 74 lived over three months, or 54 per cent, and that 20 of 67 lived over six months, or 30 per cent. On the basis of these figures he feels, I think, we are evolving out of the era of experimental surgery as has been mentioned earlier this morning.

The figures that Doctor Stinson has given from Doctor Shumway's group this morning suggest a half life of about seven or eight weeks for the usual recipient, without operation, and most of them are dead in three to four months so that these figures would confirm Doctor Freidbergs' contention. He suggested finally that there should be better matching, and that one should look for the significant antigens, and he feels these are the two large areas in selecting donors. His actual indications for surgery, I thought, were very liberal for a medical man. He was not challenged particularly on his paper, although it did contribute to the discussion.

Doctor Kahn reviewed in somewhat more detail the very nice presentation he gave this morning with the beautiful and artistic slides and he had

more of them this afternoon. He reviewed his first case with the pulmonary artery pressure of 70 mm Hg and that invoked discussion as to how high the pulmonary artery pressure or pulmonary vascular resistance could be before you consider doing a transplant, and I do not think we came to any conclusion. There were some very interesting comments made. He feels that there is no greater evidence of rejection in a cardiomyopathy and that transplantation is rather a good operation for this condition. Doctor Beanlands from Toronto challenged him and quoted a case of cardiomyopathy that had received a transplant. This patient died and at autopsy, the donor heart had all the histological appearance of the original recipient's heart with the myocardiopathy picture. This brings to mind the importance of realizing of course that cardiomyopathy is a sort of biological scientific wastebasket and I am sure that it includes a number of entities. One wonders if it is not dangerous to use the word cardiomyopathy and infer that it is one disease. There is a suggestion, if this is human experimentation and if you are transplanting a cardiomyopathy, that the value of your eventual pathological interpretation in the donor heart may be reduced, because you might wonder whether the cells you see are the result of this original disease or due to rejection.

Well if you heard a great calm descend on Salon "A", it was when Doctor Heggtveit made a very challenging and disturbing presentation. Those of us who had not read his publications were interested to find that he has drawn some very worrysome lines across the whole picture of heart transplantation. He finds, along with his studies of brain damage, and also in subarachnoid hemorrhage, that there is subendocardial hemorrhage in the heart in 50 per cent of cases, focal necrosis in 25 per cent and a variety of other lesions including infarct. Now this is in the heart. And as he was making his presentation, we immediately thought that it might be in relation with vasopressor drugs and cardiac massage but in the cases he presented, he had apparently excluded cardiac massage, vasopressors and chest injury. Of course this lead on to some questions as to how we can, in the individual with the brain damage, spot the myocardial damage. The electrocardiogram did not seem helpful and there did not seem to be any real correlation, from what I could gather from the discussion. Does this mean that the sooner we obtain the donor heart, the less likely one is to see the subendocardial hemorrhage and the focal necrosis? Is there a time limit? I think Doctor Freidberg brought up the interesting point that, if this is so, if many of the hearts that we are transplanting do have some pathology due to the brain damage, then we may be misinterpreting the pathological picture we see in the transplanted patient when he dies and we examine his transplanted heart. I guess we just have to take our chances and Doctor Lepage and I were discussing this in the 17 or 18 minutes we had to prepare this presentation. We felt that it might be wise to take a peak inside the ventricle if you have a moment to spare when you are doing a transplant. Doctor Lepage astutely said it would not change your mind as to whether you did the transplant or not, but if you saw a good deal of subendocardial hemorrhage in the donor heart that you are transplanting, it might help you interpret what happens to the patient afterwards. I am sure if there is discussion, Doctor Barnard, there may be members of the audience that would be interested in pursuing this question further.

Doctor Alksne, a neurosurgeon, discussed the soundness of the Harvard guidelines for irreversible coma. He accepted them. He said they were good. He did want to emphasize again points that had been mentioned in this report. What was the effect of sedatives, hypothermia, alcohol, anoxia and shock? He felt the Harvard report had placed insufficient emphasis on the danger of misinterpreting these things, and that there should be blood tests to correct or eliminate the possibility of the coma being affected by any of these factors. Doctor Alksne spoke about spinal and cerebral reflexes and he left me out in left field, I could not follow him. I do not think, Doctor Lepage, you quite followed him either did you? Or am I underestimating you?

Doctor Lepage:

I had the distinct impression, Doctor Bigelow, that Doctor Alksne's position was that, whereas cerebral reflexes, like movement of the eyes to turning of the head, movement to irrigation of the ear with cold or hot solutions, those reflexes were very important in determining brain death. However, spinal reflexes like deep tendon reflexes, their absence need not be among the criteria for brain death as far as he was concerned, because these can be present even with severely and completely destroyed brain. This is the impression I had, Doctor Bigelow.

Doctor Bigelow:

You did a lot better than I did, Doctor Lepage. Thank you very much. He suggested, along with the previous speaker, that there was some interesting research with which he was in contact where you stimulate the midbrain and you find myocardial damage. How is this mediated? I know Doctor James Key of Toronto has stimulated the vagus nerve many years ago and found that he induced intravascular aggregation of red blood cells or sludging. That in turn produces peripheral stasis in all the organs and one wonders if this is not one of the connecting links between organic and functional disease. It might be one of the explanations for why stimulation of the brain produces a myocardial lesion which could be vascular.

Doctor Lepage:

Doctor Alksne also pointed out that the time limit of 24 hours (two separate examination 24 hours apart as recommended by the Harvard committee) might be completely unnecessary in a good many cases and he pointed out that it was the individual physician's responsibility to determine the moment of brain death or whether brain death was present or not, and guidelines were just guidelines.

Doctor Bigelow:

The last speaker was Doctor Poirier. I had a feeling that he was teasing us, titillating us and stimulating us to think. One moment he would say that the criterion of a flat E.E.G. is a good criterion of irreversible coma, and a moment later he would explain what a very rough test this is, that it does does not indicate the refinements of cerebral function. In fact, how could a heart continue beating with a flat E.E.G., unless there was some sort of activity coming down from the brain to maintain this rhythm? That is disturbing. However I caught him after the session and I sort of got him up against the wall and I said: Now do you or do you not believe in this flat E.E.G.? And he said: Oh yes, it is the best thing you have and it is a good criterion. And I said: How long do you think it should be flat? He said: Just a few minutes. So he is on our side. I thought for a moment he was going to sink the ship this afternoon. Now I would like to ask Doctor Lepage if that was his reaction to Doctor Poirier's talk, and also I am sure he has many other interesting comments to make on the discussion this afternoon. If I may turn this over to Doctor Lepage.

Doctor Lepage:

Yes that certainly was our impression of Doctor Poirier's comments on the value of a flat E.E.G., and having lived with him for many months, I can assure you that what he says can be interpreted in a reasonable manner. He of course realizes the limitations of the instrument much better than we do, which makes him a little more eclectic than we might be. There are other comments that were made which I think bear some remarks. The size of the donor heart in relation to the recipient was discussed and it was everybody's feeling that, barring of course a tremendous difference in sizes, usually one adult heart could be implanted into another adult patient even though he is quite a bit bigger. So the size discrepancy does not make much difference as long as it is not too much exagerated.

The question of the presence of pulmonary hypertension in the recipient was also discussed. It was the general feeling that mild to moderate pulmonary hypertension, particularly since so often the left atrial pressure is very elevated in those

cases, is not of great importance. But most people present I think would be hesitant in suggesting for transplantation or accepting for transplantation a patient with a systemic pulmonary hypertension on the basis of arteriolar resistance.

I think that was the essence of our session this afternoon. Thank you.

Doctor C. Barnard:

We now have a few minutes for questions and discussion. I'd like to open this discussion by mentioning a few points that I think have not been mentioned here. When we met about a year ago discussing the same problem, we came to the conclusion, in the selection of the recipient, that we would take all comers, no matter what age they were, what they suffered from, or any other consideration. Now we have seen how poor our results are in heart transplantation, and I wonder if the time has not come, to improve our results, that we should make more of a selection of our recipients, and I think we should set an age limit. We know that in renal transplantation it has been decided that there should be an upper age limit. I think this is flexible because one man of 50 is younger than another man of 50, but I was interested to note that Doctor Kahn, who obtained those wonderful results, had none of his patients older than 50 years. We certainly have struggled more in the older age group patients. I have a patient now 65 that I have operated on and it certainly has been a real struggle to get this man through the postoperative period, especially as he had been ill for a long time. And he has now the extra disease of immunosuppression. So I'd like to hear the discussion on the age limit.

I also wonder whether a single patient demand for organ transplantation is great enough and, since facilities are still small, whether we should not take into consideration, when we select our recipient, the chances of getting a compatible donor for the antigens of this patient. What are the chances of these individual single patients getting a compatible donor? I know Doctor Botha has done some work on this and he may discuss this in a little more detail.

Another thing we have found is that all our patients in the postoperative period have developed a Herpes virus infection. I would like to hear your views on whether it is not important to know that the patient has natural antibodies against the Herpes virus, in the selection of patients for transplantation. I was also very interested in the work of the group in Houston on the immunological potential of the patient, how this varies and whether one should take this into consideration in the selection of the donor. Then there is the selection of the donor and should one accept patients of all age groups as long as they have a normal heart. We certainly have found the best immediate results in the patient who dies of subarchnoid hemorrhage, that has had some hypertension and has a hypertrophied left ventricle. He seems to have more reserve in the left ventricle than the ordinary patient. I will now leave the discussion open to you.

Doctor Vineberg:

Gentlemen. I do not see anywhere in this program any method whereby one can estimate the amount of muscle left in the heart of the recipient. Maybe some of this information may come out, it seems to me that this is a very important affair. As we have gone with our revascularization surgery, we have gone on from patients that were not in failure to patients that were truly in failure, patients that were supposed to have very bad contractile ventricles by L.V. studies and so on. Now these patients come out of failure after internal mammary implantation. Doctor Johnson in Milwaukee and others are also watching large hearts come out of failure when they transplant a vein graft into a coronary artery. Now an aspect that seems very important to me. What are the true criteria for our removal of a man's heart? After all, the human heart muscle has at least 300 per cent reserve. I think it is time that we studied each heart to see what the reserve is, plus its possible potential with regard to revascularization, versus

transplantation. We have been offering revascularization surgery to patients with no more than 40 or 50 per cent of their myocardium left according to the scar seen at operation and pre-operative studies. What percentage of the transplanted patients had less than 40 per cent of their myocardium left, would you say? These are points that I think should be brought up and I think there should be some standardization as to what kind of a patient has his heart nipped out with another one put in.

Doctor Barnard:

Doctor Vineberg, the first point of course is that all the transplantations have not been done only on patients with coronary artery disease. Many had myocardial disease of other origins which could not be helped by implantation. The second thing is that, I can only speak for myself, the patients with coronary artery disease that we have operated on I do not think you would have found muscle to implant an internal mammary in. You could look at the pictures of these patients: the whole myocardium was really replaced with scar tissue. And I think the experience of most of the surgeons who have done transplantation was that at least 80 per cent of the left ventricular myocardium has been involved with fibrous tissue. I think it is difficult to estimate beforehand how much myocardium is left. But, on studying the left ventricular action, there has been hardly any contraction of the left ventricle before we transplanted.

Are there any other discussions? We have another two or three minutes for this.

Doctor P. Grondin:

I would like to say a word about Herpes. It is true that Herpes has been a problem in our patients. I think it is possible to prepare highly selective gammaglobulin against Herpes from patients who have had this infection in the last three months. We then can have in store enough concentrated and highly selective gammaglobulin against this and other viral disease. We could cure those patients in spite of immunosuppression.

Doctor Barnard:

Are there any more comments on this Herpes point? I think this is interesting.

Doctor X:

We have looked at a lot of patients who were either born with a defect in cellular immunity or develop one, because of immunosuppression, or what not, and those are the circumstances that give you the generalization of this otherwise latent virus. Unfortunately in all of the experience that I have had and that a lot of other people have had, we were not able to definitely turn off that with antibodies. It requires cells. And I am afraid that we are not going to be able to have an effective Herpes antiserum that is really going to alter the course. Perhaps, but I would be surprised.

Doctor Grondin:

We do not have much experience with this, but we had a patient with severe necrotic intercostal Herpes Zoster who went on to develop Herpes viremia. He was then very sick and almost dying. The only thing we had was non selective human gammaglobulin. We treated him with 100 cc I.V., and in 24 hours, he recovered dramatically and was up and around. I have a feeling that this large amount of non selective gammaglobulin really prevented him from dying at that time. I am told by our bacteriologists that the amount of specific globulin against Herpes in the general population is very small. But if one takes blood from people who have recently recovered from Herpes, one could have a much more potent globulin.

Doctor Butler:

I would like to make a comment at this point not in the heart transplant program. Doctor Arthur Beall transplanted a human lung last August 31st in a patient who turned out to be particularly interesting. The recipient had a history of recurrent Herpes virus infection (fever blisters). The donor did not. To make a long story short, Doctor Gordon

Douglas did virologic studies to indicate post-operatively that the patient developed Herpes simplex infection of the transplanted lung but not of the remaining recipient lung. Now this raises some interesting theoretical implications one of which is that the patient, who had a history of recurrent Herpes infection, may have had the cellular immune mechanisms present that prevented infection in his residual lung whereas the donor lung may not have had the same mechanisms operative to prevent infection in that lung.

Doctor Barnard:

I am afraid we will have to stop this part of the discussion. We now go on to surgical technique and organ preservation chaired by Doctor Denton Cooley, Houston and Doctor Yves Castonguay, Montreal.

Doctor Cooley:

To discuss each of these papers separately we will start with the first paper by Doctor Paiement and his group from the Montreal Heart Institute on anesthesia. He pointed out that in his experience with nine patients, eight of whom did satisfactorily after transplantation, in each instance they used hypothermia in the donor down to 18° centigrade with total body perfusion. In his post-operative experience, they did not use vasopressors, Isoproterenol or cardiac stimulants, and he attributed this to the fact that these hearts were protected in this manner. I do not believe this is the uniform experience from other transplanters and I was somewhat surprised at this conclusion that he made. He emphasized the need for early extubation of the patient after anesthesia and also brought up the matter of the importance of early ambulation, a point which Doctor Barnard himself questions, in that he sees no particular benefit in early ambulation, assuming that the allograft is in a somewhat precarious position at least for the first few days after transplantation. Doctor Barnard made a comparison with patients following myocardial infarction, in which early ambulation has not been proved to be a desirable technique. These were the points that were brought out in this paper on anesthesia. Maybe Doctor Castonguay would add to this?

Doctor Castonguay:

I think that Doctor Paiement mentioned that six of the eight patients that did well after heart transplant did not receive any vasopressors. In the other two patients, a continuous drip of Epinephrine was used in one case for a period of two hours post-operatively, and in the other case Isuprel was used in the same manner for a period of about one hour post-operatively to control slow heart rate.

We do not think that early extubation is an absolute necessity, but in our experience, these patients have done so well in the early postoperative period that we just felt they did not need to be intubated for a prolonged period of time. As for early ambulation, we think that getting out of bed soon after the operation is a good moral stimulant for these patients, and prevents known complications of long immobilization. I agree that the allograft might be in a somewhat precarious position, but I do not think it can be compared to an infarcted myocardium where acute metabolic changes of the muscular fiber itself is responsible for the irritability and instability of the heart.

Doctor Cooley:

I think in this regard most of us believe that some form of cardiac stimulant postoperatively is essential. In most of our patients, not in all but in most of them, we have kept available an Isoproterenol drip which can be used in the first 24 to 48 hours and if the transplanted heart is deficient in anything, it seems to me that it is deficient in this particular drug. Vasopressors have not been utilized in our cases postoperatively and we have depended upon either Isoproterenol or injections of calcium chloride. In the majority of our patients we have also digitalized the recipient post-operatively, believing that the transplanted denervated myocardium is in need of some drug stimulant such as Isoproterenol or calcium or digitalis. In regard to the surgical technique, in my report,

I pointed out that most surgeons have now adopted the standard technique of Shumway and Lower. Most surgeons it seems are using the modification which Doctor Barnard introduced in Philip Blaiberg where an effort was made to preserve the continuity of the sino-auricular internodal pathways to the atrio-ventricular node. This incision is made from the inferior vena cava toward the right auricular appendage. I think we will recall this morning though, in Doctor Dubost's presentation, that his patient Father Boulogne had an incision between the venae cavae and from what I could see of the electrocardiogram, he does have sinus mechanism. So I do not think it is invariably true that an incision between the venae cavae is going to produce a nodal rhythm but I think the incidence should be far higher in those cases. It was reemphasized that complete excision of the right auricular appendage as well as the left auricular appendage of the recipient should be performed because these are relatively ischemic portions of the remaining atrium and may be a nidus for the formation of intra-atrial thrombosis and embolism. The question arose whether to use hypothermia or to maintain the recipient in a normothermic state. Those of you who know my attitude in this regard know that we adhere to the school which would maintain normothermia in the recipient. I think personally that the reason for the prompt recovery of the heart allograft in our cases is probably related directly to the use of normothermia. In addition to these reports we reported on a technique of total cardiopulmonary transplantation used in one moribond infant with a complete A.V. canal and extensive pulmonary damage. Doctor Barnard asked why we did a transplant there instead of a pulmonary banding, and I believe the reason we did this was the same reason he used cardiac transplantation in Doctor Blaiberg instead of an internal mammary implantation. We also reported on our two stage technique of cardiac replacement utilizing orthotopic cardiac prosthesis first and three days later replacing the prosthesis with a cardiac allograft. This technique will be shown in a movie this evening which Doctor Liotta will narrate for us.

The third paper was from the Palo Alto group and was presented by Doctor Stinson. In his paper on myocardial protection in heart transplantation, Doctor Stinson advocated the use of moderate topical cooling of the heart and did not believe that simultaneous perfusion of the allograft was necessary. They have tried both apparently and have settled on the topical cooling. He reported some interesting laboratory experiments in which they tried to determine the recuperability of the hearts which were preserved ischemic at varying temperatures: at 15° temperature he was able to ressuscitate an animal heart 230 minutes after it had been removed from the pericardium. So that it does appear that induced hypothermia in animals does preserve the contractility of the heart for prolonged periods. Doctor Barnard and several others advocated the use of both hypothermia and perfusion of the heart. In our group and in many others, simple normothermic conditions and total ischemia are used for the relatively brief period of time taken to transfer the heart from donor to recipient.

Now then, the last two papers were primarily directed toward the same subject: methods of storage and preservation of the heart. Of course this is something of vital importance to organ transplanters and particularly to heart transplanters, since we have, to this time, not found reliable or dependable methods of preservation of the heart. Doctor Proctor's presentation was particularly interesting in this regard. He had a technique with the isolated heart in a chamber with no oxygenator at all, and perfusing Krebs solution, was able to maintain the viability of the animal heart for prolonged periods of time. Again, as I said, he used no oxygenator in his system. Doctor Pitzele showed a very interesting film strip on the technique that they had used in preservation of the heart. In contrast to Doctor Proctor's group, Doctor Pitzele had removed the heart intact in the pericardium as a protection and I suppose also as support. His venae cavae were ligated so that the allograft would recirculate all of the coronary sinus blood. The technique he used involved the use of plasma as a medium and he used a membrane oxygenator with

rather low flows, about 150 cc per minute. He said that the plasma developed a partial pressure of oxygen of 500 mm Hg, a very interesting fact to me. He also related of course that the problem with these preserved hearts is to know if they have sufficient reserve to justify one using them in a human recipient. The factors which he thought were the best index of this myocardial viability or reserve were found in the level of inorganic phosphates in the perfusate and I thought that this was the principal contribution he made in this regard.

Doctor Barnard:

Well this part is open for discussion now. I might just mention a few other points. One might say the transplant team has two patients to look after, the donor and the recipient. When the donor is handed over by the other team, then I think the prime purpose of the transplant team is to keep the heart of that donor in as good a condition as possible until that heart has been transplanted in the patient and of course the best way to keep that heart in a good condition until it is taken out is to keep the best circulation possible in the donor. It is important here to correct such things as blood volume, metabolic acidosis, electrolyte imbalance and also to stimulate the heart if necessary.

Doctor Cooley questioned the advisability of perfusing the heart after it is removed. I mentioned that it is interesting to me that during the operation we preserve the brain, the liver and the kidneys and all the other organs of the patient by perfusion, yet we feel it is bad to preserve the heart by perfusion.

Another point, which was mentioned by Doctor Stinson this morning and which I think is important is that I think inguinal canulation of any kind is dangerous. I think that actually some patients were lost from infection that started at the inguinal region and spread. It is a region that does not heal well afterwards and is very near areas of infection.

One thing that I want to point out about Doctor Cooley's heart prosthesis is that I do not think that when a patient wakes up properly and has good cerebral function, it necessarily means that the patient has a good perfusion. I think he pointed out, and that is important, that the patient had very poor urinary output after the prosthesis was inserted and to me, that indicates poor circulation. This is now open for discussion.

Doctor Proctor:

I would just like to make one correction to Doctor Cooley's discussion. Our technique for storing the heart for long periods involved oxygenating the Krebs solution to a pO_2 of 400–500 mm Hg.

Doctor Heggtveit:

I would like to raise a point here that was touched on briefly at a meeting of a Study Group on myocardial infarction at the World Health Organization headquarters in Geneva in March: what is being done with these excised living, beating, ischemic human hearts? Are they being dissected conventionally and tossed in a bucket of formalin? It would seem that this is unique experimental material. Could not these hearts be kept beating *in vitro* for a period of time and their metabolism studied prior to their dissection?

Doctor X:

One word about non-perfusion of the heart during transplantation. We say the simpler the better. We do not perfuse our hearts because I think it has been demonstrated that the heart can be maintained during 40 minutes or more without harm and I think the perfusion methods now in use are not physiological so one does not know if one is harming the heart or helping it by perfusion.

Doctor Dobell:

We had an experience that may be unique. Most of these denervated hearts have sustained a reasonable heart rate after transplantation. For some reason our heart recipient, in sinus rhythm, had a bradycardia. The heart rate when the patient was awake would run around 60 and when the patient was asleep around 45. Under these conditions the patient would develop a gallop rhythm and it was

obviously not a desirable rate. Fortunately we had implanted wires into the myocardium and the patient was driven with a pacemaker for one month, postoperatively, with repeated evaluation of his own heart rate which was always slow. Finally a demand pacemaker was implanted at a rate of 85 and he continues to be paced at 85, breaking through only in periods of rather extreme exertion. I am wondering if anybody else has had this experience? Most hearts, I gather, beat at a rate of about 80 or 90, but this one chose not to.

Doctor Webb:

I would like to emphasize the problems of storage. The problems of technique have been well handled, as illustrated by some 30 centers throughout the world that have handled the technique very successfully. The problems of storage still remain to be solved: three days is not enough even with Europool. We need long term storage to allow proper matching of the recipient with the donor. Our perfusion system as described today is still incomplete. We can supply metabolites but do not get rid of the end products of metabolism because there is not a liver or a kidney in the system. Freezing remains the only way we can possibly achieve long term preservation of organs and unfortunately no one as yet has come up with any possible solution to the freezing problem. Until we do solve long term preservation, we are not going to be able to achieve the results that we would like to have. Also, of course, when our immunologists develop true tolerance, or even true enhancement, then cardiac transplantation will truly come into its own. But these two major problems still remain unsolved.

Doctor Neville:

Nothing has been said about donor and recipient committees in hospitals. In our institution, it was decreed that a separate donor and recipient committees be mandatory. It was stipulated that we could not remove the donor heart until it had ceased functioning and E.E.G. was flat. Under these conditions it is still possible to heparinize the patient and cannulate the vessels in the groin before death. Then, when the heart stops beating the donor can be placed on peripheral cardiopulmonary bypass with an oxygenator. In our only heart transplant, Doctor Pifarre, my associate, and another oxygenator team, waited until the donor heart ceased functioning. Peripheral cardiopulmonary bypass was instituted immediately and they were able to remove a beating viable heart after we had placed the recipient on bypass and excised his heart. By utilizing this technique it is possible to obtain a well oxygenated donor heart and still comply with the demands of the various peer groups.

Doctor Barnard:

I might mention that, in our first three patients, we did not remove the donor heart until the heart had stopped beating. In the last two we removed the heart while it was still beating and to me it seemed to make very little difference in the immediate postoperative take-over of this heart, whether one left it until it stopped beating or whether you took it out while it was beating. The other point that I think was stressed at this session and I think should be mentioned again for people who study preservation of hearts is this: I do not think it is enough just to see how long they can keep that heart beating, I think that means very little. I think they will have to show us that that heart can take-over the circulation properly after it has been stored for any length of time. This is the acid test, not how long you can keep it beating. Are there any other questions?

Doctor Cooley:

I would like to discuss the point that you raised about whether this prosthesis was actually providing a satisfactory cardiac output. The electromagnetic flow studies which we did directly on the pumping device revealed a cardiac output of 5 to 5.5 liters per minute. Moreover during the postoperative period, the patient's blood pressure could be ajusted at most any level that we desired, and it was usually 110 to 120 over 80. It is my conten-

tion that if there was a reduced renal function, and of course there was, it was due primarily to humoral factors rather than to hemodynamic factors. This device will pump large quantities of blood, water, or whatever you use as a medium. This patient had high plasma hemoglobin immediately upon discontinuing cardiopulmonary bypass, and I believe this led to tubular necrosis.

Doctor Liotta:

I would like to make a few comments. With the heart prosthesis, the circulatory situation can be handled according to the physician. The patient that had a prosthesis may have had too low a cardiac output, but this could have been easily corrected or controlled. If it happened in this patient, it was not a failure of the procedure or of the system, but a technical failure on our part to interpret which cardiac output would be necessary for this particular situation.

Doctor Barnard:

What was the renal function in your animal work with this prosthesis?

Doctor Liotta:

Variable. In some animals it was excellent, in others, it was poor, and sometimes related to cardiac output. It is a very well known fact that the kidney is the more sensitive and best flowmeter we can use, much better than electro-magnetic flowmeters. But in animals, mainly the calf, it is a little more difficult to obtain an acceptable cardiac output from the prosthesis.

Doctor Ersek:

In Turkey, we did the transplantation in this way. After the heart stopped, we started perfusion and then we cooled the donor down to 18° and then we removed the heart and we transplanted without any perfusion at all. After releasing the cross-clamped aorta, the heart started to beat in half a minute spontaneously. We believe that this technique has the following advantages: 1) Confirmation of legal death as accepted in the Turkish penal code, because you have to have a stopped heart before using this heart in Turkey; 2) A better preservation of the donor heart by core cooling with perfusion; 3) The preparation of the recipient is not unduly hurried since the donor is supported by total body perfusion. The decision to transplantation can be made following the direct examination of the donor heart; 4) No crowding of the operating field by cannulae and tubes since the coronaries are not perfused during the anastomoses. No suction for coronary circulation; 5) Consequently, the operation can be performed in a very comfortable manner similar to work on cadavers.

Doctor Barnard:

We now come to the session on histocompatibility in heart transplantation. Who is going to discuss this, Doctor Colombani, or Doctor Potworowski?

Doctor Colombani:

We shall summarize this session on histocompatibility in heart transplantation in two parts. The first part was devoted to the problem of ABO compatibility and the second to HLA compatibility.

In heart transplantation as well as in kidney transplantation, ABO compatibility is now always respected. Doctor Sekiguchi spoke about the problem of red blood cell antigen compatibility in heart transplantation: ABO compatibility is always respected but problems of interpretation arise with Rh and other blood group antigens compatibility. There is no evidence that Rh compatibility plays a role in heart or kidney transplantation. Nevertheless, Rh compatibility is generally respected as in the case of transfusions. From the study of red blood cell antigen compatibility, it was suggested that it might be useful to determine as many antigens as possible from donors and recipients in order to allow an *a posteriori* analysis.

The problem of pre-existing anti-red cell antibodies was presented by Doctors Sekiguchi and Dossetor. Doctor Dossetor reported one case in which anti-red blood cells as well as anti-lymphocytes cytotoxic antibodies existed and this case was terminated promptly in rejection. The role of the anti-red cell antibody in this case is questionable

because there were at the same time anti-lymphocyte antibodies. It is clear that the presence of anti-red cell antibodies reflects a general immunization of the recipient, which means that he has a better chance of having anti-tissue antibody.

For HLA, the problem was discussed mostly by Doctor Terasaki and ourselves. The description of these antigens is becoming more complete: there are now six officially recognized antigens: HLA 1, 2, 3, 5, 7, 8, and others which are under study in the different laboratories working in this field. It can be expected that in the next year, six or more new antigens will become official.

These antigens are determined by genes which are located in a chromosomal region subdivided in at least two sub-loci. The importance of these antigens in transplantation was studied experimentally with skin-grafts. It was then studied in the case of kidney transplantation and is now studied in the case of heart transplantation. The experimental skin-grafts made from child to father led to the conclusions that to obtain a good tolerance of the graft it is necessary to realize an identity for two antigens at one sub-locus, and for at least one antigen at the other sub-locus. That means that if one only had the antigens well determined at the two described sub-loci, *i.e.* a minimum of four antigens, one can accept one incompatibility but no more. The same conclusion can be obtained when the correlation between kidney graft and HLA compatibility is studied. This fact was presented by Doctor Terasaki who showed that in kidney transplantation for what he called a D match (that is two incompatibilities or more), one can expect rejection after six months to one year. Finally, this problem of HLA compatibility is now being introduced in the field of heart transplantation and from a survey made by Doctor Potworowski who sent a questionnaire to the different teams working in this field, it was observed rather surprisingly that HLA antigens were often determined before transplantation: from the 16 teams questioned, 13 said that they tried to determine tissue antigens before transplantation. That means that probably one day or another, they will take them into account. In Doctor Terasaki's study of 49 cases of heart transplants, these were classified for HLA compatibility, and the letters B, C, D, refer to the match in terms of Doctor Terasaki's nomenclature: B is a good match and D is a bad match and it seems that the B matches behaved better than the D matches. The same conclusion can be obtained from Doctor Potworowski's survey which he will comment himself.

Doctor Potworowski:

It is apparent from our survey of 51 heart recipients that over half of the B matches are still alive whereas only between 30 and 40 per cent of the C matches are alive and only 14 per cent of the D matches are alive.

The average survival time for the deceased recipients is about fourteen weeks for the B and C matches with no significant difference between the B's and the C's and, for the D matches, average survival is about four weeks. We could not find any correlation between the match and the rejection crises, either their number nor their strength.

Doctor Colombani:

Finally the last part dealt with the possibility of pre-existing anti-tissue antibodies before transplantation. It is clear now that a positive cross-match for tissue antigens, made for example by the lymphocytotoxicity technique with the serum of the recipient against the lymphocytes of the donor, means a very bad prognosis and must be considered as a straight "veto" for transplantation. The problem of organization of prospective typing in heart transplantation was also raised and it seems that the same principle should apply to heart transplantation as for kidney transplantation, that is making a pool of prospective recipients previously typed and waiting for a donor. Finally it was suggested that it would be very useful to collect the data from the teams working in heart transplantation in a heart transplant registry similar to the kidney transplant registry which gave very interesting results about the relationship between success and histocompatibility.

Doctor Barnard:

This session is now open for discussion. Being a surgeon I would not start the discussion, I will leave it to somebody else.

Doctor Kahn:

Are some transplantation antigens stronger, and therefore more important than others in heart transplantation?

Doctor Colombani:

You want to know if there are strong antigens and weak antigens? I think that even for kidney transplantation, it is not possible at the present time to answer this question but it seems likely (that's just an opinion) that all the presently described antigens have the same strength.

Doctor Barnard:

Any other questions?

Doctor X:

I would like to know...

Doctor C. Barnard:

What is your name, please?

Doctor Marius Barnard:

Marius Barnard, Sir. I would like to know, as I am listening to people discussing matches, how one can refuse to transplant, unless he has a B or a C minus match, a desperately ill patient, when we have heard about some very good results with a C plus or a D match.

Doctor C. Barnard:

Doctor Cooley, would you like to answer that question? Did you get the question?

Doctor Cooley:

My attitude and opinion about the selection of donors and recipients have not changed very much in the last twelve months since we had the conference in Cape Town. I believe that there are other extenuating circumstances which should determine the advisability of a transplantation and these extenuating circumstances are very well known to anyone who is actively engaged in any clinical program. I do not think that we can say that a man who is 50 years of age is any less likely to benefit from the operation than a man who is 45. The question we are here to answer is whether a man has a right to live longer. And it is a matter of interest about tissue matches. What does one do as we did three days ago, when he has an ideal donor in term of physical qualities, when he has a man who is at the point of death who could benefit from a cardiac transplant? Does one wait for the tissue typing? It takes two to five hours in our institution to get an answer on the tissue typing. This point came up again with us just this week, we transplanted this man, he subsequently was proved to have a D match and I think it is far better to have a D match and be alive than wait for a B match and be dead. So even though these are interesting points, they cannot be the determinants in a clinical situation. I do not think that we have yet reached that point, where we can be so rigid in our methods of selection.

Doctor Barnard:

Well, I will speak for myself. I think that I, in the future, will not select old patients anymore for cardiac transplantation. I do not think I will select these patients of 65 or old 60's for cardiac transplantation because I have a lot of other patients I can operate on and I am quite sure I can get better results in the younger patients. Secondly I will not select, if I have other patients, a patient in which my chances of getting a well matched donor are poor. I would rather select a patient in wihch my chances of getting a good match is good. Now if, with that patient, I get a D match, I will transplant, but my chances of getting a B match or a C match is much greater. I think those are my personal views and I think that is the only way, with the present poor methods of immunosuppression, we are going to obtain better results.

As we all agree on that point, I think we will thank the people who have discussed these papers and I hope you have a lovely evening.

THE USE OF ALG IN 56 CASES OF HEART TRANSPLANTATION *

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Un questionnaire sur la nature du sérum antilymphocytaire (SAL) utilisé dans les transplantations cardiaques ainsi qu'un questionnaire concernant son emploi dans chaque cas de transplantation ont été envoyés à tous les centres où une greffe cardiaque avait été effectuée avant janvier 1969. Les données recueillies à la suite de ce questionnaire nous ont permis d'évaluer le rôle du SAL au cours de 56 transplantations de cœurs humains réalisées dans 22 hôpitaux un peu partout à travers le monde.

Les sources de SAL utilisées pour les transplantations cardiaques étaient différentes quant à l'antigène utilisé, les méthodes et la voie d'immunisation, l'animal de production, le temps des saignées et les méthodes de purification.

Trois préparations de SAL sur 14 utilisées pour les greffes cardiaques se sont montrées immunosuppressives chez les singes inférieurs et les primates et cinq préparations ont inhibé chez l'homme l'immunité cellulaire vis-à-vis certains antigène. La dose de sérum utilisée a varié suivant les

(suite du résumé en page suivante)

This Second world symposium has two main objectives: first to evaluate the present situation of heart transplantation on the basis of observations gathered so far and, secondly, to foresee the prospects for future heart transplantations. My contribution to this Symposium will be to summarize the data obtained with the use of antilymphocytic serum in 56 cases of heart transplantation.

Two questionnaires related to the use of ALG were sent to each center where heart transplantations had been performed before January 1969. The first questionnaire dealt with the production of the anti-human lymphocytic sera used in the treatment of these heart transplantations: the antigens used and the techniques of production, the purity of the immunoglobulins, and the *in vitro* and *in vivo* tests performed to assess their immunosuppressive activity. The second questionnaire dealt with the use of ALG in each case of heart transplantation; we have recorded data on the administration of ALG, the incidence of local and systemic reactions, the induction of anti-horse antibodies, the number of rejection episodes, the survival time of the patients, and the role of ALG in these transplantations. The results obtained from these questionnaires, with the kind collaboration of each center, will be summarized in this presentation.

Answers to the questionnaires:

The questionnaires were sent to 32 centers, where an over-all total of approximately 100 heart transplantations had been performed before January 1969; 22 of these centers (68 per cent) completed the questionnaires. ALG was used in the treatment of 56 of the 70 cases of heart transplantations performed in these 22 hospitals. In two centers out of the 22 which answered the questionnaire ALG was not used in the treatment schedule.

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hôpitaux, entre 0,1 mg à 10 mg d'IgG par kilogramme de poids, et la majorité des patients ont reçu le SAL à partir du jour de la transplantation pour une période variant de deux semaines à plus de huit mois. La douleur locale et l'élévation de la température demeurent les principaux inconvénients de l'administration du sérum antilymphocytaire par voie intramusculaire.

Les critères sur l'évaluation du SAL dans son utilisation en clinique ont été tellement variables d'un centre à l'autre, qu'il nous a été impossible d'établir ou de nier son efficacité comme agent immunosuppressif dans les transplantations cardiaques.

Source of ALG:

The ALG preparations came from fourteen different laboratories. All but two of these preparations were produced in horses. Six different sources of human cells were used as antigens for the immunization: spleen, spleen and lymph nodes, spleen and thymus, thymus, peripheral lymphocytes, and thoracic duct lymphocytes. The mode of immunization, the time of bleeding, as well as the use of adjuvant varied from one laboratory to another. Altogether, 31 patients were treated with anti-thymus serum (3 sources) and 11 patients with anti-thoracic duct serum (2 sources). The remaining 13 patients received ALG produced with antigens from cells of the other sources listed above.

Evaluation of the immunosuppressive activity of ALG:

Of the 14 ALG preparations used, three were tested for their capacity to maintain skin grafts in monkeys. Five of the 14 preparations abolished delayed hypersensitivity type reactions to various antigens *in vivo,* including PPD, toxoplasmin, coccidiomycin, DNCB, measles, etc. Two of these ALG were tested for their ability to stimulate blastogenic transformation *in vitro,* and both were found to be positive. Four ALG preparations were not tested at all for their immunosuppressive activity before use.

Rules for the administration of ALG:

ALG was given from the time of transplantation in 92 per cent of the 56 cases and in 8 per cent for the rejection episodes only. In 10 of the patients, administration of ALG was discontinued for various reasons: development of anti-horse antibodies (3 patients); termination of the planned course of treatment (2 patients); pain, infection, allergic reaction, thrombocytopenia, and lack of ALG (1 patient in each instance).

Sensitivity to horse serum proteins prior to ALG treatment:

Sensitivity to horse serum proteins was tested prior to the injection of ALG in 45 patients, but not in the remaining 11. Among those treated, three patients (7 per cent) gave a positive response. One patient not tested prior to the first injection of ALG died of anaphylactic reaction 90 minutes after the injection.

Symptoms caused by ALG:

Table I summarizes the percentage of patients presenting symptoms due to injection of ALG. On the whole, pain and fever developed mainly in the

TABLE I

Symptoms caused by injection of ALG

SYMPTOMS	TIME AFTER INJECTION	
	0 – 6 hrs	6 – 12 hrs
Pain	73%	46%
Fever	50%	35%
Local erythema	29%	44%
Lymphopenia	62%	66%
Leucocytosis	10%	31%
Thrombocytopenia	6%	11%

first six hours following the administration of the drug, while local erythema and thrombocytopenia appeared mainly six hours or more after the injection. Lymphopenia was observed shortly after or more than six hours after the injection with equal frequency. It evolved from the data that the pain and fever produced by the intramuscular or subcutaneous route of administration were mostly seen during the first weeks of treatment and then tended to disappear.

Presence of anti-horse and anti-sheep antibodies after the administration of ALG:

Tests to detect anti-horse or sheep antibodies were performed in 22 of the 56 patients studied, using the immuno-diffusion or the passive hemagglutination technique. These tests were performed from one to 32 weeks after the first injection of ALG. Of the 22 patients tested, 6 were positive (27 per cent) and 16 were negative (73 per cent).

Rejection episodes during treatment with ALG:

The rejection episodes were classified as doubtful, weak, strong, or no rejection at all. Table II shows the percentages of the rejection episodes, according to their type, that occurred in the first twelve weeks following heart transplantation and more than twelve weeks after transplantation. The number of rejection episodes was much higher in the first twelve weeks than afterwards and there were 20 deaths during this 12-week period. Among the remaining 36 patients that survived for more than three months, 9 per cent showed no signs of rejection, even nine months after transplantation.

Survival time in 46 heart transplantations:

Table III summarizes the survival time, in weeks, in the 46 cases documented in response to this question. Among the nine patients that died during the first week following transplantation, rejection was not found to be the cause of death, with one exception. If the immediately postoperative deaths are excluded, number of deaths occurring during the first twelve weeks was similar to the number of deaths occurring between 12 and 24 weeks after transplantation.

It is very encouraging to see that nine patients (17 per cent) out of the 56 patients studied altogether survived for longer than six months.

Was ALG a potent immunosuppressive drug in heart transplantation?

The data reported in the present study do not permit an evaluation of the immunosuppressive activity of ALG. Too many factors were involved at the same time: the type of ALG used, its lack of standardization, its association with the other immunosuppressive drugs, the lack of appropriate controls, etc. ALG was employed together with the other immunosuppressive agents in all cases, in order to increase the chances of a successful treatment rather than to test the immunosuppressive

TABLE II

Classification of the rejection episodes totalling 111 in 56 cases of heart transplantation

TYPE OF REJECTION EPISODE	TIME AFTER TRANSPLANTAION	
	1 – 12 weeks	12 weeks
Doubtful	10%	> 3%
Weak	12%	2%
Strong	44%	13%
No rejection	9%	9%

TABLE III

*Survival time in 46 * heart transplants performed before January 1969 and treated with ALG*

	SURVIVAL TIME IN WEEKS			
	0 to 1	1 to 12	12 to 24	> 24
Deaths	9 (19%)	15 (33%)	11 (24%)	2 (4%)
Alive	—	—	—	9 (19%)

* Data on 10 additionnal cases unavailable.

activity of ALG. The heart transplantation was an experiment in itself; it was obvious from the questionnaire that no real attempt was made in any centers to assess in human, the immunosuppressive activity of the ALG preparation used, per se. On the whole, the preparation of ALG employed were all accepted from the start as being potent immunosuppressive drugs.

Three main sources of antilymphocytic serum were used for heart transplantation: horse anti-human thymocyte serum produced in Houston and used for treating 17 cases; horse anti-human thymocyte serum produced in Montreal and used in Canada in the treatment of 14 cases; and horse anti-human thoracic duct lymphocyte serum produced in Munich that was used to treat nine cases. With the Houston serum, as with the Montreal serum, 40 per cent of the patients survived for more than three months, while with the Munich serum, 50 per cent of the patients survived for more than three months. The means of the rejection episodes, per patient, during the first three months was as follows: 1.6 for patients treated with the Houston serum, 1.7 for patients treated with the Montreal serum, and 1.5 for patients treated with the Munich serum.

LES AVANTAGES D'UNE GLOBULINE ANTILYMPHOCYTAIRE HAUTEMENT PURIFIÉE *

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De nombreuses recherches sont faites en vue d'obtenir du sérum antilymphocitaire et des immunoglobulines pourvus du minimum de caractères antigéniques. Des IgG ont été obtenues chez le lapin et le mouton par chromatographie sur DEAE-cellulose et DEAE-sephadex. L'auteur a obtenu un bon rendement soit par le procédé d'électro-décantation, soit par le procédé Rivanol-alcool, d'une IgG de mouton antilymphocytes humains.

Le but de ces études est de perfectionner le sérum antilymphocytaire humain de façon à isoler et augmenter au maximum l'activité immunosuppressive pour un minimum de protéines hétérologues injectés. Outre le sérum, il faut également fournir les IgG présentant un maximum d'effets immunosuppresseurs pour un minimum d'effets toxiques.

Au deuxième congrès sur la transplantation, sir Peter Medawar nous invitait à garder à l'esprit que l'administration de sérum antilymphocytaire est une thérapeutique de conception archaïque, puisque l'injection de protéines de cheval à l'homme demeure un non-sens. Or, en trois ans, les nécessités de la transplantation humaine ont fait de la sérothérapie habituelle une thérapeutique continue et prolongée avec parfois des reprises et tous les risques que cela comporte. D'autre part, la transplantation du cœur a certainement ouvert à la sérothérapie la pratique presque continue de la voie intraveineuse. Après trois ans d'applications humaines, on peut constater que les incidents dramatiques de cette thérapeutique n'ont pas été ce que l'on pouvait prévoir d'une thérapeutique par sérum hétérologue, sans doute grâce aux immunosuppresseurs chimiques associés mais également aux propriétés mêmes du sérum antilymphocytaire capable d'induire une tolérance aux protéines de cheval à condition toutefois, contrairement à ce que l'on pouvait penser, d'augmenter considérablement les doses (Brendel). Cependant, il est même certain, comme Balner et Van Bekkum l'ont indiqué au cours d'études expérimentales sur des singes, que de nombreux sérums antilymphocytaires et des préparations plus pures, telles que des IgG, ne présentaient pas, bien qu'elles fussent appliquées à l'homme, les caractères de non-toxicité et d'activité immunosuppressive que nous étions en droit d'admettre. C'est chez la souris que nos connaissances sur les sérums antilymphocytaires ont été bien définies au moyen de sérums préparés chez le lapin. Il a été prouvé que l'IgG pure obtenue chez cet animal, généralement par chromatographie sur DEAE-cellulose, est très satisfaisante quant à son activité immunosuppressive (Woodruff, Lewey et Medawar, Lance, Jeejeebhoy). Monaco a montré très tôt par la greffe de peau chez l'homme et la négativation de réaction d'hypersensibilité retardée que chez le lapin immunisé contre les lymphocytes humains l'essentiel de l'activité lymphoagglutinante et immunosuppressive était concentré dans l'anticorps 7S obtenu pur par fractionnement au sulfate d'ammonium à 33 pour cent suivi d'un passage sur DEAE-cellulose.

* Travail présenté au Deuxième symposium mondial sur la transplantation cardiaque, Montréal, 6–8 juin, 1969.

Chez le mouton, il est relativement facile de produire et d'extraire des IgG antilymphocytaires par DEAE-sephadex (Halpern). Dubost nous a signalé tout l'intérêt d'une telle immunoglobuline de mouton lorsque les malades sont sensibilisés aux immunoglobulines de cheval. Récemment, dans notre laboratoire, nous avons obtenu et avec bon rendement, soit par le procédé d'électrodécantation, soit avec le Rivanol et l'alcool, une IgG de mouton antilymphocytes humains dont nous étudions actuellement les qualités immunosuppressives. Les IgG de bœuf ont été séparées par Lamoureux et utilisées à Montréal comme relai thérapeutique chez un sujet sensibilisé au cheval. Toutefois, nous voudrions faire remarquer qu'il existe des réactions croisées entre les anticorps de ces différentes espèces.

L'utilisation prolongée chez l'homme de sérum de cheval antilymphocytaire nous a incité à n'utiliser que des IgG, dans le but de concentrer les anticorps par rapport aux taux de protéines injectées et de diminuer ainsi les incidents de la sensibilisation aux protéines de cheval. Les protéines éliminées au cours des purifications sont principalement l'albumine, les α-globulines et les β-globulines. L'intérêt de cette purification réside dans le fait que les protéines éliminées (α-globulines et β-globulines) sont très immunogènes par rapport aux IgG. Le taux de précipitine anti-alpha et anti-bêta s'élève régulièrement chez l'homme au cours des traitements alors que le taux de précipitine anti-IgG reste faible, comme l'ont montré Kashiwagi et Starzl.

Nous pouvons apporter ici une expérience que nous avons effectuée sur le singe. Deux groupes de singes sont traités: d'une part, les singes 1, 2 et 3 par du sérum de cheval antilymphocytes humains préparé par le sulfate d'ammonium durant un mois; d'autre part, les singes 4, 5 et 6 par du sérum normal de cheval purifié par la méthode au sulfate d'ammonium. Ces deux sérums sont ajustés à 50 mg de protéines par ml. Les précipitines titrées nous montrent (tableau I) que les animaux traités par le sérum antilymphocytaire présentent durant le traitement un titre plus élevé en précipitines que les animaux traités par du sérum normal de cheval non antilymphocytaire. Nous constatons également que les précipitines des animaux traités par du sérum de cheval antilymphocytaire humain disparaissent plus rapidement que celles des animaux traités par du sérum normal de cheval. Ces faits ont été observés également chez l'homme, comme l'a montré Træger. Pour éviter les réactions de sensibilisation, il devenait intéressant de fabriquer des

TABLEAU I

Taux de précipitines antiprotéines de cheval

Singes *	Jours						
	−12	−6	0	15	29	81	150
6	0	0	—	0	1 : 8	1 : 16	1 : 8
5	0	0	—	0	1 : 8	1 : 16	1 : 16
4	0	0	—	1 : 2	1 : 4	1 : 16	1 : 16
3	0	0	—	1 : 32	1 : 64	0	0
2	0	0	—	1 : 16	1 : 32	0	0
1	0	0	—	1 : 16	1 : 32	0	0
				⟷ 0,7 ml s.-cut./kg 1 injection/jour			

* Singes 1, 2 et 3 : SAL purifié au sulfate d'ammonium (lymphe) (lot : 2048) (protéines totales : 50 mg/ml) ; singes 4, 5 et 6 : Sérum de cheval normal purifié au sulfate d'ammonium (protéines totales : 50 mg/ml).

IgG extrêmement pures vis-à-vis desquelles l'organisme s'immunise moins qu'avec des α-globulines et des β-globulines. De nombreuses méthodes de préparation des IgG pures ont été successivement utilisées. Terasaki utilise des précipitations successives au sulfate d'ammonium, la précipitation à l'alcool à froid, méthode (6 + 9 Cohn) que nous avons utilisée, ainsi que la précipitation par le Rivanol des α-globulines et de l'albumine suivie d'une précipitation par l'alcool à froid. Mais c'est surtout la méthode de traitement par passage sur DEAE-sephadex préconisée par Perper qui est actuellement la plus utilisée. Nous avons fait des essais de purification au moyen de la méthode M.M.E.D. (*multi-membranes electro decanter*) avec un appareil à électrodécantation qui permet la séparation des différentes fractions du sérum de cheval avec un excellent rendement. On a également essayé, après extraction de ces IgG, de les concentrer par différentes méthodes: ultrafiltration, mais également concentration par lyophilisation. Dans tous les cas les IgG produites très pures sont leuco-agglutinantes, leucocytotoxiques et blastogéniques. Si les chimistes ont obtenu des IgG antilymphocytaires extrêmement pures, on a oublié trop souvent de vérifier leur activité immunosuppressive, étant donné les difficultés de mise en œuvre et le prix des techniques de titrage chez le singe.

Dans cet effort d'obtenir des IgG antilymphocytaires pures de cheval, il fut oublié que le cheval produit en cours d'immunisation un constituant appelé constituant T, une globuline plus rapide à l'électrophorèse que les IgG. Ce constituant peut apparaître après deux mois d'immunisation par les lymphocytes et persister très longtemps chez l'animal. Starzl nous l'a rappelé et la suppression du bloc des β-globulines où se situe ce constituant T (β, α) par une purification plus poussée entraîne une diminution de l'activité immunosuppressive. Ces faits furent observés par Starzl sur un assez grand nombre de transplantés du rein où les crises de rejet furent plus fréquentes après traitement par une IgG pure obtenue sur DEAE-sephadex qu'avec une préparation au sulfate d'ammonium contenant le constituant T du cheval. Ceci fut confirmé chez le chien par Kashigawi qui observe que la lymphopénie avec un sérum de cheval anti-chien est au moins aussi importante avec le constituant T qu'avec l'IgG. Starzl nous a également signalé que l'activité lympho-agglutinante est liée au constituant T du cheval. Faut-il préparer une IgG extrêmement pure mais peut-être moins active qu'une préparation contenant également des globulines β et des globulines γ pouvant plus fréquemment provoquer des accidents de sensibilisation chez l'homme? Une longue expérience d'utilisation de préparations de sulfate d'ammonium contenant des β-globulines et des IgG fut réalisée chez l'homme en transplantation rénale, mais aucun incident grave n'a été signalé au cours de traitement chez les malades (Træger). Ces sérums au sulfate d'ammonium ont été préconisés par voie intraveineuse chez l'homme pour les transplantations cardiaques. Kaplan utilisa chez un transplanté du cœur, pendant près de deux mois, uniquement ce sérum antilymphocytaire, sans Imuran et sans cortisone, sans avoir pour autant des phénomènes d'intolérance chez le malade. Les rejets furent parfaitement contrôlés durant cette période. Ceci confirme que l'activité de cette préparation contenant le constituant T du cheval et des IgG antilymphocytaires était bonne.

Si les IgG antilymphocytaires pures du cheval, du lapin ou du mouton permettent de limiter les effets sensibilisants, voire anaphylactisants des sérums de ces espèces, il n'en reste pas moins que ces méthodes nous donnent des préparations qui, en plus de leur activité lymphocytotoxique, peuvent garder une activité antiprotéique humaine (d'où la nécessité d'absorption sur plasma humain) et également une activité antiplaquettaire avec effet thrombopénique par production d'anticorps parasites antiplaquettaires qui se situent avec les IgG antilymphocytaires. Ces effets thrombopéniques des IgG extrêmement pures ainsi préparées sont particulièrement importants avec certains sérums obtenus à partir de lymphocytes sanguins ou de lymphocytes en provenance de la rate, ce qui d'ailleurs a bien souvent limité leur production. Nous avons trouvé un moyen de déceler ces effets thrombopéniques

généralement révélés chez le singe qui est très sensible (hémorragie) ou chez l'homme, ce qui entraîne parfois l'arrêt immédiat du traitement. Le test de l'appréciation de l'effet thrombopénique des sérums antilymphocytaires est obtenu par injection à la souris des sérums antilymphocytes humains (0,5 ml par voie sous-cutanée en une seule injection). Les souris soumises à cette thérapeutique ne meurent pas, mais après 48 heures on peut déceler à l'autopsie des hémorragies sous-cutanées avec des sérums provoquant des hémorragies chez l'homme. Si l'on fait le décompte des plaquettes de la souris on s'aperçoit que ces préparations abaissent le nombre des plaquettes en dessous d'un million par mm^3 de sang. C'est peut-être une réaction croisée que l'on observe entre les sérums antilymphocytes humains et les plaquettes de la souris. Des études à ce sujet sont en cours. Nous avons pu constater que les sérums de mouton provoquent beaucoup moins de thrombopénie chez la souris que les sérums de cheval. Il est à remarquer que les sérums antilymphocytes humains (lymphe) provoquent également une chute des plaquettes chez la souris, mais cette chute n'est pas aussi importante que la chute provoquée par des sérums en provenance de sang qui cause une thrombopénie et des hémorragies chez l'homme.

Il est également à noter que les IgG extrêmement pures préparées par diverses méthodes de purification peuvent avoir néanmoins des effets pyrogènes et des effets d'intolérance. Nous avons fait des recherches sur ces différentes préparations en ce qui concerne l'effet pyrogène chez le lapin. Afin de vérifier cet effet, nous injectons deux ml par voie intraveineuse chez le lapin. Nous observons avec certaines préparations des augmentations de température jusqu'à 1,7° C. Cependant, ces préparations sont des préparations d'IgG pure. La source de ces pyrogènes peut être, par exemple, les bactéries qui se développent au cours des méthodes de préparation (DEAE-sephadex). Par contre, les méthodes à l'alcool à froid, les méthodes au sulfate d'ammonium et l'électrodécantation produisent peu de bactéries contaminantes ayant des effets pyrogènes.

Les effets pyrogènes peuvent être également dus à l'agrégation d'immunoglobulines, comme l'a montré Ishizaka pour les immunoglobulines humaines injectées par voie intraveineuse. En effet, les IgG pures peuvent donner dse agrégats moléculaires de 10S, 15S. Ces agrégats non seulement peuvent produire de la température par injection intraveineuse mais également de l'anaphylatoxine ayant des effets sensibilisants chez l'homme et le lapin. Ces protéines agrégées ont la propriété de fixer le complément mais elles sont peut-être moins réactives. Ces phénomènes d'intolérance par des IgG extrêmement pures agrégées peuvent se produire notamment au cours d'injection intraveineuse. On admet pour les immunoglobulines humaines qu'au moins cinq mg de protéines injectées doivent fixer deux unités de complément (50 pour cent). Ces essais peuvent être faits soit avec un complément de cobaye, soit avec un complément humain. Nous avons essayé de relier ces essais anticomplémentaires des IgG antilymphocytaires à l'effet pyrogène observé chez le lapin (tableau II). Donc beaucoup de méthodes de préparation d'IgG extrêmement pures conduisent sans doute à des agrégations de protéines ayant une activité anticomplémentaire. Il ne faut pas oublier non plus qu'une mauvaise conservation peut conduire à des effets de cet ordre. En étudiant différents procédés de production des IgG nous avons constaté que les méthodes de préparation d'IgG à l'alcool conduisent rarement à des préparations ayant des effets hyperthermisants chez le lapin, dont l'élévation de température n'a jamais dépassé 1° C dans ce cas. Nous avons également constaté que l'électrodécantation (M.M.E.D.) conduit à des préparations aussi acceptables que les préparations produites par l'alcool à froid. Par contre, les méthodes au sulfate d'ammonium donnent souvent des préparations ayant des effets hyperthermiques chez le lapin et ayant également des activités anticomplémentaires. Nous avons aussi constaté que les méthodes de précipitation par le Rivanol et l'alcool ou les méthodes de précipitation par le Rivanol suivies d'une ultrafiltration conduisent également à des préparations à activité anticomplémentaire à effet pyrogène.

TABLEAU II

Étude comparative du pouvoir anticomplémentaire et du pouvoir pyrogène chez le lapin du sérum purifié

SÉRUM PURIFIÉ ANTILYMPHOCYTES	TITRE L.C.T.	C' COBAYE	C' LAPIN	C' HUMAIN	EFFET PYROGÈNE SUR LAPIN (intraveineuse)
Origine : lymphe					
K 3105	2048	7,7 mg	7,7 mg	7,7 mg	0,9° C
K 0303	1024	0,28 mg	9,2 mg	1,2 mg	1,6° C
T 3001	1024	0,47 mg	15,0 mg	1,9 mg	1,4° C
T 2202	1024	0,60 mg	18,0 mg	2,4 mg	1,3° C
R 2202	2048	1,2 mg	18,0 mg	2,4 mg	1,7° C
R 0402	2048	0,6 mg	18,0 mg	1,2 mg	1,1° C
Origine : sang					
P 0404	1024	7,4 mg	29,0 mg	—	0,7° C

CONCLUSIONS

Nous devons perfectionner les sérums antilymphocytaires de façon à atteindre les objectifs suivants :

1. Isoler et augmenter au maximum l'activité immunosuppressive pour une quantité minimum de protéines hétérologues injectées.
2. Diminuer au maximum des effets toxiques qui ne sont pas négligeables surtout par voie intraveineuse (activité antiprotéique, activité antiplaquettes, activité antiglobules rouges) ; c'est le traitement de l'antigène qui nous permettra d'arriver à de tels résultats.
3. Éviter les agrégats moléculaires, tant au cours de la préparation qu'au cours de la conservation, ces agrégats pouvant être la source d'accidents hyperthermiques et d'accidents de sensibilisation.

Nous devons également essayer de produire des sérums sur d'autres espèces animales de façon à relayer les thérapeutiques lorsqu'elles ne deviennent plus utilisables.

Nous devons établir des techniques de contrôle et échanger nos sérums, créer des étalons et des standards aussi bien pour la lymphocytotoxicité que pour le pouvoir immunosuppresseur.

En effet, il est nécessaire de fournir pour la transplantation du cœur des sérums antilymphocytaires purifiés ou des IgG présentant un maximum d'effet immunosuppresseur pour un minimum d'effet toxique. Il ne faut pas oublier, cependant, que chaque individu réagira différemment et que c'est aux cliniciens d'étudier les doses et les posologies à partir d'un produit présentant des caractères connus. Il serait utile, étant donné l'importance des effets toxiques de différentes préparations utilisées pour la transplantation du cœur, de vérifier au préalable tous les lots de sérums employés. L'épreuve peut se faire chez des transplantés du rein pour lesquels l'application de ces sérums thérapeutiques pose des problèmes moins graves. C'est à ce prix que nous obtiendrons de bons résultats en transplantation du cœur, en prévenant le rejet par des préparations d'immunoglobulines actives et non toxiques.

IN VIVO TESTING OF ANTIHUMAN LYMPHOCYTE SERA *

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Le test de la survie des greffes de peau chez les singes inférieurs et les chimpanzés demeure à l'heure actuelle la méthode de choix pour évaluer l'activité immunosuppressive d'un SAL. Cependant, la valeur immunosuppressive de grands pools de SAL testés chez les primates demande encore à être confirmée par les essais cliniques chez l'homme.

INTRODUCTION

Early in 1967 already, the discussions at the Ciba Study Group on Antilymphocyte Serum (ALS) made it clear to all involved in clinical and fundamental ALS research, that this agent held high promise for use in clinical organ transplantation and possibly in other conditions requiring effective immune suppression (i.s.) (5). This conclusion gave rise to concentrated efforts in many countries aimed at the production of sufficiently large amounts of effective antihuman LS, not only to meet the demands of the clinicians, but also to permit at the same time a more exact evaluation of its i.s. properties in controlled clinical trials. During the past year these efforts have been markedly intensified — especially those at the production level — following the introduction of clinical heart transplantation, when it became gradually apparent that rejection of cardiac allografts is more difficult to prevent by conventional i.s. treatment than that of kidney allografts.

Additional stimuli were provided by the intensified attempts to transplant other organs, *e.g.* lungs and liver, and by the recent discovery of a favourable effect of recipient pretreatment with ALS in diminishing the severity of the graft versus host reaction following allogeneic bone marrow transplantation in mice as well as in monkeys (4).

In his introduction to the Ciba meeting on ALS, J. H. Humphrey pointed out that "One of the important things which ought to emerge from the discussions here is a sharing of experience regarding the practical steps needed to get a therapeutically effective serum, and what methods of assessment *in vitro* will enable its therapeutic efficiency to be predicted." It was indeed logical at that time to envisage a fast evolution of *in vitro* methods of testing, because work on the *in vitro* properties of ALS, involving a number of different systems, had already been initiated. However, none of the *in vitro* methods that seemed promising two years ago were subsequently found to possess dependable quantitative predictive value for the i.s. potency of ALS preparations.

In vivo test:

Instead, *in vivo* testing in chimpanzees and macaques has been, thus far, the only available means of evaluating and comparing antihuman lymphocyte sera. Such a development was certainly not within expectation because a high species specificity of heterologous antilymphocyte preparations had been observed by several investigators.

The susceptibility of subhuman primates to antihuman lymphocyte sera was discovered when we decided to use one of our chimpanzees to assess an

* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6–8, 1969.

Nous discutons la valeur des tests *in vitro* ayant une corrélation positive entre la valeur immunosuppressive de SAL chez les primates, et ces analyses. Nous cherchons à savoir si la valeur immunosuppressive obtenue chez le primate à la suite de greffes de peau est un bon indicateur du pouvoir immunosuppressif de ce sérum quand il s'agit de la greffe de d'autres organes comme le rein, le foie, le cœur.

Nous discutons de la possibilité d'induire chez l'homme la tolérance immunologique vis-à-vis les immunoglobulines normales, avant l'emploi du sérum antilymphocytaire. Le but de ce procédé est d'empêcher la formation d'anticorps circulants dirigés contre les protéines actives du SAL. Nous discutons, enfin, la nécessité de produire de grands *pools* de SAL pour faire, sur un seul lot, toutes les analyses essentielles *in vivo* ainsi que des essais cliniques chez l'humain, avant l'utilisation de ce SAL dans les greffes d'organes.

ALS preparation before making it available for patients. It was then found that chimpanzees not only respond to several of the toxic side effects but also that a significant prolongation of skin allograft survival as well as suppression of a delayed-type skin test (DNCB sensitivity) following the administration of certain antihuman lymphocyte sera could be obtained (2). These studies were then extended to lower monkeys and it was found that M. rhesus and M. speciosa also respond to antihuman lymphocyte sera, be it with a less pronounced immunosuppressive effect. This unexpected interspecies susceptibility is ascribed to the fact that chimpanzees, and to a lesser extent macaques, share a number of leukocyte antigens with man (3 and 9).

During the past two years we have been engaged in comparing the properties of more than 30 antihuman lymphocyte sera and globulins in chimpanzees and monkeys, using the effect on free skin allograft rejection time and the DNCB reaction (in chimps only) as an index for i.s. potency. The results (Table I) show that there is a rough correlation between the responses of macaques and those of chimpanzees, in the sense that the sera that show strong i.s. in chimps always give rise to a clearcut response in monkeys. Sera having no activity in monkeys show no or only a weak activity in chimpanzees. It seems reasonable to assume, and to use as a working hypothesis, that this trend will extend to man, so that sera with outspoken i.s. potency in chimpanzees will probably be also highly active in humans. It has to be stressed however that the final proof of this assumption is still lacking and in fact seems difficult to obtain. There are at least two reasons for this gap in our knowledge. The first is the difficulty to get a reliable quantitative assessment of the i.s. effects of ALS in clinical situations, where the disease itself as well as the simultaneous administration of other i.s. agents tend to cloud the picture. Only by the use of rigid protocols in a series of controlled clinical trials can we hope to overcome this obstacle. The second difficulty is caused by the limited amounts of anti-human LS available for simultaneous clinical use and laboratory assessment. Very large pools of sera are obviously required for such critical studies and as long as clinical demands for ALS continue to exceed supplies, such pools will not be made available. Fortunately Medical Research Councils and other government organizations in a number of countries have recently taken steps to assure such critical evaluations in the future.

Another reason for urgently establishing the relation between the responses of chimpanzees and humans to the same anti-human LS preparation is the development and perfection of *in vitro* assays which could proceed much more efficiently and faster by comparing them with the standardized results of tests in subhuman primates and monkeys.

In vitro tests:

For routine testing, required for several steps in the large scale production of anti-human LS, *in vitro* assays are obviously to be preferred to the elaborate, time-consuming and expensive *in vivo* testing. Already duringt he past year, two *in vitro* assays have emerged that seem to offer good possibilities. Among many of the tests that were proposed, the rosette inhibition assay (1) and the opsonisation test (8) showed an excellent correlation with the *in vivo* i.s. activity in experiments involving rodents. Both are being tested with many of the anti-human agents that have become available for *in vivo* testing and suggestive positive results have already been obtained with one of them (rosette inhibition, J.F. Bach *et al., Transplantation,* in press). A number of other "candidate" *in vitro* tests have been found to bear insufficient relation to the results of the *in vivo* tests (Table II).

TABLE I

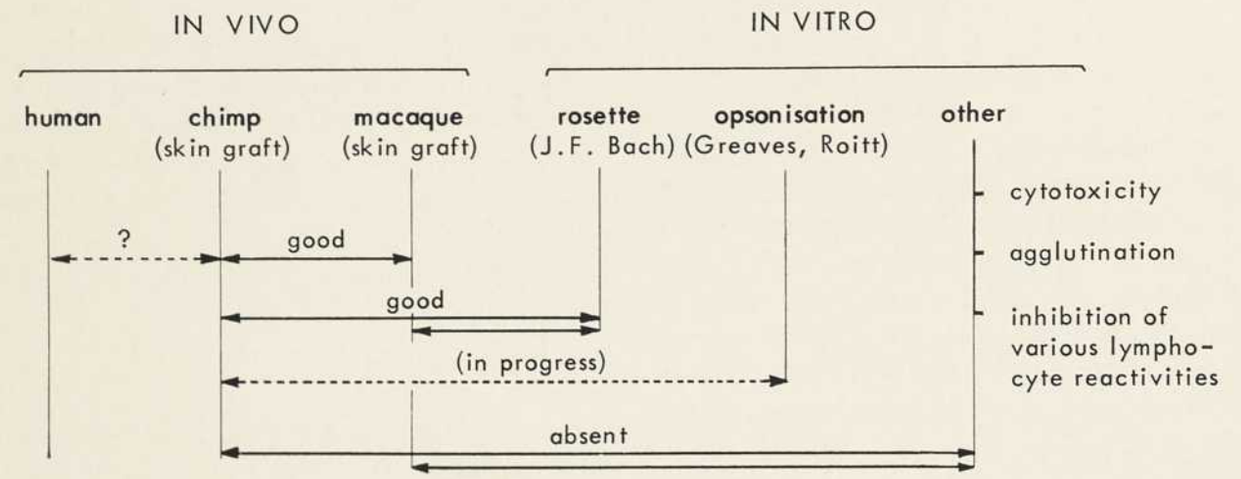
Horse anti-human lymphocyte sera and globulins

Antigen	Code and origin		Suppression of allograft reactivity		
			In monkeys	In chimps	Clinical impression
Spleen	IX	Louvain	—		Favorable
	XII	Paris	—		Favorable
	V *	London	—	—	
	VI *	London	—	—	
	XI *	London	—	(+)	
	II	Leiden	—	+	
	VII	Denver		+	Favorable
Blood lymphocytes	I	Amsterdam	+	++	
	XIII	Lyon	—		
	XVII *	Los Angeles	—		
	XXVI – 1	Bilthoven	—		
	XXVI – 2	Bilthoven	—		
	XXVI – 3	Bilthoven	—		
	XXVI – 4	Bilthoven	—		
	XXVI – 5	Bilthoven	—		
Thymus	III	Leiden	—	(+)	
	XVI *	Montreal	—		
	XXIV *	Toronto	+	++	
	XVIII	Kalamazoo	—	+	
	XX	Kalamazoo	+	++	
	XXVIII	Houston	+		Favorable
	XXXIII	Marburg	++		
Thoracic duct cells	IV	Lyon	+	++	Favorable
	XV	Lyon	+		Favorable
	VIIIa	Munich	+		Favorable
	VIIIb	Munich	++		Favorable
	XXXII	Marburg	+		
Node Lymph. leuk. Cult. cells	X	Nijmegen	—		
	VIII	Munich	+		Favorable
	XIX	Minneapolis	—		Favorable

* Pool of sera from 2–5 horses.

TABLE II

Correlation between assays for I.S. efficacy of anti-human L.S.



Significance of in vivo tests:

Free skin allografts have been widely employed to measure the degree of suppression of the homograft reaction. It should be noted, however, that the two agents most widely employed in clinical organ transplantation today, Imuran and prednisolone, have little if any effect on skin allograft survival, unless the histocompatibility barrier between host and donor is very low indeed.

The question might be asked whether the results obtained with skin grafts may be interpreted quantitatively to predict the degree of suppression of organ allograft rejection. In the rejection pattern of organ allografts two components have recently been recognized to respond differently to i.s. agents (10). One, represented by the damage of large vessels, seems to be more susceptible to inhibition by Imuran, the other, represented by lymphoid cell infiltration and damage of parenchymal cells, seems to be more specifically inhibited by ALS (Table III). These observations provide a theoretical basis for the combination therapy of ALS and Imuran, which has been found to yield superior results in a rat model of kidney allografting by de Bruin of our group (7).

TABLE III

Kidney homografts in rats (M. J. de Vries et al., 1968)

I.S. TREATMENT	INCIDENCE OF VASCULAR REJECTION %	GRADE OF GLOMERULAR DAMAGE
Imuran	0	Severe
ALS	30	Slight/moderate
Imuran + ALS	0	Slight

The damage of large vessels, one of the characteristic aspects of rejection in organ allografts, is not an integral part of the rejection process in skin allografts, the information obtained from assays involving skin grafts may therefore be incomplete. The i.s. potency of ALS as determined by these tests may still require correction before being applicable to the organ allograft situation. To clarify these complicated and highly practical problems *in vivo* studies involving comparison between responses of skin grafts and organ grafts are needed, because organ grafting is too elaborate a procedure to ever become a routine test method by itself.

In this respect it should be noted that it is not even justified, *a priori*, to extend the findings obtained with grafting one particular organ to other organs, since striking differences have been observed between for instance heart and kidney grafts with

regard to their response to i.s. agents under standard conditions in the rat (Table IV), (6). Needless to say that *in vitro* assays will never be able to provide an answer to this type of question.

Defining optimal application of ALS:

In devising optimal treatment schedules for ALS at least two factors have to be taken into account. One is the influence of the agent on the rejection process, the complicated nature of which has been pointed out above. The second is the reaction of the recipient to the agent, this is immunological in nature and may result in the production of antibodies against the active principle, thereby preventing or minimizing its further efficacy as well as creating the hazards of allergic reactions following its continued administration. Suggestive evidence is accumulating that the simultaneous administration of other i.s. agents, in particular of Imuran, may inhibit or even prevent the immunological reaction of the recipient against ALS. If that would prove to be of critical importance and if the combined administration of ALS and Imuran would become indicated on the grounds discussed in the previous section, the current procedure of *in vivo* testing of anti-human LS may need modification.

The development of less immunogenic or even non-immunogenic anti-human LS preparations would of course simplify matters considerably, not only from the point of view of testing, but chances that this could soon be accomplished seem to be remote. On the other hand the induction of tolerance to heterologous anti-human lymphocyte gamma globulin may prove to be feasible in patients and such developments would probably necessitate similar adaptations of the *in vivo* testing procedure.

Finally, the establishment of optimal modes and routes of administration still presents many problems, which will only be solved by systematic *in vivo* experiments, preferably in subhuman primates.

TABLE IV

Effect immunosuppressive regimens in rats
Per cent graft survival beyond 30 days

TYPE OF ALLOGRAFT		IMURAN WITH OR WITHOUT STEROIDS	ALS WITH OR WITHOUT IMURAN
Free	Skin	No effect	90% (9)
Large vessels anastomosed	Kidney	53% (32)	80% (34)
	Heart	10% (21)	26% (19)

Kidneys orthotopic, hearts heterotopic (From de Bruin *et al.*, 1968, and van Bekkum *et al.*, 1968).

Side effects:

Apart from the hazards arising from sensitization to ALS mentioned above, antilymphocyte preparations have already shown a rather discouraging variety of other undesirable side effects (Table V). Some of these can be avoided or diminished to acceptable levels by absorption and purification. Others, *e.g.* the induction of thrombocytopenia seem to be inherent properties, to a certain extent probably inseparable from the i.s. activity. It is clear that *in vitro* tests will at best provide only partial information concerning toxic effects of ALS preparations and that therefore *in vivo* toxicity studies will be indispensable for some time, to provide the ultimate safeguards required for all preparations before their routine clinical application is justified. Presently available information on the predictive value of the various *in vitro* and *in vivo* tests for toxicity of anti-human LS is summarized in Table VI. Because of the close biological

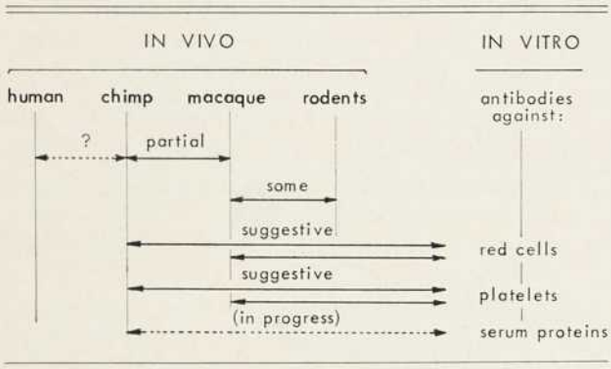
TABLE V

Main hazardous side effects of ALS

IMMEDIATE :	Thrombopenia Hemolysis	
LATER :	Serum anaphylaxis nephritis arthritis	} due to AB formation
(high doses) :	Infections (virus)	
LONG TERM :	Malignant lymphomas	

TABLE VI

Predictive value of toxicity tests for anti-human L.S.



similarity of chimpanzees to man, the chimp remains the subject of choice for these toxicity checks. However, practical considerations are severely limiting the use of these animals so that maximal efforts have to be made to devise other systems for toxicity evaluations.

As regards the assessment of the long-term effects of ALS and of the effects of long continued administration, for the time being there seems to be no reliable substitute for the subhuman primate. The long-term hazards of ALS are clearly not the main concern at present, because the conditions for which treatment with ALS is required are all of a rather urgent nature. As with many other promising therapeutic agents even their large scale application will not be prevented by the absence of reliable information concerning the long-term hazards. One can only hope that governmental agencies will have the foresight to promote initiation of programs for the evaluation of Long term studies on ALS in animal models. It seems advisable to include studies on the effect of antihuman LS in subhuman primates into such projects.

CONCLUSION

In vivo testing of antihuman LS in apes and monkeys is as yet the only reasonable possibility of predicting its i.s. potency. The exact value of the results of these tests urgently needs to be established by a systematic comparison with the results of controlled clinical trials using large pools of ALG.

It is to be expected that reliable *in vitro* tests for use in the various steps of ALS production will soon become available, so that *in vivo* testing could be restricted to a final assay of each large pool before it is made available for clinical application.

The study of the rejection mechanisms for skin and organ allografts and their respective susceptibility to i.s. agent has shown important differences and similar but less significant differences have been found between various types of organ grafts *e.g.* between kidneys and hearts. Consequently, the interpretation of *in vivo* assays based exclusively on skin allograft rejection, in terms of their predictive value for the suppression of organ allografts will have to be reinvestigated.

There are indications that the clinical application of ALS may in the future be predominantly in combination with Imuran. The latter drug may not only enhance the i.s. effect by an action of its own but also indirectly by inhibiting antibody formation by the recipient to the ALS preparation. Alternatively, the successful induction of tolerance to the effective principle in ALS preparations may become feasible. In both cases, the *in vivo* testing procedure could be perfectionated accordingly, although the current unmodified *in vivo* testing would still be a valuable screening procedure.

For the assessment of toxicity, *in vivo* assays remain essential but replacement of chimpanzees and monkeys by lower animals has to be sought.

The late effects of ALS administration and the effects of long term administration will have to be carefully studied in view of the prospects for increased use of ALS. It seems advisable to obtain pools of anti-human lymphocyte preparations large enough to do all the essential *in vivo* studies in subhuman primates as well as clinical trials with the very same agent.

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INTRAVENOUS USE OF HIGH DOSAGE OF ALG *

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L'auteur décrit une méthode de traitement avec des doses élevées de globulines antilymphocytaires associées à l'Imuran et à la Cortisone. Au cours des transplantations, les doses recommandées sont de 20 ml par jour au cours de la première semaine. Le premier jour, on donne la moitié de la dose avant l'opération. Les doses de corticostéroïdes et d'Imuran sont diminuées plus tard, alors que le traitement aux globulines antilymphocytaires se poursuit pendant trois mois. Si une crise de rejet se manifeste des semaines après l'arrêt des globulines antilymphocytaires, un nouveau traitement identique aux mêmes doses est institué.

Les manifestations du traitement aux globulines antilymphocytaires sont :

1. Une lymphopénie, qui porte le plus souvent sur les petits lymphocytes. On note cependant l'apparition, après quelques jours, de lymphocytes plus grands, et de formes irrégulières, dont la présence signe l'action des globulines antilymphocytaires ;
2. Une destruction des granulocytes ; (suite du résumé en page suivante)

Yesterday we heard of the clinical results obtained with heart transplantations round the world. You will agree with my general statement that these results are not yet optimal. The mean survival rate is low even if we exclude those patients who died from surgical complications and calculate only such cases who died from rejection.

On analysing all heart transplants one particular group does not fall within this picture. The survival rate in those patients that were treated with high doses of high grade ALG and then by the intravenous route was significantly higher.

I cannot give you the exact details of those patients that were treated with ALG by other teams, but Tables I and II will show you the results in nine cases — with the last case in London, ten — that were treated with our ALG according to a schedule worked out by us. Our ALG was raised in cooperation with the Behring Company, Marburg. Two of the patients were treated with our ALG after the appearance of a rejection crisis, eight were treated from the very beginning of the operation. Eight of these ten patients are still alive and did not show signs of serious rejection as long as the ALG-therapy was continued. One patient died four months after the operation on account of a brain embolus, but no histological signs of rejection of the heart could be detected. One patient died after three months of sepsis. Those results were obtained in spite of the fact that most of these patients had a mismatch in two or three transplant antigens.

These results as well as our experience in the treatment of kidney transplants and autoimmune diseases seem to indicate that our scheme of treatment with high doses of ALG additional to Imuran and cortison will be the method of choice.

I would like to describe now the criteria and the side effects involved. Mostly we used antilymphocytic globulin which had a high agglutinating and

* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6–8, 1969.

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3. Une thrombopénie qui retourne à la normale malgré le traitement continu aux globulines antilymphocytaires. Ceci est probablement dû à une agglutination des leucocytes et lymphocytes qui englobent les thrombocytes, ou plus encore à l'action des substances produites par la destruction des leucocytes ;
4. Une augmentation de la température, de la fréquence cardiaque, et parfois une légère chute de la tension artérielle, au cours des premiers jours. Ces réactions diminuent et disparaissent après la première semaine. Elles sont également produites par les substances toxiques de destruction des leucocytes.

Les doses élevées de corticostéroïdes données au cours des premiers jours sont nécessaires pour leur effet immunosuppressif, mais également pour supprimer les réactions dues aux substances toxiques libérées par la destruction des leucocytes.

En vue d'éviter le syndrome de Cushing et l'ulcère de stress, on substitue une partie des glucocorticoïdes par des doses élevées d'Aldostérone et de Spironolactone, qui se sont avérés efficaces chez l'animal d'expérimentation et dans les transplantations rénales humaines.

cytotoxic titre of about 1:2000 to 1:4000 respectively. This ALG was raised by using lymphocytes from the thoracic duct, lymphocytes from leukemic patients or thymocytes. The immunesuppressive effect of this ALG was tested on primates by Doctor Balner. We recommend in the first week of the transplantation 20 ml of ALG daily, starting already before the operation, then 10 ml. In the first few days high doses of corticosteroids up to 500 mg per day should be given. Later on the dosage of corticosteroids and Imuran can be diminished as long as the treatment with ALG is continued. In cases with a pronounced sensitivity against Imuran this drug could be omitted for one or two weeks without serious complications. This therapy was usually carried on over a period of three months. There were no rejection crises as long as the ALG therapy lasted. If there was a rejection crisis weeks

TABLE I

Adjuvant therapy in rejection episodes following heart transplantation *

PATIENT	TYPE OF INJECTION	AVERAGE DOS. (ml/day) / DURATION OF TREATM. (days)	TITER OF ALG		CLINICAL RESULTS	SURGEONS PHYSICIANS
			leuko-aggl.	cytotox.		
Dr. Ph. B. Cape Town	acute (cellular)	5 – 7,5 / 72	1 : 4096	1 : 4096	reversal	Ch. Barnard S. C. W. Bosman
M.E.P. Vina del Mar	acute (cellular)	10 / 15	1 : 4096	1 : 4096	reversal	J. Caplan R. Eberhard

*** Heart transplant treated with ALG (intravenously), corticosteroids and Imuran.**

after the interruption of the ALG therapy, then it was easily suppressed by increasing the conventional therapy. In order to avoid serious complications one should test for an eventual high sensitivity against horse serum proteins before treatment by injecting 0.1 ml ALG intradermally. If there is no reaction within one hour, we start the treatment by infusing the first half of the ALG in 250 ml physiological saline in a slow drip over one hour. The second half are given in the evening.

The following effects of ALG should be kept in mind:

1. A lymphopenia involving mostly the small lymphocytes which fall to 5 to 10% in the peripheral blood (Figure 1). After some days there appear, however, bigger, sometimes bizarrely formed lymphocytes which must be counted separately in order to avoid errors. Those cells have a pronounced ergastoplasma and look somehow like plasma cells. We assume that these cells are

TABLE II

Adjuvant therapy from day of operation in heart transplantation *

PATIENT	PAT. SURVIV. TO DATE	AVERAGE DOS. (ml/day) / DURATION OF TREATM. (days)	TITER OF ALG		CLIN. RESULTS		PHYSICIANS
			leuko-aggl.	cytotox.	during treatm.	after treatm.	
H.O. (Sao Paulo)	10 months	10 – 15 / 180	1 : 4096	1 : 4096	no reject pericard effusion	no reject	E. J. Zerbini E. Antonaciu
J.S. (Cape Town)	10 months	10 / 100	1 : 256	1 : 512	no reject	no reject	Ch. Barnard S.C.W. Bosman
N.O. (Valparaiso)	8 months	10 – 15 † / 200	1 : 4096	1 . 4096	no reject		J. Caplan N. Adriasola
C.P. (Sao Paulo)	3 months	10 – 15 / 80	1 : 4096	1 : 4096	no reject	+ septic.	E. J. Zerbini E. Antonaciu
W.K. (Cape Town)	2 months	5 – 10 / cont.	1 : 4096	1 : 1024	no reject	—	Ch. Barnard S.C.W. Bosman
D.F. (Cape Town)	2 months	10 – 20 / 30	1 : 2048	1 : 8000	no reject	—	Ch. Barnard S.C.W. Bosman
E.H. (Zurich)	2 months	10 – 20 / cont.	1 : 2048	1 : 8000	no reject	—	A. Senning F. Largiardèr E. Linder

* Heart transplants treated with ALG (intravenously), corticosteroids and Imuran (case 10, London, operated by Dr. Ross, not on table).

† Patient treated also with Lyon serum (Carraz-Tracger).

transformed lymphocytes. We tend to consider the appearance of such cells as a positive sign of the ALG treatment. In the thoracic duct lymph they may represent 50 per cent of the existing lymphoid cells.

2. Since ALG destroys the granulocytes with the same intensity as the lymphocytes, we normally find young forms of polymorphous cells. This is a normal reaction.

3. In spite of the fact that our ALG has no titre against thrombocytes, a thrombopenic effect can always be observed (Figure 2). The lower curve shows the mean thrombocyte values of nine patients. It can be seen that in the beginning the values decrease. When the therapy is continued, the values return gradually to normal in spite of continued high ALG quantities. Only twice we experienced such a heavy thrombopenia that ALG had to be discontinued for one or two days. This phenomenon might be due to an aggregation of leucocytes and lymphocytes which enclose thrombocytes. Furthermore, we have some indications that some substances of the destroyed leucocytes produce a thrombocytopenia.

4. In the first days we observed an increase in temperature and heart rate and sometimes a slight fall in blood pressure in most of the patients. Simultaneously rigor appears (Figure 3). Those reactions diminished from day to day to disappear usually after the first week. This too is caused by toxic products of the destroyed leucocytes. The speed of infusion also plays an important role. Therefore, we recommend to give the ALG diluted in saline in a slow drip over one hour. Even this cannot prevent the reactions entirely, but antipyretica and antihistaminica help considerably. In some of the patients the mentioned ill effects were not observed at all.

In 70 patients including kidney transplants, leukemia and autoimmune diseases we observed in about 10% urticaria and three times signs of anaphylactic shock. Only in the latter case ALG had to be discontinued. This shows the advantage of the intravenous route, as only by this method ALG administration can be stopped instantaneously.

In the first days high doses of corticosteroids are not only required because of their immunesuppressive effect, but they are also indispensible in order to suppress the reactions due to the toxic substances which are released by the destroyed leucocytes. In order to prevent a Cushing syndrome and stress ulcera we prefer now to substitute part of the glucocorticoids with high doses of aldosterone and spironolactone which have proved to

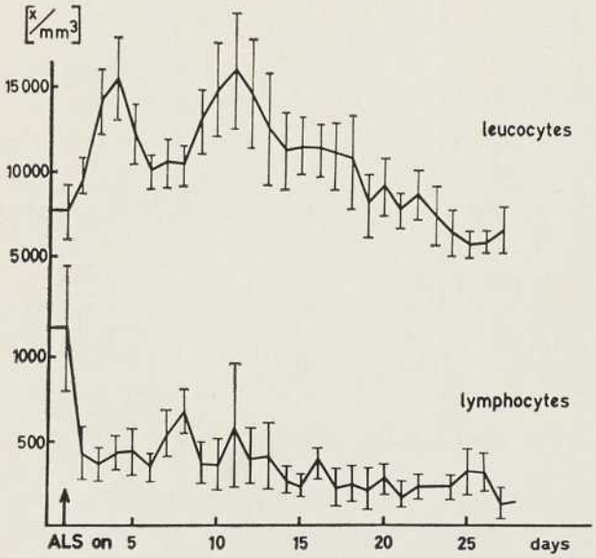

Figure 1 — Mean values of WBC and lymphocytes in 8 heart transplants during ALG treatment.

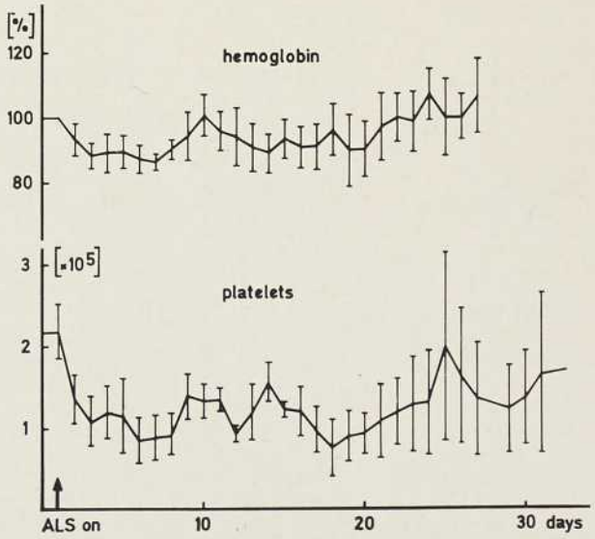

Figure 2 — Mean values of Hb and platelets in 8 heart transplants during ALG treatment.

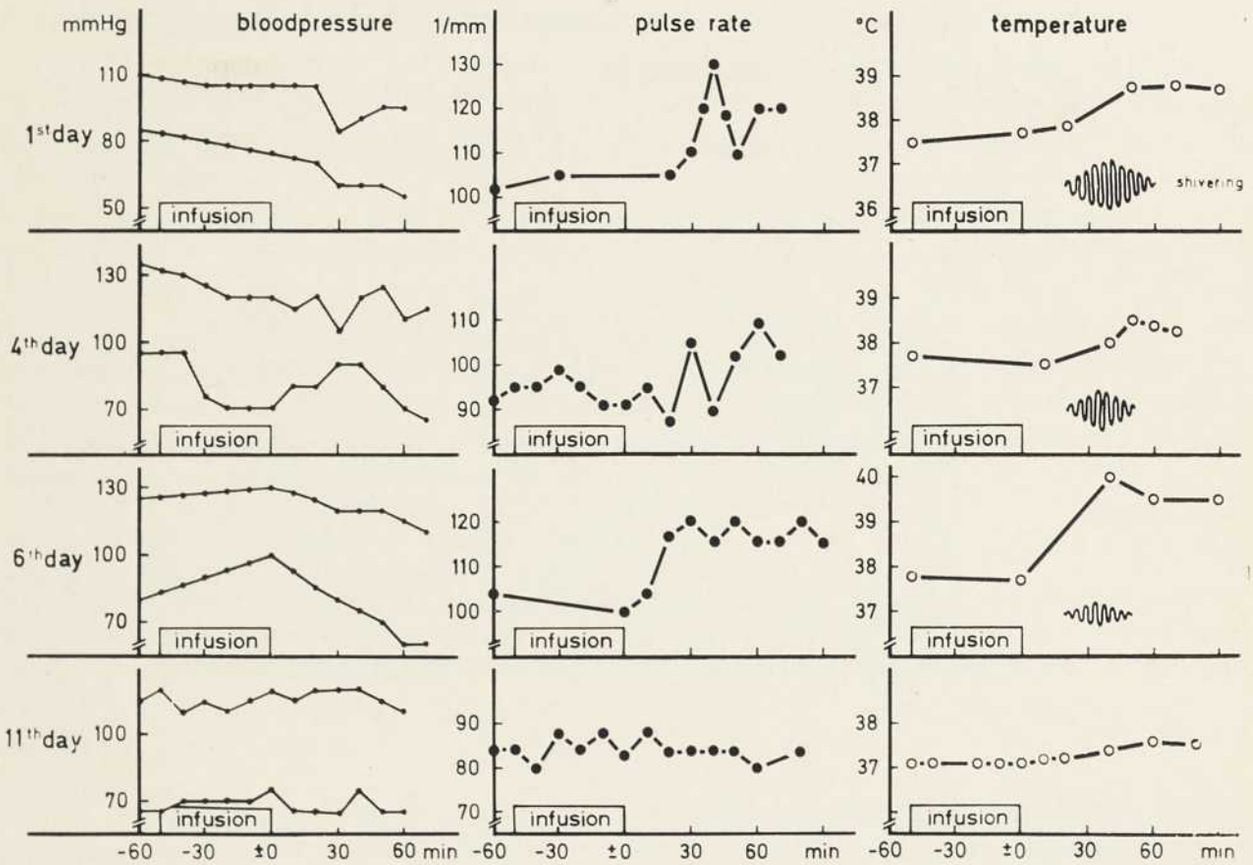

Figure 3 — Blood pressure, puise rate, temperature and rigor in one patient under ALG infusion.

be efficient by us in animal experiments and human kidney transplants.

Symptoms of serum nephritis were seen in one case of an autoimmune disease, who did not receive Imuran or corticosteroids and in one heart transplant. In the last case the batch of ALG used contained a certain amount of precipitating antibodies against human serum proteins, which were apparently not sufficiently absorbed.

We apply such high doses of ALG intravenously additional to the conventional Imuran and steroid therapy not only to increase the immunesuppressive effect. Much more we believe that with such high doses of ALG one can prevent a sensitizing of the host against at least some transplant antigens. This can only be achieved if important cloni of lymphocytes can be destroyed immediately before and maybe also a few days following the transplantation. Therefore we recommend now high doses of ALG already one or two days before the operation. With other words, the aim of this therapy is not only the suppression of the sensitized lymphocytes, but also a partial prevention of sensitizing. It seems to us that one might achieve a partial tolerance against some transplant antigens at least in some cases. Otherwise we cannot explain the phenomenon of the long time survival of the transplants even after the ALG therapy had been discontinued and the patients were maintained on relatively low doses of Imuran and corticosteroids.

SUCCESSFUL TREATMENT OF ACUTE CARDIAC HOMOGRAFT REJECTION WITHOUT ANTILYMPHOCYTE GLOBULIN *

H.M. KAUFFMAN, Jr., M.D.[1], **Derward LEPLEY**, M.D., **Dudley JOHNSON**, M.D., **and Donald KUBAN**, M.D.

Les auteurs relatent le cas d'une malade qui a subi une homotransplantation, et qui a présenté un phénomène de rejet au sixième jour. Alors qu'elle recevait 3 mg/kg d'Isotiaprine et 200 mg de Methyl prednisone par jour, on augmenta au moment de la crise de rejet la Methyl prednisone à 300 mg/jour. En plus, on administra 100 microgrammes d'Actenomycine C par voie intraveineuse et des radiations du cœur avec une dose de 150 r aux sixième, huitième, dixième et douzième jours après la transplantation. On nota que le voltage du QRS s'améliorait, de même que le retour à la normale de la tension artérielle et de la pression veineuse. Ultérieurement, le dosage de la Methyl prednisone fut réduit. Après sept mois et demi, la malade présente une activité complètement normale. Elle reçoit 3 mg/kg d'Isotiaprine et 30 mg de Methyl prednisone par jour.

Since most investigators performing clinical cardiac transplantation have used antilymphocyte globulin as part of the immunosuppressive therapy, it is important to document the successful treatment of cardiac homograft rejection in a patient who has received no antilymphocyte globulin during her entire postoperative course.

The patient is a 49 year old white female with a ten year history of chronic myocarditis of unknow etiology. Catheterization data revealed profound left ventricular failure with no evidence of valvular disease or coronary artery disease. Because of inhability to control the patient's congestive heart failure in the hospital environment, transplantation was recommended. The patient was started on Azathioprine, 1 mg per kg, while a search was made for a suitable cadaver donor. She remained on this dose for three weeks before a cadaver heart was available.

Histocompatibility test was performed by Doctor Kuban who obtained a B match and by Doctor Terasaki who obtained a D match. Unfortunately, because of the cadaver situation, a retrospective checking of this discrepancy in the two matches was impossible. Cardiac homotransplantation was performed on October 21, 1968 and no technical difficulties were encountered. The patient received Azathioprine 3 mg per kg and 200 mg of methyl prednisone, which is approximately 4 mg per kg on the day of transplantation. The initial function of the cardiac transplant was quite satisfactory. On the fourth postoperative transplant day a diagnosis of acute ilio-femoral venous thrombosis of the right leg was made. Rather than re-explore the patient's right inguinal incision, it was elected to perform a vena caval clipping and treat the patient's thrombosis with heparine. This surgery was tolerated well but on the 6th post-transplant day a decrease in the voltage of the QRS complex was noted. Concurrently with this decrease in voltage there was a decrease in systolic blood pressure and a rise in venous pressure. There was also a decrease in the creatinine clearance as well as an increase in weight.

* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6–8, 1969.

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The rejection episode was treated by increasing the dose of methyl prednisone to 300 mg per day, by the administration of 100 micrograms of actinomycin C intravenously and by the administration of 150r of radiation to the heart on the 6th, 8th, 10th and 12th post-transplant days. The QRS voltage was noted to improve along with a return of systolic blood pressures and venous pressures to more normal levels. At the same time the patient's creatinine clearance again improved and she underwent a diuresis. The methyl prednisone was subsequently tapered and the patient had no other manifestations of rejection episode.

The patient was discharged from the hospital on the 33rd postoperative day but returned on the 55th day with symptoms of malaise. She was readmitted with a questionable rejection episode that was never proven and it was suspected that the patient had a flux-like syndrome without undergoing a true rejection episode. She was subsequently discharged for outpatient follow-up but was readmitted a third time on the 142nd post-transplant day with a tender right groin mass. This was drained under general anaesthesia and resulted in the development of a right groin lymphatic fistula. This lymphatic fistula drained for 21 days during which time the patient developed a mild lymphopenia in spite of reduction of her Azathioprine dosage. It was also noted at this time that her QRS voltage improved. The significance of this lymphatic fistula is still not clearly understood. The patient was again discharged on the 170th post-transplant day.

The patient is currently thirteen months and a half post-transplantation and has returned to a completely normal activities. There have been no further clinical or laboratory data suggestive of rejection episodes and she currently is receiving 3 mg per kg of Azathioprine per day and 20 mg of methyl prednisone per day.

DIAGNOSTIC CRITERIA OF REJECTION IN HUMAN HEART TRANSPLANTATION *

Ihor DYRDA

Cet article relate les critères de diagnostic du rejet cardiaque chez 57 transplantés cardiaques, parmi lesquels on observa 64 épisodes de rejet, dont 30 pour cent furent fatals.

Trente pour cent des premiers épisodes furent diagnostiqués entre le cinquième et le quinzième jour. Après quoi, la fréquence diminua fortement mais persista de façon constante et proportionnellement au nombre de patients survivants. Il est impossible de dire si la fréquence de rejet diminuera après six mois.

La sensation de malaise est très souvent le signe précoce du rejet, tandis que le tableau de « grippe » est généralement un signe tardif.

The aim of this presentation is to summarized the known facts about the diagnosis of rejection of the human heart allograft and thus clear the field for more thought provoking presentations by the panelists that will follow.

With this in mind a questionnaire was sent to the different transplant groups that so far have grafted a total of 130 human hearts. We received information about 57 that is 45 per cent of the total. The source of this data is analysed in Table I. It may be summarized as follows: 34 from the U.S.A.; 14 from Canada; 5 from Europe; 3 from South America and 1 from Australia. The sample appears to be quite representative of the world experience. The mean survival time was 80 days; 25 per cent of the patients had survived 150 days and 10 per cent were surviving more than 210 days. During the first month after surgery there was a mortality of 40 per cent (most of it during the first 15 days) and during the following months the mortality ranged from 12 to 22 per cent of the survivors per month. Rejection was the main cause of death in 50 per cent of the cases.

Figure 1 shows the distribution of the rejection episodes. In 57 heart allografts 64 rejection episodes were diagnosed of which 30 per cent were fatal. Twenty-nine other rejection episodes were suspected. These were not included in the data that will follow.

Thirty per cent of the episodes were diagnosed between five and fifteen days after transplant that is during the first set rejection period. Thereafter, the incidence sharply decreased and remained constant being proportional to the numbers of surviving patients each month. From the data available it is not clear if the incidence of rejection will decrease after six months.

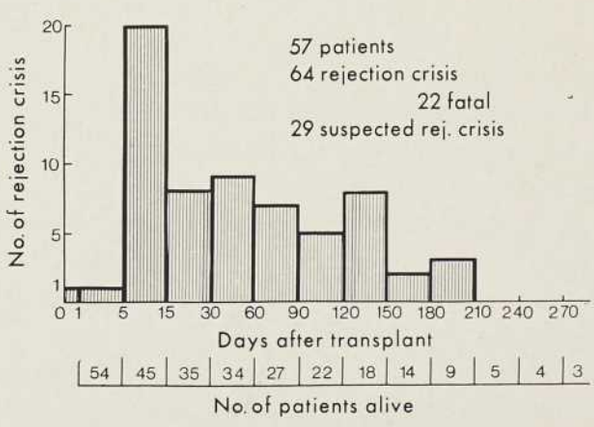

Figure 1 — Distribution of the rejection episodes.

* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6-8, 1969.

Les signes et symptômes de défaillance cardiaque sont peut-être parmi les premiers notés, mais ils signent déjà une atteinte myocardique fonctionnelle et anatomique importante.

L'augmentation de l'indice cardio-thoracique peut être secondaire à la dilatation du cœur et à l'épaississement des parois myocardiques, secondaires à l'œdème. Il n'y a pas moyen de dissocier ces deux facteurs.

Les signes électrocardiographiques du rejet sont certainement parmi les premiers qui apparaissent. On note une réduction du voltage de l'onde QRS, une déviation de l'axe du QRS et une augmentation de la fréquence cardiaque.

Parmi les données hématologiques, on peut noter une modification de la formule blanche, avec augmentation des lymphocytes. La vitesse de sédimentation et le nombre de plaquettes ne sont pas des indices valables.

Les modifications enzymatiques sont tardives, et l'augmentation initiale peut bien être secondaire à la thérapie et à la nécrose, tandis que l'augmentation tardive peut être secondaire à la défaillance cardiaque.

Signs and symptoms during rejection:

The systemic changes during rejection (Table II) are very non specific and may in some cases be secondary to the therapy. The feeling of not being well is sometimes a very early manifestation of rejection, on the other hand the complete "flu like" picture is usually a late sign.

The signs and symptoms of heart failure during rejection are shown in Tables III and IV. These may well be among the first to be noted but they are certainly not early since their presence means that enough functional if not anatomical myocardial damage is present to nullify the cardiac reserve and produce overt failure. The incidence is quite impressive but one must remember that during a fatal rejection crisis sooner or later all these signs will be present. Since in this serie 30 per cent of the episodes were fatal one must substract 30 per cent from these values in order to have the picture during reversible episodes of rejection.

Table V shows the X-rays signs. The increased C/T index may be secondary to heart dilatation and to an increased thickness of the myocardial wall secondary to œdema. We have no means to dissociate these two factors since ultrasound measurements of the myocardial wall are not yet generally done.

The electrocardiographic signs of rejection (Table VI) are certainly among the first to make their

TABLE I

Information was received regarding 57 patients with transplanted heart from the following centers

ANN ARBOR	USA	3	BORDEAUX	FRANCE	1
CHICAGO (Kittle)	USA	1	CHICAGO	USA	1
DALLAS	USA	2	DENVER	USA	1
HOUSTON (Cooley)	USA	19	JOHN HOPKINS	USA	1
LYON	FRANCE	1	MILWAUKEE	USA	1
MONTREAL (Dobell)	CANADA	1	MONTREAL (Grondin)	CANADA	9
MUNICH	GERMANY	2	PARIS (Cabrol)	FRANCE	1
PITTSBURG	USA	1	RICHMOND	USA	4
TORONTO (Baird)	CANADA	3	TORONTO (Bigelow)	CANADA	1
SIDNEY	AUSTRALIA	1	VALPARAISO	CHILE	3

appearance thus making the E.C.G. the most important tool in rejection diagnosis and follow-up. The reduction in QRS voltage, the shift of the QRS axis and the acceleration of the heart rate are the most frequent and the most easily measured changes. This is well illustrated in Figure 2. This patient died of rejection 47 days after transplant. You will notice the first change in the axis and voltage beginning at the 5th day, the second change at the 25th day and the final change at the 40th.

The questionnaire regarding the biochemical and hematological changes during rejection was inadequate for this reason we have no cumulative

TABLE II

General signs and symptoms during rejection

Asthenia	78%
Myalgia — malaise	48%
Fever	46%
Anorexia	46%
Nausea	28%

TABLE III

Signs and symptoms of heart failure during rejection

Decreased work tolerance	90%
Fall in blood pressures	83%
Tachycardia	77%
Dyspnea	72%
Weight gain	65%
Peripheral œdema	60%
Distention of jugular veins	55%
Congestive rales	52%
Hepatomegaly	52%
Pleural effusion	26%

TABLE IV

Cardiac examination in rejection

Tachycardia	77%
Arrhythmia	55%
III heart sound	57%
Muffled heart sounds	57%
Pericardial friction rubs	35%
Loud S_2P	17%
Mitral insufficiency murmur	0%

TABLE V

X-rays signs of rejection

Increased C/T index	62%
Pulmonary congestion	51%
Pleural effusion	32%
Pericardial effusion	13%

TABLE VI

E.C.G. signs during rejection

Decreased in QRS voltage	93%
Tachycardia	81%
Right axis deviation	38%
Left axis deviation	13%
QRS duration increase	28%
R.B.B.B.	13%
PR internal increase	13%
PVC's	20%
Atrial flutter — Fibrillation	7%
P, ST-T, QT	?

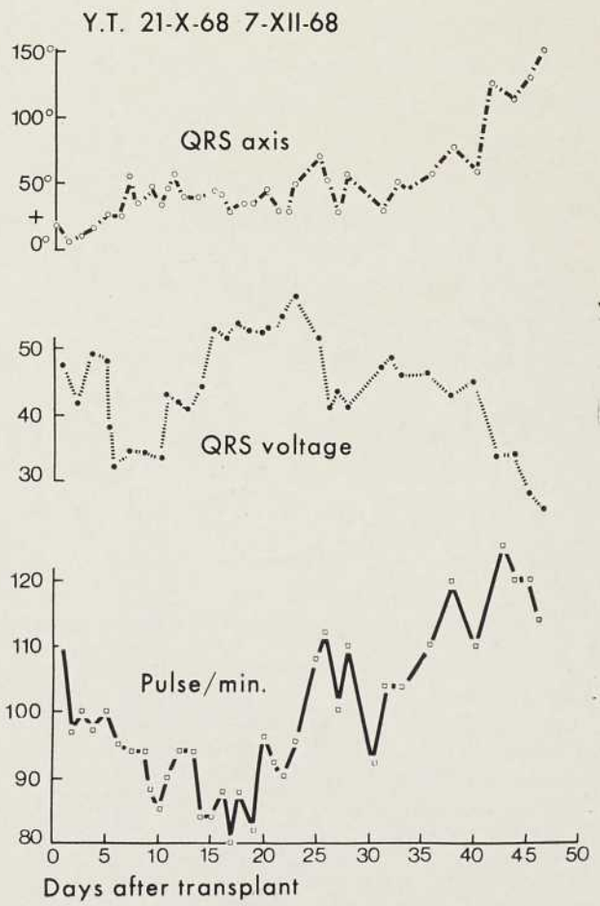

Figure 2 — Changes of the E.C.G. after heart transplantation. This patient died of rejection 47 days after transplant.

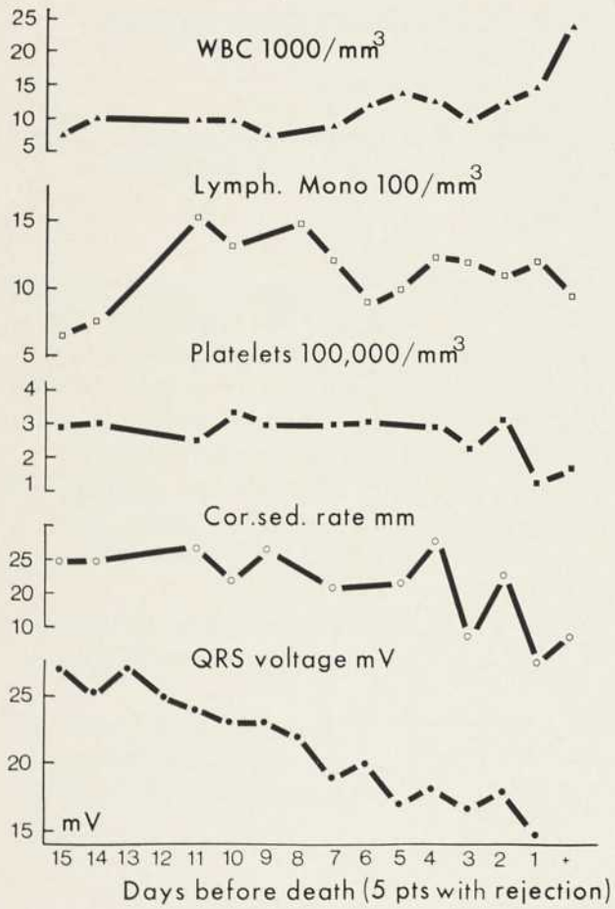

Figure 3 — Changes of the blood before 5 fatal rejection crisis.

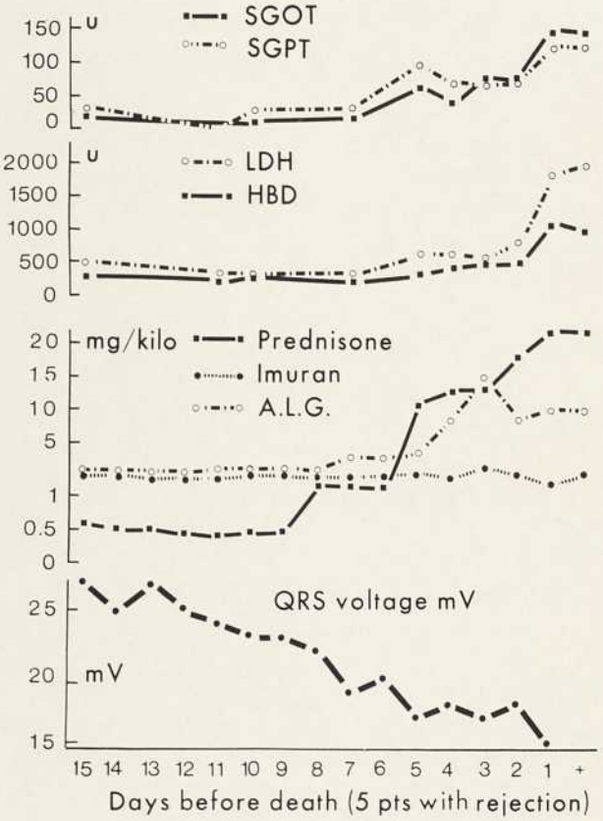

Figure 4 — Enzymatic changes before 5 fatal rejection crisis.

data to present. Figures 3 and 4 will illustrate our own experience with five fatal rejection crisis.

The WBC changed only six days before death (Figure 3). Note the rise in the lymphocyte count between the 14th and the 7th day. The drop at this point is secondary to increased therapy. The platelet count and the sedimentation rate were of no value.

Figure 4 shows the enzymatic changes. Note that these changes are late and the initial rise may well be secondary to the therapy and only this final sharp increase may be secondary to necrosis and heart failure.

In summary it would not be wrong to say that after twelve years of animal experience and eighteen months of human experience there still is no good way to make an early diagnosis of heart allograft rejection and that the electrocardiogram is still the only trustworthy tool at our disposal.

CLINICAL AND HEMODYNAMIC CHANGES DURING REJECTION OF THE TRANSPLANTED HUMAN HEART *

James J. NORA, M.D.,[1] Michael R. NIHILL, M.D., M.R.C.P.,[2] and Robert D. LEACHMAN, M.D.[3]

Les données cliniques les plus souvent rencontrées au cours des épisodes de rejet sont dans l'ordre de fréquence : la défaillance cardiaque, le frottement péricardique et le malaise. Parmi les examens de laboratoire quotidiens : les LDH, l'électrocardiogramme, l'examen hématologique, la détermination du débit cardiaque, l'examen radiologique du thorax, l'étude des stimulations leucocytaires phytohémaglutiniques.

Du point de vue histocompatibilité, trois facteurs sont déterminés : la compatibilité du système ABO, le *cross-match* des lymphocytes pour la recherche des anticorps préformés, et la détermination des antigènes lymphocytaires utilisant la méthode de Terasaki.

Au cours de la thérapie immunosuppressive, trois agents furent utilisés, ce sont l'Azathioprine, les adrénocorticostéroïdes et les globulines antilymphocytaires.

Les signes typiques de rejet cardiaque précoce, c'est-à-dire dans les quatre premières semaines, sont : la réapparition ou l'augmentation du frottement péricardique, l'augmentation radiologique du volume du cœur,

Reliable indices of cardiac rejection in the human subject must be defined to provide optimal clinical management. Methods for recognizing the acute episode at the earliest stages are required to prevent irreversible destruction of the allograft. Episodes of chronic rejection must also be appreciated as early as possible to arrest progressive changes which could compromise the physiologic competence of the transplanted heart.

CLINICAL MATERIAL AND METHODS

Nineteen human cardiac allografts have been performed in 18 patients at the Texas Heart Institute of St. Luke's Episcopal and Texas Children's Hospitals in Houston, Texas. A number of clinical and laboratory variables were selected as potential indices of cardiac rejection on the basis of previous canine and murine studies, and on theoretical grounds. These indices were modified as our clinical experience increased (3, 4, 11 and 12).

Clinical and laboratory indices. Clinical findings encountered repeatedly during cardiac rejection episodes have been evaluated in terms of their frequency of association with rejection: 1) congestive heart failure; 2) pericardial friction rub; and 3) malaise. Laboratory indices were monitored prospectively to guide immunosuppressive therapy. Most of these indices have also been reviewed retrospectively, and a judgment made as to when each parameter taken independently should be accepted as diagnostic of rejection:

1. Lactate dehydrogenase isozymes, studied daily for the first two postoperative weeks and reduced

* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6–8, 1969.

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l'augmentation du volume du foie avec œdème périphérique. D'habitude, il y a une température qui s'élève à 101°, une augmentation de la fréquence cardiaque et des malaises sous forme d'anorexie, parfois des nausées et douleurs généralisées. Deux ou trois jours avant les signes cliniques, on constate une augmentation des LDH. L'électrocardiogramme montre une ischémie souvent sous-endothéliale et une diminution du voltage du QRS. Parfois, on note une arythmie et une déviation axiale droite du vectocardiogramme dans le plan frontal. Ces signes électrocardiographiques précèdent les signes cliniques, mais apparaissent après les modifications des LDH.

On note une augmentation subite des globules blancs, allant jusqu'à 25 à 40 000.

Si l'épisode ne peut être contrôlé par les immuno-suppresseurs, on note une chute de la pression sanguine qui sera contrôlée avec de l'Isoproterenol.

À l'autopsie, il y a une infiltration interstitielle du myocarde, avec des lymphocytes, un petit nombre d'histiocytes et un nombre variable de leucocytes polymorphonucléaires. Cette infiltration est plus marquée dans la région périvasculaire et se rencontre également dans l'intima, épaissie, des artères coronaires.

Parmi les signes de rejet tardifs, on note à l'électrocardiogramme une diminution progressive du voltage du QRS, de même que des changements ischémiques sous-endothéliaux. Le malade ne se sent pas bien. On notera progressivement une augmentation du volume du cœur, une augmentation du poids, une diminution de la tolérance à l'exercice,
(suite du résumé en page suivante)

gradually to twice weekly by the end of the second month, using microzone electrophoretic separation (13).

2. Daily electrocardiograms.

3. Daily hematological evaluation, including routine complete blood count, alkaline phosphatase, stain of peripheral smear, periodic Rebuck window (15), and bone marrow aspiration when indicated.

4. Hemodynamic evaluation, beginning with a baseline dye dilution cardiac output 24 hours after transplantation and followed by sequential cardiac outputs and full cardiac catheterization studies including response to exercise and cardioactive pharmacological preparations (7).

5. Daily chest roentgenograms, for the first two weeks, then reduced in frequency as indicated.

6. Weekly phytohemagglutinin leukocyte stimulation studies (10).

Histocompatibility. Prospective histocompatibility studies performed locally at Texas Children's Hospital have been compared with retrospective studies on the same patients obtained through a collaborative study with Doctor Paul I. Terasaki (6). The three constituents of the donor-recipient match are: 1) ABO red blood cell compatibility; 2) lymphocyte cross-matching for preformed antibodies; 3) lymphocyte antigen matching using the Terasaki microdroplet lymphocyte cytotoxicity testing technique and grading scale.

Immunosuppressive therapy. Three basic immunosuppressive agents have been employed in the management of patients in this series. Several modifications in the earlier reported use of these agents (3, 4 and 11) have been made on the basis of increasing clinical experience (12).

Azathioprine. This agent is still considered to be corner-stone of the immunosuppressive program. At the time of decision for cardiac transplantation (usually only hours before the procedure), 4 mg/kg body weight is administered orally. This dose is

et des malaises. La fièvre et le frottement péricardique ne sont pas caractéristiques.

On note une diminution du débit cardiaque et une augmentation progressive de la pression télédiastolique du ventricule droit et de la pression de l'oreillette droite. À l'examen clinique il y a une diminution de la pression artérielle et une augmentation du rythme cardiaque. Les LDH sont augmentés, mais non de façon significative. La leucocytose est aux environs de 10 à 15 000.

À l'autopsie, les artères coronaires présentent un épaisissement de l'intima et une fibrose avec une infiltration de lymphocytes, plasmocytes et histiocytes contenant des vacuoles lipidiques. On trouve souvent une infiltration d'érythrocytes par rupture des petits vaisseaux. On trouve également des foyers de nécrose myocardique.

Il y a donc cinq indices principaux dans le rejet cardiaque, ce sont la défaillance cardiaque, le frottement péricardique, les malaises, les modifications des LDH et du tracé électrocardiographique. Parmi ceux-ci, trois constituent des indices fiables dans la détection du rejet tardif : ce sont la défaillance cardiaque, les malaises et les modifications électrocardiographiques.

Prévention du rejet :

1. Histocompatibilité. L'histocompatibilité tissulaire des groupes C et D a un pronostic très réservé ;
2. Thérapie immunosuppressive : Azathioprine, adrénocorticostéroïdes et globulines antilymphocytaires.

reduced to 3 mg/kg for the next two days and further reduced to 2 mg/kg, which is maintained throught the first postoperative month. The final maintenance dose has been dependent on bone marrow and systemic tolerance of the drug, and has varied from 25 mg to 100 mg per day in adults discharged to return home and to work. Cyclophosphamide has been used intravenously as a supplement to azathioprine during the most severe acute rejection crises.

Adrenocorticosteroids. At the time of decision for transplantation prednisolone, 1 mg/kg is administered orally and 5 mg/kg of hydrocortisone is given intravenously. These two agents are administered in the same dosage every six hours throughout the first postoperative day, at the end of which the prednisolone is reduced to 0.75 mg/kg per dose and the hydrocortisone to 2 mg/kg per dose each every six hours. The hydrocortisone is then discontinued and reserved for use during rejection crises in doses as high as 10 mg/kg every two hours. Prednisolone is given orally throughout the first two weeks at a level of 0.5 mg/kg every six hours. At the end of the first month prednisolone has been gradually reduced to 0.25 mg/kg every six hours and before discharge from the hospital the adult patients have been further reduced to levels between 15 mg and 30 mg daily, divided into three or four doses. Other synthetic adrenocorticosteroid preparations have been used for maintenance therapy in doses comparable to the above prednisolone schedule.

Antilymphocytic globulin. ALG prepared in Houston for the patients in the present study is produced in horses against human thymus and purified to a final product which consists entirely of IgG fraction. This agent is given daily for two weeks, intravenously or intramuscularly, and then administered every other day. The dosage is determined by the cytotoxic titer of the product and the rate of clearance of the isotopically labeled doses.

RESULTS

Before reviewing the clinical and laboratory findings of rejection from the point of view of assessing the validity of each individual parameter as an indicator of cardiac rejection, it would be useful to describe what might be called the typical findings in early rejection (first four weeks) and late rejection (over four weeks). These descriptions are based on the observation of 26 episodes of cardiac rejection (Table I) occurring in 14 of the 18 patients in this series.

Early human cardiac allograft rejection. What we interpret as a first set rejection is clinically detectable as early as the fifth postoperative day with the reappearance or increase in the pericardial friction rub, roentgenographic increase in heart size and often an increase in liver size, with peripheral edema. There is usually a temperature elevation to about 101°, and increase in heart rate and a distinct malaise with anorexia, sometimes nausea and generalized aching.

Prior to the appearance of these clinical findings, as early as the second or third day, serum LDH-1 is significantly elevated (see LDH-1 criteria in subsequent section). The electrocardiogram shows ischæmia, often subendocardial injury, and loss of QRS voltage; occasionally arrhythmia and rarely a right axis shift of the frontal plane QRS vector. These ECG changes may precede the clinical findings of rejection, but usually occur later than the LDH-1 abnormality.

At about the same time the pericardial friction rub, fever and malaise begin, the white blood count is suddenly increased, often rising to levels of 25–40,000 WBC/mm^3. The cells are almost all polymorphonuclear, mature and have a normal alkaline phosphatase stain count. The Rebuck window shows persistence of polymorphonuclear cells rather than normal transition to mononuclear. The phytohemagglutinin leukocyte stimulation is usually normal, without ever having reflected suppression.

If the episode cannot be adequately controlled by the immunosuppressive program, the pulse pressure drops and an intravenous isoproterenol drip (and recently glucagon) may be required to maintain the

TABLE I

Clinical experience with cardiac transplantation

CASE NO.	DATE OF OPERATION	AGE (YRS)	SEX	DIAGNOSIS	TISSUE MATCHING GRADE	NO. OF REJECTION EPISODES	FIRST REJECTION EPISODE BEGAN	SURVIVAL	CAUSE OF DEATH
1. a)	5- 2 68 (1st op.)	47	M	Rheumatic Multivalvular	C	2	8 days	200 days	—
b)	11-21-68 (2nd op.)	"	"	Cardiac rejection	C	1	Immediately	3 days	Rejection
2.	5- 5-68	48	M	Coronary		0	—	3 days	Infection
3.	5- 7-68	62	M	Coronary	C	0	—	8 days	Pre-existing diseases
4.	5-21-68	54	M	Coronary	C —	2	50 days	145 days	Rejection
5.	7- 2-68	46	M	Coronary	D	6	6 days	149 days	Rejection
6.	7-20-68	58	M	Coronary	C +	1	—	267 days	Rejection
7.	7-23-68	57	M	Coronary	C	3	13 days	170 days	Rejection
8.	7-29-68	49	F	Coronary	C	1	11 days	55 days	Infection
9.	8-18-68	5	F	Endocardial Fibroelastosis	D	1	5 days	8 days	Rejection
10.	8-19-68	50	M	Coronary	C —	1	5 days	68 days	Infection
11.	9-15-68	2 m.	F	A-V Canal (cardio-pulmonary transplant)	C —	0	—	14 hours	Pulmonary insufficiency
12.	10-25-68	52	M	Coronary	C	3	23 days	126 days	Rejection
13.	11- 5-68	50	M	Cardiomyopathy	D	1	5 days	7 days	Rejection
14.	11- 9-68	55	M	Coronary	C	0	—	48 days	Infection
15.	11-16-68	50	M	Coronary	D	1	7 days	188 days	Living
16.	11-29-68	54	M	Coronary	D	1	6 days	13 days	Rejection
17.	3- 4-69	56	M	Coronary	D	2	5 days	80 days	Living
18.	4- 7-69	47	M	Coronary	D	0	—	3 days	Infection

cardiac output. In three patients an acute first set rejection could not be reversed by aggressive immunosuppression and cardiovascular support. Death occurred in these patients between the seventh and thirteenth postoperative days.

The findings of acute rejection at autopsy (9) show a myocardial interstitial infiltrate consisting of lymphocytes, a small number of histiocytes and varying numbers of polymorphonuclear leukocytes. The infiltrate is more concentrated in perivascular areas. A similar infiltrate is found in thickened intima of coronary arteries.

Late human cardiac allograft rejection. It is most important to appreciate that late rejection begins with a gradual loss of QRS voltage, which is not clearly recognized as a trend for several days, because of the day to day variability in this parameter. Ischæmic changes increase and subendocardial injury may appear. The patient often insists that he feels well. These electrocardiographic abnormalities may be reversed by one or two days of massive intravenous hydrocortisone supplementation of the immunosuppressive regimen. If the electrocardiographic changes are not recognized early, further progression occurs which includes increase in roentgenographic heart size, increase in weight with peripheral edema, decrease in exercise tolerance and malaise. Fever and pericardial friction rub are not characteristic of late cardiac allograft rejection.

The hemodynamic changes encountered during episodes of clinical rejection include decrease in cardiac output as measured by indicator dye-dilution technique. There is progressive increase in right ventricular end-diastolic pressure and right atrial pressure. A consistent clinical reflection of the failing myocardium and decreased cardiac output is drop in systemic blood pressure accompanied by narrow pulse pressure and increased heart rate.

LDH-1 is increased, but not diagnostically so, and LDH-1 is not greater than LDH-2. The white blood cell count may become slightly elevated (10,000–15,000 WBC/mm^3) which is not to the extent found in early acute rejection. Immature granulocytes and atypical mononuclear cells are found. The Rebuck window may show a normal migration of cells with some decrease in mononuclear elements. Phytohemagglutinin leukocyte stimulation frequently returns to normal before the onset of a rejection episode.

The findings at necropsy of chronic rejection (9) reveal coronary arteries exhibiting intimal thickening and fibrosis with an infiltrate of lymphocytes, plasma cells, and histiocytes containing lipid vacuoles. There are varying degrees of luminal compromise. Acidophilic degenerative change may be present in the tunica media of the medium and small coronary arteries. There is a myocardial interstitial infiltrate of lymphocytes, plasma cells and histiocytes. Extravasation of erythrocytes due to rupture of small vessels is common. Focal areas of myocardial necrosis are found.

RECOGNITION OF REJECTION

Congestive heart failure. All patients, except one, required digitalis in the immediate postoperative period, and fluid retention with peripheral edema has been experienced by all patients in the first weeks after surgery. However, florid congestive heart failure with increasing cardiomegaly, hepatomegaly, peripheral edema and dyspnea is what is more characteristic of patients having cardiac rejection. Severe congestive heart failure was obvious in all patients with early rejection (Table II) and was prominent in all but two late rejection episodes. The specificity of this sign increases with the number of postoperative weeks to the point that an episode of congestive heart failure beginning after four weeks must be regarded as cardiac rejection.

Pericardial friction rub. Table II shows that nine of eleven patients having rejection episodes during the first four weeks had a reappearance or significant increase in a pre-existing pericardial friction rub. After four weeks, a pericardial rub rarely accompanied cardiac rejection. It may be argued that many patients have the recrudescence of a pericardial friction rub following other types of open heart surgery, but this does not depreciate

the immunological implication of this finding in cardiac rejection or the post-pericardiotomy syndrome. We regard the pericardial friction rub as a direct and clinically detectable sign of host-graft immunologic interaction.

Malaise. Although malaise may sound vague and non-specific, we have come to regard it as a reliable index of cardiac rejection. Loss of appetite, nausea, pain in muscles, joints, chest and abdomen, and progressive lethargy has been found in all patients with acute rejection, whose sensorium permitted such an assessment. Heart transplant patients are not inclined to complain over minor discomforts, and we become deeply concerned when they say they "feel sick". Infection is also accompanied by malaise, but is distinguishable by the detection of findings supporting infection, such as positive blood culture, abscess and clinical and roentgenographic evidence of pneumonia.

A word of caution should be offered regarding the use of malaise as an index of rejection. In the early stages of chronic rejection there is apparently no malaise, and even in later stages there is a tendency to deny the presence of symptoms. In a retrospective analysis of clinical and laboratory parameters, we concluded that one of our patients had begun chronic rejection of his heart approximately three months before he died. Yet during that time he was working a full day as a salesman and swimming daily up to one week before his death.

Lactate dehydrogenase (LDH) isozymes. LDH isozymes, specifically LDH-1, which predominates in myocardium, have been studied as an independent index of cardiac rejection (13). During the first month following surgery the mean level of LDH-1 is significantly higher in patients rejecting than in those not rejecting. After one month the difference is no longer significant. The percentage of LDH-1 always exceeded 35 per cent of total LDH during the first four weeks after surgery. After four weeks, however, clinical rejection was not accompanied by LDH-1 levels in excess of 35 per cent. LDH-1 was greater than LDH-2 in nine of eleven patients with cardiac rejection during the first four weeks, but after the sixth postoperative week, 13 out of 15 episodes of clinical rejection occurred without this finding.

The criteria we derived from this study as suggestive, if not diagnostic, of human cardiac rejection during the first post-transplant month are:

TABLE II

Five most reliable indices of cardiac rejection and their occurrence in rejection episodes (Rej) or in apparent absence of rejection (Non)

		11 EPISODES 0 – 4 WEEKS		15 EPISODES > 4 WEEKS	
		Rej.	Non	Rej.	Non
CHF ↑	Present	11	3	(12)	0
	Absent	0	4	(3)	10
Rub	Present	9	0	(3)	1
	Absent	2	7	(12)	9
Malaise	Present	10	2	(12)	4
	Absent	?	?	3	6
LDH-1 ↑	Present	10	1	2	0
	Absent	1	6	(13)	10
ECG abnorm.	Present	11	4	(15)	(4)
	Absent	0	3	0	7

( ) = more than one episode in the same patient.

1. LDH-1 > LDH-2;
2. LDH-1 > 35 per cent of total LDH;
3. LDH-1 > 100 International Units.

Using these criteria LDH-1 was elevated in 10 of 11 acute rejection episodes during the first four weeks and in only one patient was there a diagnostic LDH-1 elevation without obvious clinical rejection. After four post-transplant weeks, LDH-1 ceases to be a useful index of rejection. Lactate dehydrogenase isozymes are most reliable as an index of rejection in the early postoperative period, at a time when the electrocardiogram is least reliable due to non-specific changes related to heart surgery obscuring signs of rejection.

Electrocardiogram. The electrocardiographic findings consistent with cardiac rejection are listed in what we would consider to be the order of importance and specificity:

1. Progressive decrease in QRS amplitude;
2. Sub-endocardial injury;
3. Ischaemia.

The ECG tracings were studied daily as a guide to immunosuppressive therapy and were later studied in a retrospective analysis of this parameter as an independent index of rejection. During the first four weeks all patients surviving more than three days, whether or not they had other findings of cardiac rejection, had electrocardiograms diagnostic of rejection (Table II). However, after four weeks the electrocardiogram became more discriminating and the frequency of false positive diagnoses of rejection (based solely on ECG criteria) decreased. What is perhaps the most specific ECG index of rejection, right QRS axis, is not listed in the above three criteria, because it does not occur as frequently as the other signs, which lessens its diagnostic importance. Arrhythmias are also useful but not consistent clues to rejection.

The two deficiencies in the use of the electrocardiogram as a clinical guide to rejection are: its lack of discrimination in the first postoperative weeks between rejection and non-specific changes related to open heart surgery; and its rather wide range of variability, day to day, and even hour to hour. These deficiencies are compensated for by relying on another parameter in the first weeks after surgery (LDH-1), and by plotting ECG changes daily searching for the earliest evidence of an established trend.

At this time, despite the above-noted inadequacies, we consider the electrocardiogram to be the most useful guide we are currently employing to judge rejection of the transplanted human heart.

Other laboratory parameters. The results of the hematological and phytohemagglutinin studies are still being evaluated to determine their prognostic value as independent parameters of rejection. As soon as conclusions are possible these data will be reported.

A certain number of other clinical and laboratory indices of rejection suggested in earlier reports from this and other transplantation centers (1, 3, 4, 5 and 11) have not been discussed because they have not proved useful. For example, erythrocyte sedimentation rate appears to be of no value as an index of rejection, at least in the presence of our usual immune suppressive program. Enzyme determinations other than LDH-1 have not shown consistent and reproducible patterns as yet.

At the present, there appear to be five reliable indices of early acute cardiac rejection: 1) congestive heart failure; 2) pericardial friction rub; 3) malaise; 4) elevated LDH-1; 5) ECG changes. Three of these parameters are reliable in the detection of late or chronic rejection: 1) congestive heart failure; 2) malaise; 3) ECG changes. Supporting evidence for both acute and chronic rejection is found in the hematological, hemodynamic and phytohemagglutinin studies, but their reliability as independent parameters of rejection is still being assessed.

PREVENTION OF REJECTION

Histocompatibility. The relevance of tissue typing to renal allograft survival was predictable from the extensive background of mammalian investigations (16) and confirmed in clinical studies involving related and unrelated donors (14 and 18). However, a number of years of experience with

human renal transplantation has been required to reach the conclusion that lymphocyte typing is correlated with clinical outcome. The length of time required to establish this correlation has been related to the development of methods for leukocyte typing and antigen identification which are reproducible. The relative immunologic incompetence of the uremic patient may also have delayed the appreciation of histocompatibility differences by permitting survival of patients with degrees of mismatch which cannot be tolerated in cardiac transplantation.

Recognizing the limitations in analysis of small samples, a trend is nonetheless becoming apparent. Length of survival and quality of survival of human cardiac allografts appear to correlate directly with the closeness of histocompatibility match detectable by present methods. Table III summarizes our observations on histocompatibility and clinical outcome in patients surviving seven days. The decrease in mean survival and mean survival without a rejection episode with decrease in grade match from C + to D is shown. Also documented is the relationship between matching grade and rejection episodes per patient day with one rejection episode per 29 patient days being found in D grade matches and one episode per 267 patient days being found in C + grade matches.

Immune suppression. The results of immunosuppressive therapy were highly satisfactory in the early course of the patients of this series. The regimen of azathioprine, adrenocorticosteroids and antilymphocytic globulin was patterned after the use of these agents in unrelated donor-recipient renal allografts (17). An effort was made to reduce immune suppression to levels which would permit full employment and restoration of activity. Four patients returned home to a high level of activity including swimming and hunting. Two were fully employed, one as a certified public accountant and one as an automobile salesman. No patient discharged from the hospital on the immunosuppressive level which we required for discharge has developed a significant infection. These doses (see Methods) were based on the adequacy of suppression of clinical and laboratory evidence of cardiac rejection achievable with bone marrow and systemic tolerance of the medications.

Antilymphocytic globulin was first introduced into human cardiac transplantation in the patients in this series, and its effectiveness and optimal methods of use are still being assessed. We do not feel that maximal benefit has yet been achieved from this agent, if it fulfills an early prediction by Sir Peter Medawar (8) of being "... not only the most powerful but also the only true immunosuppressive agent." We are making efforts to discover optimal dosage, route and frequency of administration, and to devise methods of preventing increasing immunologic clearance. Presently our dosage regimen is based on cytotoxic titer and rate of clearance of isotopically labeled doses (2) and no patient has yet had his treatment with this medication discontinued. However, by all routes of administration, the plasma half-life of 125_I labeled ALG decreases, in some patients very slowly over

TABLE III

Clinical course related to histocompatibility grade in 14 human cardial allografts surviving 7 or more days

	C +	C	C —	D
Mean survival (days)	267	106	98	69
Mean survival without rejection (days)	267	22.5	19	5.7
Rejection episodes per patient day	1/267	1/92	1/65	1/29

a period of months, and in others very rapidly over a period of weeks (2). These data suggest that ALG is antigenic in man and that there is need to develop methods to prevent formation of antibodies against ALG to prolong its effectiveness as an immunosuppressive agent. An encouraging observation has been made in one of our patients who began to demonstrate an increasing ALG half-life in his eighth postoperative month after first manifesting the customary decrease in 125_I plasma half-life of ALG. This may represent the development of tolerance to ALG.

DISCUSSION

Human cardiac allograft rejection is a real and dramatic event, the clinical features of which may be readily described. An important difference between cardiac and renal rejection which is becoming apparent is that there does not seem to be the quiescent period of graft tolerance after three postoperative months in poorly matched heart patients as there is in kidney patients. The rejection of the heart appears to be inexorably progressive in patients with poor histocompatibility matches. Far from being a privileged organ, the heart seems even more vulnerable to rejection than the kidney.

Several factors contribute to this vulnerability. The critical physiologic function of the heart will not permit periods of shutdown without death as in the transplanted kidney. Nor can a rejected heart be removed and the patient maintained for many weeks by extracorporeal support while seeking another organ. In addition, the cardiac patient may not have the recognized diminished immunologic competence of the uremic patient.

It must be appreciated that human cardiac transplantation is an investigative procedure which has minimal present clinical application. Those who may derive enough benefit from the procedure at our present level of understanding to justify the enormous expenditure of human and fiscal resources would seem to be patients with excellent histocompatibility matches and all other presently accepted clinical criteria for heart transplant recipients (3 and 4). Opportunities for better histocompatibility matching may be found through regional and national pooling of prospectively-typed donors and recipients, with emergency transportation of patients to selected centers. Recipients of histocompatible hearts should hopefully receive both genuine clinical benefit and provide a background of needed immunologic and physiologic information concerning long-term graft survival.

From this series we have learned that rejection and infection in patients with grades C and D histocompatibility matches constitute significant deterrents to immediate clinical application of cardiac transplantation. However, if cardiac transplantation is going to have wide clinical application in the future, new methods will have to be devised for overcoming poor histocompatibility matches. Present methods of immune suppression have not proved to be as adequate as anticipated. A logical next step would be vigorous investigation into possible methods for induction of tolerance. Further frontiers worthy of consideration are long-term organ preservation and possibly, at some distant time, crossing the formidable species barrier.

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ELECTROCARDIOGRAPHIC AND VECTOCARDIOGRAPHIC SIGNS OF REJECTION *

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Les auteurs présentent les modifications électrocardiographiques rencontrées au cours du rejet chez deux malades qui ont bénéficié d'une transplantation cardiaque. Parmi ces signes, on notait :

1. Des arythmies sous forme de bloc sino-auriculaire et auriculo-ventriculaire ;
2. Des troubles de conduction auriculo-ventriculaires sous forme d'une augmentation de l'espace PR, et des signes de troubles de conduction intramyocardiques, sous forme d'un élargissement du QRS, puis du QT.

Analysis of the E.C.G. tracing following heart transplantation in the dog (4) has enabled us to recognize the following signs of rejection:

Arrhythmias;

Troubles of the intramyocardial conduction;

Low voltage;

Troubles of repolarization.

Since the beginning of our experimentation, the most important E.C.G. variations announcing rejection seem to be arrhythmias and intramyocardial conduction troubles; low voltage comes later and its pronostic is very bad. This is due to the very disseminated character of the histological lesions as observed on acute or chronic myocarditis (5 and 6).

These data have been verified on two human cases.

A. The first case concerns a 59 year old man transplanted on May 12th 1968, for ischemic disease (1, 2 and 3).

1. The E.C.G. survey and its comparison to the titration of serum enzymes derived from heart muscles (7), has been normal from the first to the 204th day, where abruptly occurred a sino-auricular 2/1 block which was registered for only one minute with a left rotation of the ventricular axis. This arrhythmias has not been followed by any more troubles during a month (Figure 1).

2. The second E.C.G. change, on the 240th day gave more evidence of rejection, by the increase of the PR interval; of the QRS and QT duration, and a variation to the left of the ventricular axis; but there were no arrhythmia, nor low voltage; and at the same time fever occurred and the myocardial enzymes increased. The adjustment of therapy was followed in a few days by restitution to normal data. Then during three months we observed no ECG changes.

3. On April 25th, eleven months after the transplantation, for a four hour period an arrhythmia occurred. It consisted first on a sino-auricular 2/1 block then a 3/1 block which reduced the heart rate to about 38/mn, then occurred an auriculo-ventricular block of variable degrees until it reached a complete auriculo-ventricular block. Diffused repolarization troubles, as seen in pericarditis, were present for a week. Enzymes derived from heart increased in the same period. Then the E.C.G. returned to normal.

4. Troubles of repolarization have occurred between the second and the third E.C.G. changes,

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Quant au rejet s'annonçant par une diminution du voltage, les auteurs considèrent ce facteur important, mais il faut tenir compte qu'il peut apparaître dans le cas d'un épanchement péricardique et qu'en fait, ce signe est tardif et précédé par d'autres modifications électrocardiographiques. Son apparition signifie un rejet actif. Les changements ischémiques, lorsqu'ils sont localisés et d'apparition brutale, peuvent être le fait d'un rejet. Des modifications diffuses, telles que dans les péricardites, peuvent se rencontrer également au cours du rejet ou dans le syndrome post-péricardotomie. Les changements progressifs et diffus de l'onde T, conséquence du traitement à long terme et à haute dose aux corticoïdes, disparaissent avec une thérapie au potassium. Il semble que les signes prémonitoires du rejet soient les arythmies et les troubles de conduction intramyocardiques, tandis que la présence d'un bas voltage signe la présence du rejet. Notons que dans l'évaluation du bas voltage, il est important que le tracé soit pris sur la même machine, avec le même technicien, à la même heure, et en disposant les électrodes de la même façon.

while an osteoporosis was noted with an hypokaliemia due to cortisonic treatment. With potassium *per os*, all repolarization troubles disappeared.

The actual E.C.G. is like the first E.C.G. after transplantation, with a 90/mn sinusal rate and an incomplete right bundle branch block, as shows the vectocardiogram.

We must note that the voltage has never changed, even during the most important rejection crisis (204th day) out of normal variations due to position, breathing... The three E.C.G. changes were arrhythmias on the first and third crisis, and auriculo-ventricular and intra-myocardial conduction troubles on the second crisis.

B. The second case concerns a 48 year old man transplanted on November 24th 1968, for ischemic disease and complete heart failure.

The first E.C.G. after transplantation shows a vertical position and a dextrorotation of the heart, an incomplete right bundle branch block, and signs of posterior infarction, as confirmed by vectocardiogram. We believe that a coronary air embolism occurred.

During the four first days, voltage was low, and it then increased to normal values, 25–30 mv, where it remained until now (seven months).

However, on three times, we noted a slight decrease of voltage following minor variations of the zymogram (Figure 2).

On the end of the fourth month, progressively appeared a variation of repolarization on leads V5 and V6 which might be interpreted as subepicardic ischemia. No more E.C.G. change was noted.

DISCUSSION

These facts suggest some comments.

1. Arrhythmias occurred twice, during registration of the E.C.G. This calls for a daily E.C.G. survey and a program of continuing E.C.G. tracings once the patient has returned home. We try to realize such a program.

Such arrhythmias occurred, in a patient transplanted in Bordeaux (Pr. Fontan). The administration of heparine was followed by the suppression of arrhythmia (access of auricular fibrillation which called for several electric shocks during a two month period). This fact, and the knowledge of histological coronary thrombosis with rejection are an argument for the anticoagulant therapy.

2. The most important crisis of rejection has given multiple signs of rejection, such as in myocarditis: auriculo-ventricular conduction time increased (PR interval from 0.16 to 0.20 sec.), intramyocardial conduction troubles (enlargement of

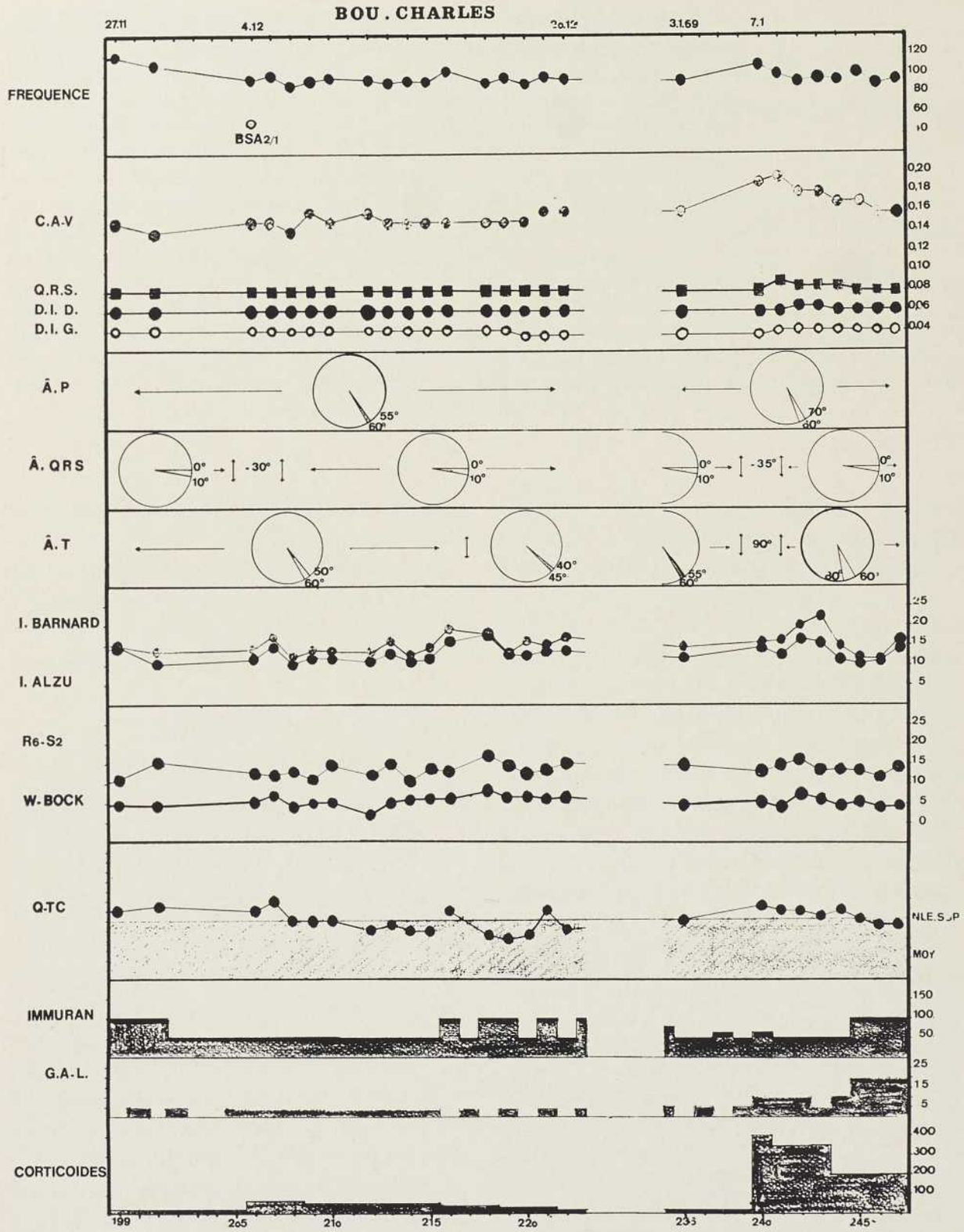

Figure 1 — Diagram of the first transplanted man. On December 4, 1968, occurs a sino auricular block 2/1 and the axis of QRS turns to the left. No more changes on the following E.C.G. On January 7, 1969, rejection crisis with increase of PR interval (from 0.16 to 0.20 sec), of QRS duration (0.08 to 0.10), and of QT duration by comparison to QT corrected time while QRS axis, turns to the left. In four days the E.C.G. returns to normal.

QRS) and later repolarization troubles (QT duration increased). We believe that all these variations are the reflect of the very disseminated character of the lesions seen on histology. In animal experimentation we have given both the E.C.G. and histologic proof.

3. We have not observed rejection crisis announced by low voltage. We have often seen in

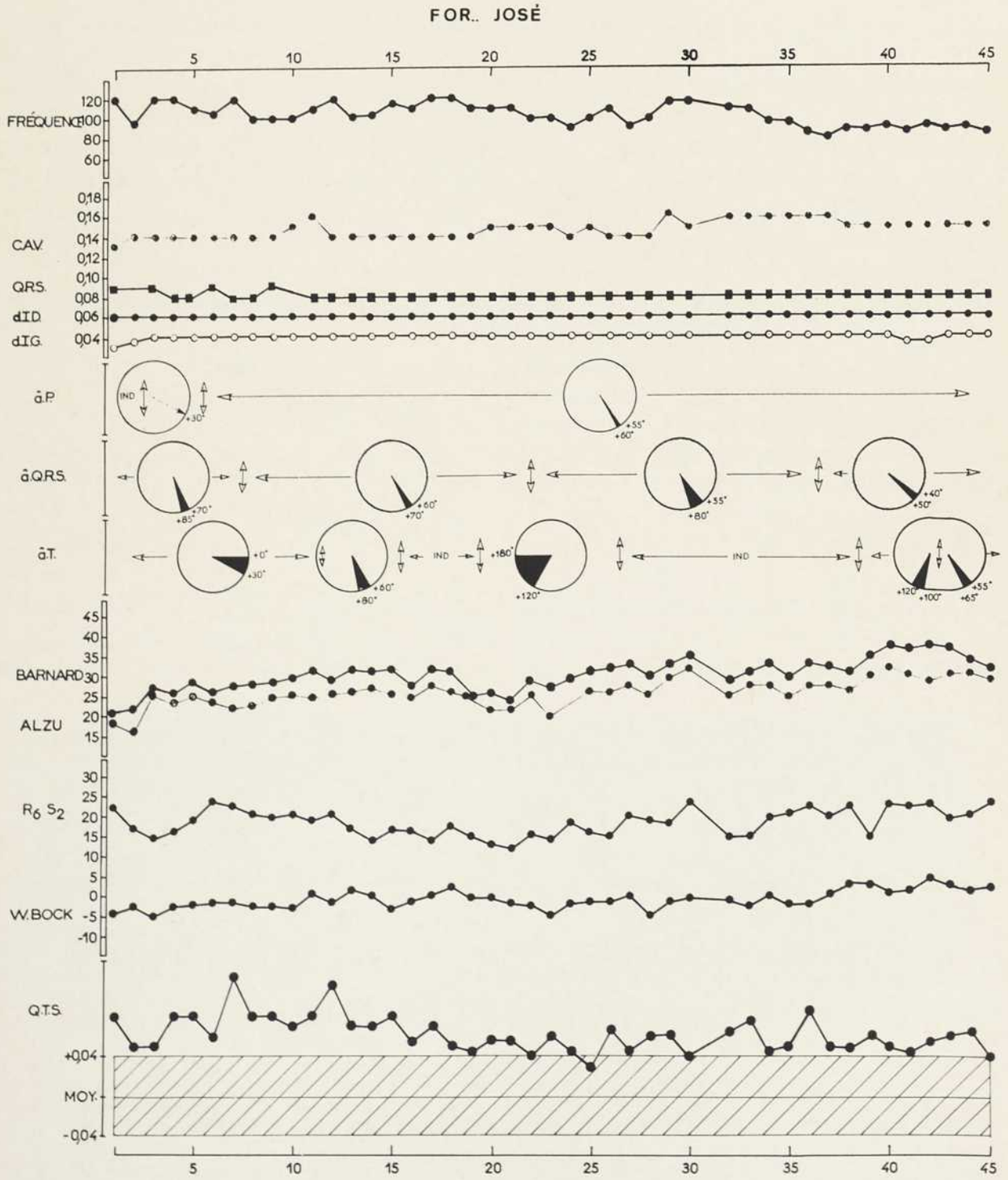

Figure 2 — Diagram of second transplanted man during the first 45 days. Normal data except variation of repolarization due to surgery.

dogs, and found on the second case, an immediate postoperative low voltage which increased to normal values a few days later. This immediate low voltage seems to be due to surgery and not to rejection.

We followed the voltage on three indexes: on leads I, II and III (Barnard's Index), on aVL-aVR-aVF (Alzu's Index) and on horizontal plan (sum of R5-S2 or R6-S2). We think that a low voltage is present if encountered in the three indexes. It must be known that, position, breathing, location of precordial electrodes may be responsible of voltage variations. As a matter of fact we did require the following precautions: same E.C.G. set, same technician, same hour of tracing, and marking of the position for precordial electrodes.

We believe that low voltage is a most important sign of rejection. But two remarks must be made: it can be the fact of an intrapericardial effusion such as in postpericardotomy syndrome. In our experimentation on dogs, we noted it as a late sign occurring at the terminal period of evolution, and usually preceeded by other E.C.G. changes, even minor. Its appearance signs an evolutive rejection. After this we never waited to induce the adequate therapy.

4. The repolarization troubles call for discussion:

a) *Ischemic changes*, when localizated and abruptly occurring, can be the sign of a rejection crisis, as shown by histology (coronary thrombosis).

b) *Disseminated changes*, as in pericarditis may be seen, even in the course of a rejection crisis (intramyocardial conduction troubles) or a postpericardotomy syndrome.

c) *Progressive and disseminated changes of T wave*, may be related to an important and prolonged decrease of serum potassium and to high corticoid therapy doses. Return to normal might be attained by a potassic therapy.

CONCLUSION

Analysis of animal experimentation and survey of two human cases show that the most important signs of acute rejection are arrhythmias and intramyocardial conduction troubles. The low voltage is mostly always present on rejection crisis but appears to be a later sign, and a very bad prognosis. Preceeded by other E.C.G. changes, even minor ones must not wait to increase therapy.

Isolated troubles of repolarization must be analysed by comparison to metabolic changes of serum.

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CLINICAL RECOGNITION OF REJECTION OF THE TRANSPLANTED HEART *

Velva SCHRIRE †

L'auteur étudie le diagnostic clinique du rejet dans la transplantation cardiaque :

1. Modifications générales sous forme d'anoxie, fièvre, augmentation de la vitesse de sédimentation, malaises et fatigue.

2. Changements subjectifs sous forme de dyspnée d'effort avec diminution de la tolérance à l'effort.

3. Données objectives : augmentation du volume du cœur, arythmies, extrasystoles ventriculaires et élévation de la pression veineuse jugulaire.

Les modifications des bruits cardiaques apparaissent tardivement, tandis que le frottement péricardique paraît précocement. Il est difficile de dire si ce frottement est dû à la chirurgie ou au rejet.

4. Modification radiologique : cardiomégalie.

5. Modifications électrocardiographiques : réduction du voltage et altérations des ondes ST et T ; la réduction du voltage des complexes QRS semble être secondaire à une détérioration de l'état clinique. La réduction du voltage est probablement en relation avec le rejet, bien qu'il faille tenir compte de l'épanchement péricardique possible. L'interprétation est compliquée en raison de la péricardite postopératoire et de l'administration de la digitale.

As of the present moment, clinical recognition of rejection of the transplanted heart is primitive to say the least. Evidence at present available suggests that complete tolerance with no histological or functional abnormality does not exist and that a modified but continuous rejection is to be expected. Rejection can thus be encountered in varying degrees of severity and probably in one and the same patient, peaks and troughs in the active rejection process commence with the insertion of the allograft and continue unabated thereafter. Superimposed on these acute and chronic episodes

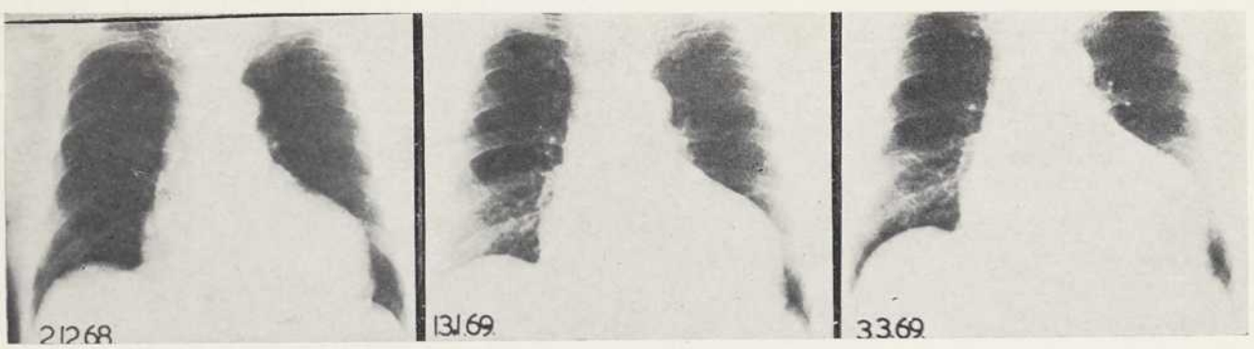

Figure 1 — Serial X-rays in patient P.B. show progressive increase in heart size

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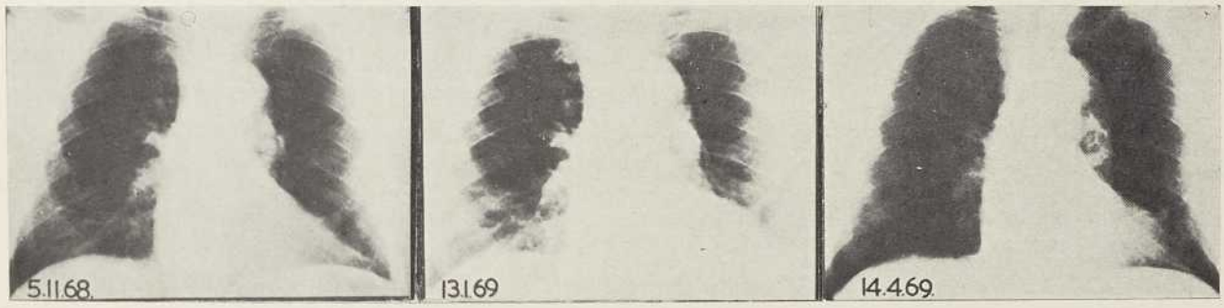

Figure 2 — No significant change in heart size (patient P.S.)

are the effects of immunosuppressive therapy and incidental infection.

Systemic changes noted have been fever, anorexia and raised E.S.R. Subjective complaints have been loss of sense of well-being, fatigue and dypnœa. Elevation of jugular veins and hepatomegaly are difficult to elicit at the bedside because of steroid effects. Cardiomegaly is best recognized radiologically. Arrhythmias have been strikingly absent. Alteration in heart sounds and murmurs have only appeared at a late stage in our longest surviving patient. Triple rhythm, however, was an early sign in our last subject. The significance of a pericardial rub in the early postoperative period is difficult to determine. Long persistence of pericarditis suggests rejection.

Radiological evidence of cardiomegaly with or without pulmonary congestion appears to be an important sign. This occurred early in our second transplant patient and never subsided although some fluctuation in size with acute rejection episodes occurred (Figure 1). Cardiomegaly soon after surgery was noted also in our last patient, but this was associated with triple rhythm and signs of cardiac failure. The absence of cardiomegaly throughout the course of our third patient parallels his smooth trouble-free course (Figure 2).

Electrocardiographic changes should be looked for both from the point of view of voltage changes and changes in configuration of the ST-T waves (Figure 3). In our second patient (P.B.) daily oscilloscopic records showed gradual reduction in voltage in all leads, most likely due to rejection

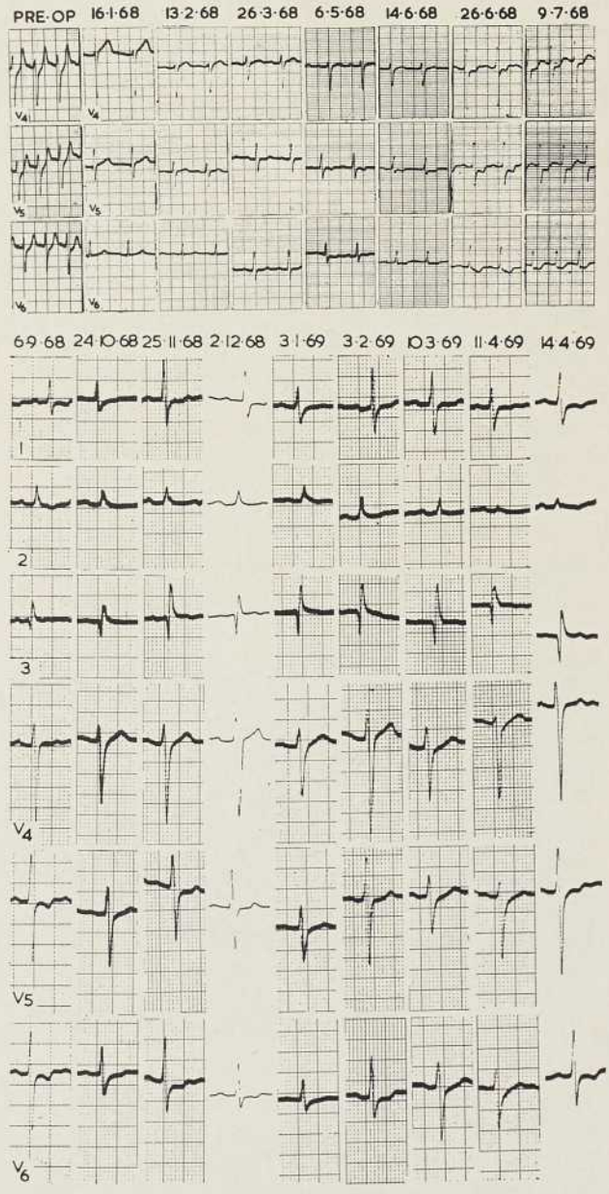

Figure 3 — Serial ECG tracings in patient P.B. showing progressive changes in the ST-T waves over 15 months with the development of RAD.

although the effects of pericardial effusion cannot be excluded. The voltage promptly increased when more steroids were administered.

In the early stages after operation fluctuation in voltage is common however and interpretation difficult. Marked fluctuation from hour to hour and day to day may be significant. Interpretation is complicated by postoperative pericarditis and the administration of digitalis. This applies even more to the ST-T changes but persistence of T wave inversion in the left chest leads is probably significant (Figure 4).

Once the acute effects of operation and possibly acute rejection have disappeared voltage changes may be more meaningful. After an initial fall associated with rejection, there was a rapid return to normal when the steroid dosage was increased (patient P.B.) and this pattern was repeated during the subsequent major clinical rejection episode. Our third patient (P.S.) who has remained well throughout has showed no significant major change. Similarly in our second patient progressive ST-T wave changes developed (Figure 3) whereas in our third patient these abnormal changes regressed (Figure 5).

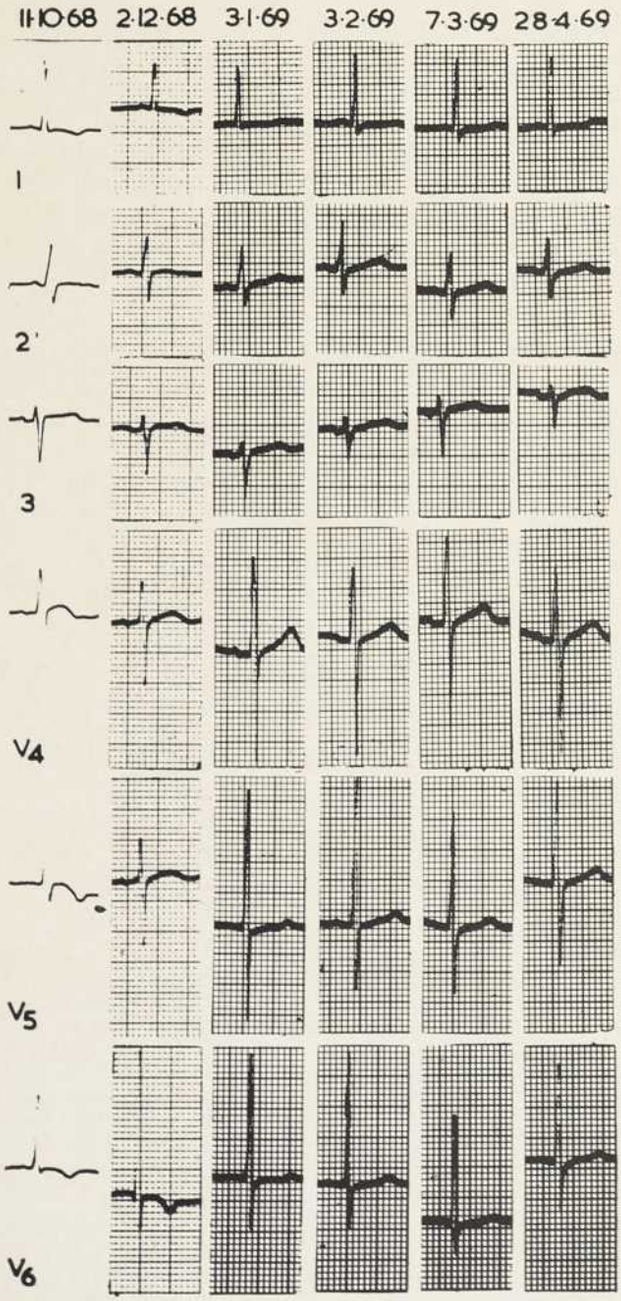

Figure 4 — Serial records early in postoperative course of patient D.F. showing fluctuation in voltage and progressive ST-T wave changes.

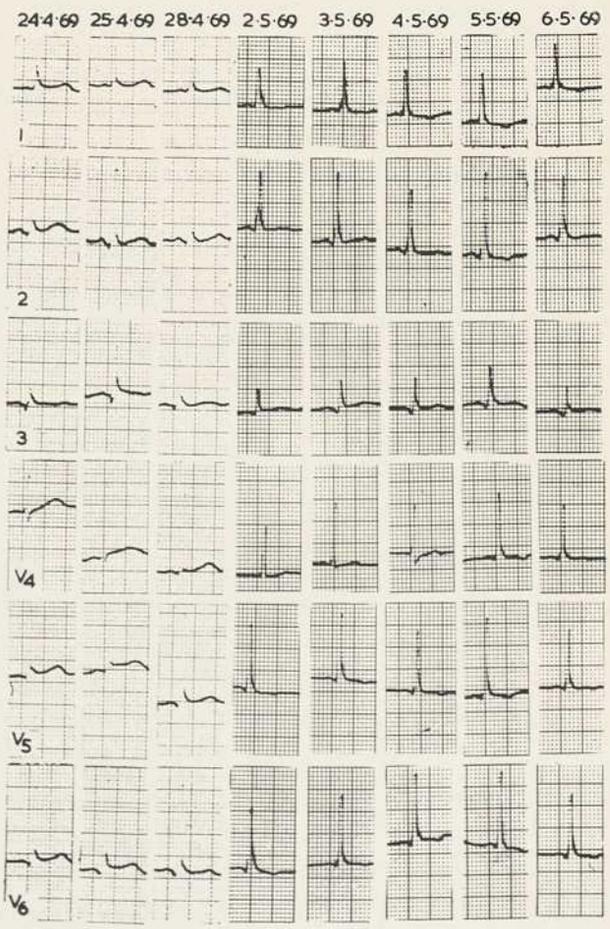

Figure 5 — Serial ECG changes in patient P.S. The initial tracing shows severe left ventricular hypertrophy with progressive restitution and return towards normal.

SUMMARY

The diagnosis of rejection after transplantation of the human heart is extremely difficult. This is not only due to the complex nature of rejection but also to the difficulty between distinguishing changes caused by rejection, those following the use of drugs, those resulting from common complications of cardiac surgery such as pericardial effusion and the post-cardiotomy syndrome not to mention the effects of superadded infection.

Repeated, regular and careful examination of the patient with special reference to cardiac function is essential. Changes in the electrocardiogram particularly in voltage and in the ST-T segments may be important.

PREVENTION OF INFECTION AFTER CARDIAC TRANSPLANTATION *

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L'infection au cours de la transplantation cardiaque constitue la complication la plus fréquente et la plus sévère.

Parmi les facteurs prédisposants l'on note l'acte chirurgical lui-même, l'environnement hospitalier et la thérapie immunosuppressive. Les corticoïdes favorisent la dissémination de l'infection, et inhibent la phagocytose cellulaire. Les globulines antilymphocytaires augmentent le degré d'infection virale. Notons également l'administration intraveineuse des liquides et médicaments comme facteur prédisposant. Les agents infectieux les plus courants sont les *Staphylococcus aureus,* type 3, qui sont les staphylocoques d'hôpital, les *Pseudomonas aeruginosa,* les mycoses, notamment le *Candida albicans* et d'autres agents comme le *Pneumocystis carinii.*

Au cours d'une infection antérieure par des streptocoques β-hémolytiques, certains composants capsulaires et membraneux peuvent produire des réactions d'immunisation croisée avec les tissus cardiaques et avec les constituants musculaires artériels. De sorte que les agents bactériens sont non seulement capables d'avoir une action directe, mais aussi capables de déterminer un processus de rejet dû à un phénomène d'immunisation croisée. (suite du résumé en page suivante)

The experience of heart transplantation in different surgical centers throughout the world demonstrates that infection is one of the most frequent and severe postoperative complications. In this presentation we will discuss the prevention and the treatment of infection in three patients submitted to heart transplantation.

A. PREDISPOSING FACTORS

1. *Surgical handling of the patient.*

In spite of every precaution, surgical management offer every opportunity for contamination. Therefore, it seems advisable to restrict surgery to a minimum. For instance, direct aortic cannulation for the arterial blood return, avoiding the inguinal incision, is probably a wise step.

2. *Hospital environment.*

It is a widely known fact that general hospitals, where many heart transplantations were performed, are contamined by pathogenic agents, specially staphylococci and Gram-negative bacteria. Infectious processes, in order to be eliminated, are counterattacked by hypersensitivity phenomena.

3. *Immunosuppressive therapy.*

Immunosuppressive agents depress the immunological process that regulates the hypersensitivity phenomena. Their action is less marked on early hypersensitivity and more intense on delayed hypersensitivity. Immunosuppressive agents, specially corticosteroids, present prominent inhibitory action on cellular phagocytosis. Furthermore, corticosteroids favor the spread of infection due to its

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Certaines mesures prophylactiques sont envisagées : éliminer les foyers infectieux, protéger la peau et les muqueuses, confiner le malade dans un espace restreint, et sélectionner le personnel non infecté.

Parmi les mesures pour enrayer l'infection, notons l'emploi des antibiotiques, et parmi ceux-ci le Céphalotin, qui est également administré au donneur. Dans le cas où le Céphalotin ne peut être utilisé, d'autres antibiotiques tels que la Gentamycine et la Cloracilline sont utilisés. Notons en outre l'isolement dans une chambre stérile, la stérilisation des aliments et les cultures du sang, des urines et des expectorations.

protective action on the lipoproteic lisossomic membrane.

Bacterial infection occurring early after cardiac transplantation is controlled mainly by reactions related to early hypersensitivity phenomena. Immunosuppressive agents, including antilymphocytic globulin, inhibit delayed hypersensitivity and, probably, immediate hypersensitivity in the level of IgM and IgG. Furthermore, ALG enhances viral infections. As a consequence, administration of high doses of immunosuppressive agents in the early postoperative period is a contributing factor in the spread of bacterial infection. Greater danger, however, is the appearance of infection in the late postoperative period, due to the depressive action of the immunosuppressive drugs on delayed hypersensitivity phenomena.

4. *Intravenous fluid administration.*

Intravenous administration of fluids and drugs kept in refrigerators must be always regarded as a possible source of infection.

B. INFECTIOUS AGENTS

The agents listed below are capable to produce infection in any phase of the cardiac transplantation postoperative course, but there is a tendency for some of them to start earlier than others.

Among the infectious agents appearing early after surgery are:

1. *Staphylococcus aureus,* type III ("hospital staphylococcus").

2. *Pseudomonas aeruginosa,* Gram-negative bacteria with the following characteristics:

a) resistant to routine methods of sterilization;

b) able to grow even in low temperatures, for instance in fluids kept in refrigerators;

c) frequent in patients with indwelling urinary catheters, and

d) present in the tracheo-bronchial tree of tracheostomy patients.

3. *Other Gram-negative agents* of the Enterobacteriaceae family (Klebsiella, Aerobacter, Serratia) and the Proteus and coli groups.

Among the most frequent infection agents responsible for the late appearing processes are:

4. *Fungi,* such as *Candida albicans,* whose proliferation is aided by the combined use of antibiotics and corticosteroids, as well by prolonged intravenous administration of glucose solutions.

5. Other agents, such as *Pneumocystis carinii,* responsible for severe respiratory infections, and cytomegalic virus, present sometimes in extracorporeal equipment.

C. INDIRECT CONSEQUENCES OF PREVIOUS INFECTIONS
(Biological mimicry phenomena)

Certain capsular (1) and membrane (3) components of β-hemolytic streptococci, such as *M* protein, *C* carbohydrate and glycoprotein, are able to produce cross immunization reactions with heart tissues (myocardium, valves, endocardial smooth muscle), as well as with arterial muscular components.

On the other hand, Rapaport and collaborators (2) demonstrated the possibility of certain bacterial antigens, specially staphylococcal, to be comparable to transplant organ antigens.

Therefore, bacterial agents are able not only to course direct attack on the patient but also to determine rejection process through cross immunisation phenomena.

D. PROPHYLACTIC MEASURES

According to the aforementioned considerations, certain prophylactic measures were observed in our cases.

1. *Preoperative care.*

The preoperative care included:

a) removal of infectious foci elicited by clinical and laboratorial investigation;

b) care of skin and mucosae;

c) confinement of the patient to a restricted area, to avoid contact with other patients or with contaminated surrounding;

d) selection of non-infected personnel, taking care to treat or remove infectious foci in the individuals involved in the patient's care.

2. *Postoperative care.*

Among the measures to avoid infection in the postoperative period were:

a) *Use of antibiotics.* The characteristics of the "ideal" antibiotic in the prophylaxis of infection after cardiac transplantation were considered to be: *i*) wide spectrum of action against frequent infections agents; *ii*) bactericidal activity; *iii*) reduced possibility of developing bacterial resistance.

Taking in account these points, cephalotin was one choice. This antibiotic, derived from the cephalosporanic acid, presents some advantages:

i) intense bactericidal action, interfering with the bacterial membrane synthesis;

ii) good control of different strains of staphylococci, even penicillinase producers;

iii) active against Gram-negative bacteria, except *Pseudomonas aeruginosa;*

iv) possible to administer even in patients with poor renal function.

Cephalotin was administered in doses of 1.0 g every six hours, during four days. Administration started before extracorporeal circulation. The antibiotic was discontinued after four days in order to avoid superinfection. Cephalotin was given to the heart donor (2.0 g before surgery).

In cases where cephalotin cannot be used, the alternatives are gentamycin and cloxacillin.

Gentamycin has the following characteristics:

a) good activity against Gram-negative bacteria and against penicillin — resistant staphylococci; *b*) small likelihood of inducing superinfection. Cloxacillin is active against penicillinase producing staphylococci.

b) *Confinement to sterile area.* After cardiac transplantation the recipient was kept in a sterile unit during one week. After that period of time, confinement of the patient was gradually released.

c) *Sterile diet* by antoclaving was instituted in the period of confinement. Special care was dispensed to vegetables and fruits during this period.

d) *Routine check* of blood, urine, sputum and feces was instituted in order to detect early infection. Repeated blood cultures were performed, without waiting for the previous results. Blood cultures for anaerobic agents, fungi and « L » forms of bacteria were likewise performed.

E. TREATMENT OF INFECTION

In three cases of heart transplantation, in spite of the prevention, infection was present in two cases.

1. In Case I there was no infection.

2. In Case II the following infections processes were observed:

a) Staphylococcal pyodermitis in the fingers. This complication appeared 100 and 135 days after transplantation. Serum enzymes increased only when the infectious process was well established.

Treatment was drainage and administration of cloxacillin.

b) Genital herpes simplex.

3. In Case III the following events were observed:

a) The inguinal incision disrupted in consequence of infection. Positive cultures were obtained for *Escherichia coli* and *Proteus vulgaris*, sensitive to cephalotin;

b) Mycotic arteritis of the left femoral artery, followed by rupture and hemorrhage. The femoral artery was ligated;

c) Infection in the inguinal incision persisted, and the patient had septic embolization to the right lung;

d) Suggestive signs of bacterial endocarditis;

e) Necrotizing papilitis;

f) Bilateral pyolonephritis and renal failure;

g) Pulmonary artery thromboembolism, followed by irreversible cardiac arrest.

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LE TRAITEMENT DES INFECTIONS VIRALES PENDANT L'IMMUNOSUPPRESSION *

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Au cours des transplantations, les infections virales sont à craindre, d'autant plus que leur prévention est aussi difficile que leur traitement.

Ce sont avant tout des virus de la famille des Herpès-viridae : Herpès-virus-varicellae responsable autant de la varicelle que du zona, Herpès-virus-hominis à l'origine des différentes formes cliniques de l'herpès, et cytomegalia-virus.

Étant donné l'utilisation de sang au cours des transplantations, le risque d'hépatite sérique n'est pas négligeable.

Mesures prophylactiques et thérapeutiques :

1. Application rigoureuse des techniques d'asepsie et isolement dans une unité stérile ;
2. Sélection du personnel hospitalier.

Dans le domaine de la prévention spécifique, il n'existe pas de vaccin efficace contre ces virus. (suite du résumé en page suivante)

Les conditions actuelles des thérapeutiques immunodépressives font courir à l'opéré d'une greffe d'organe un risque infectieux accru. Outre les infections bactériennes, mycotiques et parasitaires, des infections virales sont à craindre, d'autant plus que leur prévention est aussi difficile que leur traitement.

Chez ces opérés, les virus les plus opportunistes sont ceux auxquels nous ont habitués les malades atteints d'hémopathies ou de réticulopathies malignes traités par corticoïdes et antimitotiques. Ce sont, avant tout, des virus de la famille des Herpès-viridae : Herpès-virus varicellae responsable autant de la varicelle que du zona, Herpès-virus hominis à l'origine des différentes formes cliniques de l'herpès, et Cytomegalia-virus.

C'est ainsi que parmi les neuf malades qui ont subi une transplantation cardiaque à l'Institut de cardiologie de Montréal, l'un d'eux a présenté un zona qui a grandement contribué à assombrir le pronostic. Ce malade a subi la greffe d'un cœur le 26 septembre dernier ; le 16 octobre, est apparue l'éruption érythémato-vésiculeuse d'un zona au niveau de l'hémithorax moyen gauche, deux jours après que des signes de rejet aient nécessité l'utilisation de doses massives de corticoïdes. Les lésions cutanées, très extensives, sont rapidement devenues bulleuses, ecchymotiques, hémorragiques, nécrotiques et, malgré la corticothérapie, elles étaient extrêmement douloureuses. Des vésicules varicelloïdes sont apparues à distance sur les téguments et la muqueuse buccale ; le malade présentait des céphalées, il était fébrile, asthénique, réellement déprimé. Malgré l'isolement en unité stérile et des techniques d'asepsie aussi rigoureuses que possible, en dépit du traitement local et de l'antibiothérapie générale, une surinfection par le Pseudomonas s'est greffée sur les lésions cutanées. À son décès, le 2 décembre, outre les lésions cutanées qui persistaient, il existait de multiples abcès pulmonaires et cérébraux à Pseudomonas et Candida albicans.

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L'utilisation des immunoglobulines a été un échec probablement parce que leurs titres d'anticorps contre le virus varicelle-zona sont constamment très faibles, alors que ces mêmes titres sont suffisants contre les virus de la rougeole, de la rubéole, de la poliomyélite et de la vaccine.

La plupart des inhibiteurs viraux connus à ce jour n'ont qu'un intérêt expérimental. Les interférons n'ont jusqu'alors pas répondu aux espoirs qu'ils avaient suscités, pas plus que les nombreux agents chimiques et les antibiotiques qui ont été expérimentés.

La 5-iodo-2-desoxyuridine (I.D.U.) a été utilisée sans succès dans le zona.

Trois autres greffés ont présenté des éruptions d'herpès périlabial et des ulcérations disséminées dans toute la cavité buccale et sur la langue.

Voici les maladies virales que nous avons observées chez les greffés de l'Institut de cardiologie. Nous savons, par nos collègues d'autres équipes de transplantation, que nous ne sommes pas les seuls à avoir eu à faire face à ces infections par Herpès-viridæ.

Il faut remarquer aussi que les transplantations cardiaques, nécessitant l'utilisation de sang, le risque d'hépatite sérique n'est pas négligeable. Cinq de nos opérés ont franchi le cap de l'incubation minimum de l'hépatite sérique, soit deux mois; aucun d'eux n'a présenté d'hépatite.

Quelles sont les ressources prophylactiques et thérapeutiques que nous avons actuellement à opposer à ces infections virales?

Il va de soi que les méthodes générales de prophylaxie — isolement dans une unité stérile et application rigoureuse des techniques d'asepsie — permettront d'éviter un certain nombre d'infections virales, de la même façon qu'elles protègent contre les infections bactériennes d'origine exogène.

Tout membre du personnel hospitalier atteint ou suspect d'infection virale — syndrome grippal, herpès... — sera évincé de l'entourage de l'opéré, ainsi que tout sujet vacciné contre la variole, durant la phase de contagiosité de la lésion vaccinale. (La fréquence actuelle des voyages exigeant cette vaccination rend nécessaire le rappel de cette précaution.)

Par contre, les méthodes générales de prophylaxie seront sans action sur les infections qui, comme le zona et l'herpès, peuvent être d'origine endogène. Il est très probable, en effet, que le zona soit dû à la réactivation de l'Herpès-virus varicellae demeuré sous une forme inactive dans un ganglion sensitif, depuis la varicelle remontant à l'enfance. Le zona apparaît lorsque la réactivation du virus survient alors que le taux d'anticorps circulant tombe à un niveau trop bas pour neutraliser le virus régénéré.

Le virus du zona, tout comme celui de l'herpès ou de l'hépatite sérique, peut donc pénétrer clandestinement avec le malade dans la chambre stérile, débordant ainsi les méthodes générales de prophylaxie.

Dans le domaine de la prévention spécifique, on sait qu'il n'existe pas de vaccin efficace contre ces virus-ci. On sait également que les immunoglobulines ont été largement essayées, dans la prévention de la varicelle, notamment chez des enfants, contacts soumis à la corticothérapie pour diverses affections. Ces essais se sont soldés par des échecs dont la cause peut être due au fait que, si les immunoglobulines standards utilisées habituellement ont des titres suffisants et assez homogènes contre les virus de la rougeole, de la rubéole, de la poliomyélite et de la vaccine, leurs titres d'anticorps contre le virus Varicelle-Zona sont constamment très faibles. (C'est tout au moins ce qui ressort d'une étude faite sur les titres de ces anticorps dans 26 préparations commerciales d'immunoglobulines, par Netter et ses collègues, au Laboratoire nationale de la santé publique à Lyon.)

Il paraît donc souhaitable que nous puissions disposer d'immunoglobulines spécifiques préparées à partir de sérums de convalescents de zona ou de varicelle. Un projet est en cours à cet effet : il permet d'espérer que nous serons mieux en mesure de prévenir des zonas du type de celui que nous avons observé.

La valeur préventive des immunoglobulines pour l'hépatite sérique est très controversée. Cependant, dans un travail portant sur 1 500 transfusés, Mirrick, en 1965, a trouvé que les immunoglobulines diminuaient de façon significative le risque d'hépatite qui est passé de quatre pour cent à un pour cent.

Quant au traitement des maladies virales chez les greffés, il n'est pas différent de celui des maladies virales en général ; c'est dire qu'il est encore très pauvre. La plupart des inhibiteurs viraux connus à ce jour n'ont, à de rares exceptions près, qu'un intérêt expérimental. Les interférons n'ont pas jusqu'alors répondu aux espoirs qu'ils avaient suscités, pas plus que les nombreux agents chimiques et les antibiotiques qui ont été expérimentés. La-5-iodo-2-desoxyuridine (IDU) a donné de bons résultats dans le traitement de la kératite herpétique, mais elle a été utilisée sans succès dans celui du zona, comme l'a été aussi l'ABOB, un dérivé diguanidé.

Aussi, a-t-on recours aux immunoglobulines à de très fortes doses. Elles n'ont pas entraîné chez notre malade d'amélioration notable. Des immunoglobulines spécifiques auraient peut-être donné de meilleurs résultats, comme c'est le cas dans le traitement des vaccines généralisées.

En somme, il n'est pas facile de protéger les greffés des infections par les micro-organismes les plus opportunistes. Souhaitons que des agents antiviraux et spécifiques efficaces soient découverts, mais nos espoirs vont également vers la découverte de méthodes moins débilitantes et moins déprimantes que celles dont nous disposons actuellement, pour rendre tolérant l'organisme du receveur au cœur greffé.

COMPLICATIONS OF PROLONGED CORTISONE THERAPY *

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Les complications que l'on rencontre avec le traitement aux corticoïdes sont en relation avec le dosage et la durée de la thérapie. Dans le but d'éviter ces complications, de nombreuses approches sous forme de dosages intermittents, doses élevées pour une courte durée, ont été utilisées.

N.B. Les complications en elles-mêmes, présentées en diapositives, n'ont pas été reproduites dans le manuscrit.

In the management of rejection, the value of glucocorticoids as an adjunct to cytotoxic drugs has been well documented (4, 6 and 7). Steroid hormones play a major role in the immunosuppressive therapy utilized by most transplantation centers.

Although the experimental and clinical evidence indicate that glucocorticoids acts in several ways inhibiting the antibody formation and the transplantation immune response (3, 5 and 9), the mechanisms of action of these steroids in producing an immunosuppressive effect, is only fragmentarily understood. However, the use of glucocorticoids in man, for prolonged periods of time, has by no means been free of complications. The incidence of undesirable side effects varies from series to series (1, 2 and 8) (Figure 1), the general consensus being that these complications are related principally to dosage and duration of therapy. In organ transplantation the use of cytotoxic drugs and ALS with their side effects add new problems to the use of glucocorticoids and very often, the need of diminution or suppression of them is life-threatening to the patient. Figure 2 shows the complications due to prolonged glucocorticoid therapy in a patient submitted to cardiac transplantation. Figure 3 refers to a post-transplantation lung syndrome occurred in the 95th postoperative day with good remission (clinical and radiological) after reassuming cortisone treatment. In the search to avoid complications due to the use of glucocorticoids various approaches have been attempted: intermittent dosage; large doses for short terms. Reduction of dosage has its schemes according to experience of different centers.

Advances in biochemical and other investigative technics focus the influence of adrenal corticoids on metabolic functions of single cell and their constituents. Furthermore, actions of glucocorticoids must be considered in the light of their ability to

Figure 1 — Complications of prolonged treatment with corticosteroids (six months to twelve years).

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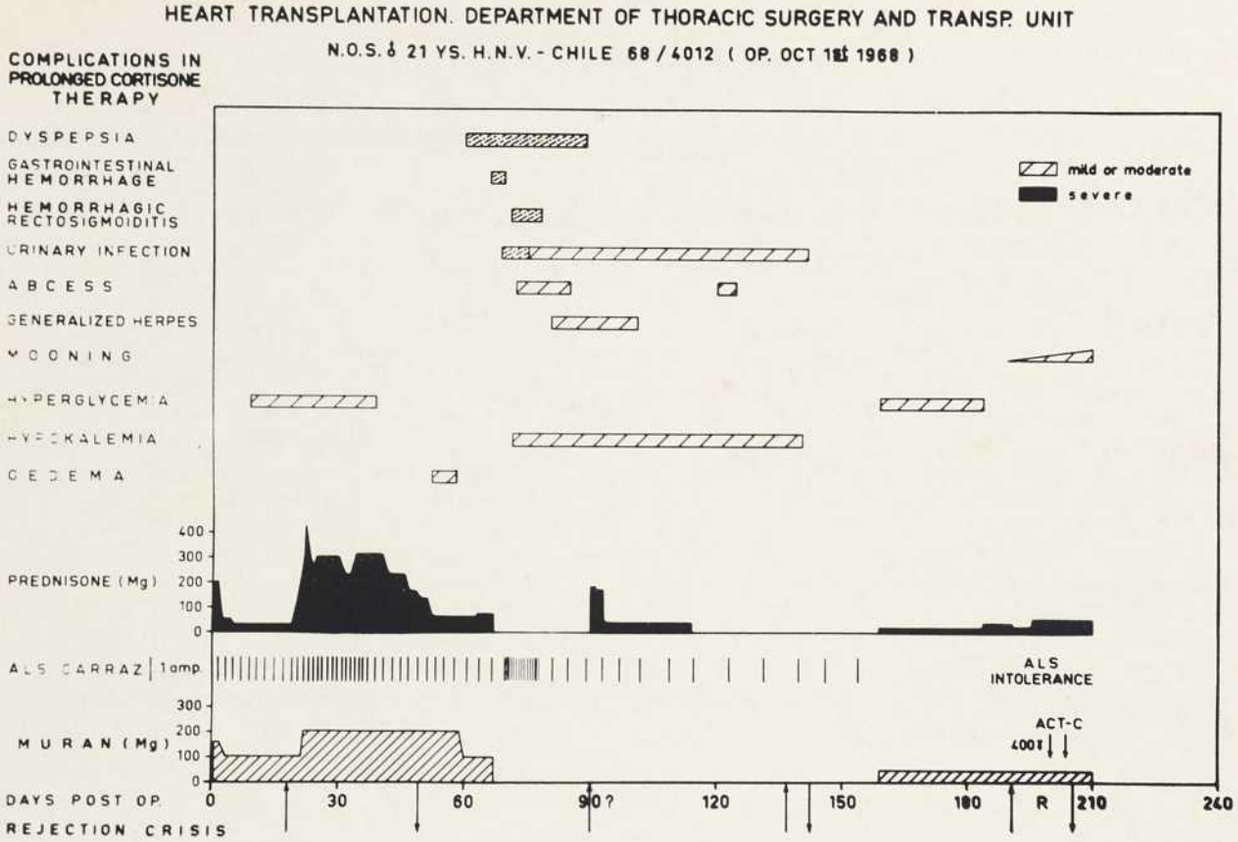

Figure 2 — Complications in prolonged cortisone therapy

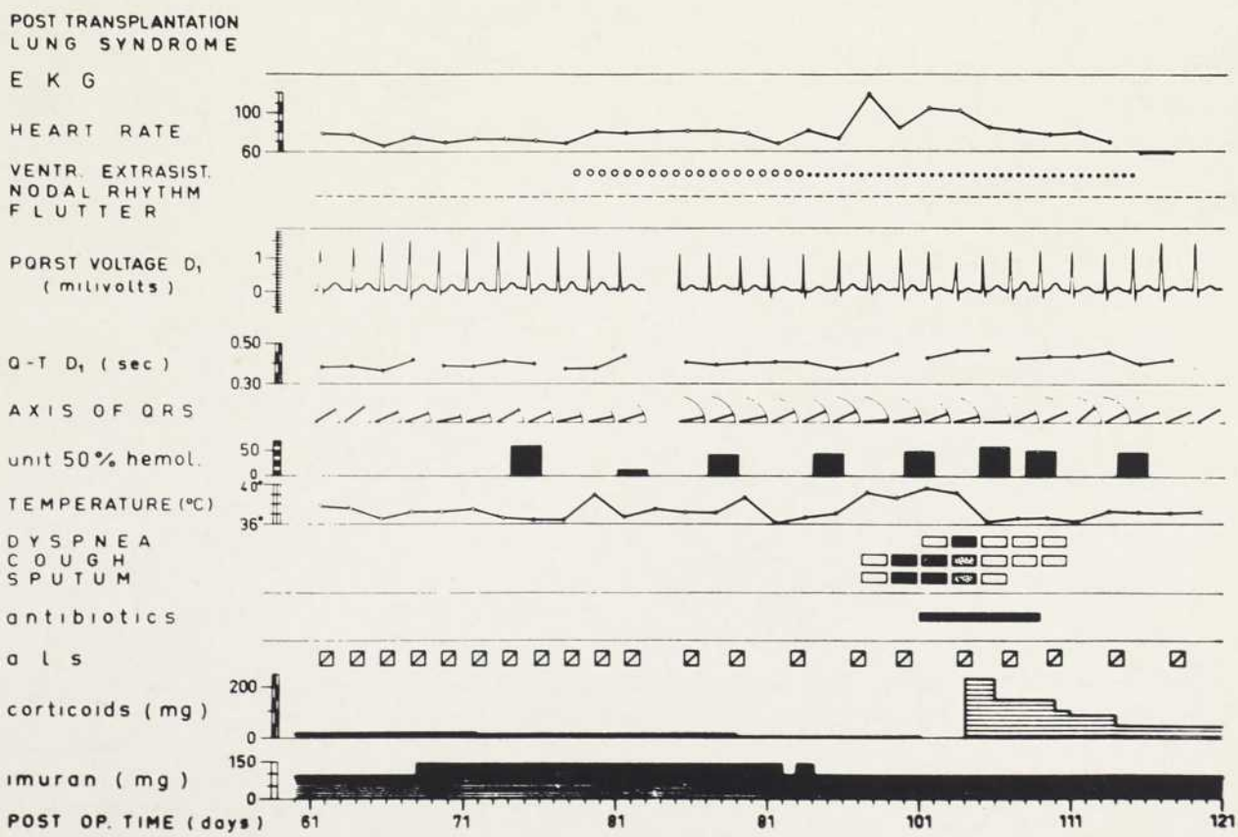

Figure 3 — Post-transplantation lung syndrome

stimulate molecular functions in certain cells while simultaneously inhibiting those of other cells. These investigations, undoubtedly will be of great value in finding drugs with greater potency and fewer undesirable effects to be used in transplantation of organs.

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TOXICITY OF AZATHIOPRINE *

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L'azathioprine (Imuran), substitut dérivatif de la 6-mercaptopurine (6-MP), constitue l'agent immunosuppressif de base pour les transplantations rénales chez l'homme.

Pharmacologie. L'azathioprine est très facilement réabsorbée par le tractus gastro-intestinal. Sa voie d'élimination est rénale. Sa période d'action est d'environ quatre jours. Dans la transplantation d'organes, la dose sera de 4 mg/kg au cours des quatre à sept premiers jours. Après quoi, la dose sera réduite à trois mg par kg de poids.

Mécanismes biochimiques de la toxicité. Les manifestations cliniques de la toxicité de l'azathioprine sont parrallèles à celles de la 6-MP. Il y a deux modes d'action : d'une part la transformation de la 6-MP en 6-MP-ribotide, qui bloque de novo la synthèse des purines, et d'autre part l'activité de neuf substituts de la 6-MP.

Toxicité hématologique. La complication la plus souvent rencontrée est la leucopénie. La dose d'azathioprine sera en fonction de cette formule leucocytaire. La vitesse avec laquelle les leucocytes diminuent est
(suite du résumé en page suivante)

INTRODUCTION

In 1951, Elion, Hitchings, and Vanderwerff (21) reported that 6-mercaptopurine (6-MP), which had been synthesized as a part of a systematic search for purine antagonists, inhibited the growth of bacteria in culture. In 1958, Schwartz, Stack, and Damashek (52) demonstrated that 6-MP prevented the formation of antibodies to bovine serum albumin in rabbits. Within two years, it was discovered that it would prolong the survival of skin allografts in rabbits (39 and 51) and renal allotransplants in dogs (5 and 59). In 1961, it was reported that a renal homotransplant in a bilaterally nephrectomized dog treated with 6-MP had survived for over one year (46). As the result of these studies, the major modality of immunosuppression for organ transplantation in man was changed from irradiation to chemotherapy. Unfortunately, 6-MP depressed hematopoiesis, caused jaundice and produced denudation of the epithelium of the intestinal tract in experimental animals (9 and 44) and these complications were frequently observed at dosage levels used for the prevention of rejection (6, 46 and 60). Azathioprine (Imuran), the S-imidazolyl substituted derivative of 6-MP (Figure 1), was less toxic and immunosuppressive at lower doses in mice (1 and 20). In dogs, it also appeared to be more effective than 6-MP in prolonging the survival of renal allotransplants (11) and it was subsequently universally adopted as the basic immunosuppressive agent for renal allotransplants in man. As the result of extensive clinical experience, it has been established that at

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plus importante que son nombre absolu. Lorsqu'il existe une leucopénie, il faudra prendre des précautions en vue d'éviter les infections. On donnera de l'acide folinique et des préparations multivitaminiques. La thrombocytopénie est rare en l'absence de leucopénie comme complication de la thérapie à l'azathioprine.

On surveillera également le nombre des globules rouges et le taux d'hémoglobine.

Toxicité hépatique. Elle se caractérise par l'apparition d'un ictère dans 20 pour cent des homotransplantations rénales.

Toxicité gastro-intestinale. Les doses élevées provoquent de l'anorexie, perte de poids et diarrhée. On rencontre également des ulcères des membranes muqueuses, des lèvres, de la langue et de la bouche.

Les effets duodénaux que l'on rencontre sont attribués à la thérapie aux corticostéroïdes.

Sensibilité accrue pour les tumeurs malignes. L'azathioprine, en association avec la Prednisone, favorise l'implantation de métastases et de tumeurs malignes transplantées avec l'organe. Ces complications peuvent être évitées en sélectionnant les donneurs et receveurs. Même dans ce cas, on a rapporté que l'azathioprine et les globulines antilymphocytaires

therapeutic levels azathioprine also has significant toxic effects, but that careful management can largely eliminate deaths from these effects.

Pharmacology

Azathioprine is readily absorbed from the gastrointestinal tract. The sodium salt which is available for investigational use may be administered intravenously. Once absorbed, the imidazole group is split off to yield 6-MP which is probably responsible for most if not all of the toxic and immunosuppressive actions of the drug, and because of this, data on both drugs will be discussed. This cleavage is promoted by sulfhydryl compounds and not enzymatically mediated (18). The drug has a relatively long period of action, approximately four days. Cumulation of successive doses over this period readily occurs and unless this possibility is appreciated, a toxic level of drug may easily be reached. Also, when azathioprine is discontinued because of leukopenia, it is often four days before an appreciable recovery of the leukocyte count is observed.

The chief route of elimination of azathioprine is in the urine. In man, 13.0 to 21.4 per cent of an oral dose was excreted as 6-thiouric acid, 1.3 to 2.4 per cent as 6-MP, and 0 to 2.25 per cent unchanged in 24 hours (18). In addition, approximately 7 per cent is excreted as 5-mercapto-1-methyl-4-nitroimidazole in 24 hours (8). Because the urine

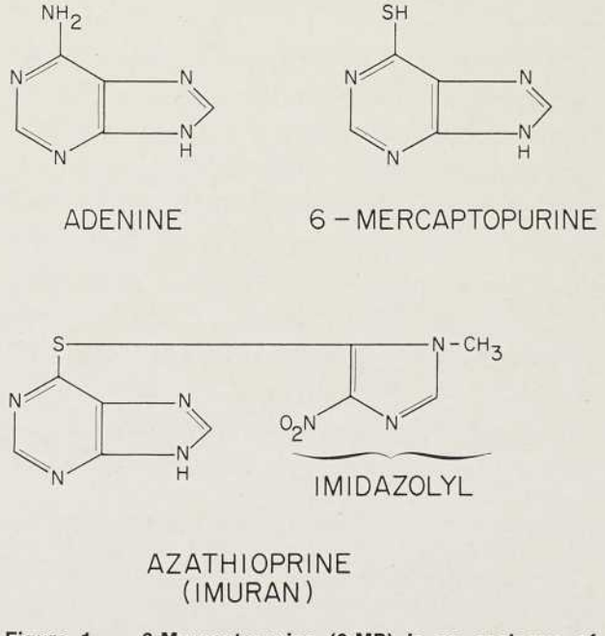

Figure 1 — 6-Mercaptopurine (6-MP) is an analogue of adenine. Azathioprine (Imuran) is an analogue of 6-MP and after absorption, the imidazole moiety is cleaved to release 6-MP.

pouvaient en effet promouvoir l'induction de tumeurs malignes inhérantes à la thérapie immunosuppressive. La fréquence de ces tumeurs spontanées n'est pas suffisamment large pour abandonner la thérapeutique, mais elle souligne l'importance d'une surveillance continue de ces patients.

Toxicité du système reproducteur. L'azathioprine a un effet tératogénique sous forme de rupture des chromosomes et autres anomalies.

Complications squelettiques. On rencontre des nécroses aseptiques de la hanche, du genou et de l'épaule, ostéodystrophie, ostéoprose et autres lésions squelettiques. Il semble que ces lésions squelettiques soient en relation avec la maladie rénale, l'hyperparathyroidisme secondaire et la thérapie corticostéroïde, plutôt qu'avec la toxicité de l'azathioprine.

Sensibilité accrue pour les infections. Celle-ci est en relation avec la suppression des mécanismes immunologiques cellulaires et humoraux. L'administration concomitante des corticostéroïdes, notamment aux doses élevées, augmentera fortement cette sensibilité. S'il se développe une infection en l'absence d'une dépression de la moelle osseuse, insuffisance rénale ou atteinte hépatique, la dose d'azathioprine sera maintenue aux doses thérapeutiques. Le traitement de l'infection bactérienne se fera avec un antibiotique à spectre étroit, étant donné le développement de mycoses avec les antibiotiques à large spectre.

is the principal route of excretion of azathioprine and its degradation products, the dose should be reduced if renal function is impaired. Also when allopurinol is given concomitantly, the dose of azathioprine should be reduced because the conversion of 6-MP to 6-thiouric acid, which is one of the chief degradative pathways, is blocked (19, 49 and 57).

For immunosuppressive in organ transplantation, the dosage schedule of azathioprine which we employ is 4 mg per kg which is given for four to seven days after which it is tapered to approximately 3 mg per kg. The dose is then adjusted according to the patient's leukocyte count. By 120 days after transplantation, 33 per cent of the patients are receiving from 2.28 to 2.87 mg per kg, 33 per cent of patients are receiving less, and 33 per cent are receiving more.

Biochemical mechanisms of toxicity

The clinical manifestations of toxicity of azathioprine closely parallel those of 6-MP and it is likely that they result in a large part, if not entirely, from the release of 6-MP by azathioprine. At the biochemical level, there are two possible pathways observation that 6-MP binds tightly to RNA (29). of action of 6-MP (45). In the first of these, 6-MP is initially converted to 6-MP ribotide and in the second, this step is not required. In the first pathway the 6-MP ribotide blocks purine *de novo* synthesis at a number of sites, the most important of which are the initial step in purine synthesis, the formation of phosphoribosylamine from phosphoribosylpyrophosphate and glutamine; the conversion of inosinate to succinoadenylate; and the dehydrogenation of inosinate to form xanthine-monophosphate (17). A second possible pathway of action of 6-MP has been suggested by the observation that 9-substituted 6-MP's are still active (30, 33 and 34). Although one explanation for the activity of these analogues has been that they were converted to 6-MP, 9-arabinosyl-6-mercaptopurine, prolonged skin allograft survival in rats despite the absence of 6-MP and its breakdown products in the urine (35). The activity *in vivo* of these 9-substituted 6-MP derivatives may be related to the When the SH group of 6-MP was blocked by a methyl group, binding was prevented.

Hematological toxicity

The most common complication of azathioprine therapy is leukopenia (leukocyte count less than 2,000 per mm^3) which occurred one or more times in 40 per cent of the renal homotransplant recipients at the Medical College of Virginia. The occurrence of leukopenia in patients with a blood urea nitrogen greater than 30 per cent which usually indicated that significant rejection had occurred was a particularly ominous sign because less than 30 per cent of these patients were alive with functioning transplants four months later. Terminal depression of the bone marrow characterized by severe leukopenia, thrombocytopenia (platelets less than 100,000 per mm^3), and decreased reticulocytes (less than 1.5 per cent) occurred in 22 per cent of leukopenic patients. This usually occurred in patients with significant pre-existing infections who were receiving doses of prednisone greater than 30 mg per day. Severe rejection reactions occurred in other leukopenic patients when azathioprine was discontinued, especially if previous rejection episodes had already damaged the transplant. In still other patients leukopenia was only one manifestation of a debilitated state associated with progressive downhill course. The first episode of leukopenia was most likely to occur during the initial two months after transplantation (50 per cent of the patients) but did occur as late as 18 months after transplantation. It was twice as likely to occur in splenectomized as in unsplenectomized patients, but it was no more likely to occur in recipients of kidneys from living related donors than in recipients of cadaver kidneys.

The management of leukopenia consists first of all in preventing a fall to dangerous levels by daily monitoring of the leukocyte count and tapering the dose of azathioprine gradually as the leukocyte count falls. This might have prevented the leukocyte depression illustrated in Figure 2. The rate of fall of the leukocyte count is more important than the absolute level. Many patients have been maintained on azathioprine for years with leukocyte counts less than 5,000 per mm^3 except when infection or operation stimulated a leukocytosis. When the leukocyte count falls as much as 40 per cent in one day or to a level below 3,000 per mm^3 it is advisable to discontinue azathioprine entirely. The patient is placed on gown and mask reverse precautions when the leukocyte count falls below 2,000 per mm^3 and in a sterile room (48) when the leukocyte count falls below 1,000 per mm^3. The lowest total leukocyte count which a patient has had followed by recovery was 225 per mm^3. In addition to a standard multivitamin preparation, it is also our custom to administer folinic acid to severely leukopenic patients because increased urinary formiminoglutamic acid after histidine administration has been observed in a leukopenic renal transplant recipient on azathioprine who had normal serum folate and vitamin B_{12} concentrations suggesting that reduction of folic acid was blocked (40).

Thrombocytopenia of a significant degree rarely

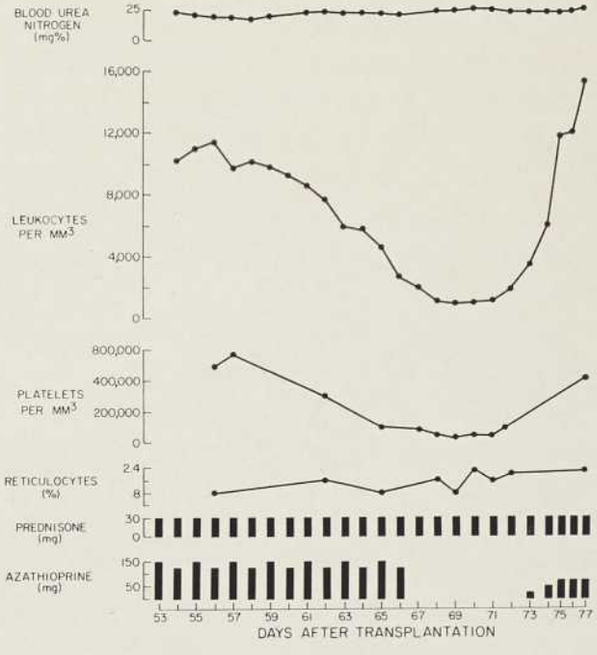

Figure 2 — Leukopenic episodes such as the one illustrated in this renal transplant recipient usually respond promptly to a decrease or discontinuance of azathioprine. If the organ transplant is functioning well as in this case, the azathioprine may be discontinued temporarily without precipitating rejection, but if it has already been impaired by rejection, the transplant or the patient is often lost. Although thrombocytopenia often accompanies leukopenia as in the case illustrated here, it rarely occurs in the absence of leukopenia as a result of azathioprine toxicity.

if ever occurs in the absence of leukopenia solely as the result of azathioprine therapy.

Anemia (Hb less than 11.0 g per 100 ml) occurred in 18 per cent of kidney recipients with normal renal function who were more than four months after transplantation. One of these patients who had a megaloblastic anemia responded dramatically to treatment with folinic acid and not with folic acid, but the remaining patients failed to respond to either drug. Cautious reduction of the azathioprine dosage may be helpful in this latter group.

Hepatic toxicity

In toxicity studies in normal animals, 6-MP caused hepatic necrosis in rats and intracanalicular bile stasis, jaundice, and bromsulphalein retention in dogs (44). In rats, 6-MP increased the urinary excretion of d-amino levulinic acid and coproporphyrins and it also increased the d-amino levulinic acid and or porphobilinogen in the liver (23). Eales observed significant disturbances in porphyrin metabolism in two heart transplant recipients, both of whom were receiving azathioprine (15).

In adults with acute leukemia treated with 6-MP, the incidence of jaundice was 34 per cent. At postmortem examination, a variety of lesions were found in different patients: bile stasis in canaliculi, hemosiderosis, cirrhosis, periportal fibrosis, central fatty changes, infiltration with leukemic cells or tumor, and hepatoma (4). In another study of leukemic patients, 42 per cent developed jaundice while taking 6-MP, but only 9 per cent developed it who were not receiving it (16). In renal homotransplant recipients at the Medical College of Virginia the incidence of hepatitis was 20 per cent. The incidence in recipients of cadaver transplants, however, was 35 per cent while in recipients of kidneys from living donors, it was only 8 per cent. Because cadaver kidney recipients were often on dialysis for prolonged periods of time while awaiting a suitable kidney, they received more blood transfusions than recipients of kidneys from living related donors and were, therefore, more likely to be exposed to serum hepatitis. Also the possibility of transmission of hepatitis by the transplant is greater when the donor is a patient dying from a head injury from whom a complete medical history may be unavailable. Both recipients of kidneys from a common cadaver developed jaundice in two instances in our series. Frozen section examination of the cadaver liver would probably have detected hepatitis which was not evident upon gross examination.

The extent to which azathioprine and 6-MP directly cause liver disease and the extent to which they predispose to viral infections which in turn cause liver damage is unknown. Regardless of the exact mechanism, it is necessary to reduce or discontinue these drugs in the face of impaired liver function. The bromsulphalein test has been the most sensitive mode of detecting dysfunction and the serum total bilirubin and serum glutamic oxalacetic transaminase levels have proved useful in following such patients subsequently. When the function of the transplant was good, *i.e.* when it was not undergoing chronic rejection, it was possible to stop azathioprine for periods up to eight months without precipitating rejection of the organ in such patients. However, if the function of the kidney was impaired as the result of chronic rejection, discontinuation of azathioprine in the face of jaundice usually resulted in early rejection of the kidney just as in leukopenic patients. After recovery from hepatitis it was often necessary to reduce the maintenance dose of azathioprine to the range of 25 to 50 mg per day for many months to avoid recurrence of jaundice.

Gastrointestinal toxicity

Large doses (6.3 to 50 mg per kg per day) of 6-MP in dogs caused anorexia, weight loss and diarrhea. There was extensive denudation of the epithelium, capillary congestion in the tips of denuded villi, and leukocytic infiltration throughout the mucosa in the small intestine. In the large intestine, there was only moderate glandular atypia and some mucosal hemorrhages (9 and 44). In adults with acute leukemia treated with 6-MP at dosage levels of 4.0 mg per kg or more, gastrointestinal symptoms of anorexia, nausea, and vomiting were common (16 to 32 per cent of patients).

But with lower doses and in children, the incidence was much lower. Stomatitis occurred in 24 per cent of patients (28). At the doses of azathioprine used in renal homotransplant recipients (up to 4.0 mg per kg) anorexia, nausea, vomiting, and diarrhea have not been significant problems in the absence of other complications. Ulcers of the mucous membranes of the lips, tongue, and mouth have been frequent problems, however. When these have been extensive they were usually accompanied by denudation of the esophageal epithelium which on occasion involved virtually its entire surface.

Duodenal ulcers have occurred in 22 per cent of renal homotransplant recipients at the Medical College of Virginia who did not have a prior history of ulcer (36), but this complication is probably attributable to the corticosteroid therapy which these patients also received rather than to azathioprine. Other lesions of the small intestine and colon have been insignificant. Fatal pancreatitis has occurred in one patient in the Medical College of Virginia series and two cases have been reported (31 and 55). Pancreatic pseudocysts occurred in three of 55 patients reported by Ginn (24).

Increased susceptibility to malignant tumors

Azathioprine together with prednisone has very likely promoted the transplantation of malignant tumors in organs transplanted from patients dying of tumors (37 and 38). The effectiveness of immunosuppressive therapy in promoting the implantation and metastasis of malignant tumors transplanted with organs was confirmed in two other instances in which the tumors were rejected along with the organ transplant when immunosuppressive therapy was discontinued (24, 58 and 61). Azathioprine may also contribute to the recurrence of "naturally" occurring neoplasms in transplant recipients (31 and 53).

Although these untoward effects of azathioprine can be avoided by selecting donors and recipients who have not had cancer, there have been recent reports suggesting that azathioprine and antilymphocyte globulin may actually promote the induction of malignant tumors and that this is inherent in all immunosuppressive therapy. Both 6-MP (14) and azathioprine (7) have been reported to induce lymphomas in mice. A malignant primary lymphoma of the lung developed in a cadaver kidney recipient in our series two years after transplantation, who had received azathioprine, corticosteroids, and actinomycin C, but not antilymphocyte serum. Eight other cases of lymphoma occurring in renal homotransplant recipients while on immunosuppressive therapy have been reported (12, 13 and 43). In addition, one of our patients developed a carcinoma *in situ* of the cervix three years after receiving a renal homotransplant from her sister. Her immunosuppressive therapy consisted of azathioprine, corticosteroids, actinomycin C, and local irradiation of her kidney. A squamous cell carcinoma of the ear occurring two and one half years after transplantation (31) and an anaplastic carcinoma of the lung occurring seventeen months after transplantation (61) have also been reported. The incidence of spontaneous tumors in these recipients on immunosuppressive therapy is not large enough to negate the present therapeutic advantages of renal transplantation. They do indicate the importance of a continuous surveillance of these patients have been apparently cured by timely treatment (12, 31 and 43).

Reproductive system toxicity

When administered to pregnant rats early in gestation, azathioprine caused a high percentage of fetal reabsorption and those fetuses which survived were usually severely stunted. Although azathioprine was less toxic than 6-MP for the mothers, it was more toxic for the fetuses (54). Azathioprine was also teratogenic in mice (25), rabbits (56), and dogs (41). In patients treated with azathioprine, chromosome breakage and other abnormalities were observed in bone marrow aspirates (32), and in patients treated with 6-MP, polyploidy and chromatid breaks were observed in peripheral blood leukocytes (42). These observations suggest that azathioprine is teratogenic in man as well. Nevertheless, two patients in our series, of whom one has been reported (2), carried infants to term with no

interruption of azathioprine therapy. The children were completely normal at birth and have continued to develop normally, one and three years later. One patient had a miscarriage at three months. In addition, eight normal children have been sired by fathers who were on azathioprine and prednisone. Despite these and normal offspring of patients in other series who were conceived while one of the parents was receiving azathioprine therapy, it would still seem prudent to warn patients of the potential teratogenic effects of azathioprine.

Skeletal complications

Aseptic necrosis of the hip, knee, and shoulder, osteodystrophy, osteoporosis, and other skeletal lesions frequently complicate renal homotransplantation (11, 26 and 27). In weanling rats, azathioprine caused irregularity of the cell columns, an extended hypertrophied zone, and cellular distortion in the epiphysial plate of the proximal tibia, but it did not significantly increase slippage of the plate when it was subjected to shear stress (3). These skeletal lesions in patients are probably related to renal disease, secondary hyperparathyroidism, and corticosteroid therapy rather than to azathioprine toxicity.

Increased susceptibility to infection

Because of the capacity of 6-MP and azathioprine to suppress both cellular and humoral immunological mechanisms — see the excellent review article by Schwartz (50) — infections by bacterial, fungal, and viral organisms frequently complicate the use of these drugs (10, 22, 24, 31 and 47). The addition of corticosteroids, especially in high doses, greatly increases susceptibility to infection. Broad spectrum antibiotics such as the tetracyclines and chloramphenicol are particularly likely to induce overgrowth of fungal organisms in the gastrointestinal tract with subsequent systemic dissemination and, therefore, treatment of bacterial infections is limited to antibiotics with a narrow spectrum of activity whenever possible. If bone marrow depression, or renal failure, or liver dysfunction do not occur simultaneously with an infection, and if the invading organism is sensitive to an antibiotic, the dose of azathioprine is usually maintained unchanged during treatment.

SUMMARY

Azathioprine (Imuran) is cleaved to 6-mercaptopurine (6-MP) after absorption and therefore the toxic manifestations of these two drugs are very similar. In organ transplant recipients, increased susceptibility to infection is the most serious untoward effect of these agents. More specific toxic effects include: leukopenia, which occurs in 40 per cent of renal homotransplant recipients; thrombocytopenia; normocytic and macrocytic anemia; hepatitis which occurs in 20 per cent of recipients; and ulceration of the mucous membranes of the lips, tongue, mouth, and esophagus. In addition, azathioprine has promoted the metastasis of tumors which were transplanted together with renal transplants and it appears to have induced lymphomas *de novo* in some recipients. Although there have been no reported cases, experiments in animals and the observation of chromosome damage in man suggest that it may also be teratogenic. The incidence of toxic manifestations may be reduced by careful adjustment of dosage in relation to the total leukocyte count and by the use of lower doses in patients with renal failure and patients receiving allopurinol.

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COMPTE RENDU DE LA DEUXIÈME RÉUNION PLÉNIÈRE — PROCEEDINGS OF THE SECOND GENERAL SESSION :

— Role of ALG in heart transplantation ;
— Clinical and laboratory diagnosis of rejection ;
— Infection and other complications of immunosuppression.

Chairman : Denton COOLEY, Houston.
Reporters : H. E. TAYLOR, Ottawa, and Gilles LAMOUREUX, Montréal ; Edward B. STINSON, Palo Alto, and Ihor DYRDA, Montréal ; Donald ROSS, London, and André LEDUC, Montréal.
Transcript Editor : Gilles LEPAGE.

We will first call on Doctor Taylor and Doctor Lamoureux to report on the session regarding the role of ALG in heart transplantation.

Doctor Taylor:

Doctor Cooley, ladies and gentlemen. I feel rather like a medical student right now, my mind is full of facts but I am not sure how they are going to drop-out in this oral exam.

It really is very difficult at the present time to scientifically evaluate the effectiveness of ALG as an immunosuppressive agent since we have as yet no method of accurately standardizing it. One of the most urgent problems to be solved is the development of an *in vitro* or *in vivo* method of defining the potency of ALG and thus to develop a standard international unit which would allow valid comparisons between one center and another. Another difficulty is related to the variety of antigens used for its preparation. This was discussed by several people this morning. On the one hand Doctor Lamoureux, from the answers to a questionnaire that he had distributed to all centers doing heart transplants, came up with figures that suggested an ALG prepared either against human thymus cells or against thoracic duct lymphocytes might be more effective than ALG prepared against other lymphoid tissues. On the other hand, Doctor Starzl reported experiments in his laboratory where his model was the intra-abdominal heart transplant in which he used ALG prepared from three antigens, one using spleen, one using the spleen and lymph-node cells, and the third one using thymus. And he found no difference in the survival time of the heart transplant as far as these three were concerned and concluded that the type of antigen may not be quite as important as we presently think. However, contamination of the antigenic material with other cells, such as red cells and platelets, will of course give rise to antibodies against these two cell types and therefore one may encounter the problem of having a high titer hemaglutinin or a high titer anti-platelet antibody which would result in the production of hemolytic anemia and/or thrombocytopenia. I gathered the general impression, from the clinicians who have used ALG in significant number of patients, that thrombocytopenia certainly may occur, particularly if the titer is over 1/32. In the Medical Research Council of Canada ALS program about which I may speak briefly later, we now have a highly immunosuppressive ALG prepared against human thymocytes. This had a lymphocytotoxic titer of 1/9 000 and after fractionation, absorption with red cell stroma, ultraconcentration and lyophylization, it still had a hemaglutinin titer of 1/256. Doctor Starzl did the platelet antibodies for us and this was 1/32. This particular product has been highly immunosuppressive in the hands of Doctor Balner when tested in both the speciosa monkey and the chimpanzee. In the latter it significantly prolonged the four allografts from other chimps and one human heterograft. However the

chimpanzee on the 15th day had a hematocrit of 26 and a platelet count of 40 000. Although this did not come out in the meeting this morning, I have been told by other colleagues that one cannot positively make a correlation between the effect of these agents in the chimpanzee directly to the human. Doctor Van Bekkum did speak of this this morning and he believes that there is some degree of correlation although further experimental work has to be done along that particular line. So I think we can conclude then, as far as antigen is concerned, that the contamination of these other cells is a very important factor in the methodology used to get the most pure form of cell or cell fraction and there is evidence, again not discussed this morning, that the cell membrane fraction may be possibly the best antigen.

Speaking of the species of animals used to produce the ALG, horses are the common ones, simply because they give masses of serum and also are good antibody producers. Other workers have used rabbits, goats, sheep, and cows. And I think the important point concerning this is to have sufficient quantities of ALG prepared in a variety of species so that if one encounters the clinical problem of serum sickness or anaphylaxis, then one can immediately change over to one of the other species ALG.

Various protocoles for immunization have been used for a considerable length of time. I think the groups that are interested in the mass production of ALG were perhaps influenced by the work of Medawar and Levey with the two-pulse method and their statement that any ALG produced with Freund's adjuvant was likely to be toxic. This does not seem to be borne out in the human work. I think that everybody now agrees that the most effective ALG can be produced by the initiation of immunization with adjuvant therapy, followed by intravenous whole cell or membrane fractions.

Methods of fractionation were discussed at some length by Doctor Carraz and here again there is a tremendous variation from one center to another. In his earlier work, he found the ammonium sulphate method of preparing his gammaglobulin gave him a very sound immunosuppressive product. On the other hand we know that DEAE-sephadex methods, either the batch or columns method, also give a very sound immunosuppressive agent. And from this general discussion, without too much chemistry involved, on the preparation of ALG, it became obvious that the immunosuppressive activity from the stand point of prolongation of skin grafts is largely in the IgG fraction but also in the T equine fraction. Now this had not been recognized and therefore, at one stage in the development of ALG, there was a tendency to over-purify and so to speak, throw the baby out with the bath water. With the present methods of using batch DEAE-sephadex and two fractionations you do end-up with a 90 per cent pure gammaglobulin IgG and T fraction. It is interesting that the mass producers such as Connaught Laboratories and the Institute of Microbiology who have been making tetanus and diphteria antitoxin for years, have known this. In fact Cinader showed this some years ago with tetanus antitoxin. When you acutely immunize a horse, you tend to have almost all your immunosuppressive activity in the IgG fraction. When you chronically immunize and drain-off by plasmaphoresis over a period of months or even years, as in the case of tetanus antitoxin producers, then you get a shift towards the T fraction. Doctor Starzl pointed this out in a paper originally given I think in Vancouver at the Royal College meeting and he emphasized this point again today. So I think it is of some importance that in fractionation, one must be aware of losing the immunosuppressive activity by over-purification.

We came to the different therapeutic regimens that have been used in the various centers. And again there is a great deal of controversy. I gathered from the papers given by Doctors Treager, Starzl and Brendel, that long term maintenance therapy is probably a must. I think each of these speakers emphasized the fact that if you stop ALS, an escape occurs very rapidly from its effect. Doctor Treager, in experiments using skin allografts, showed us very nicely that as long as

he maintained the dose, the skin allograft was beautiful. Stop the ALG therapy and very rapidly the rejection phenomena set-in. For heart transplantation, it became very obvious that ALG is probably a must in the immunosuppressive therapy of such patients. This may not necessarily apply as far as renal transplants are concerned. The intravenous versus the intramuscular methods of introducing the serum were discussed by Doctor Brendel and also by Doctor Monaco from the audience. Doctor Brendel is convinced that the intravenous method is the one of choice: a high dose can thus be given rapidly and painlessly. Doctor Barnard also spoke of his own patients in South Africa who had been treated in this manner for rejection crises.

The synergistic effect of the other immunosuppressive agents, Imuran and the steroids with ALG led, not to a controversy, but to some differences of opinion. It has always been assumed that ALG and cortisone worked synergistically, but Doctor Lamoureux showed preliminary experiments that suggested that administration of cortisone with ALG may in fact not be synergistic in the mouse skin allograft model. In his experiments anyway, the survival time of the grafts when he used cortisone and ALG together was shorter than with ALG alone. However, this was not agreed to by other members of the panel.

This brings up the next problem, that of the toxicity of ALG. In relation to this, we have already mentioned the effect upon the hemopoietic system, particularly platelets, with thrombocytopenia, the possibility of a bleeding tendency, purpura, internal hemorrhage, and so on. This can be overcome by adequate absorption of the ALG by using platelets, a difficult problem. You can also diminish significantly the hemaglutinins by absorbing with either whole red cells or red cell stroma. Now theoretically serum sickness should be a real problem: we are chronically administering to a patient a foreign serum protein and yet, for some peculiar reason, this does not seem to have reared its ugly head in the clinical use of ALS over long periods of time. It has been reported that one can demonstrate a binding of the horse gammaglobulin to various basement membranes, particularly the glomerular basement membrane and yet I gathered this morning that so far, there have been no significant examples of serum sickness nephritis. Is this related to the simultaneous immunosuppression using steroids and Imuran along with the ALG? This I do not think anybody can answer at the moment. When one thinks of giving the large doses that Doctor Brendel spoke of intravenously, where you have a mass of antigen confronting your immune system, one would suspect, one would expect in fact, that one would rapidly get immune complexes and deposition of these complexes when you get certain states of equivalence between antigen and antibody, and this should give rise to the lesions of serum sickness. These are the classical experiments of Frank Dixon many years ago. And yet I understood that, while you can demonstrate by immunofluorescence methods the binding of the protein to basement membranes, this does not seem to be of any significant pathogenetic significance at the moment.

No one mentioned neoplasia this morning. However this is a real hazard and at the Brook Lodge ALS conference held about a month ago, there was given incontrovertible evidence that ALS or ALG in experimental models not only will increase the incidence of transplantable tumors or virus induced tumors, but that spontaneous tumors will appear in highly significant numbers in animals usually not susceptible to the appearance of tumors. In my own laboratory, we have a transplantable myeloma tumor. If you put in a fragment of the tumor, it grows beautifully. But the injection of a suspension of the myeloma cells showed no apparent growth. Then by accident, testing ALS for another purpose, we used these animals that apparently were not producing tumors, they were given two doses of ALS a week apart and on the 21st day, the tumors at the site of the original injections were 1.25 cm in diameter. So here is evidence that ALG can indeed stimulate a latent tumor to become typically malignant and invasive. This is somethting that we have to bear in mind.

I know Doctor Starzl and his group have drawn together certain figures from around the world and I think that one has to accept this as a very definite hazard in the long term use of ALS.

I would like to conclude, Mister Chairman, with just a few remarks about our own Canadian program and this is based on my starting premise here that you cannot at the present moment scientifically compare anything that is going on in one center with that in another. A committee of the Medical Research Council was formed about a year and a half ago and they drew up a careful protocol. Last summer we started to prepare for this clinical trial using renal transplantation as the method of assessment. What we are attempting to do is to produce a common pool of ALG that has met all the criteria that we can presently use such as lymphocytotoxicity, various *in vitro* tests, and the Balner chimpanzee test (in other words standardizing at least on the basis of the available *in vitro* and *in vivo* tests) and then utilize this standard product in 100 patients with renal transplants and compare these with another group of patients who will not receive ALG. We will immunize 24 horses, starting one group of 12 here at the Institute next week and then another group in Connaught Laboratories. Human thymus will be used as antigen. This will give us a pool of some 500 liters of crude ALS which will be tested for cytotoxicity. Satisfactory samples will then be pooled, absorbed and fractionated and finally tested by Balner. This mass production of a common pool of ALG has raised many problems, however it should be ready for the clinical trial early in 1970.

Doctor Cooley:

Thank you Doctor Taylor, would Doctor Lamoureux care to add anything to this? No? I have been impressed personally with Doctor Kahn's results in three consecutive myocardiopathy patients in which he did not use ALG. Is there any significance to the fact that his program did not include ALG, did it have any relationship to the success of his cases?

Doctor Taylor:

Mister Chairman, this was mentioned by Doctor Sarzl. I had posed the question: in what way can we prove that ALG is really effective anyway? Doctor Starzl pointed out there are many experimental models and now human cases, particularly in the kidney transplantation field, in which there is a great deal of argument. Should you use ALG anyway? They are doing fine without it. However the general impression was that the cardiac transplant really should have ALG and in particular, of course, if there is any evidence of any rejection. I believe there was no sign of any rejection at any time in those three cases Doctor Kahn mentioned yesterday.

Doctor Cooley:

Any question or contribution from the floor? I would like to announce the arrival of two other outstanding surgeons in this field. I see Doctor C. Walton Lillehei, from New York, and Doctor Henry Bahnson, from Pittsburg, have just arrived.

The next report will be that of Doctor E. B. Stinson from Palo Alto and Doctor Dyrda, from Montreal, on clinical and laboratory diagnosis of rejection.

Doctor Stinson:

In response to Doctor Dyrda's questionnaire which was sent to all transplantation centers, 57 cases were reported back. In these 57 cases a total of 64 definite rejection episodes were reported. The highest incidence of diagnosed rejection occurred in the first two postoperative weeks with a gradual decrease in incidence after this time until about four months postoperatively. However, rejection was reported out to about 200 days. Of these total 64 episodes, 30 per cent were fatal and all in all, rejection caused approximately 50 per cent of postoperative deaths.

The clinical and laboratory manifestations of cardiac rejection can be categorized in three broad groups. The first would include the manifestations of local organ dysfunction. The second would

include the primary immunological factors, and third, the systemic consequences of either or both of these. Furthermore the manifestations of local dysfunction of the graft can be subdivided into those of electrophysiology, anatomical changes, physiological changes, biochemical changes, including myocardial enzymes, and alterations in physical cardiovascular examination. It is apparent that most people have been more successful in utilizing these parameters for the diagnosis of rejection than in utilizing either the immunological or systemic alterations. It is somewhat gratifying to find one point of uniform agreement in the Symposium, and that is that the electrocardiogram is of paramount importance in the diagnosis of rejection. In both early and late acute rejection the most common changes encountered include decreasing electrocardiographic voltage, deviation in the mean frontal plan axis, usually toward the right, and various atrial arrhythmias. These include paroxysmal atrial tachycardia, simple atrial premature beats, atrial flutter and atrial fibrillation. A gradual decrease in voltage may occur with chronic rejection changes. It was mentioned that a transient decrease in voltage occurs commonly during the first postoperative week. Unfortunately, very little control data are available on this. It is apparent however that more and more centers are willing to initiate therapy for rejection on the basis of electrocardiographic changes alone.

Some of the physiological alterations that occur with acute cardiac rejection, including diminished cardiac output and arterial pressure, cannot be demonstrated until nearly the terminal episode. However with various provocative tests, earlier myocardial changes can be demonstrated. With both heterotopic and orthotopic transplantation, one can, by controling heart rate either through linear or coupled pacing document a diminished ability of the rejecting heart to increase its stroke volume. Doctor Heimbecker presented data with the heterotopic preparation showing changes in left ventricular function during rejection as measured by force development during isometric contraction from varying ventricular volumes. But in the early postoperative period, measurement of cardiac hemodynamics has contributed very little to either diagnosis or clinical management. Later on, in the course of chronic rejection, clinical impressions can be confirmed by alterations in resting cardiac output or the myocardial response to exercise.

As will be demonstrated in the session on the pathologic changes that occur with cardiac rejection, there are early alterations in cardiac anatomy. The plain chest X-rays serve only to confirm changes in size of the cardiac silhouette which are greater than systolic-diastolic variations, but these changes do not generally occur early during rejection. By ultra-sound cardiographic measurements of various cardiac dimensions, however, more early changes can be detected. These include measurement of wall thickness, presumably contributing evidence of myocardial edema, changes in chamber size predominantly right ventricular diameter, and changes in overall heart size. In addition, ultrasound cardiographic measurements can show diminished amplitude and rate of systolic movement during rejection (similar to the fluoroscopic observations described by Doctor Lower).

In terms of the physical diagnosis of rejection, there is some disagreement because of the nonspecificity of many of these signs. However, the earlier signs apparently include a pericardial friction rub. Doctor Nora from Houston was more enthusiastic about this than most. It should be noted however that pericardial friction rubs are common following all types of heart surgery. In our own experience early physical evidence of rejection has included changes in the character of right ventricular contraction (palpation) and also an increase in the amplitude of the neck vein pulsations without an increase in venous pressure (V waves). Later on more severe changes include obvious venous distension, right and left ventricular heaves and/or a murmur of mitral insufficiency. Abnormal heart sounds do tend to occur earlier than the other signs, particularly the S-3 or early diastolic gallop.

There was a significant dichotomy of opinion regarding the enzyme diagnosis of cardiac rejection.

I think this results primarily from theoretical considerations, namely that immunologic damage to the heart severe enough to cause cellular necrosis or even damage to cellular membranes sufficient to elevate the serum levels of myocardial enzymes must theoretically be a relatively late event. Doctor Nora again was more enthusiastic than others about the fast moving fractions of lactic acid dehydrogenase as an index of rejection, and presented evidence that in the great majority of their cases alterations in the first band of LDH were quite suggestive.

In regard to primary immunological factors in cardiac rejection, I think the situation is similar if not identical to that obtaining in renal transplantation. Doctor Botha from Cape Town defended very elegantly the negative results that have been recorded and indicated that a detectable level of circulating cytotoxic antibodies has omnious prognostic significance. In particular, though, he urged continuation of the studies that have been initiated mostly for the sake of obtaining more data.

Most of the systemic consequences of cardiac rejection are non specific and serve as suggestive phenomena. The most common occurrence in response to Doctor Dyrda's questionnaire was a decrease in exercise tolerance as reported by the patients or as observed clinically, occurring in more than 90 per cent of definite rejection episodes. Other symptoms and signs include malaise, fever, anorexia and fatigue.

The clinical relevance of all these considerations is significant only in so far as they permit the early diagnosis of cardiac rejection and the application of effective therapy. In summary, it appears that there is nearly uniform agreement that the electrocardiogram serves as the primary index of acute cardiac rejection. Other early phenomena include a diastolic gallop rhythm, changes in cardiac dimensions as determined by ultra-sound techniques, and possibly the elevation of the myocardial fractions of lactic acid dehydrogenase. It should be emphasized that short of biopsy, none of these indices is entirely specific and this consideration has pointed out the need for controlled studies which at this point do not exist.

Doctor Cooley:

Thank you Doctor Stinson. Doctor Monties from Marseille had asked to be permitted to make some remarks at this time. Doctor Monties.

Doctor Monties:

La ressemblance anatomo-pathologique des lésions de rejet et de coagulation intravasculaire disséminée d'une part, la fréquence des phénomènes thrombo-emboliques lors des transplantations d'autre part, nous ont amené, au cours de l'évolution d'un malade opéré depuis six mois, à surveiller de très près sa coagulation sanguine. Nous avons fait deux constatations. La première, une hypercoagubilité très importante qui est apparue au troisième jour et qui a nécessité des doses très importantes d'héparine (415 mg) dans les 24 heures. La deuxième, c'est l'apparition, lors d'un épisode de rejet aigu le 31 décembre 1968, de signes biologiques de coagulation intravasculaire disséminée avec une chute des plaquettes à 70 000, un épistaxis, et un thrombo-élastogramme qui montrait un aspect typique de coagulation intravasculaire disséminée. Cette chute des plaquettes a été interprétée comme signe de rejet, car nous avons à ce moment-là augmenté la globuline antilymphocytaire et traité le malade par l'héparine. En six heures les plaquettes sont revenues à un taux normal, l'état clinique du malade s'est amélioré, en particulier l'épistaxis, les douleurs thoraciques ont disparu, et l'amplitude du QRS a augmenté. Ces deux constatations nous amènent à faire deux commentaires. Tout d'abord, le phénomène d'hypercoagubilité postopératoire que nous avons vu cadre très bien avec tous les phénomènes thrombo-emboliques qui ont été décrits après transplantation en particulier par Starzl. D'autre part, les rapports tant anatomo-pathologiques que biologiques entre la coagulation intravasculaire disséminée et le rejet paraissent dans cette observation avoir été établis. Ceci nous amène à deux conclusions. Dans la surveillance du transplanté cardiaque, une appréciation régulière des tests de coagulation, en particulier le thrombo-élastogramme et la numération des plaquettes,

peut être un apport intéressant dans le dépistage d'un rejet et, *a posteriori,* nous nous sommes aperçus que sur le thrombo-élastogramme, on aurait pu depuis dix jours prévoir l'apparition des signes de rejet. Deuxièmement, le traitement à l'héparine a permis vraisemblablement, en évitant des lésions de thrombose intracardiaque ou intracoronaire, de laisser au traitement classique le temps d'agir.

Doctor Cooley:

Is Doctor Lower here? Would you make a remark to this question about whether heparin might protect the allograft from developing these occlusive changes in the coronary circuit?

Doctor Lower:

We have no experience, but Doctor Kahn is using heparin. Perhaps he could tell us.

Doctor Cooley:

We are getting off the main subject but I think we would like to hear what Doctor Kahn will say.

Doctor Kahn:

We studied the effect of heparin on coronary blood flow in pig heart using ^{131}Cesium chloride. Heparin markedly increased coronary blood flow in the transplanted heart producing less vascular changes than in control pigs. This fact led us to clinically use heparin in our three human cardiac transplants.

Doctor Cooley:

I think this is an interesting observation. I know from our two longest survivors which Doctor Milam will present today in the pathology session, that there was extensive evidence of coronary occlusive disease as a principal sign of rejection. It seems that this might be one area where we could use preventive measures and if heparin would be one of the preventive measures, I think it should be used more liberally. Are there any other questions or comments that someone would like to make at this point?

Doctor C. Barnard:

For the past nine months we have studied the value of the impedance electrocardiogram in the diagnosis of rejection, as suggested to us by Doctor Kubicek and his group in Minneapolis. It is a little early to come to any definite conclusions, but my impression is that it is not going to be of value in the diagnosis of acute rejection. However, it seems to be of value in studying the gradual deterioration in the cardiac muscle of the patient, the chronic damage, the reduced reserve of the heart. We have seen in some of our patients that there is a gradual deterioration in the various parameters studied with impedance electrocardiogram. So this may be a useful way to follow the chronic damage that takes place in the myocardium, due to subclinical rejection that goes on all the time.

Doctor Stinson:

Doctor Cooley, I think this is an important point, for the diagnosis of chronic rejection is extremely unsatisfactory. We, and other centers, have seen patients coming to autopsy with severe narrowing of nearly all the coronary arteries. Yet, until the last few days of life, these patients were apparently normal in terms of cardiovascular examination, exercise tolerance, exercise electrocardiograms and balistocardiograms. The diagnosis of chronic rejection is an area that surely needs intensive investigation.

Doctor Cooley:

Our longest survivor, who went for nine months, had not a single recognizable epsidoe of rejection, yet he died rather abruptly and the autopsy revealed classical findings of graft rejection and a good deal of evidence of coronary occlusion. We are inclined to believe that he died from a Stokes-Adams attack, which would be a coronary type of death rather than the usual type of chronic rejection.

Let us now move on to the next report which will be given by Mister Donald Ross on infection and other complications of immunosuppression.

Mister Donald Ross:

Mister Chairman, I would like to ask your indulgence because I am strictly a surgeon and not an expert on infection, bacteriology or immunosuppression. All I can do is try and report as objectively as I can what was discussed at this morning's meeting.

I think there was general agreement among all transplanters that we should get rid of immunosuppressives if we can, or keep them to a minimum, but that they are here to stay for some time in the future.

It is clear also that all the immunosuppressives contribute to the general problem of infection in one way or another. Infection of course can be of a specific nature, the one we are usually confronted with in surgery is due to pathogenic organisms of gram positive and particularly of gram negative type. These we can do something about. This is what Doctor Zerbini spoke about, and he emphasized the importance of prevention of infection in cardiac transplantation. He spoke about the need to minimize surgical trauma, and one of the points that was brought up yesterday in connexion with this was the avoidance of groin incisions. In other words one should limit skin incisions as much as possible. There was a vogue for cannulating the venea cavea from the groin in the past. This is a bad region for infection and it has been shown in transplantation of the heart to be somethig to avoid. Discussing sources of infection which were not normally considered, Doctor Zerbini warned us about intravenous fluids even if kept in a refrigerator, and also emphasized the dangers of indwelling urinary catheters and tracheostomy tubes. He spoke about the need to clean up foci of infection particularly in skin, and to exclude from the operating environment all infected personnel, whether they had overt sepsis or a history of infection in the last days or weeks before the transplant.

He discussed the question of antibiotic cover in the postoperative period? There was general agreement among all the people in the room that antibiotics should be used, although I thought I heard Doctor Dubost yesterday say that he had never used antibiotics in any of his transplants. The generally recommended antibiotic was cephaloridine, and Doctor Zerbini suggested its use for four days postoperatively. He also mentioned the need to give it to the donor if possible. That is perhaps a point that most people do not remember in the heat of the moment. As an alternative antibiotic regime, he mentioned the possibility of using gentamycin and cloxacillin. All speakers agreed that they used antibiotic cover, and I think Doctor Cooley suggested that he would use it for a longer period of time than four days. Our own policy has also been to use it for three to four days only.

The other infective organisms that have come into prominence in relation to immunosuppression in transplantation are the so-called opportunist organisms, which include all the viruses, the cytomegalic virus, the pneumocystis carinii and the fungi. Doctor Fontaine spoke to us about those and gave a rather gloomy prognosis in that we are unable, at present, to control the viruses very adequately. The non specific gammaglobulin fractions which are available are not very helpful, but he raised some hope for the future by suggesting more specific preparations. He discussed the experience of the Montreal group and pointed out that four of their nine patients had had important viral infections, one of them a Herpes Zoster infection, which he showed pictures of, and which had become secondarily infected and certainly contributed to the death of the patient. He warned us also about the risk of serum hepatitis from the use of blood during the by-pass and postoperatively. I imagine that would please Doctor Cooley who has long ago proposed a non-blood prime. He also again emphasized the need to avoid all infected personnel, particularly those who have had recent viral infection. He mentioned that immunoglobulins were of doubtful value as prophylaxis against serum hepatitis. He did however show that there was a diminished risk among general cases, not necessarily transplant cases, who had had immunoglobulin therapy, the risk being reduced from 4 per cent to 1 per cent. So there may be a case for using immunoglobulins

as a prophylactic measure, although we certainly need to have more specific types of globulins.

Doctor Kaplan spoke about the problems associated with the use of cortisone and again there was general agreement that cortisone is probably the most useful immunosuppressive we have, although it is also felt that its mode of action is not entirely clear. It certainly contributes to infection. He did point out that it was the second killer, second only to rejection as a cause of death in transplanted patients. He also listed the long list of complications which most people recognize in relation to the use of cortisone including gastro-intestinal hemorrhage, osteoporosis, particularly of the hip and of the vertebral bodies, and so on. Fluid retention in relation to heavy immunosuppression with cortisone has been a problem with our patients, and as Doctor Marius Barnard has mentioned it is also a problem with the Cape Town patients. Other effects of immunosuppression with cortisone include diabetes, hypertension, euphoria and the depression which comes when the dose is reduced and this can present a serious management problem. Doctor Kaplan suggested that withdrawal of immunosuppression with cortisone could be a cause of so-called transplant lung and pseudo-rheumatism, but I do not think there was general agreement on this.

Doctor Pierce spoke at length and with considerable expertise on the toxicity of Imuran, relating his experience mainly to his knowledge of the use of Imuran in renal transplantation. He pointed out that Imuran is broken down to 6-Mercaptopurine and that the toxic manifestations may in fact be related to this drug. Imuran, of course, contributes like the other drugs to infective problems, particularly viral infection. He also discussed its toxicity in relation to the liver and here again there is no general agreement, some believing that there is a direct toxic effect of Imuran on the liver, others believing that it is a viral hepatitis facilitated by the use of Imuran. He believed that there was a 20 per cent incidence of liver problems in cases receiving Imuran suppression. He spoke about its effect on the bone marrow, the gastro-intestinal tract and the incidence of tumors. He did point out that in relation to the bone marrow, the incidence of leucopenia was higher in patients without splenectomy although I do not think this is likely to have much effect on the heart transplantation program. In relation to gastro-intestinal toxicity, he had noted extensive ulceration of the mouth, nose and throat even extending down into the œsophagus with long standing Imuran therapy. He brought up the important question of malignancy in relation to Imuran. This has been discussed in relation to ALG, but he pointed out that Imuran could also promote the incidence of tumors in a number of ways: it could promote the transplantation of cancer from a donor, it could promote its growth or it could promote the recurrence of a tumor, for instance in cases which had been operated on for hepatoma or Wilms' tumor. This of course would relate more to liver and kidney transplantation. He also emphasized that it could induce tumors, for instance lymphoma tumors, even in patients not reveiving ALG. He also said that there were sporadically mentioned cases of carcinoma developing in patients on Imuran. And so there seems to be little disagreement that Imuran is a definite source of tumor formation. He mentioned something which I believe is not generally recognized, that Imuran has potential effects on the fetus and that perhaps it should not be used in pregnant patients, or conversely, patients who had received a transplant and were on Imuran therapy should perhaps be dissuaded from becoming pregnant. However, he showed a picture of a young woman with a baby in her arms having successfully completed her pregnancy during therapy with Imuran without any ill effects on the baby.

In the general discussion, there was a question about how long people should be kept in sterile conditions following a transplant. According to Doctor Barnard's group they should be kept in sterile conditions until they leave hospital and I think we would agree with this. We believe that one of the most dangerous places for a patient to be is inside a general hospital in relation to infection. There was talk on the recommended doses

of steroids in heart transplantation patients and Doctor Kaplan advocates starting on a dose of 120 mg per day whereas Doctor Barnard mentioned their massive initial dosage of about 500 mg a day.

That, mister Chairman, was the main burden of the message. I did not hear any discussion, although I would have liked to, on tissue typing and whether improved tissue typing methods and better matches could enable us to reduce our immunosuppression dosage, but perhaps this will come out in the general discussion. Thank you.

Doctor Cooley:

It is interesting that of all the viruses that might affect the recipient, the Herpes virus is the most difficult to control once it starts. Also remarkable to me is the low incidence of hemologous serum jaundice in these patients and one wonders if the Prednisone is not masking the presence of hepatitis or whether it is just the fact that some of the patients do not live long enough to go through the incubation period for this complication to appear.

I would now like to ask Doctor Lillehei if he would comment on their series in New York since he was not here yesterday for the report on the world experience. I would like to hear impressions of the importance of histocompatibility and immunosuppressive therapy.

Doctor Lillehei:

We have done six cardiac transplants in the period since May 1st 1968. One aspect that, I think, is unique about our series is that in the group of six donors, there were 29 organs that were transplanted along with the heart at the same time. This is a point we think of some significance for the future because of the obviously limited supply of donors.

The surgical technique utilized in the first cardiac transplant involved the use of coronary perfusion. That heart was the only one that did not take over and support the circulation postoperatively. I hasten to add, however, that this donor was in very poor condition at the time because I was attending a meeting in California when he was admitted and I had, of necessity, to take a devious route back home so that the donor's cardiovascular status was preserved inordinately long and he had several arrests before the heart could be harvested. In the next five, we used the technique of moderate hypothermia in the donor and recipient (30°C) with non-coronary perfusion. The hearts were placed in Ringer's lactate at 6°C immediately after removal from the donor and for transport to the recipient's operating room. All of these hearts took over well with excellent blood pressure and no instances of heart block after implantation.

There were two deaths in the second week, one from infection in a patient who had a pre-existing purulent empyema which had been undiagnosed prior to the transplant procedure. He was thought to have a pleural effusion and had not been tapped because he had gone into shock with the previous tap some months earlier while hospitalized on the cardiology service. Immediately after the transplantation operation, in the I.C.U. we did tap it and got 1 400 cc of pus with a positive culture of mixed gram negative organisms and thus treated this patient rather conservatively with immunosuppression, but by the eighth day, he had both septicemia and rejection. The other patient who succumbed early, at nine days, was a patient with very severe pulmonary hypertension associated with terminal failure due to a chronic myopathy. She also had had a ventricular septal defect closed twelve years earlier. Two other patients died of rejection, one after 115 days and the other after 63 days. I think knowing what we know now, we simply did not observe these patients closely enough. They had been discharged from the hospital and were seemingly doing very well. Both had had a very benign postoperative course and we were lulled into a false sense of security. The patient succumbing at 63 days was one in whom the donor heart was removed in another hospital three blocks away, placed in Ringer's lactate at 6°C, and carried by a surgical fellow to the recipient's operating room where it was sutured in without coronary perfusion.

The total ischemia time was 35 minutes for removal and transportation and 53 minutes for suturing. The heart resumed beating spontaneously and the patient made a completely uneventful recovery. He was discharged from the hospital 52 days postoperatively. There is then, currently one patient alive at approximately one month postoperatively.

I might comment on the tissue matching which was done prospectively in all by our laboratory. The one patient who is alive now was a D match, he has had one severe episode of rejection just recently, approximately one month postoperatively, which is currently being treated. There were in the six, 3 B matches, 2 C matches and 1 D match. Of the three liver transplants that were done from these donors, two of them are still alive, at approximately five months and three months. I think that summarizes our experience.

Doctor Cooley:

Thank you Doctor Lillehei. Doctor Bahnson, would you like to make a remark about your case?

Doctor Bahnson:

I am sure that the one case we had would not allow one to make or draw any sort of conclusion. He has been treated like all the rest and I think operated upon like most of the rest. He has received ALG and I suppose the only difference is that ALG was made from a horse in our back yard that had previously dumped Doctor Cooley when he came to visit us. Although we often have heard of instances in which the recipient has rejected the transplant, I think this is the only instance in which a transplanter had been rejected. Thank you.

Doctor Cooley:

Would anyone like to make a comment about infection or some other point that should be raised at this time? Doctor Botha?

Doctor Botha:

Doctor Cooley, I did not attend the session on anti-lymphocyte globulin this morning. I had to take part in another session at the same time. Therefore, I would like to make a comment now, particularly since I am not altogether happy about your categoric statement, when introducing this general discussion, that complications from ALG such as might affect the kidneys for example, have not yet been observed. We should consider a very recent observation made in one of our patients which may be relevant and important.

A diagram which I showed at the International Symposium on pharmacological treatment in organ and tissue transplantation, held in Milan during February demonstrated a rather remarkable drop in complement level at the time when ALG was given intravenously to a heart transplant recipient. As some of you know, this patient improved rather dramatically at the time when he received ALG. Doctor Turk from London, who had previously commented on the effect of ALG on complement levels, suggested in Milan that our patient's rapid clinical improvement was possibly due to the fact that ALG was a binding complement, either through its antibody action against the lymphocyte antigens, or perhaps by some other means; and this resulted in relief for the graft itself in the sense that complement could not longer damage the graft by participation in a rejection process.

Another observation emerges from this diagram; we note that really low levels of complement were first measured at the time when this patient developed demonstrable antibodies to horse globulin. We should consider the possibility that antigen-antibody reactions between horse serum and human serum, in either of two possible directions, may bind complement. This is relevant to the serological and clinical course of another heart recipient.

A remarkable event in the early post-transplant course of our fifth patient was the almost total disappearance of complement approximately seven days after the heart transplant. This observation caused me considerable concern. In this instance, the precipitous fall of complement level did not prevent the patient, who had rather an unsatisfactory leucocyte antigen match in my opinion, from

exhibiting two early episodes of cardiac rejection in spite of enjoying what was probably the most energetic regime of prophylactic immunosuppression employed to date. This patient received 20 ml of ALG intravenously daily, in addition to high doses of steroids and azathioprine.

Another unusual feature of this case is that before the transplant operation (and therefore before ALG was administered) the patient had a weak antibody to horse globulin. This antibody titre rose from 1 in 2 after ALG was commenced, to a titre of 1 in 16 at the time when the complement level commenced falling precipitously. We were not initially aware of the presence of this antibody, since we were not able at any stage in this patient to demonstrate antibodies to horse protein with our regular test method, *i.e.* the latex fixation test. Retrospectively the antibody could be demonstrated readily with the passive haemagglutination method.

Thirty days after ALG was commenced, the clinical picture of serum sickness emerged. Retrospective analysis then revealed that from about the tenth day onwards (*i.e.* approximately three days after the complement level had reached a very low peak), there is a slow rise in serum creatinine, and a more notable rise in the total protein excretion per 24 hours. This patient is now seriously ill with serum sickness.

Finally, I want to mention just briefly that the batch of ALG given the latter patient contains a precipitating antibody to human serum, demonstrable by the Ouchterlony method. Therefore, in theory we are dealing here with at least two possibilities: the formation of antigen-antibody complexes either between horse antibody and human antigen, or between human antibody and horse antigen.

Perhaps this is too early a stage to make any really valuable comment on the possible effect of ALG on the clinical course of this recipient. However, I think we should very certainly bear in mind that we may here be witnessing serious deterioration in kidney function as a direct result of ALG. Thank you.

Doctor Cooley:

Thank you, Doctor Botha. Are there any others who would like to comment before we adjourn?

Doctor Barrett:

I would like to draw attention to some work in another field which might have applicability in the assessment of the future of ALG. It has been known for some time that one can use a human fraction of factor 8 in the treatment of hemophilia. Some years ago Biggs and Bidwell developed sheep and pig factor 8, and they used it in a crude form in humans and in every instance it was found that the factor 8 in the patient was subsequently inactivated. In a few instances, there was frank anaphylaxis. One of the commercial firms developed this product further, purified it and the same results were obtained. I make these remarks to draw attention to the fact that perhaps this work has not been fully realized by people who are working on ALG.

Doctor Cooley:

Are there other comment? Doctor Aguirre from Chili.

Doctor Aguirre:

I think of interest to say something about discontinued periods of use of ALG. In one of our patients, we had problems because of a bleeding peptic ulcer and a subcutaneous abcess. Then we discontinued Prednisone and Imuran therapy. In this period of six weeks, we maintained our patient only with ALS from l'Institut Pasteur de Lyon, Professor Carraz, and in this period we had no signs of rejection. Only in the few last days did we see ST and T alterations and these were transitory and improved by using ALG exclusively. Afterwards we saw a sensitization to the ALG and had to again use Imuran and Prednisone therapy.

Doctor Cooley:

Than you, Doctor Aguirre. Doctor Treager, would you like to make a remark?

Doctor Treager:

Was this ALG used by the intravenous route or by the intramuscular route?

Doctor Aguirre:

Intravenously.

Doctor Treager:

I must say that in more than 70 patients using the same ALG that was used in Valparaiso, we had only one serum sickness and it was necessary in only one case to stop ALG. Maybe using the intravenous route, you may have more serum sickness and more anaphylactoid reactions of this type.

Doctor Carraz:

Au sujet de l'observation du docteur Botha, je voudrais signaler ici ce que j'ai signalé ce matin sur les différentes méthodes de préparation des ALG. Certaines peuvent former des aggrégats moléculaires qui fixent le complément, et ces faits sont très connus en ce qui concerne les immunoglobulines humaines utilisées par voie intraveineuse. Ces immunoglobulines peuvent abaisser le taux de complément sérique de manière extrêmement importante. Cela a également été démontré chez le lapin. J'ai présenté ce matin le problème que posent les aggrégats moléculaires suivant les différentes méthodes de préparation. Ces aggrégats peuvent amener une intolérance à l'ALG qui n'est pas due uniquement aux anticorps antiprotéiques mais à la forme moléculaire de l'ALG présenté.

Doctor Cooley:

Doctor Brendel, would you like to make a remark?

Doctor Brendel:

I only want to say that in this batch of ALG about which Doctor Botha was talking, we had a certain amount of precipitating antibodies which were not absorbed sufficiently, due to a technical error. In those cases we had serum sickness with high doses of intravenous ALG. In 55 other patients with organ transplantation, we did not see yet any signs of serum sickness. In some other patients not with organ transplantation, we had anaphylactic reactions, but these were cases with auto-immune diseases, and we know that those patients have a special allergic hypersensitivity. In such cases we never will use ALG alone without high doses of steroids and Imuran. Of course, if you stop ALG treatment after several injections, you first have to look if you have antibodies against your ALG in the serum before you can start ALG again. But if you do not find any antibodies, then you can use ALG from the same batch twice or three times as we and our group of surgeons have done several times.

Doctor Cooley:

Thank you Doctor Brendel. We have not heard from our colleagues from the Soviet Union and I would like to invite Doctor Vishnevsky or one of his associates to make a remark if they would care to... No? Are there any other comments? Well then, we will adjourn.

THE CLINICAL COURSE OF PATIENTS WHO HAVE SURVIVED HEART TRANSPLANTATION * †

V. SCHRIRE, M.Sc., Ph.D.,M.D., F.R.C.P.E., F.R.C.P., F.A.C.C.,
and C. N. BARNARD, M.Med., M.D., D.Sc., M.S., Ph.D., F.A.C.S., F.A.C.C,

Les auteurs ont présenté quatre malades qui ont survécu à une transplantation cardiaque. Le premier malade était âgé de 57 ans lors de l'intervention et il a reçu le cœur d'une personne de 25 ans. L'évolution postopératoire immédiate fut relativement simple et le malade a montré une amélioration importante de sa fonction cardiaque. Au cours de son évolution postopératoire de 15 mois le malade a présenté deux épisodes de rejet aigu, qui furent contrôlés par le traitement immunosuppresseur et globalement l'état du malade est amélioré comparativement à ce qu'il était avant son intervention.

Le deuxième cas est un homme de 52 ans ayant reçu le cœur d'une personne de 35 ans. Son évolution postopératoire immédiate fut particu-
(suite du résumé en page suivante)

Our first surviving patient was Doctor Philip Blaiberg — retired dental surgeon of 57 — who was operated upon on 2.1.68 for excision of his heart and heart transplantation. The donor's heart was 25 years old and as far as we can tell, was a normal organ for a patient of this age, sex, race and weight. The immediate postoperative period was uneventful and the rapid disappearance of signs and symptoms of heart failure was impressive. Chronic subclinical subacute rejection probably began at an early stage but it was not until an acute exacerbation of rejection which occurred on the 25th day that clinical evidence became manifest. The salient features at this stage were a rise in temperature, pulse and sedimentation rate. There was also evidence of a deterioration in cardiac function with a rapid reduction in effort tolerance and a diminution in his feeling of well-being. Radiological examination showed enlargement of the cardiac silhouette. It was difficult to determine whether this was due to cardiac dilatation, pericardial effusion or both. Four hundred ml fluid with a lymphatic count of 2,000/cc was aspirated from his pericardial sac but repeat examination showed that both pericardial effusion and cardiac dilatation were present.

Intensification of the immunosuppressive regime produced a gratifying clinical response and he was discharged from hospital 74 days after his operation, on digitalis and diuretic therapy. In his own home he was able to live a quiet and reasonably active life. He no longer complained of undue effort dyspnoea nor did he have paroxysmal dyspnoea or any urgent symptoms. One cannot say, however, that at any time the new heart was functioning as well inside his body as it had in the donor.

Assessment of his cardiac status by the routine conventional methods was always difficult. Because of his thick neck aggravated by the distorting effects of steroid therapy in large doses, no clinical assessment of his venous pressure or liver size could be made with any degree of accuracy; at no time did a triple rhythm appear at this stage but

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† From the Cardiac Clinic, Groote Schuur Hospital, the Council for Scientific and Industrial Research Cardiovascular-Pulmonary Research Group, and the Departments of Medicine and Surgery, University of Cape Town.

lièrement bonne. L'amélioration clinique du malade fut immédiate et il put sortir de l'hôpital au 38e jour après l'opération. Le malade mène une vie active sans symptôme et n'a jamais présenté d'épisode de rejet aigu après sept mois d'évolution.

Le troisième malade était âgé de 63 ans. Il souffrait d'une défaillance cardiaque secondaire à une maladie rhumatismale et il reçut le cœur d'un homme de 54 ans. L'évolution postopératoire fut plus lente que dans les autres cas mais le malade fut tout de même dans l'ensemble amélioré. Six semaines après son intervention le malade a présenté des complications d'infection pulmonaire sans toutefois qu'il y ait d'évidence de rejet aigu.

La quatrième malade est une jeune femme de 37 ans souffrant d'une cardite rhumatismale. Le cœur d'un jeune hypertendu de 33 ans lui fut transplanté. Dans son cas l'amélioration postopératoire fut également rapide mais quatre semaines après l'intervention la malade a fait une défaillance droite importante. Elle a également présenté une réaction d'hypersensibilité au sérum antilymphocytaire qui a nécessité son arrêt. Il est intéressant de noter que les deux derniers malades ont subi une infection herpétique en cours d'évolution.

there was ample radiological evidence of persistent cardiomegaly.

We were very loath to submit him to laboratory tests because of our fear of infection and it was not until the 157th day after operation, shortly after his second rejection episode that central venous pressure was measured and a mean pressure of + 15 mm Hg was obtained with a cardiac index of 1.6 l/min/m^2. This venous hypertension was not clinically recognisable.

In the fifth month after transplantation clinical evidence of deterioration of cardiac function again became evident and an acute episode of rejection became recognizable by the 135th day. The clinical picture was obscured and clouded by an intercurrent infection with Listerella Monocytogenes within twelve days after treatment for the rejection episode, which produced a septicaemia with meningitis, hepatitis and a localised lesion in the upper zone of the right lung. The hepatitis was caused or aggravated by azothioprine which was temporarily discontinued. The septicaemia was cured with appropriate antibiotics and the rejection controlled with increased steroids and antilymphocyte serum. After many vicissitudes he weathered the storm and was finally discharged following a second hospital stay of 124 days. He was now free of jaundice, felt much improved and was able to return to his sedentary life. He was able to live a restricted life, exercise within his limitations, wrote his autobiography and took on a new lease of life.

It has always been difficult to determine just how disabled he is because this is mainly a subjective assessment. The euphoric effects of steroid therapy have kept him cheerful throughout and he has learnt himself what he can do and what is beyond his reach. He lives a useful life within his limitations, is very active in his writings, and hobbies and enjoys every moment of his day. Serial electrocardiographic tracings are presented to demonstrate the progressive evolution and persistence of ST-T wave changes which probably reflect the chronic rejection process (Figures 1 and 2).

Current status – Objective evidence:

On 16.4.69, fifteen months after operation several tests for return of cardiac innervation were made. His mean venous pressure was 14 mm Hg with a cardiac index of 2 l/min/m^2. Auscultation

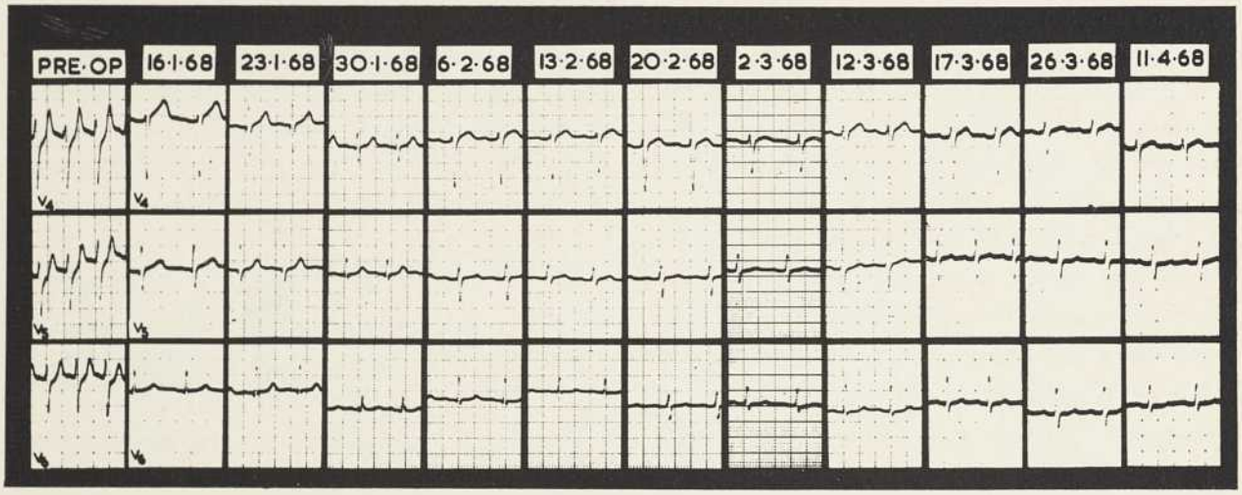

Figure 1 — Serial electrocardiograms from the second heart transplant patient (P.B.) showing the progressive development of ST-T wave changes in the left ventricular leads over a period of 6 months. Digitalis has been administrated throughout.

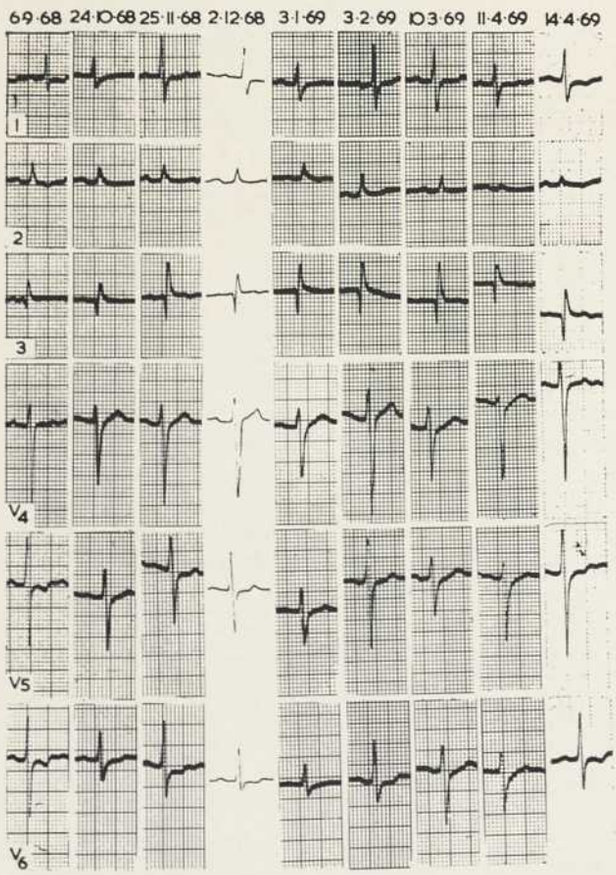

Figure 2 — Serial electrocardiograms from the same patient (P.B.) from 8 to 15 months after operation. Right axis deviation with marked "clockwise rotation" has occurred reflecting right ventricular dilatation, T wave inversion in the left ventricular surface leads persists. Fluctuation in the voltage is marked. Digitalis has been administered throughout.

now shows a persistent triple rhythm (Figure 3). Latterly he has developed a pansystolic apical murmur suggestive of mitral incompetence associated with more overt signs and symptoms of heart failure with attacks of acute paroxysmal cardiac dyspnoea.

The current electrocardiogram and X-ray chest are shown in Figures 4 and 5 respectively.

Comment:

This patient has shown the features of chronic cardiac rejection which has gradually impaired

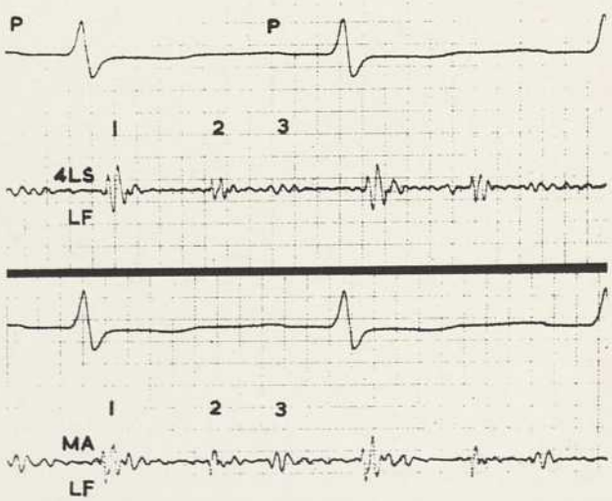

Figure 3 — Low frequency recordings from the fourth left intercostal space parasternally (4LS) and apex (MA) showing the loud third heart sound. (Patient P.B.)

cardiac function until he has developed chronic congestive cardiac failure with all the features of a chronic cardiomyopathy. Two clinical recognizable episodes of acute rejection were actively treated and controlled. Subjectively the patient has obtained enormous benefit and has enjoyed the extra span of life granted to him to the full. This is a tribute to the personality of the individual and the treatment he has received.

Our second surviving patient, Mr. Petrus Smith, a man of 52 received his new heart on 7.9.68. He is particularly fortunate in that his donor, a woman of (?) 35 suffered from malignant hypertension. He has therefore started with a supernormal, massively hypertrophied heart. This became immediately manifest in the postoperative period. The improvement was dramatic and he was out of hospital in 38 days. His physical capacity quickly returned

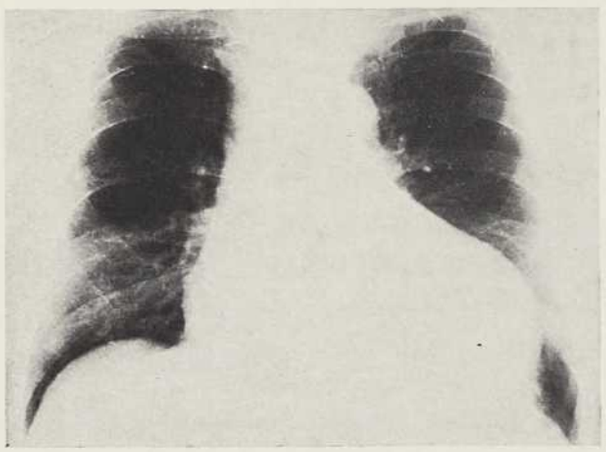

Figure 4 — X-ray chest 16 months after operation in the second heart transplant patient (P.B.) showing the gross cardiomegaly.

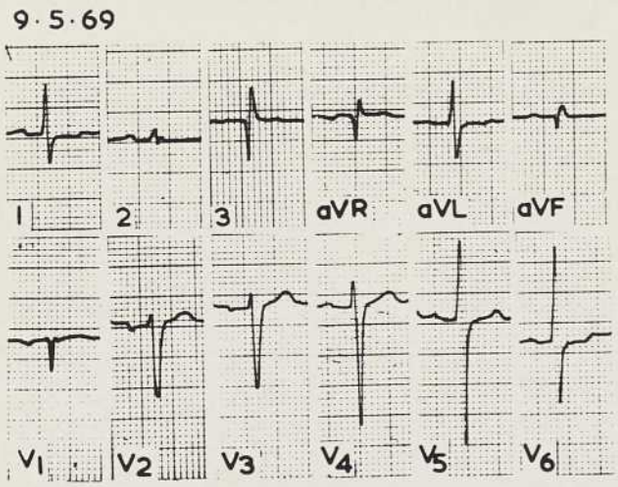

Figure 5 — Electrocardiogram 16 months after operation in the same patient (P.B.) showing terminal intraventricular conduction disturbance with clockwise rotation presumably due to right ventricular dilatation. ST-T wave changes are present in the left ventricular leads. Patient is on digitalis.

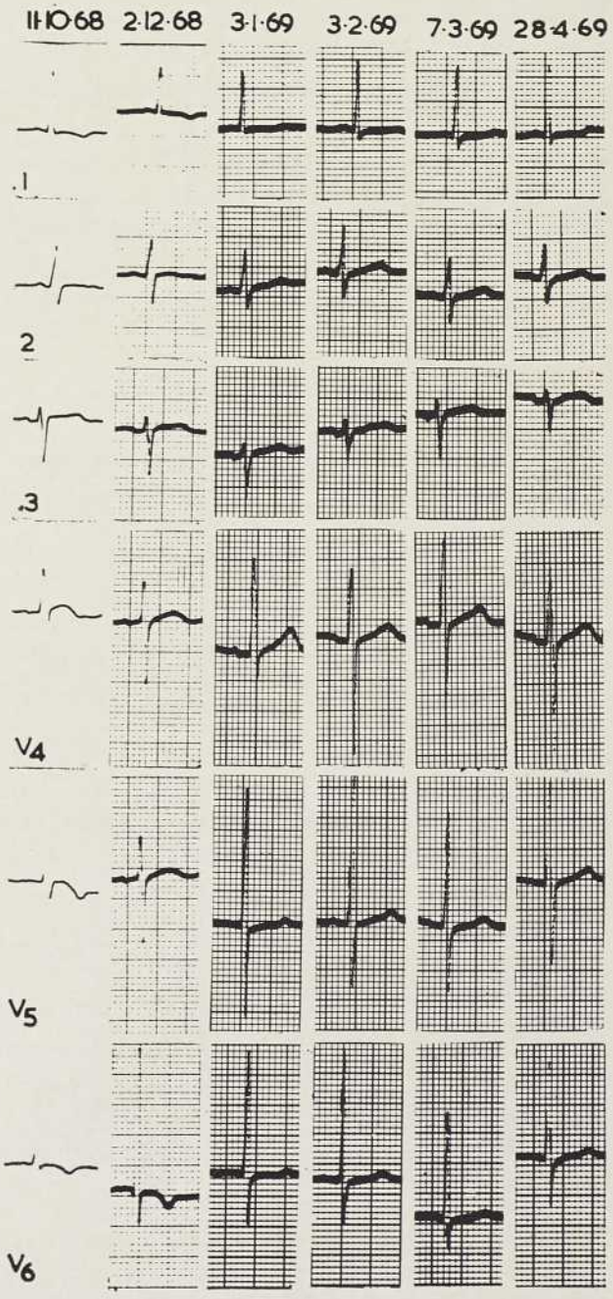

Figure 6 — Serial electrocardiographic tracings from the third heart transplants patient (P.S.). The initial record shows the changes of severe left ventricular hypertrophy. Over a period of 6 months there is progressive improvement of the ST segment depression towards normal.

and he was able to resume an active life with a good effort tolerance and freedom from all cardiac symptoms. He recovered sufficiently to take part in an exhibition tennis match against professional tennis stars. At no time has there been any episodes to suggest acute rejection over a period of seven months

Like the previous patient, his thick neck, obesity and Cushingoid appearance makes clinical assessment of his venous pressure or liver size impossible to gauge. At no time has he developed any murmurs or a triple rhythm. He has refused any objective measurements. The serial and current electrocardiograms are shown in Figures 6 and 7 respectively.

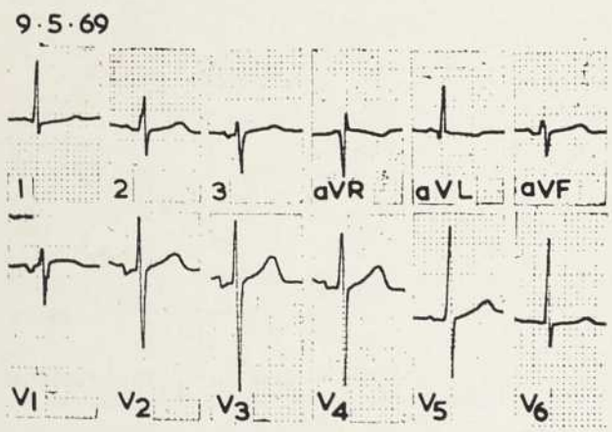

Figure 7 — Electrocardiographic tracings 8 months after operation (patient P.S.) has virtually returned to normal.

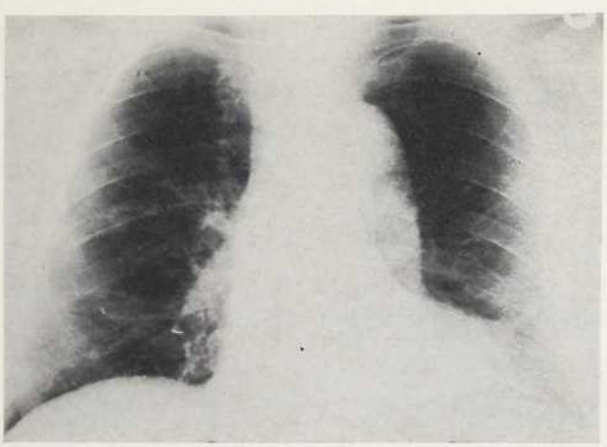

Figure 8 — X-ray chest 8 months after operation (patient P.S.) shows the dilated arteriosclerotic heart of the recipient and the relatively normally-sized donor heart. The lungs are clear.

Current status:

There are no abnormal clinical findings. The current X-ray is shown in Figure 8.

This patient has made a rapid and dramatic recovery. He lives a normal active life. His only problems are those due to his own personality defect. There have been no clinically recognizable acute episodes of rejection. How much chronic subclinical rejection has taken place is impossible to tell but he starts with a considerable advantage and a great deal of muscle mass will have to be destroyed before this becomes manifest. He has not required digitalis therapy and has received intermittent and occasional oral diuretic therapy.

Mr. William Killops, a man of 63, was operated upon on 7.4.69 in an extremely depleted terminal condition. Aortic valve replacement had been performed on 28.9.67 for severe aortic valve disease but he had received little benefit. Chronic progressive unremitting heart failure continued from the day of his operation, and study in February 1969 confirmed the presence of total heart failure due to myocardial disease. He could not wait for a good heart, he had to take the first available. The donor was a 54 year old arteriosclerotic female who died of a cerebral haemorrhage but had no frank evidence of cardiac disease. Macroscopically the organ was flabby and not as vigorous as our previous three cases.

The postoperative course has been much slower. The postoperative diuresis has been less impressive. Digitalis and diuretics have been continued. He has been particularly troubled by herpes simplex a common complication in our patients. However, his progress, although slow, has been towards steady improvement. Six weeks after operation he developed pulmonary complications due to palatal anaesthesia associated with a herpetic infection, but this responded to treatment. There has been no evidence of acute rejection. However, probably associated with steroid therapy he has shown marked mental changes initially severe depression and later a delusional state. We do not anticipate any dramatic restoration of function towards normality.

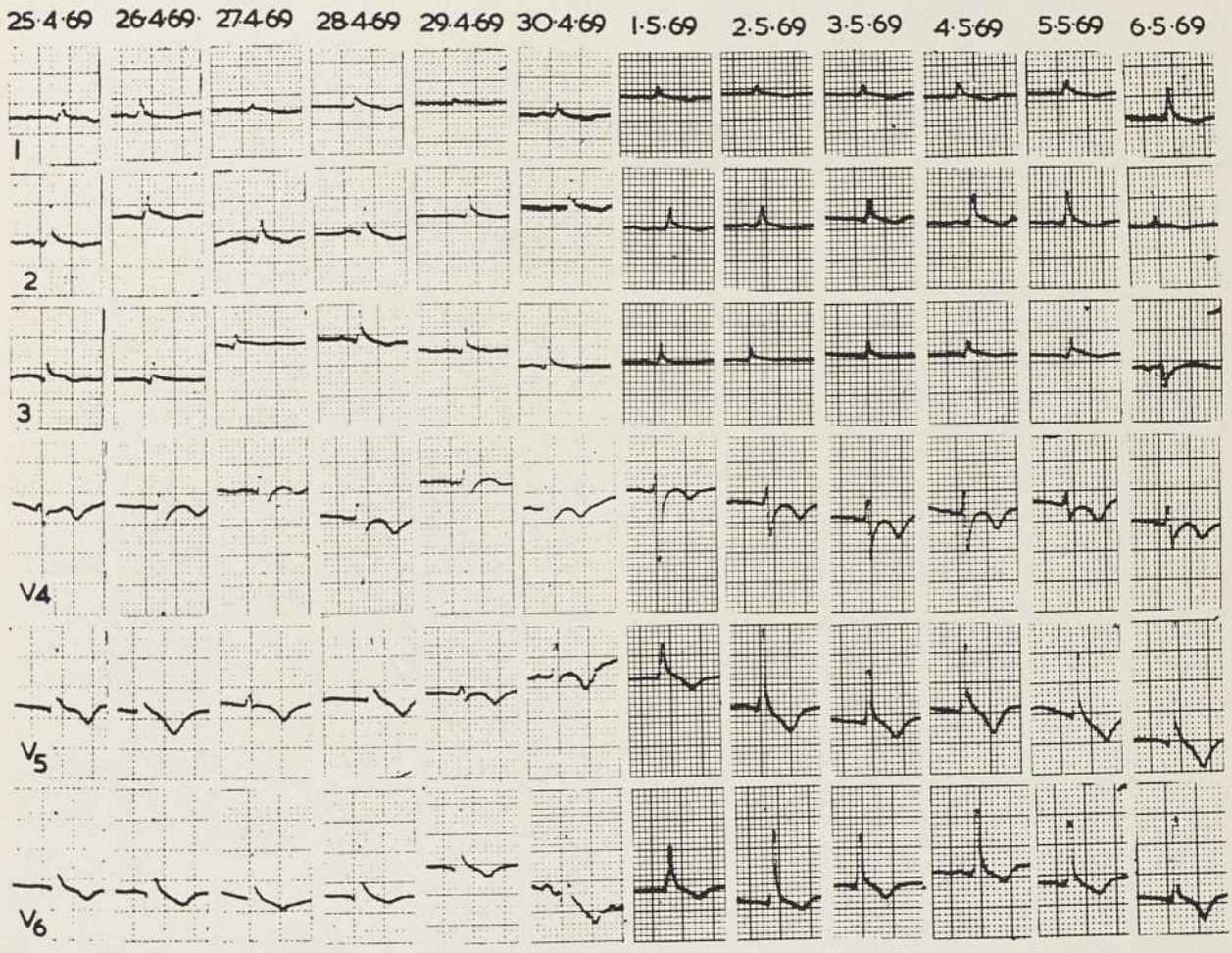

Figure 9 — Electrocardiographic tracing from the fourth heart transplant patient (W.K.) a month after operation showing the T wave inversion in the left ventricular leads. Whether these are due to postoperative pericarditis, rejection processes, or digitalis is uncertain.

The current electrocardiogram and chest X-ray are shown in Figures 9 and 10 respectively.

Miss Dorothy Fisher, a coloured female of 37, received her new heart on 17.4.69. In 1954, she had her first mitral valvotomy, for severe mitral stenosis. A repeat valvotomy was performed in 1959. A mitral xenograft was inserted in August 1968. Following the last operation she made no improvement. Her main symptom was recurrent acute attacks of paroxysmal œdema unrelieved by therapy and her activities were reduced to sitting in a chair. Investigation showed poor myocardial function with a normal functioning valve.

She, too, was fortunate in receiving a heart from a young hypertensive male of 33. Operated upon only ten days after Mr. Killops the immediate post-operative course was strikingly different. Her initial improvement was rapid. However, three to

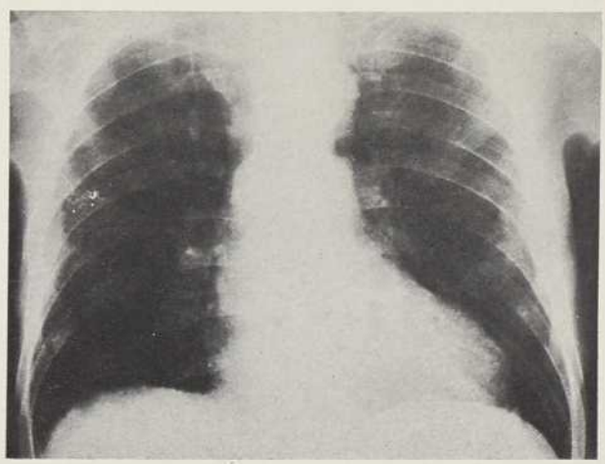

Figure 10 — X-ray chest 1 month after operation (patient W.K.) shows a virtually normal-sized donor heart, an arteriosclerotic elongated recipient's aorta and clear lungs.

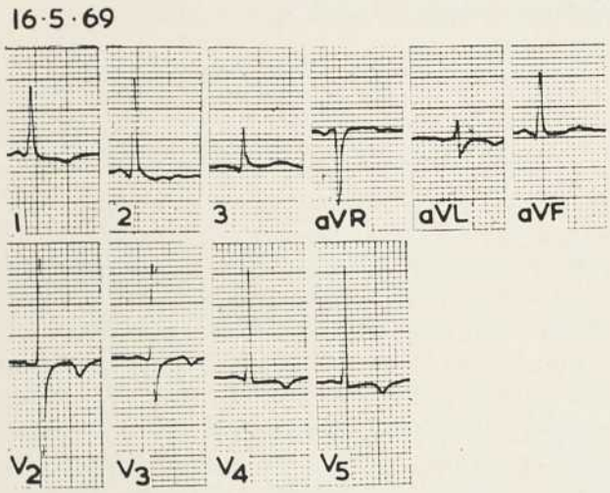

Figure 11 — Electrocardiographic tracings from the fifth heart transplant patient (D.F.) shows the increased voltage due to the persistent left ventricular hypertrophy of the donor heart. The T wave inversion is probably a result of pericarditis but could be associated with rejection. The patient has not received digitalis.

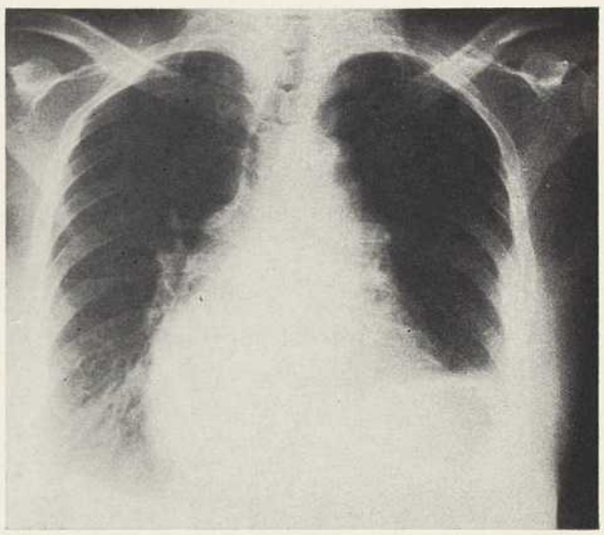

Figure 12 — X-ray chest from the same patient and at the same time as Figure 11. The left diaphragm is elevated due to phrenic nerve paralysis (surgical trauma). The donor heart appears to be enlarged but the lung fields are clear.

four weeks after operation she began to manifest signs of persistent right heart failure with jugular venous hypertension, hepatomegaly and a triple rhythm. The electrocardiogram showed marked fluctuation in voltage from day to day and during the day with progressive ST-T wave cheanges (Figure 11). Radiologically cardiomegaly was noted at an early stage and has persisted. She developed hypersensitivity to ALS necessitating discontinuance of the serum. Massive doses of steroids are required and to complicate the issue she has developed severe persistent herpes simplex infection. The current X-ray is shown in Figure 12.

Acknowledgements:

We wish to thank the Council for Scientific and Industrial Research and the City Council of Cape Town for their continued financial assistance and Doctor J. G. Burger, Medical Superintendent, Groote Schuur Hospital, for permission to publish.

MANAGEMENT OF LONG TERM SURVIVORS AFTER CARDIAC ALLOGRAFTS IN HUMANS *

J.-P. CACHERA and Ch. DUBOST

Cet article décrit l'évolution de deux malades qui avaient subi une transplantation cardiaque, l'un treize mois, l'autre six mois auparavant. Ces patients devaient leur survie à un traitement immunosuppresseur au long cours qui comprenait l'Imuran par voie buccale (1,5 à 3 mg par kg et par jour), la Prednisone par voie buccale (0,25 à 0,50 mg par kg et par jour), le sérum antilymphocytaire par voie sous-cutanée (cinq cm^3 trois fois par semaine). En dépit de ce traitement, les patients ont présenté des accidents de rejet aigu, qui furent jugulés rapidement par l'injection intraveineuse de doses élevées de Prednisone et de sérum antilymphocytaire. Les auteurs considèrent que des troubles électrocardiographiques, même mineurs, intéressant la conduction intramyocardique ou la repolarisation, traduisent une crise de rejet à son début et imposent un traitement approprié immédiat.

Early detection of the rejection phenomenon is the first and the main axis of long term management in survivors after cardiac allogenic graft (1 and 2); early and intensive therapy specifically directed against the acute rejection attack is the second main topic; the third is the prevention and eventually the care of the deleterous side effects of the immunosuppressive agents. The experience acquired in that field with two patients surviving respectively thirteen and six months after cardiac transplantation is the purpose of the present report.

SCHEDULE OF CONTROLS

The schedule of the controls routinely used in the grafted patients has already been published (3).

DETECTION OF REJECTION

Three episodes suggesting the onset of a rejection crisis were observed in the patient n° 1; in the patient n° 2, so far the hypothesis of the beginning of an acute rejection process could not be supported by any modification of the various criteria.

Patient n° 1

(R.P. Boul... operated on the 12.5.1968)

The first episode occured on the 4.12.1968 (8^{th} month postoperatively) and was detected only by the routine E.C.G. tracings: a short episode of nodal bradycardia was recorded during a few minutes; the rhythm was about 38 to 40 per minute; no P waves of the grafted heart were present; the other parameters of the E.C.G., and namely the voltage of QRS, did not demonstrate any change. The clinical status of the patient at the time was perfect; no fever, no systemic symptoms were present; the cardiac sounds were normal.

Despite the fact that such nodal arrhythmias have been frequently observed after auto-transplantation in animals (4), the immunosuppressive therapy was immediately modified. The daily dose of Prednisone was increased from 15 to 60 mg per os; A.L.G. was injected daily at the dose of 30 ml, instead of the routine dose of 5 ml each other day (Figure 1).

* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6-8, 1969.

The dose of Imuran was not modified regarding the bone marrow toxicity; at that time, anemia and leukopenia were present and did not allow higher dosage of the drug.

The patient was kept under continuous monitoring of the E.C.G.; however the tracings remained normal and none recurrence of the nodal bradycardia was observed.

The dose of Prednisone and of A.L.G. could be progressively decreased.

The second episode of rejection was observed one month later, on the 7.1.1969 (nine months postoperatively). Suddenly, during the night, the patient felt discomfort, asthenia and anxiety; rectal temperature reached up to 38°5 C, with shivers.

Cardiac sounds appeared dull; no friction rub was present; arterial pressure was normal, E.C.G. was immediately recorded (Figure 2) and slight but obvious alterations of the intramyocardial conduction were present, *i.e.*:

- enlargement of the QRS complex, the duration of QS increasing from 0.08 sec to 0.09 sec,
- lenghtening of the PR interval from 0.16 to 0.19 sec. There was no changes in the voltage and axis of QRS but the magnitude of T waves was diminished in the precordial leads.

The diagnosis of incipient rejection was assessed on similar changes in the E.C.G. observed in animals during periods of rejection (4):

- the immunosuppressive therapy was modified two hours after the onset of the first symptoms: the patient received 30 ml of A.L.G. subcutaneously and 400 mg of Prednisone intravenously during the night.

The next day, the same E.C.G. changes were present; moreover, it was noticed a slight drop of the voltage and alterations of the repolarization (T waves inverted in the precordial leads). After five days, E.C.G. returned progressively to normal;

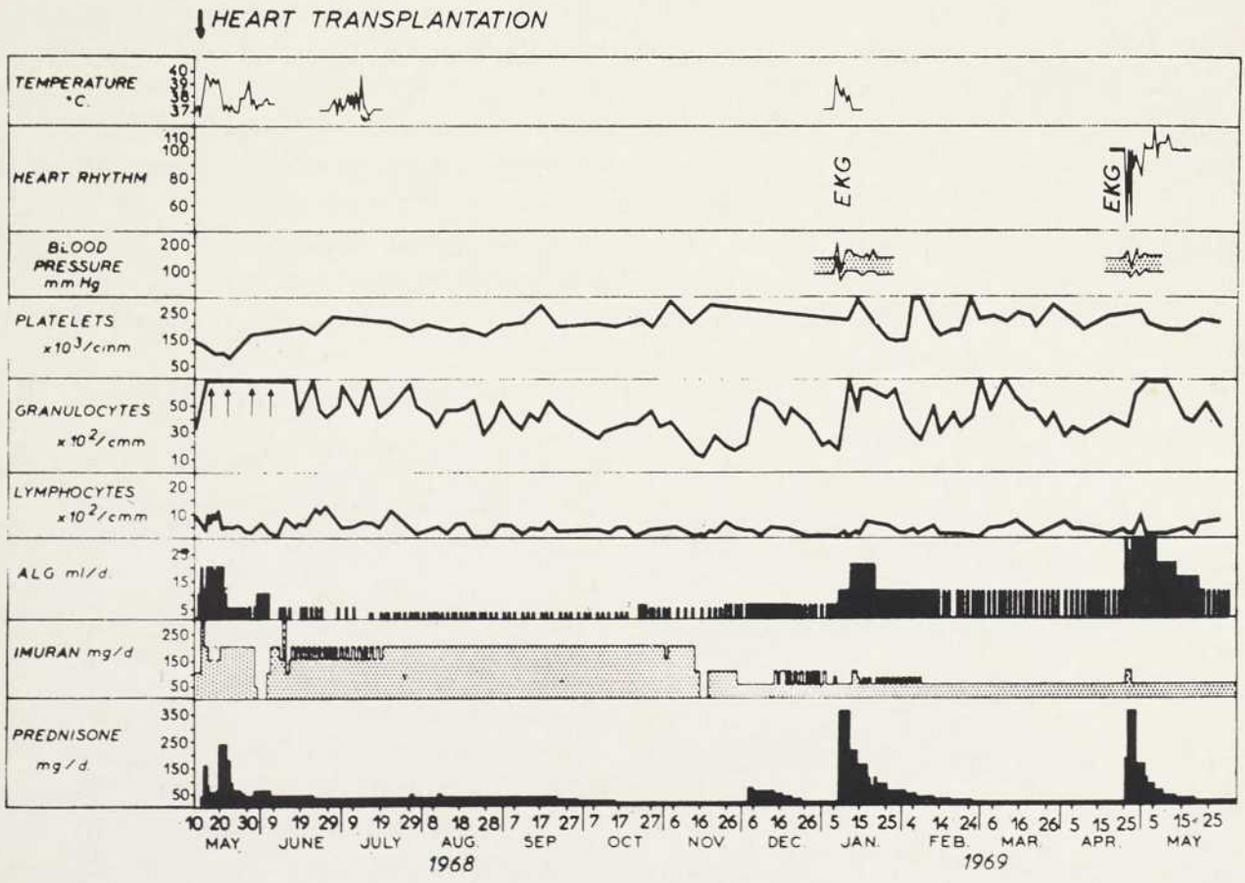

Figure 1 — Clinical parameters and immunosuppressive therapy in patient n° 1.

the dose of Prednisone was progressively decreased (Figure 1).

The third episode of rejection occured on the 24.4.1969 (12th month postoperatively). This episode was detected, as the first one, only by the routine E.C.G. tracing. The changes observed were:

- sequences of nodal rhythm with bradycardia around 38 per minute,
- sequences of A.V. bundle block,
- ventricular ectopic beats.

There was no systemic symptoms, no changes in cardiac sounds. Intensive anti-rejection therapy was undertaken without any delay, involving:

- 30 ml of purified A.L.G. given intravenously,
- 400 mg of Prednisone intravenously.

Continuous monitoring of the E.C.G. was settled on, pulse and arterial pressure were controlled each 30 minutes. The arrhythmias were alternating with sequences of normal sinus rhythm during the first day; the next day, definitive return in normal sinus rhythm was observed. Thus, the therapy could be progressively decreased.

Definite sequelae were not observed in this patient after those three episodes:

- the clinical condition is now satisfactory,
- the cardiac sounds are normal on the phonocardiogram,
- the E.C.G. patterns are similar to those recorded few days after the transplantation,
- the systolic ejection time of the left ventricle measured from the indirect carotid pulse tracing is normal;
- the cardiac flow evaluated from isotopic dilution curves is close to normal values.

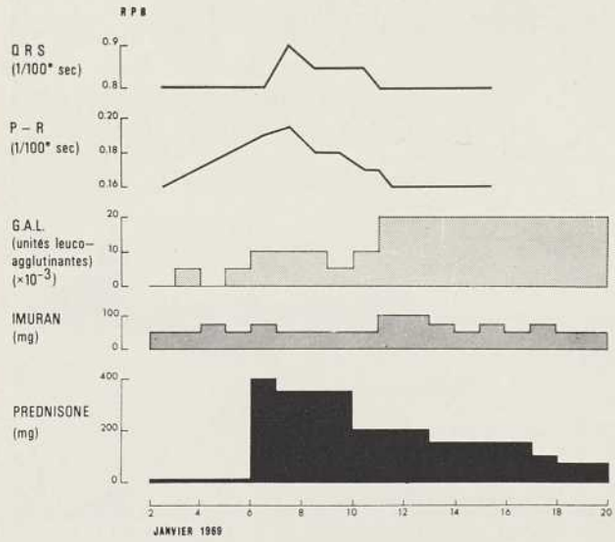

Figure 2 — Diagram showing the changes in the E. C. G. and the treatment of the second episode of rejection in patient n° 1.

X-ray morphology of the heart is normal and did not demonstrate any change during the episodes of rejection; no enlargement of the cardiac chamber was observed. Correlating with this observation, X-rays of the heart taken in animals during rejection of a grafted heart may suggest that radiologic changes of the heart occurs relatively late after the onset of the rejection attack and therefore must be considered most as sequelae rather than symptoms of rejection.

Lastly, enzyme seric levels in this patient have been routinely followed and may suggest some remarks. Despite enzyme profiles are probably highly influenced by the immunodepression, significant changes have been noticed during the episodes of rejection; variations in enzyme curves should not assess if considered alone the early diagnosis of rejection; nevertheless, confronted with other parameters, enzyme profiles probably may provide a useful corroboration (6) especially P.C.K. (phosphocreatine-kinase) plasmatic levels.

Patient n° 2

(J. For... operated on the 23.11.1968)

In the second patient, no obvious signs of rejection were so far observed. However, the clinical course during the first two postoperative months was not actually uneventful. On the sixth day, intense pericardial reaction developed with friction rub and slight enlargement of the cardiac shadow; simultaneously, a systolic murmur appeared, of medium intensity, maximum at the apex. E.C.G. tracings showed normal voltage, normal conduction values, but T waves deeply inverted in the leads V1 to V4. Enzymes levels were higher during the same period than it was observed on controls after standard open cardiac surgery.

For this reasons, the immunosuppressive therapy was maintained at high dosages during the first two weeks postoperatively (Figure 2).

The systolic murmur and the changes in the repolarization on the E.C.G. decreased progressively during the following weeks. Nevertheless, systolic murmur was still recorded on the phonocardiogram on the 7th week postoperatively.

However, the clinical status of the patient improved obviously and no signs of mitral insufficiency or left ventricle failure were present.

On the 46th day, a sudden drop in the arterial pressure occured at rest; the pressure dropped from 130–80 mm Hg to 75–40 mm Hg; the patient felt no symptoms, except anxiety and sweats; cardiac sounds were not modified; E.C.G. was not modified except a moderate sinus tachycardia. The patient received immediately 200 mg of Prednisone intravenously. After two hours, the arterial pressure returned to normal. The hypothesis of a acute rejection phenomenon is here questionable (Figure 3).

Six months after the procedure, the systolic murmur has now disappeared; repolarization changes are still present in the E.C.G., X-ray of the heart is normal. The cardiac flow is almost normal; systolic ejection time on the carotidogram is normal; enzyme levels are in normal ranges. However, vectocardiogram shows patterns compatible with sequelae of rejection.

TREATMENT OF REJECTION

Long term immunosuppression is considered to be a permanent prophylaxy of the potential chronic rejection. In our experience, this long term therapy involved:

- Imuran per os, at the daily average dose of 1.5 to 3 mg per kg,
- Prednisone per os (Methylprednisolone) at the daily average dose of 0.25 to 0.50 mg per kg,
- Anti-lymphocyte immuno globuline (AL. Ig. G.) prepared by immunization of horses or sheeps by lymphocytes extracted from peripheral blood of human donors. The routine average dose was of three injections weekly of 5 ml each subcutaneously. The dose was subsequently modified according to the titer in leucoagglutinins of the batch used.

Despite this chronic immunodepression, acute rejection crisis should occur at any time; the success of the treatment of such acute rejection episodes is subordinate to his intensity and precocity.

In our experience, it was felt that massive increase in immunodepression should be undertaken as soon as possible after the detection of E.C.G. abnormalities. In the present status of our knowledge, it was thought that any variation, even minimal, in the E.C.G. should be significant; clinical symptoms, roentgenologic changes of heart volume, severe alterations in the E.C.G. with low voltage must not be waited for. The emergency treatment of rejection in our hands involves:

1. High dosages of Prednisone given intravenously: 400 mg of Hydrocortisone diluted in 250 ml of normal dextrose and infused within six hours. A complementary dose of Prednisone per os is useful: 200 to 300 mg of Methylprednisone.

2. Administration of a purified A.L.G. intravenously; a dose of 30 ml should be given within the first six hours of the treatment.

The patient must be placed under continuous monitoring of the E.C.G. with alarm systems; pulse rate, arterial pressure and cardiac auscultation should be controlled every 30 mn.

The treatment is then adapted subsequently to the analysis of the different parameters of control. If high dosages of steroids are to be maintained during several days, it seems desirable to isolate the patient under bacteriological supervision.

SIDE EFFECTS OF THE TREATMENT

Long term immunodepression usually induces deleterious side effects and sometimes provokes severe events.

In case n° 1: the toxicity of Imuran was restricting the use of the drug in two instances; four weeks after the transplantation, increase is bilirubin levels

and transaminase activities were due to hepatic intolerance to the drug; on the seventh month postoperatively, anemia and leukopenia (2068 cells/mm^3) were noticed; Imuran was not given during two weeks; in order to counterbalance this defect in the therapy, dosages of Prednisone and A.L.G. were increased (Figure 1).

Deleterious effects of high dosage of Prednisone were resulting in cushinoid appearance, gain in weight, diabete and osteoporosis.

Osteoporosis was judged especially dangerous and decalcification was carefully followed by X-rays of bones at regular intervals; despite those controls, severe vertebral decalcification occurred. On the other hand, A.L.G. was well tolerated in this patient: no pain to the sites of injection, no anaphylactic shocks, neither anti-erythrocytic, nor antithrombocytic side effects.

In case n° 2: no major problems were encountered in the use of Imuran and of Prednisone.

On the contrary, the patient developed after two weeks a progressive intolerance to the horse xenogenic proteins; each injection of A.L.G. was followed by fever and hypotension; gradually, the hypotensive reaction became more severe and episodes of shock were observed; seric levels of anti-horse-proteins antibodies were substantially increased. Then the horse xenogenic anti-lymphocyte globulin was relieved by a sheep globulin; this product was perfectly tolerated and no disadvantage have been so far observed using it (Figure 3).

SUMMARY AND CONCLUSIONS

Two patients have been followed under close controls respectively thirteen and six months after receiving a cardiac allogenic graft. In the first patient, three episodes of electrocardiographic changes were considered significant of a beginning acute rejection crisis. Emergency treatment was undertaken, involving intravenous infusion of high doses of steroids and of a purified A.L.G., and resulting in promptly return to normal of the E.C.G. In the second patient, no definite criteria of rejection

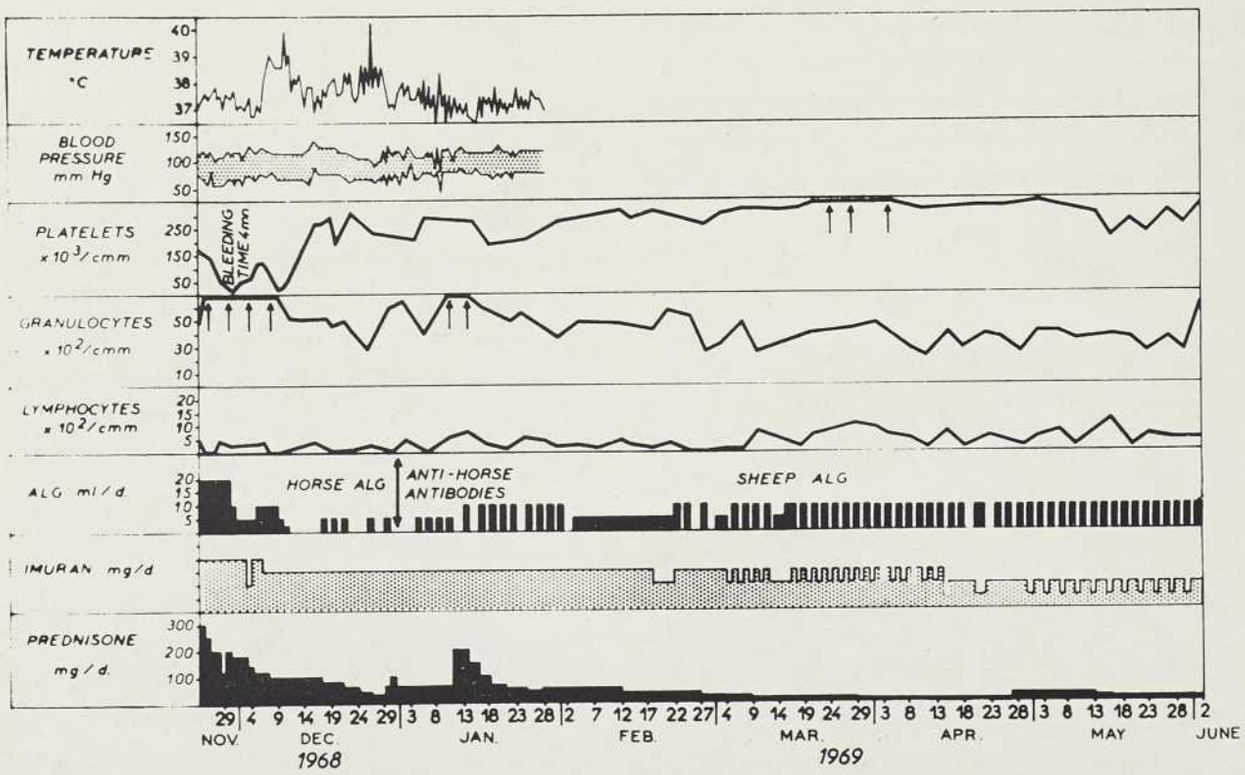

Figure 3 — Clinical parameters and immunosuppressive therapy in patient n° 2.

were so far observed, but severe intolerance to the horse A.L.G. necessitated the use of a sheep A.L.G.

The management of long term survivors after cardiac allografts in humans needs careful and multiple controls on the patients and permanent flexibility of the immunosuppressive therapy; immunodepressor agents in use for the present may induce, if used chronically, severe troubles which themselves involve controls and therapy.

In present status of our knowledge, such conditions are available only in hospital environment, and appears incompatible with early discharge of the patients.

ADDENDUM

Since this paper was submitted, the two grafted patients died suddenly, respectively, 17 and 13 months after the procedure.

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HEMODYNAMIC RESPONSES OF THE TRANSPLANTED HUMAN HEART * †

R. D. LEACHMAN,[1] James J. NORA, Donald G. ROCHELLE, Louis L. LEATHERMAN, Dennis COKKINOS, Reinaldo CABRERA, and O. ZAMALLOA

Les auteurs revisent l'état fonctionnel du cœur humain dénervé après transplantation orthotopique. Dix-neuf malades ont été ainsi étudiés. L'exercice induit un changement du rythme cardiaque qui est plutôt discret comparativement à la normale et qui correspond au niveau de noradrénaline et d'adrénaline circulantes. Chez les dix-neuf malades étudiés les auteurs n'ont retrouvé aucun signe de réinnervation. Les études hémodynamiques sériées ont démontré qu'il y a une diminution du débit cardiaque ainsi que de la compliance ventriculaire droite qui coïncide avec l'apparition de signes cliniques de rejet chronique. Ces altérations fonctionnelles peuvent aussi apparaître au cours de l'exercice même si au repos l'état hémodynamique est normal. Les réponses du cœur humain dénervé à la digitale à l'isoprotérénol et au glucagon sont semblables aux réponses normales. Le cœur dénervé peut maintenir un débit cardiaque approprié même si les mécanismes régulateurs neurologiques sont interrompus.

Orthotopic allografting of the human heart separates the heart from all its nervous connections. The application of heart transplantation to clinical situations emphasizes the need for knowledge of the function of the transplanted heart and its response to frequently used pharmacologic agents.

Extensive studies of the denervated animal heart have been made and provide a basis for phylogenetic comparison with the recently accumulated human data.

MATERIAL AND METHODS

Since May, 1968, 19 patients have had cardiac allografting as a palliative treatment of end-stage heart disease and have been available for study. In some patients, serial hemodynamic studies, at rest and following three minutes of horizontal exercise at 25 watts, have been done. In others, the hemodynamic response to selective drug injection has been monitored. Changes, in heart rate, right atrial, ventricular, pulmonary arterial, and pulmonary capillary blood pressures, left ventricular systolic and end-diastolic pressures, first derivatives of the left ventricular pulse wave, cardiac output, cardiac index, and stroke index were measured. Pressures were measured through a cardiac catheter with a P23d5 strain gauge and recorded photographically. Cardiac output was determined by indocyanine green dilution technique. The first derivative LV dp/dt was measured by an RC differentiating circuit from the LV pressure pulse transmitted through the cardiac catheter.

In addition to the serial hemodynamic observations, the effect of acute digitalis administration (1.25 mg digoxin I.V.) in two patients, 12 and 15 weeks postoperative, isoproterenol, 0.0008 mg/min. infused continuously into one patient 27 weeks postoperative, and glucagon 1.0 mg/min. infused

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† From the Departments of Medicine and Pediatrics, Baylor University College of Medicine and The Texas Heart Institute of the St. Luke's and Texas Children's Hospitals.

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to a total dose of 4 mg in one patient 12 weeks postoperative were measured.

RESULTS

The hemodynamic data derived from 6 of the 19 patients are presented in Table I.

Heart rate. The heart rate at rest was faster than normal and varied between 84 and 115 beats per minute. Following three minutes of horizontal exercise, the heart rate increased in all subjects tested, but in delayed fashion as compared to normal (Figure 1). The heart rate increased from an average of 108/min. to 125/min. Maximum increase in heart rate was attained after three to four minutes of exercise in contrast to normal subjects who achieved maximum rate in less than one minute with this same level of exercise.

Resting heart rate was recovered only after 10 to 12 minutes as compared to a two minutes or less recovery time for normal subjects.

In one patient, circulating arterial norepinephrine and epinephrine were measured at rest, at the termination of three minutes of horizontal exercise and ten minutes after exercise. Resting arterial norepinephrine level was 0.018 mg/100 ml and this rose to 0.026 mg/100 ml during exercise and returned to 0.018 mg/100 ml ten minutes following exercise. These values correlated with heart rate changes of 92/min. resting, 128/min. during exercise and 112/min. at ten minutes following exercise (Figure 2).

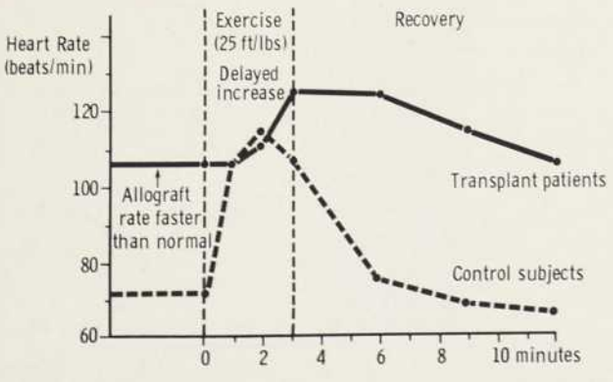

Figure 1 — Effect of exercise on heart rate.

SERIAL HEMODYNAMIC CHANGES

Six patients had serial hemodynamic study. Cardiac output and intracavitary pressures determined at varying periods following cardiac transplant are included in Table I. In all but one of these patients, the cardiac output and pulmonary and systemic arterial blood pressures were within the normal range in the first weeks following allografting. In one patient (E.T.) who received a transplant for multivalvular rheumatic heart disease, the elevated pulmonary arterial pressure persisted following transplantation, even though the cardiac output returned to normal. In the same six subjects, the cardiac output increased following exercise. A delay in the increase in cardiac output was prominent in H.J. who had decrease in cardiac output from 3 l/min. to 2.5 l/min. following three minutes of exercise, but an increase to 5.2 l/min. after six minutes of exercise. In patients E.T., G.D., and H.J., hemodynamic study was done four weeks or less prior to death from chronic rejection. Coincident with the decrease in voltage of the QRS complexes which heralded the onset of rejection, the cardiac output decreased, and right ventricular and left ventricular filling pressures increased. In these patients with chronic or late rejection, striking obliterative changes in the coronary arteries and edema of the myocardium were seen at the time of death.

Valsalva maneuver. There was no alteration of the heart rate during or following the Valsalva maneuver. The frequency of the P wave originating

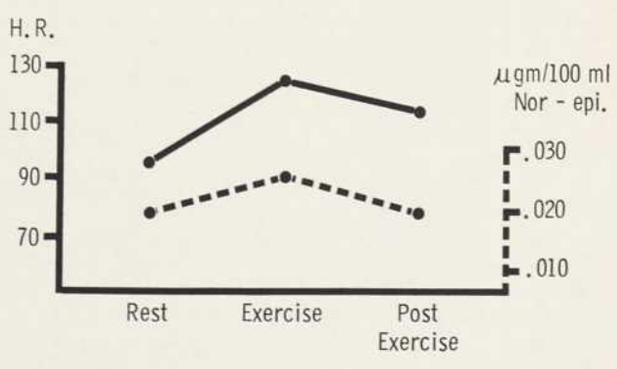

Figure 2 — Circulating catecholamine and heart rate at rest, during exercise and 10 minutes following exercise.

TABLE I

Hemodynamic data derived from six patients

PATIENT	TIME	H.R. R	H.R. E	C.O. R	C.O. E	C.I. R	C.I. E	S.I. R	S.I. E	PAP R	PAP E	PCW R	PCW E	PVR R	PVR E	DATE TRANSPLANT	STATUS
E.T.	Pre-Op	—	—	2,5	—	—	—	—	—	75/25	—	—	—	16 * TPVR	—	5- 2-68	Exp. 29 Wks.
	4 Wks.	108	120	5,9	6,0	3,5	3,7	32	30	65/20	—	—	—	5,9 * TPVR	—		11-29-68
	6 Wks.	112	142	4,9	6,0	2,7	3,4	24	23	55/25 40	—	12	17	5,7	—		
	12 Wks.	111	120	5,6	—	3,0	—	27	—	55/30 37	—	27	22	5,71	—		
	27 Wks.	120	—	2,5	—	1,3	—	11	—	45/18 30	—	14	—	6,4	—		
L.F.	Pre-Op	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5-21-68	Exp. 21 Wks.
	24 Hrs.	—	—	9,1	—	5,4	—	—	—	—	—	—	—	—	—		10-14-68
	1 Wk.	90	115	5,6	9,6	3,4	5,8	37	51	—	—	—	—	—	—		
	3 Wks.	96	110	5,8	—	3,5	—	36	—	43/15 26	50	12	30	2,4	—		
	9 Wks.	96	108	4,6	8,1	2,7	4,7	28	43	35/15 28	48	17	34	2,4	1,7		
G.D.	Pre-Op	—	—	—	—	—	—	—	—	—	—	—	—	7	—	7- 2-68	Exp. 21 Wks.
	48 Hrs.	—	—	3,9	—	2,2	—	—	—	—	—	—	—	—	—		11-28-68
	6 Wks.	96	102	3,5	5,3	2,0	3,1	21	30	20/10 12	—	5	—	2,0	—		
	16 Wks.	108	114	2,7	5,3	1,6	3,1	15	27	26/10 20	26	11	20	3,3	1,1		
F.E.	Pre-Op	90	—	2,8	—	1,6	—	18	—	53/27 35	—	21	—	5,0	—	7-20-68	Exp. 40 Wks.
	1 Wk.	78	90	4,8	13,5	2,9	8,1	37	90	27/13 19	—	15	12	0,8	—		4-13-69
	20 Wks.	104	108	5,1	7,2	2,9	4,2	28	38	27/10 15	17	5	12	1,9	0,66		
	34 Wks.	90	—	4,1	—	2,3	—	26	—	24/10 14	—	7	—	1,7	—		
H.J.	11 Wks.															7-23-68	Exp. 25 Wks.
	3" exerc.	114	132	3,0	2,5	1,6	1,3	14	10	35/15 22	—	13	—	3,0	—		
	6" exerc.	114	138	3,0	5,2	1,6	2,7	14	20	—	—	—	—	—	—		
	21 Wks.	108	—	3,5	—	1,8	—	17	—	40/12 23	—	18	—	1,4	—		
L.B.	3 Days	110	—	4,86	—	2,37	—	22	—	—	—	—	—		—	11-16-68	Living
	14 Wks.	108	120	5,9	10,6	2,8	5,1	27	43	19/7 12	—	—	—	2 * TPVR	—		

Legend :
HR : Heart rate
CO : Cardiac output L/min.
CI : Cardiac output L/min/M²
SI : Stroke volume ml/stroke/M²
PAP : Pulmonary artery pressure
PCW : Pulmonary capillary wedge pressure
PVR : Calculated pulmonary vascular resistance
TPVR : Total pulmonary vascular resistance
R : Rest
E : Exercise
X : Mean pressure

of atrial stump of the recipients' heart did, however, vary in normal fashion to this maneuver (Figure 3). Pain and cold stimuli have failed to alter the heart rate.

Digitalis. Acute administration of digitalis failed to alter the heart rate or systemic arterial blood pressure. In one patient, the LV dp/dt increased from 1,873 mm Hg/sec. to 2,497 mm Hg/sec. In both patients, there was a significant increase in cardiac output, achieved by increased stroke volume (Figure 4).

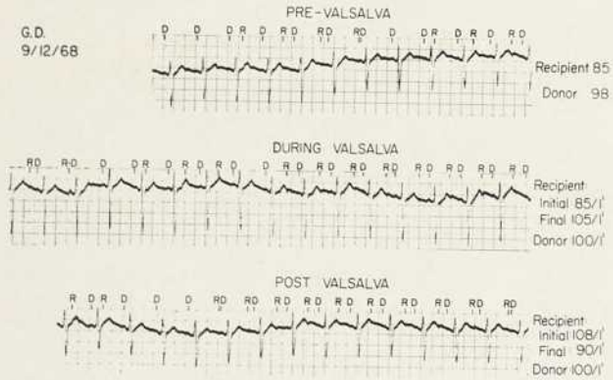

Figure 3 — Valsalva maneuver.

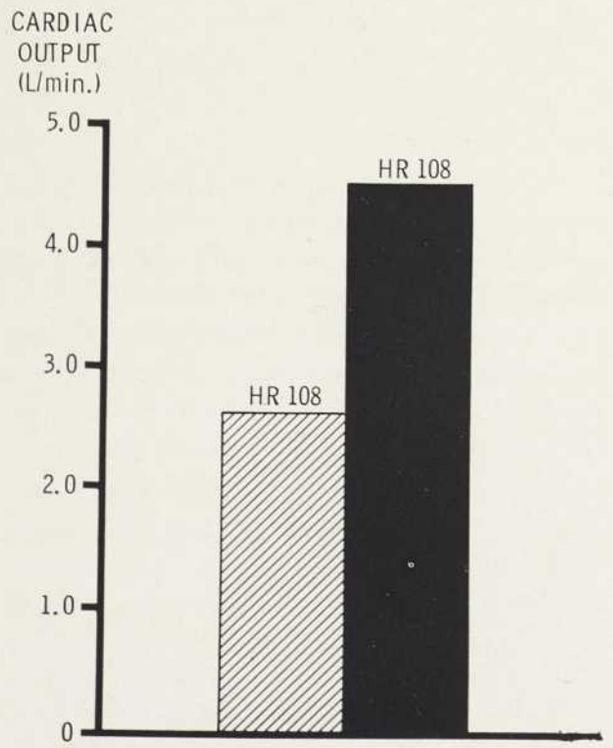

Figure 4 — Digoxin (1.25 mg I.V.) increased cardiac output with no change in rate.

Isuprel. After stabilization with continuous infusion the cardiac output increase from 2.5 l/min. to 4.9 l/min. and there was an increase in heart rate from 120/min. to 160/min. This increase in cardiac output was achieved by both an increase in heart rate and stroke volume (Figure 5).

Glucagon. In one patient, the slow infusion of glucagon resulted in an increase in cardiac output from 4.16 to 6.49. This increase was achieved primarily by an increase in stroke volume. Stroke index increased from 26 cc to 35 cc per beat, although the heart rate increased from 90/min. to 102/min. The LV dp/dt increased from 2,000 mm Hg/sec. to 2,850 mm Hg/sec. (Figure 6).

DISCUSSION

The transplanted heart is separated from all of its nervous connections and functions quite adequately in the denervated state.

In 1914, Patterson, Piper and Starling (12) presented evidence of intrinsic myocardial mechanisms

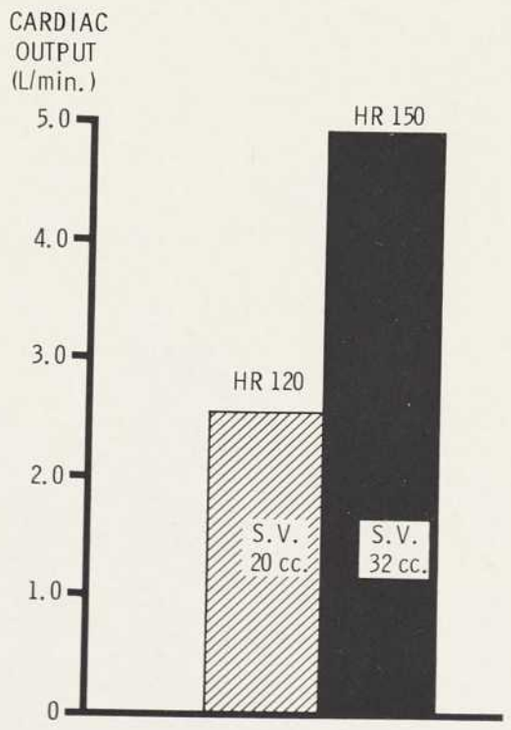

Figure 5 — Isoproterenol (0.00115 mg/min. for 35 min.) produces normal prompt response in heart rate and cardiac output.

which regulate cardiac output in the isolated animal heart. Early studies of the denervated heart in intact dogs by Gasser and Meek (10) in 1914 and Samaan (13) in 1935 presented evidence of decreased exercise capacity.

Cooper (3) and others developed a technique for selective denervation of the dog heart which could be confirmed by nerve stimulation and drug response. Subsequently they (4) found evidence of myocardial catecholamine depletion and increased sensitivity to circulating catecholamines.

Donald (6, 7 and 8) and others have studied the response to exercise of the denervated dog heart. They found that there was a slow increase in heart rate with exercise which reached a maximum after one minute and a half and then slowly decreased to resting levels. There was also an adequate increase in cardiac output which was achieved primarily by an increase in stroke volume.

In our patients, as shown in Table I, it can be seen that the increase in cardiac output was achieved through both an increase in stroke volume and heart rate. This correlates well with the measured increase in circulating norepinephrine and is consistent with the observations of Angell *et al.* (1), Donald *et al.* (7), and Dogget *et al.* (5).

The explanation for the high resting heart rate observed in these patients remains obscure. An elevated right atrial pressure was suggested by Blinks (2) as an explanation of this phenomenon. Donald and Shepherd (9), however, were unable to change the heart rate by infusion and could not correlate changes in right atrial pressure during exercise with changes in heart rate. In our patients, we could find no correlation between heart rate and right atrial pressure at differing times at rest or with exercise.

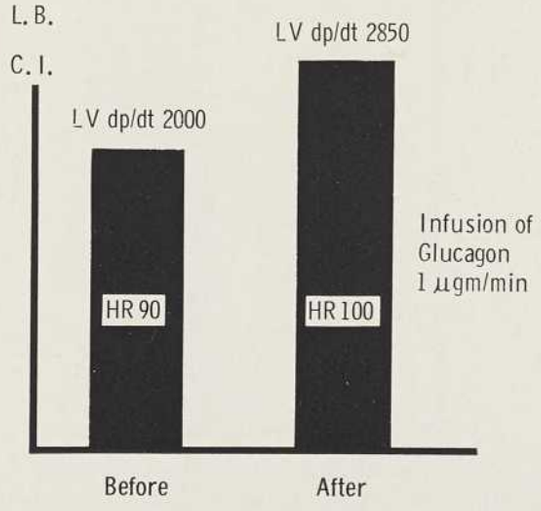

Figure 6 — Influence of a slow infusion of glucagon.

The absence of rate change of the transplanted heart to usual reflex stimuli confirms the absence of denervation. None of our patients have evidence of return of reflex control of the transplanted SA node even after eight months (F.E.).

The only patient in our series suffering from multivalvular rheumatic heart disease (E.T.) had severe pulmonary artery hypertension prior to surgery. Following transplantation, the pulmonary arterial pressure did not immediately return to normal although it did decrease. In spite of the pulmonary hypertension a satisfactory cardiac output was maintained and he ultimately returned to work, only to return to the hospital with signs of allograft rejection. A second patient (F.E.) suffered from coronary artery disease and had pulmonary hypertension secondary to left ventricular failure. His pulmonary artery pressure returned to normal within one week after transplant.

The first of these patients undoubtedly had pulmonary vascular changes secondary to long-standing pulmonary hypertension. In general, those patients with pulmonary venous hypertension associated with pulmonary arterial hypertension can be expected to decrease the pulmonary arterial blood pressure when the pulmonary venous hypertension is relieved by the transplanted heart.

Three of the patients who had serial hemodynamic study were catheterized within four weeks of their death from rejection. In these patients, E.T., G.D., H.J., there was a decrease in cardiac output and an increase in right ventricular end-diastolic pressure. These changes correlated with the electrocardiographic and clinical findings of rejection and seemed to occur almost simultaneously with them (11). On histologic examination of the

rejected allografts in these patients, coronary arterial obliterative changes and edema of the myocardium have been found. These histologic changes seem to correlate with the functional changes, that is, a decreased ventricular compliance and reduced contractile state. As noted in Table I, the transplanted heart which appeared to function well at rest, responded less well to exercise. Even early after transplantation, there was evidence of a decreased ventricular compliance under stress.

In response to acutely administered cardiotropic drugs, the denervated heart responds in predictable fashion. Digoxin increases cardiac output, primarily by increasing stroke volume. Isoproterenol increases cardiac output both by increasing stroke volume and heart rate and glucagon increases cardiac output and contractility as measured by LV dp/dt. In two patients who developed atrial flutter or fibrillation, increasing digitalis dosage did not result in an increase in AV blockade, an observation consistent with the idea that digitalis effect on the AV node is mediated through the vagus nerve. At least one patient with atrial flutter following transplant has responded by increasing AV block however (14). In the presence of decreasing cardiac function, the administration of digitalis, isuprel, or glucagon may be expected to improve the function of the transplanted human heart.

SUMMARY

The functional state of the denervated orthotopic human heart allograft has been reviewed. With stress, changes in heart rate occur sluggishly as compared to normal and correlate with circulating nor-epinephrine and epinephrine levels. No evidence of renervation has been found in any of 19 patients studied.

On serial hemodynamic study, there is a decrease in cardiac output and right and left ventricular compliance coincident with clinical signs of chronic rejection. Under conditions of exercise, these functional abnormalities may be seen even though the resting dynamics are normal.

The response of the denervated human heart to digitalis, isoproterenol, and glucagon is similar to normal.

The denervated heart can maintain adequate cardiac output even though the regulatory mechanisms are interrupted.

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REHABILITATION FOLLOWING CARDIAC TRANSPLANTATION *

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Les auteurs rapportent le cas de leur malade qui est l'un des survivants à long terme. Ce patient de 54 ans souffrant d'insuffisance coronarienne sévère a reçu le cœur d'un malade de 18 ans. La classe d'histo-compatibilité était B-. Le patient a présenté un premier épisode de rejet aigu au 12e jour après l'intervention. Il a reçu le traitement immunosuppresseur standard consistant en globuline antilymphocytaire, azothioprine et prednisone. L'étude de la fonction pulmonaire deux mois après l'intervention et à répétition par la suite a montré une image de pneumopathie obstructive. La fonction cardiaque fut évaluée cinq mois après l'intervention. A ce moment les pressions et le débit cardiaque étaient normaux, l'exercice modéré amenait une augmentation du débit cardiaque par l'augmentation du volume d'éjection systolique sans modification de la fréquence cardiaque. A l'effort

The early mortality from cardiac transplantation has been high. One hundred and thirty-three operations have been performed since December 1967 (1). Sixty patients (45 per cent) have died in the first month following operation. Twenty-two of the first 100 patients are alive six months or more following transplantation.

This report will briefly outline the course of C.P.J. who is one of the 22 "long term" survivors (3).

The patient was 54 years old at the time of his transplantation on November 17, 1968. He had an eleven year history of coronary artery disease. His physical activity was markedly limited for three years prior to operation. He had several admissions to hospital for treatment of acute left ventricular failure.

One month prior to operation he was admitted to hospital in gross heart failure and for about ten days it was feared that he would not survive. After thorough discussion cardiac transplantation was felt to be justified.

The donor was an 18-year old boy who died of a head injury. Tissue typing revealed a matching grade of B – based on Terasaki's classification (2).

Details of the operation have been reported elsewhere (3). The donor heart was anoxic for 59 minutes at normothermia. The recipient heart showed several old infarcts and severe diffuse coronary artery disease.

A pericardial friction rub was heard within 24 hours of operation. It became louder on the 12th day, thereafter decreasing in intensity but not clearing until the 40th day.

Serial electrocardiograms have consistently shown sinus rhythm. Nonspecific T wave abnormality developed at the end of the first week. There has been a steady shift in the mean QRS electrical axis toward the right (Figure 1). The QRS voltage in lead I has fluctuated to a lesser degree than the voltage in lead V5 or V6. There was an interesting dissociation in the voltage changes in these leads

* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6–8, 1969.

extrême cependant le débit cardiaque augmentait surtout à cause de l'augmentation de la fréquence cardiaque. L'évolution du malade au point de vue électrocardiographique montre un axe qui dévie progressivement vers la droite et une baisse progressive du voltage qui peut être le témoin possible d'un rejet chronique.

Au point de vue clinique le malade a connu une évolution tout à fait satisfaisante avec une nette amélioration de son état. Quatre mois et demi après l'intervention le malade a démontré une réaction d'hypersensibilité à la globuline antilymphocytaire qui a donc dû être cessée à ce moment. Le malade n'a accusé aucun trouble psychologique subséquemment à l'intervention. Dans l'ensemble de son évolution il n'y a donc eu qu'un seul épisode de rejet dont le diagnostic fut posé par les modifications électrocardiographiques à ce moment.

between the fourth and eleventh days. The voltage was decreasing in V5 but increasing in lead I. In the past three months there has been a steady fall in voltage in the chest lead whereas lead I has remained essentially unchanged. Decreasing voltage in the electrocardiogram has not been associated with other evidence of rejection and the patient has remained clinically well.

Standard doses of immunosuppressive therapy have been used. Antilymphocytic globulin injections were stopped after four months and a half because of severe reactions. Maintenance therapy consists of azathioprine 150 mg and prednisone 20 mg daily.

Rejection:

On the twelfth day, the patient probably experienced an episode of acute rejection (figure 2).

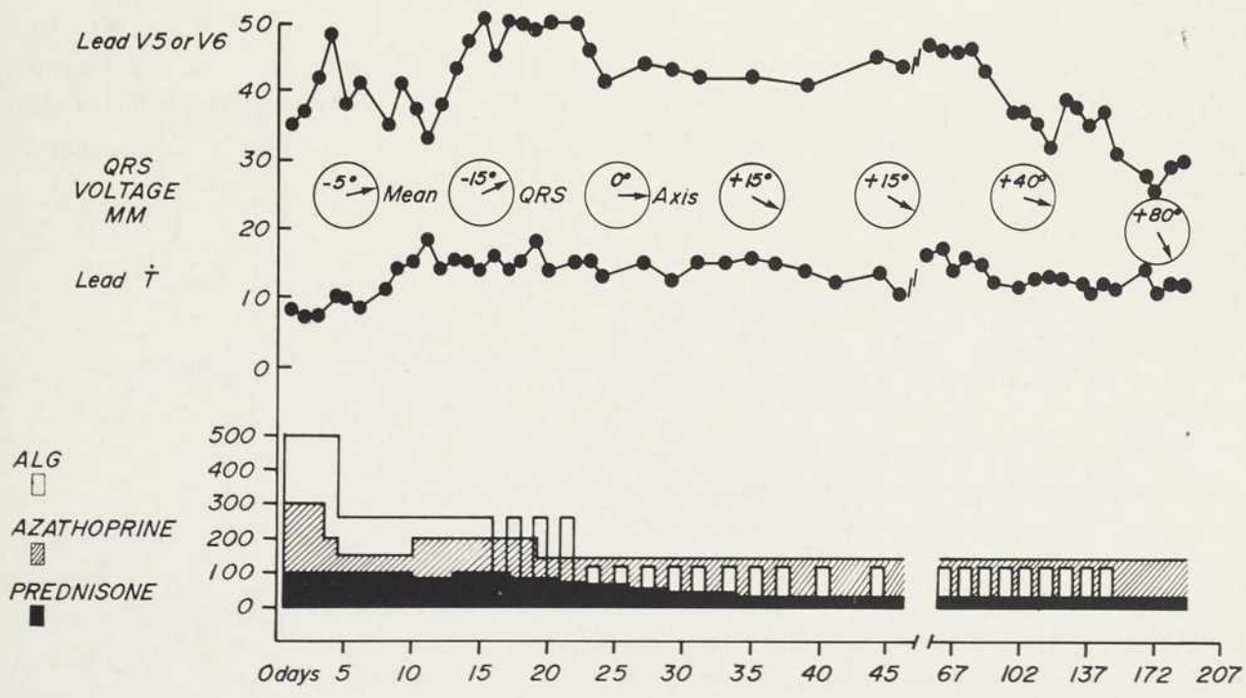

Figure 1 — Changes in QRS voltage in lead I and in lead V5 or V6 (maximum QRS voltage in either lead). Note the steady fall in voltage in the chest lead over the past three months but relatively stable voltage in lead I. There has been a progressive shift in the electrical axis to the right.

He had a chill, the pre-existing pericardial friction rub intensified and the leukocyte count and erythrocyte sedimentation rate increased markedly. The QRS voltage in the chest leads had been decreasing (Figure 1). In the preceding four days, there was a minimal rise in the percentage of LDH_1 to 42–46 per cent. Slight elevation of jugular venous pressure was the only indication of heart failure. The patient felt well and he continued to have a good appetite.

Haemodynamic and pulmonary function studies[1]:

Five months after transplantation, right and left heart catheterization was performed at rest and during supine exercise at various work loads. At rest, pressures and cardiac output were normal.

During mild exercise (100 kpm/min.) there was an increase in cardiac output from 5.7 l/min. to 9.1 l/min. This occurred as a result of an increase in stroke volume, without change in heart rate. With more strenuous exercise (300 kpm/min.) the cardiac output increased to 10.2 l/min. This additional rise in cardiac output was due to an increase in heart rate without a further increase in stroke volume. During moderate exercise (300 kpm/min.) the pulmonary artery pressure increased to 46 mm Hg. The left ventricular end diastolic pressure during mild exercise (100 kpm/min.) rose to 18 mm Hg.

Pulmonary function studies were done two months after surgery and repeated at intervals. An obstructive ventilatory defect and considerable limitation of maximal breathing capacity were demonstrated.

On exercise, at more than 100 kpm/min. heart rate and oxygen uptake increased linearly with each increment in work load. Maximal work achieved was 500 kpm/min. and at this level minute ventilation approximated the patient's maximal breathing capacity. Thus his maximal work capacity may be limited by his ventilatory defect.

Comments:

The patient has made steady progress since operation. He has gradually gained weight and enjoys an excellent sense of well being. He has not experienced dyspnoea or chest pain and regular examinations have shown no evidence of heart failure.

Three months after operation, he was walking two miles each day and jogging 100–200 yards at a time. His physical activity was temporarily reduced when he experienced severe ALG reactions necessitating withdrawal of the drug at four months and a half. In the two months since ALG was stopped, he has increased his activity so that he

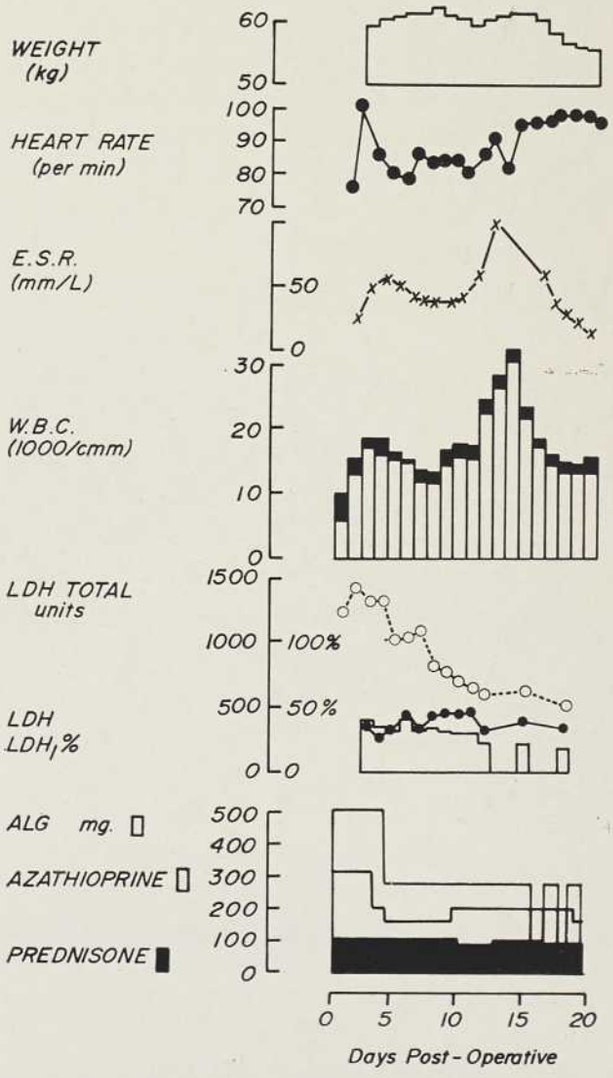

Figure 2 — Changes associated with a probable episode of acute rejection on the 12th day.

1. **The haemodynamic and pulmonary function studies will be reported in detail in a separate communication.**

now walks three to four miles per day. He can climb three flights of stairs without dyspnoea or fatigue.

He has experienced only one possible episode of acute rejection. One can only speculate on the significance of the gradually decreasing voltage in the chest lead of the electrocardiogram. Is it an indication of chronic rejection ?

There has been interest in the psychological reaction of heart transplant patients since the report from Palo Alto which indicated psychotic reactions in five of their survivors. Our patient's psychological state has remained normal.

ADDENDUM

At 13 months the patient continues to be well and to enjoy normal activity. There has been no evidence of heart failure. The chest X-ray shows a normal sized heart and clear lungs.

The QRS voltage in the electrocardiogram did not change in response to an increase in the dose of prednisone at six months, and it has remained stable over the past seven months.

There has been no evidence of reinnervation of the heart. Maintenance therapy consists of azathioprine 150 mg per day and prednisone 15 mg daily.

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PATHOGENESIS OF HEART REJECTION PHENOMENON *

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Nous rappelerons, ici, quelques notions de base sur la comprehension des mécanismes immunologiques dans le rejet des greffes d'organes.

La présence des antigènes, dits de transplantation, sur toutes les cellules nuclées de l'organe greffé, est à l'origine d'une série de processus immunologiques qui aboutiront, à plus ou moins brève échéance au rejet de la greffe. Ces antigènes de la transplantation peuvent être mis en évidence à l'aide d'antisérums spécifiques pour chacun de ces antigènes. C'est ce que l'on appelle le typage.

Une fois reconnus comme étrangers par certaines cellules de l'organisme, petits lymphocytes ou macrophages spécialisés, il se produit une série de processus successifs aboutissant à la production d'outils immunologiques spécifiques ; les anticorps et les cellules sensibilisées. Ce sont les agents responsables du rejet de greffes.

INTRODUCTION

With a few exceptions, the grafted hearts have been the site of the rejection episodes that have caused the death of the majority of patients. Since the beginning of this century and particularly during the last ten to fifteen years, it has been demonstrated that the rejection phenomenon of a transplanted organ is entirely due to immunological mechanisms [Medawar (13), Brent (4), Gowans (6)]. Despite all the immunosuppressive means so far employed, the transplanted organ is always the site of the immunological mechanisms of rejection. These rejection episodes may happen at any time, from the moment of the transplant until years after the graft is performed.

However, because the chances of a one-year survival time in well-matched kidney recipients has reached 85 per cent and afforded the patient a better life, similar results were expected for human heart transplantation. The latter procedure was therefore, undertaken with these considerations in mind. Unfortunately, the heart seemed to be more vulnerable to rejection than the kidney or the liver, in the sense that its function cannot be interrupted while measures are being taken to overcome a rejection episode.

This paper will deal with the following problems involved in the pathogenesis of the rejection phenomenon; why and how sensitization occurs, what rejection is, and how the immunological products mediate the tissue injury referred to as rejection.

Why and how does sensitization occur?

The term "sensitization" implies the development of immunological processes subsequent to the introduction of a foreign antigen into the body. These sensitization processes lead to the production of specific humoral antibodies and specific sensitized cells capable of reacting specifically with the same antigen present on the cell membranes of the transplanted organ. Such reactions cause the cellular injury or the episodes of rejection.

Transplantation antigens: Like any other organ, the heart is composed of nucleated cells. The cellular membranes of these cells are made up of numerous chemical structures, some of which are peculiar

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Ces produits immunologiques spécifiques pénètrent, via la circulation, dans l'organe greffé et induisent les premiers signes histo-pathologiques du rejet. Ceci se traduit par une infiltration de lymphocytes sensibilisées et de leurs formes blastiques, d'abord intravasculaire, puis successivement s'étendant dans l'espace périvasculaire et parenchymateux.

Il y a deux types de rejet. L'un, acellulaire, est causé par des immunoglobulines qui se fixent aux antigènes de la cellule greffée et, en présence des enzymes du complément, induisent le rejet. L'autre, cellulaire, est peut-être le plus important. Il est causé par les réactions directes des lymphocytes sensibilisés avec les antigènes de la greffe. Cette réaction entraîne la libération d'un médiateur chimique, toxique pour les cellules de la greffe. Ce facteur est capable de provoquer la lyse des cellules de la greffe en l'absence des enzymes du complément.

to a species, while others are characteristic of the organ and of an individual member of a given species. These substances are immunogenic and the sensitization that occurs following transplantation is caused precisely by these chemical structures. The substances are the histocompatibility antigens, also called the "transplantation antigens".

Characteristics of the transplantation antigens: The transplantation antigens are genetically determined and are controlled by dominant genes. This pre-determination implies that there are genetic structures controlling their synthesis and reproduction. The antigens are present, in different concentrations in all cellular and cytoplasmic membranes of nucleated cells and some are unique to each individual within a species.

Typing of the transplantation antigens: Our knowledge of the transplantation antigens is relatively new. It has been shown that many of these antigens are different from those present on the surface of red blood cells, but comparatively little is known as regards their composition and the method of extracting and purifying them.

Specific antibodies of these antigens are found in the serum of some multiparous women and in the serum of patients who have been given repeated blood transfusions. These specific antisera are used to determine the presence or absence of the antigens on the cells of a particular individual. This determination of the antigenic pattern of an individual is called "typing". Unfortunately, sera for detecting all the antigens are still lacking as are uniform, reproducible and standardized universal techniques for their detection. In spite of this, the actual data on the histocompatibility of heart transplantation (Potworowski *et al.*, *Laval méd.*, **41** : 217, 1970) have shown that a well-matched heart has a better chance of survival than a poorly matched one. These results have confirmed the experience of many investigators as regards kidney transplantation.

Lymphoid organs involved in sensitization: The transplantation antigens are the agents that induce, in the recipient, the sensitization processes leading to the production of the specific immunological products and the graft rejection. The sensitization processes take place mainly in the spleen and the lymph nodes, but the steps leading to the production of specific immunological products are not yet completely known. Cells giving rise to the production of plasma cells and humoral antibodies are believed to transform and divide in the lymphoid follicles. The participation of this humoral mechanism of defence in organ rejection is still debated. Antibodies are definitely involved in the immediate type of rejection occurring in the early minutes following the transplantation and in the late or chronic acellular type of rejection occurring months and even years after the transplantation.

On the other hand, cell-mediated immunity seems to play a major role in rejection episodes. It has been observed that lymph nodes draining the site

of a graft increase in size and are subject to considerable cellular changes. Large pyroninophylic cells, first described by Scothorne and MacGregor (18) and later studied in detail by Turk's group (22), appear in the paracortical area of the draining nodes. It is believed that these large cells transform into sensitized blastic cells and then into sensitized lymphocytes.

Sensitizing steps: Figure 1 summarizes the processes occurring after an antigenic stimulation and leading to the production of the specific immunological products.

Burnet and Fenner (5) were the first to postulate, in 1949, the existence of a mechanism that, after antigenic stimulation, can distinguish its own antigens from foreign antigens. Billingham *et al.* (3) have proven the existence of such a mechanism, which is now called the "recognition process"

It is not known which cells are involved in this recognition processus, or how and where it takes place. However, the results of experiments reported by Medawar and Strokes in 1965 and also by Gowans (6) indicate that some small lymphocytes circulating in the graft could recognize foreign antigens from their own. The actual hypothesis is that once "activated", these lymphocytes come into the paracortical area of the lymph nodes and, consequently, transform into sensitized lymphocytes. Another type of experiments led Nossal (15) to believe that macrophages of a particular type, the dendritic macrophages, also have this capacity to distinguish between foreign antigens and their own.

Once it is identified as foreign, the antigen is degraded into its immunogenic components in specialized types of macrophages. The information is then believed to be passed on to a thymic dependent, "antigens-sensitive cell", which divides and transforms into specialized productive cells.

These cells then enter into a productive phase The mature plasma cell responsible for the synthesis of humoral antibodies is an end result of the transformation of these antigen-sensitive cells. It synthesizes the immunoglobulins. The sensitized

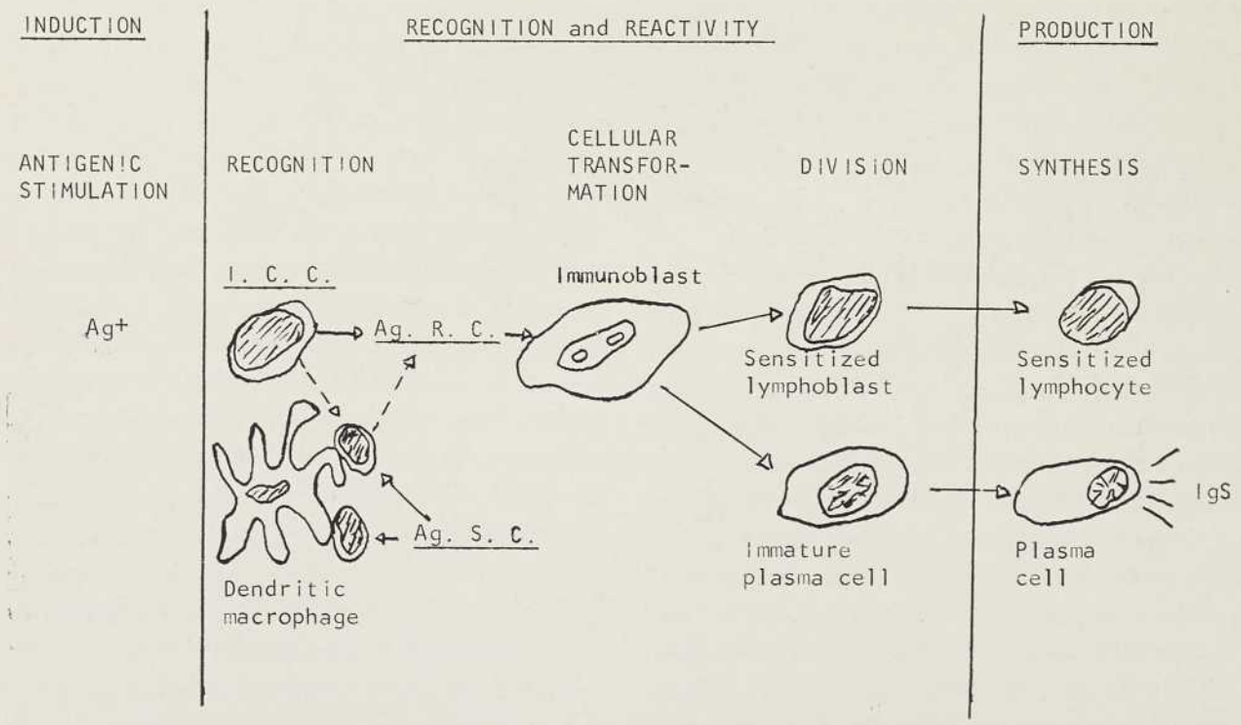

Figure 1 — Phases of sensitization. Legends : I.C.C. : Immunological competent call. Ag.R.C. : Antigen reactive celle. Ag.S.C. : Antigen sensitive cell.

lymphocyte is another end result of the cell transformations. It is responsible for "cell-mediated immunity", which plays a major role in cellular injury and tissue rejection.

What is rejection?

At the end of the sensitization process, many sensitized lymphocytes and antibodies specifically trained to react with the foreign antigens of the graft are present. Via the circulation, these immunological products can easily reach the foreign antigens of the graft and react with them. From an immunological point of view, rejection is the result of these immunological reactions between the foreign antigens of the graft and the specific immunological products. The intensity of the reaction varies with the quantity of immunological products available and capable of reacting with one or more different antigens contained in the grafted tissue. The degree of the reaction varies with its localization in the organ and the avidity of the antibodies and sensitized cells for the antigen. Clinical and laboratory signs may be completely absent, in spite of a profound cellular injury. Only histopathological changes can disclose the earliest signs of rejection, sometimes long before functional impairment of the organ is manifested, and long before any detectable changes have occurred in the serum and circulating cells.

Histological signs of rejection: Table I summarizes the first histopathological signs of an immunological reaction. Accumulation of sensitized cells in the blood vessels of the graft is often one of the first histopathological signs of rejection. Infiltration of the blood vessel wall and, later, of the perivascular area with mononuclear cells often constitutes the first immunopathological signs of organ rejection. Progressively and rapidly, these cells invade the surrounding parenchymal tissue. The sensitized lymphocytes react with the foreign antigens of the graft in such a way that they may cause complete disintegration of the parenchymal cells. Macrophages and large phagocytes then appear, to remove the cellular debris.

How do immunological products produce damage?

Many workers, including Snell (19), Amos (1), and Waksman (23), have described the histopathological changes occurring in grafted tissues and organs after transplantation. The rejection phenomena may be divided into two different types, one of which is acellular, while the other is cellular.

The acellular type of rejection is caused by preformed antibodies capable of reacting with the antigens of the graft. The antigen-antibody reaction taking place causes thrombosis characteristic of an Arthus-like reaction. There is no mononuclear infiltration, but polymorphonuclear leucocytes appear in this type of rejection, which sometimes occurs in the hours immediately following the transplantation. Such an acute type of rejection is probably due to cytotoxic antibodies similar to those described by Merrill (14) and Kissmeyer-Nielsen *et al.* (8) in dogs presensitized with leucocytes and platelets. In man, this type of rejection may be predicted if care is taken to look for anti-leucocyte or anti-organ antibodies before transplantation. Barnes *et al.* (2) have also described a type of rejection that takes place without the intravascular formation of thrombi. The cytotoxic antibodies can produce damage of this kind when they combine with the antigens and some of the factors of complement.

TABLE I

Histopathological characteristics of rejection cellular infiltration : histiocytes – lymphoblasts – lymphocytes immature and mature plasma cells

SITE OF INFILTRATION		SIGNS OF REJECTION
Intravascular :	Cell accumulation Hyperplasia Oedema	Proliferative Endarteritis
Perivascular :	Infiltration Obstruction	Vascular Obstruction
Intraparenchymal :	Cellular damage Phagocytes Fibrosis	Cellular lysis and desintegration

The cellular type of rejection is characterized by a specific cellular infiltration of lymphocytes, histiocytes, plasma-blasts, and plasma cells inside the blood vessel wall and in the perivascular area. Obliteration of blood vessels often follows this stage and further infiltration and disintegration of parenchymal tissue is the rule. This type of rejection is caused by a cellular infiltration that is similar in many aspects to the one found in the delayed hypersensitivity type reaction.

The role of sensitized lymphocytes in the production of lesions is much more complex. The lymphocyte is a multipotent cell capable of transforming (Holub 1962; Nossal 1963) into histiocytes, plasma cells, and perhaps into other similar lymphocytes.

Sensitized lymphocytes alone can cause cellular damage without the participation of antibodies or complement in target cells *in vitro* as well as *in vivo* (10). Cellular damage has never been reported to occur after transfer of antibodies, but it is possible that both mechanisms, the cell-mediated immunity and humoral antibodies, may join together to produce a more serious tissue injury.

The direct participation of lymphoid cells in the production of cellular damage has been shown by *in vitro* and *in vivo* experiments. Rosenau and Moon (17) demonstrated that, upon short contact, sensitized lymphocytes can destroy target cells in tissue culture. Taylor and Culling (21) found that homologous and heterologous spleen cells from animals sensitized with L fibroblasts were capable of destroying L fibroblasts in tissue culture. Both Weaver *et al.* (24) and Snell (19) showed that lymphocytes attach themselves to the target cells before destruction of the cells appears and, according to the latter author, both cells perish in this cell to cell interaction. Lawrence and Pappenheimer, in 1956, and Maurer (12) found that human blood leucocyte extracts are capable of transferring only the delayed hypersensitivity type reaction and are unable to transfer the capacity to synthesize serum antibodies. Many other types of experiments have shown that the lymphocytes are key cells responsible for a large percentage of rejections of grafted organs. The exact mechanism by which the lymphocytes kill the cells is not fully understood. However, recent experiments have led us to believe that, once fixed to its specific antigen, the lymphocyte secretes a factor that is toxic for the target cells.

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MANAGEMENT OF ACUTE REJECTION IN HEART TRANSPLANTS *

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Le début est ordinairement insidieux, puis le processus évolue rapidement de manière logarithmique.

La réversabilité de la crise dépend : *a*) de la précocité du diagnostic, et *b*) de l'intensité du traitement.

Même en face d'un diagnostic douteux, il est recommandé d'entreprendre le traitement. Celui-ci fait appel à l'Azathioprine, aux corticoïdes et au sérum antilymphocytaire à des doses variables selon le cas.

La corticothérapie à dose élevée s'avère le facteur thérapeutique initial le plus important.

Si aucune réponse favorable n'est obtenue dans les 24 heures, la dose quotidienne de corticostéroïdes devra être doublée, et celle du sérum antilymphocytaire augmentée.

Au cas où la crise de rejet s'accompagne de thrombocytopénie, ASA, Anturan et Phenylbutazone devront être envisagés.

INTRODUCTION

Although there are certain significant differences between rejection crises in renal and cardiac transplants, in that cardiac rejection is more difficult to recognize and to reverse, I think the same basic philosophy of management applies to each.

At a time when no rejection is occurring, we are usually dealing with a state of artificial tolerance induced by immunosuppressive agents. We have therefore a very delicate balance between ''tolerance'' and rejection. When the balance breaks down in favour of rejection, the process usually begins slowly and rapidly gains momentum in a wave-like or almost logarithmic fashion. Whether or not the tide can be reversed therefore depends upon 1) how early rejection is detected and 2) how vigorously it is treated.

Therefore, the major principles of treatment are 1) early recognition 2) early and vigorous anti-rejection therapy. If the diagnosis is doubtful, as it often is, it is better to institute full treatment, rather than to withhold treatment to a point where the rejection is irreversible. These principles are obviously more important in acute rejection crises, than in so-called chronic insidious rejection.

In regard to specific treatment, the patient would, of course, be already receiving azathioprine, corticosteroids and ALS, the dosage of each depending upon the length of time post transplant, the degree of previous rejection and other factors, such as leucopenia and infection.

Large doses of corticosteroids are certainly the most important initial therapy. The actual dosage employed varies from center to center and also depends upon the dosage at which rejection occurred. In order to obtain initial and sustained high blood and tissue levels, the intravenous route every four to six hours is preferable. Hydrocortisone as opposed to cortisone analogues is preferred by some groups in that there is evidence

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suggesting that it has a greater immunosuppressive effect.

The dosage of azathioprine should be increased to maximum tolerated levels. This in itself usually will not act in time to reverse the rejection but it will maintain a higher level of immunosuppression if the rejection is reversed by other means.

If there is no obvious response within twenty-four hours the daily dose of corticosteroid should be doubled and ALS should be either increased or restarted if it has been discontinued. It has been suggested that ALS should be given intravenously for established rejection, if not at all times. Routine postoperative local irradiation of the graft as introduced by Hume et al. has not been statistically significant in prolongation of cadaveric renal transplants, but in some cases seems to have helped to reverse rejection crises, particularly those of the cellular type. We have not employed it in our cardiac cases.

In renal transplants, we have found a three to four day course of Actinomycin C or D, effective in reversing resistant rejection episodes. However, the beneficial effect is usually not evident for three or four days. This would seem to be worth trying in cardiac transplants.

If the rejection crisis is accompanied by significant thrombocytopenia, thus suggesting an acute vascular type of rejection, agents which inhibit the tissue response to immunological injury, such as ASA, anturan and phenylbutazone, should be considered.

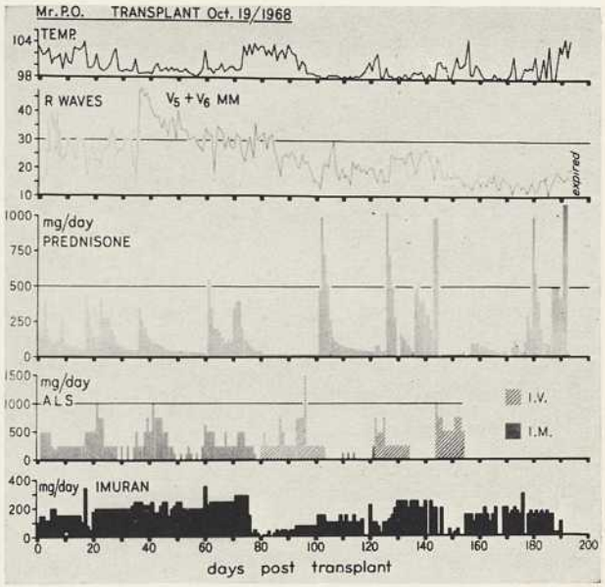

Figure 1 — Management of several rejection crises in one patient.

An example of how we attempted to manage several rejection crises in one of our patients are shown in Figure 1. It can be seen that initially decreases in R wave voltage, which were interpreted as rejection, responded to large doses of prednisone. Subsquently, the R wave voltage fell progressively in spite of intermittent massive doses of prednisone. The patient died seven months following cardiac transplantation of an undiagnosed infectious illness. At autopsy pneumocystis carinii were found in the lungs. The heart showed moderate chronic vascular rejection and fibrosis. It is felt that excessive immunosuppressive therapy, in part falsely based on electrocardiographic signs of rejection, led to the patient's death.

CHEMICAL PREVENTION OF MYOCARDIAL NECROSIS *

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Des nécroses cardiaques métaboliques, sans occlusion coronarienne, peuvent être produites expérimentalement chez l'animal par un traitement simultané de corticoïdes (gluco-minéralocorticoïdes) et de sels de sodium (Na_2HPO_4). Cette cardiopathie électrolyte-stéroïde avec nécrose (CESN) est aggravée par l'exposition subséquente au stress ou par l'ingestion de lipides. La CESN est accompagnée d'une diminution marquée du potassium sérique et myocardique et peut être inhibée chez l'animal par l'administration orale de KCl ou de $MgCl_2$.

L'industrie pharmaceutique a mis au point plusieurs préparations (organiques et inorganiques) de sels de potassium et de magnésium, recommandées pour la prophylaxie de l'infarctus du myocarde et même pour la période « post-infarctoïde ». Une autre application clinique de ce principe est l'utilisation de solution polarisante : insuline, glucose et potassium, mais l'action bénéfique de cette « solution polarisante » est très controversée. De tous les sels utilisés, le chlorure de potassium apparaît le plus efficace, mais une trop forte dose peut provoquer des troubles gastro-intestinaux et son action sur la kalémie est de courte durée. En fait, tous ces procédés présentent de nombreux désavantages.

Although this congress is primarily concerned with cardiac transplantation in relation to the rejection phenomenon, it might be in order also to consider recent progress in the prevention of other forms of myocardial damage by drugs. In animal experiments, it has been possible to protect the myocardium against various forms of injury by potassium-sparing agents; hence it may be worthwhile to explore the possibility of offering similar protection against the rejection phenomena.

Metabolic infarctoid necroses without occlusive vascular lesions have been produced by various combinations of steroids, electrolytes, stress and lipids (14 and 16). However, for the routine screening of potentially antinecrotic agents, we usually employ those variants that are produced by glucomineralocorticoids (*e.g.*, methylchlorocortisol, fluorocortisol) in combination with Na_2HPO_4, stressors, or lipids. These models of metabolic myeardial necroses have been selected because, unlike those elicited by cardiotoxic drugs (plasmocid, papain, etc.), they depend upon factors likely to play a role also in cardiac diseases of man.

The ESCN is associated with a sharp drop in myocardial and serum potassium (12). Furthermore, this type of experimental cardiac necrosis can be prevented by the oral administration of KCl or $MgCl_2$ (14). Hence, industry has made available a large number of potassium and magnesium preparations, among them several organic salts, recommended for the prophylaxis of myocardial infarction and even for treatment during the post-infarction period. Numerous investigators reported favorable results with this form of medication (2, 7, 8, 10, 11, 23 and 25), but in the absence of a truly significant large-scale double-blind study, a definite conclusion as to its clinical usefulness would be premature.

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D'autres approches nous semblent plus indiquées pour résoudre ce problème. Poursuivant nos études, nous avons pu démontrer l'inhibition de la CESN par la spironolactone, un antagoniste de l'aldostérone. De plus, ce composé inhibe les lésions cardiaques et les autres manifestations de l'intoxication à la digitale et aussi l'anesthésie produite par divers stéroïdes sans action corticoïde. Comme toutes ces activités protectrices se manifestent même après la néphrectomie bilatérale, il semble que l'effet prophylactique de la spironolactone contre les nécroses cardiaques soit dû à un mécanisme d'action autre que le seul maintien du potassium sérique.

Une autre solution au problème de fournir suffisamment de potassium au cœur endommagé est le traitement avec des antikaliurétiques. Sous ce rapport, le triamtérène et l'amiloride se sont révélés très efficaces pour prévenir la formation de nécrose cardiaque par diverses techniques chez le rat. L'amiloride est encore une drogue au stade expérimental, mais la spironolactone et le triamtérène sont déjà disponibles pour usage clinique et se sont révélés tolérables par des patients qui les recevaient pour des traitements différents.

Dans le passé, on a surtout mis l'accent sur l'emploi de dilatateurs coronariens et, malgré l'extraordinaire activité de la digitale, peu d'attention a été accordée à l'emploi d'autres drogues myotropes. Le potassium a déjà été utilisé avec succès contre les arythmies cardiaques. Nous croyons qu'il serait maintenant désirable d'entreprendre des études cliniques sur l'utilisation de certains composés prophylactiques, de préférence par voie orale, non toxiques en usage prolongé par des « cardiaques en puissance » et qui sont, de ce fait, en danger constant. Nous croyons que l'amiloride, le triamtérène et la spironolactone possèdent ces propriétés.

It must be emphasized that, at least in the experimental model, organic salts of potassium or magnesium are not superior to the chloride; in fact, even chlorides of other cations (*e.g.*, NH_4Cl, $CaCl_2$) possess some prophylactic effect, perhaps by virtue of their acidifying property (14 and 16). In any event, as judged by animal experimentation, KCl and, to a somewhat lesser extent, $MgCl_2$ appear to be the most active prophylactic electrolytes.

Another clinical application of the same principle was recommended by Sodi-Pallares and his associates who use intravenous infusions of potassium, insulin and glucose, the so-called "polarizing solution" (20 and 21). Although the authors credit the animal experiments on the ESCN for the stimulus to attempt this form of treatment, it must be pointed out that we did not demonstrate any enhancement of the potassium effect by additional treatment with glucose and insulin. Furthermore, even with KCl we obtained only a prophylactic, not a curative effect. Though many investigators confirmed the beneficial action of the "polarizing solution" (4, 5, 6, 9 and 13), others denied it (1, 3, 22, 23 and 24) and — at least in its present form — it does not appear to be of striking value.

Of course, oral treatment with KCl is likewise not ideal for clinical use; the salt is unpleasant to take, it may cause gastro-intestinal irritation and even jejunal ulcers, and besides, its effect is of short duration. After a single oral dose of KCl, the blood potassium rises sharply, often to dangerous levels, but soon returns to normal. Thus, this medication does not lend itself well to the prophylaxis of cardiac necroses which may occur unpredictably at any time and therefore require chronic preventive measures. In our animal experiments, KCl was very effective, but only because we could predict the onset of myocardial necrosis within hours and hence had to provide the necessary potassium merely during a very limited period.

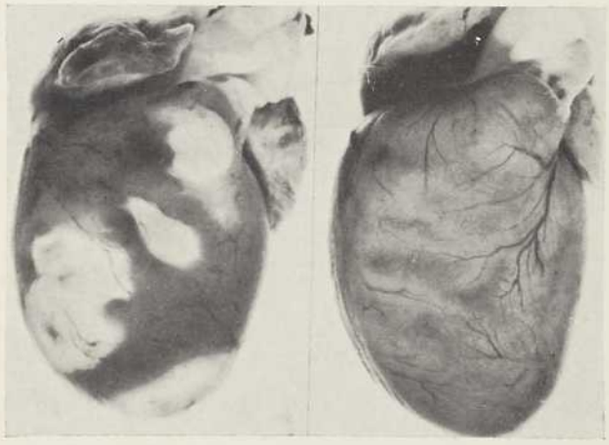

Figure 1 — Infarctoid myocardial necroses of the ESCN type (left) and their prevention by amiloride.

It seemed reasonable therefore to look for more convenient and more lasting ways to provide the myocardium with the necessary amount of potassium. Again using our experimental models of infarctoid cardiac necroses, we were able to accomplish this first by using spironolactone (15). This compound has been chosen because we felt that through its mineralocorticoid blocking action, it would abolish the effect of the corticoids which are used as part of the conditioning procedure in our test. However, as previously stated, spironolactone also possesses other blocking actions; in fact, we observed that it protects the rat even against the production of myocardial necroses by combined treatment with digitoxin and Na_2HPO_4 (19).

It will be remembered that the aglycones of cardiac glucosides resemble the corticoids in that they possess a steroid nucleus and since, in addition, they have a lactone ring at C_{17} they are structurally even more closely related to spironolactone than is desoxycorticosterone or aldosterone. It was tempting to assume, therefore, that in the digitoxin-induced cardiopathy spironolactone acted again by virtue of its steroid (although not mineralocorticoid) blocking effect. However, more recent observations have shown that spironolactone prevents not only the cardiac but also the extracardiac manifestations of digitoxin poisoning; indeed it prevents even the anesthesia produced by various steroids possessing no corticoid potency. Since all these actions are manifest also after bilateral nephrectomy, it is dubious whether the prophylactic effect of spironolactone against cardiac necrosis should be ascribed to its classic effect: the blockade of mineralocorticoid-induced sodium retention and potassium elimination at the level of the renal tubule (18). In any event, in spironolactone we found another compound which antagonizes experimental myocardial necroses produced by various means. Its antidigitoxin effect should also be kept in mind because the compound is often given to patients digitalized because of cardiac failure.

Yet another approach to the problem of providing sufficient potassium to the failing heart is treatment with potassium-sparing agents. In this connection, it is noteworthy that both amiloride (17) and triamterene (18) pretreatment can prevent infarctoid necroses produced by various techniques in the rat. Amiloride is still only an investigational drug but both triamterene and spironolactone are available for clinical use. They have been found to be well tolerated by patients who received them for other reasons (mainly as adjuncts to diuretics that cause excessive potassium losses). Hence, double-blind studies concerning their possible prophylactic value in patients predisposed to myocardial necrosis would be highly desirable.

I think you will all agree that cardiac transplantation must remain a procedure of last resource. Cardiac infarction claims about 500,000 lives a year in the United States alone ! What we need to combat this hazard — one of the greatest of contemporary life — is an effective prophylactic. This could be a dietary measure, perhaps combined with certain remedies, preferably agents that can be taken by mouth and are not toxic even when used over long periods by "coronary candidates" whose life is in constant danger.

In the past, major emphasis was placed upon coronary dilators and, despite the extraordinary effectiveness of digitalis, little attention was given to other myotropic drugs which affect the cardiac muscle cell directly. Potassium has been used with

success to combat arrhythmias and it undoubtedly plays an important part in the mechanism of digitalis action. Using the ESCN as a model, potassium as well as magnesium chlorides were found to possess also an antinecrotic effect which is shared by various potassium-retaining (amiloride, triamterene) and antimineralocorticoid (spirinolactone) agents. Perhaps now the time has come to initiate clinical studies along these lines.

ACKNOWLEDGEMENTS

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ANTILYMPHOCYTE SERUM AND PROCARBAZINE COMPARED AS IMMUNOSUPPRESSANTS IN MICE, RATS AND RABBITS *

P. Brian STEWART and Rosalind BELL †

Des expériences pratiquées sur des souris et des lapins ont permis d'établir que la Procarbazine est un immunosuppresseur puissant agissant sur les réactions immunologiques d'origine cellulaire et d'origine humorale. Chez le rat, elle supprime la formation d'anticorps circulants en réponse à l'injection d'albumine sérique de bœuf, et elle diminue considérablement le niveau des hémaglutinines circulantes résultant de l'injection de globules rouges de mouton. Chez la souris, la Procarbazine prolonge la survie des allogreffes de peau en dépit d'une puissante incompatibilité tissulaire. La Procarbazine, que l'on utilise déjà comme agent anticancéreux chez l'homme, semble donc mériter un essai prudent, pour vérifier si la puissante action immunosuppressive observée chez l'animal se retrouve chez l'homme.

Progress in transplantation research has been held up especially in man by the failure to prevent the eventual rejection of grafted organs. Several ways to solve this problem are currently under intensive study, such as tissue typing to ensure the best histocompatibility match (4); identification, solubilization and injection of transplantation antigens into potential recipients to ensure tolerance prior to grafting, perhaps combined with the use of enhancing antibody (21); and lastly (the field we happen to be interested in), the search for new agents or chemicals to suppress the immune response. At the moment, no one can tell which approach will turn out to be the most successful, as all have major problems and disadvantages to be overcome.

Antilymphocyte serum (ALS) was a landmark in the search for new immunosuppressant agents, and gives a glimpse of the potential of this approach. Experimentally, it is the best immunosuppressant, and has become the standard by which to judge others.

No previously known chemical immunosuppressant would prolong the survival of skin grafts as strikingly and reliably as ALS, until the discovery of procarbazine, which Floersheim (9) claimed could do this across strong histocompatibility barriers. This study was undertaken to ascertain the effect of procarbazine on both the humoral and cell mediated immune responses in several species, and to compare its potency with that of ALS.

MATERIALS AND METHODS

Animals:

The mice were obtained from the Jackson Laboratories, Bar Harbor, Maine; the Sprague-Dawley rats and the rabbits from Canadian Breeding Laboratories, Montreal. All animals were given water and regular laboratory chow *ad libitum*. If for any reason antibiotics were needed to treat one animal, then all the animals in the test and the corresponding controls were given them.

Preparation of the antilymphocyte sera:

The antiserum to mice was produced in white New Zealand rabbits, using the method of Gray

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et al. (10). The antiserum against rats was produced by immunizing white New Zealand rabbits with a mixture of thymus and lymph node cells from Sprague-Dawley rats (16×10^7 cells/rabbit), suspended in complete Freund's adjuvant and injected into the four footpads. Three weeks later, a booster dose of a suspension of 10×10^7 of the lymphoid cell mixture per rabbit was administered intravenously without Freund's adjuvant. One week later, the rabbits were exsanguinated under sterile conditions and the blood pooled.

The antiserum against rabbits was produced by immunizing a horse. The first injection of a mixture of 2×10^9 thymus and lymph node cells obtained from Himalayan rabbits was mixed with incomplete Freund's adjuvant and given intramuscularly in several sites. Seven days later, the same number of lymphoid cells, this time obtained from white New Zealand rabbits, suspended in incomplete Freund's adjuvant was given, followed 14 days later by a similar immunization. One week later, the horse was bled by arterial catheterization. This same horse had also been simultaneously immunized with mouse lymphocytes so that it was a bispecific or bivalent ALS. The anti-mouse component was active as an immunosuppressant.

All antisera were heated to 56° C for 30 minutes to destroy complement, and stored at −20° C. They were sterilized before use by passage through a Seitz filter. The appropriate normal heterologous serum was treated in the same way.

Method of skin grafts:

The donors and recipients were always the same sex. The method of skin grafts of mice was that described by Bliss (2) and his criteria were used to assess rejection. For the rabbits, pinch ear grafts were placed in the dorsum of the ear and secured in place with sutures. The dressings were removed on Day 4, and the grafts inspected daily. They were considered rejected when they had become hard with complete lack of elasticity.

Immunization and dosage:

Day 0 was the day of immunization and grafting, and events occurring before this are a negative and thereafter are a positive figure. The rabbits were immunized intravenously with 100 mg of bovine serum albumin (BSA) (Armour Laboratories, Chicago) dissolved in 5 ml of saline. The rats were immunized with 1.0 mg BSA in 0.2 ml of complete Freund's adjuvant given into the footpads of the hind paws. Immunization with sheep red blood cells (SRBC) was carried out with 0.1 ml in mice, 0.2 ml in rats of a thrice washed, 30 per cent suspension, given intraperitoneally.

ALS was given in a dosage of 10 ml/kg i.p. to mice, and 10 ml/kg s.c. to rats, on Days −2, −1 and 0, and then intermittently on Days + 3, 6, 9 and 12. Rabbits could only tolerate a dose of ALS of 3 ml/kg s.c. on Days −2, −1, 0, + 2, 4, 6, 8 and 10. A similar dosage scheme of either normal horse or rabbit heterologous serum was given to appropriate groups as controls.

Procarbazine (N-isopropyl-α-(2-methylhydrazino)-p-toluamide) was kindly supplied by Hoffman-La Roche as the hydrochloride. In mice the maximally tolerated dose was 150 mg/kg by intraperitoneal injection. A few mice were given 300 mg/kg i.p., but this had to be lowered after a few days. The maximum dose tolerated by rats was 35 mg/kg s.c. Some rats were started at 75 mg/kg s.c., but the dose had to be reduced within a few days because of toxicity.

The maximum daily dose for rabbits was 7.5 mg/kg s.c.

Procarbazine was given in either of two dosage schemes. In the first, procarbazine was given daily from Day −21 to Day 0 at the maximum tolerated dose, and then the dose was halved until Day 7. This scheme is referred to in the text as the procarbazine Day −21 regimen. In the second, the maximum tolerated dose of procarbazine was given from Day −2 to Day 13 (or until Day 3 in the PFC test), and is referred to in the text as the procarbazine Day −2 regimen.

Measurement of humoral antibody:

The amount of humoral antibody to BSA was measured using the Farr test (6). The ABC_{33} results were expressed as the γ ^{131}I BSA N bound per ml of undiluted serum when 0.02 γ ^{131}I BSA N was the amount of antigen added.

The direct hemagglutination titers to SRBC were determined using a microtiter technique (17). The results were expressed as the mean negative logarithm to the base 2.

The plaque forming cell test was performed by the technique described by Jerne *et al.* (11). The results were expressed as the percentage suppression of the total number of plaque forming cells per spleen in the treated group compared to the immunized controls, after adjustment for the non-specific plaques in unimmunized controls. In the ALS treated mice, the comparison was made with controls given the same amount of normal heterologous serum.

RESULTS

Comparison in mice:

The procarbazine Day −21 was equally as effective as ALS in prolonging skin graft survivals exchanged between strains incompatible at the H_2 locus (Table 1). In strains of mice compatible at

TABLE I

Comparison of immunosuppressant action of ALS and procarbazine in mice

		ALS	PROCARBAZINE
		Mean Survival in Days & S_{EM}	
Skin transplants			
H_2 Incompatible			
C57Bl/10 ⟶ AKR			
($H2^b$) ($H2^k$)	Controls	10,3 ± 0,2 (36) *	10,3 ± 0,2 (36) *
	Test	21,3 ± 2,45 (12) *	20,8 ± 4,9 ** (15) *
H_2 Compatible			
C3H/He ⟶ AKR			
($H2^k$) ($H2^k$)	Controls	12,5 ± 0,18 (32) *	12,5 ± 0,18 (32) *
	Test	44,7 ± 2,0 (18) *	51,3 ± 3,3 ** (18) *
			30,5 ± 1,7 † (28) *
Humoral antibody (Day 13)			
Anti-SRBC — mean-$\log_2$ titer			
	Controls	6 (1/8) ††	5,7 ** (0/8) ††
	Test	2,1 (4/10) ††	0 (8/8) ††
Plaque forming cell test (Day 4)			
% Suppression		95,0 (10) *	99,9 (10) *

*** Number in parentheses is mice in the group.**
**** Procarbazine Day — 21 regimen.**
† Procarbazine Day — 2 regimen.
†† Ratio of group making no antibody.

the H_2 locus, the prolongation of skin graft survival was even more striking, having a mean of 51.3 days compared to 44.7 days when ALS was used. The procarbazine Day −2 regimen was less effective although still significant in this model, prolonging skin graft survivals with a mean of 30.5 days compared to 12.5 days fort he controls.

Procarbazine Day −21 treatment completely suppressed humoral antibody to SRBC and was much more effective in this parameter than ALS (Table I). Although the average titer was profoundly depressed by ALS treatment, 6 of the 10 mice made detectable amounts of humoral antibody.

Both ALS and the procarbazine Day −2 regimen were highly effective in suppressing the antibody-producing plaque forming cells, ALS producing a 94 per cent suppression and procarbazine a 99.8 per cent suppression.

The mice tolerated both the ALS and procarbazine Day −2 therapy well. With the procarbazine Day −21 regimen, the mice tended to lose weight, although this loss stabilized after a week or two and recovered slowly after cessation of therapy on Day 7. Two mice in the H_2 compatible group died, one at Day 88 and one at Day 148 with intact grafts, and postmortem revealed no gross abnormalities. Neither was used in computing the average.

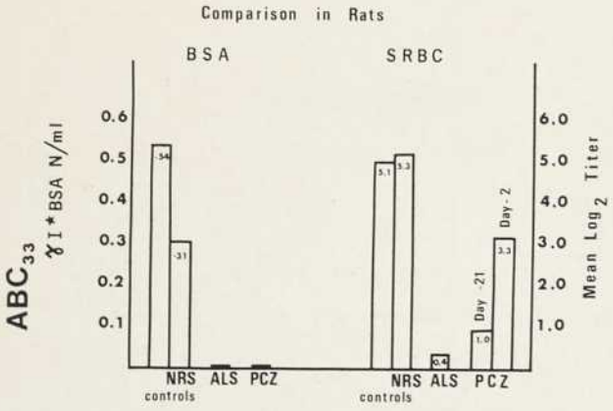

Figure 1 — EFFECT OF ALS AND PROCARBAZINE ON THE HUMORAL ANTIBODY RESPONSE TO BSA AND SRBC IN RATS. The amount of antibody to BSA is expressed as the ABC_{33} shown on the scale on the left ordinate. The figures on the bars represent the mean of each group. The amount of circulating hemagglutinins to SRBC is expressed as the negative logarithm to the base 2 on the scale on the right ordinate. The figures on the bars represent the mean of each group.
NRS – Normal rabbit serum
ALS – Antilymphocyte serum
PCZ – Procarbazine
For dosages, see text.

Comparison in rats:

The effect of ALS and procarbazine in suppressing the production of humoral antibody was tested in rats against two antigens, BSA and SRBC. Against the weaker antigen BSA, ALS and procarbazine were capable of suppressing the humoral antibody response completely as measured by the Farr test, at Day 14 there being 14 and 15 rats in each group respectively (Figure 1).

With the stronger antigenic stimulus of SRBC, neither agent was able to completely suppress the humoral antibody response (Figure 1). ALS was the more effective, and 20 out of 23 in the group made no antibody. With the procarbazine Day −21 regimen, 5 of the 10 were completely suppressed. It was again apparent that the procarbazine Day −2 regimen was less effective, and only 4 of the 15 rats were completely suppressed, while the others made a reduced but appreciable amount of humoral antibody, compared to the controls.

Comparison in rabbits:

Procarbazine and ALS were tested in rabbits for their effect in prolonging skin allografts and their ability to suppress humoral antibody to BSA.

ALS prolonged the survival of skin allografts from black New Zealand rabbits on eight white New Zealand rabbit recipients from an average in the controls treated with normal horse serum of 9.5 days to 17.8 days (Figure 2). However, in these same rabbits, ALS was completely ineffective in suppressing humoral antibody production. The animals made a normal primary response to BSA and after a second immunization of Day 21, a normal secondary response at Day 28. All eight test rabbits produced antibodies to BSA. The rabbit thus seems the best species to demonstrate the selective effect of ALS in suppressing cell mediated immunity only (Figure 2).

The procarbazine Day −21 scheme was effective in prolonging the survival of skin grafts and suppressing humoral antibody to BSA.

All six control white New Zealand rabbits rejected their black New Zealand skin grafts on Day 8, and procarbazine prolonged the skin grafts between the same strains to an average of 12.4 days in five animals (Figure 3). These same rabbits were immunized on Day 0 and Day 21 with BSA. Procarbazine had a profound effect on the humoral antibody response. None of the five rabbits had any detectable antibody on Day 14 but by Day 21, four of the five made a reduced amount, approximately 20 per cent of the controls. After a second stimulus, the difference between the controls and procarbazine treated animals was even more apparent. The controls made on the average an ABC_{33} of 727 γ ^{131} BSA N/ml and the test animals made an average of 11.5 γ ^{131}BSA N/ml. All five test rabbits produced detectable antibodies to BSA (Figure 3).

A striking finding in treating rabbits with procarbazine was the narrow dosage range. Preliminary experiments showed that 10 mg/kg s.c. killed all animals within three to four weeks. A dose of 5 mg/kg s.c. was almost without effect on the immune response, but also non-toxic as all animals appeared healthy and none died. The dosage used in this study was 7.5 mg/kg s.c. given daily for three weeks before grafting and immunization, and then reduced to 3.5 mg/kg s.c. until Day 7, when

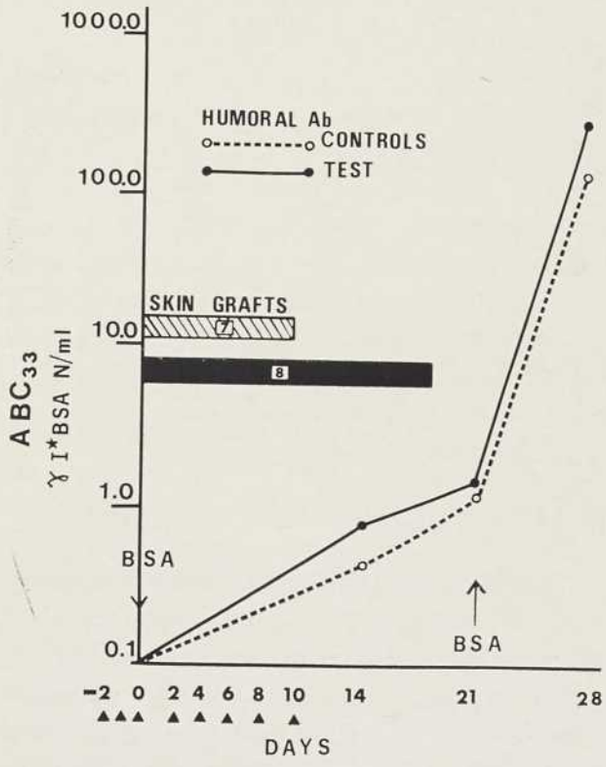

Figure 2 — THE EFFECT OF ALS TREATMENT ON THE LEVEL OF HUMORAL ANTIBODY AND SKIN GRAFT SURVIVALS IN THE SAME RABBITS. ***Grey bar :*** **Mean skin graft survival in days of controls treated with normal horse serum.** ***Black bar :*** **Mean skin graft survival in days of ALS treated rabbits. Numbers in bars represent the animals in each group.** ***Triangle :*** **Intermittent treatment of rabbits with either normal horse serum or horse anti rabbit antilymphocyte serum. For dosage see text. The same rabbits were immunized with BSA on Day 0, and again on Day 21. The amount of circulating antibody to BSA was expressed as the ABC_{33} as the γ^{131}I BSA N/ml, as shown on the logarithmic scale on the left ordinate. ALS treatment prolonged the survival of skin allografts from an average in the controls of 9.5 days to 17.8 days in the test group, but had no effect on the humoral antibody response to BSA.**

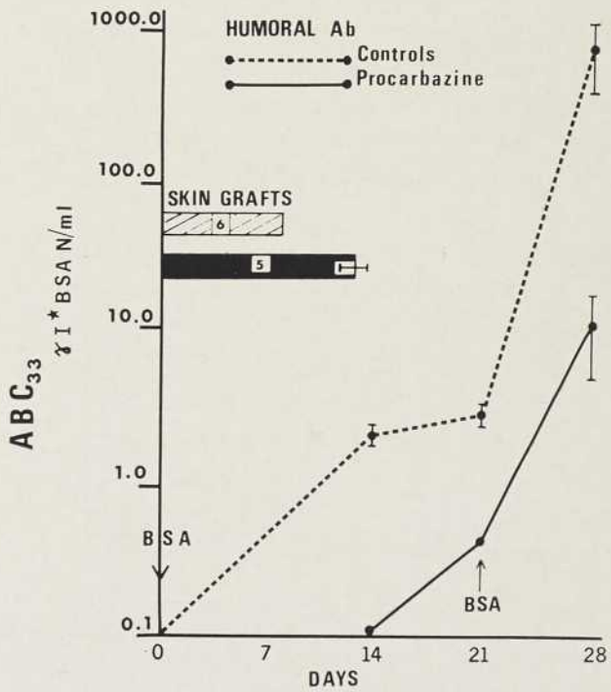

Figure 3 — EFFECT OF THE PROCARBAZINE DAY −21 REGIMEN ON THE SURVIVAL OF SKIN ALLOGRAFT AND CIRCULATING ANTIBODY IN THE SAME ANIMALS. Same symbols as in Figure 2. The *vertical line on dots* represents standard errors of the mean. Procarbazine treated animals had a prolongation of skin graft survivals of 12.4 days, from 8 days in the controls. The primary humoral antibody response to BSA was delayed and reduced, and the secondary response was markedly reduced.

it was stopped. This had a profound effect as shown above on the immune response, but there were signs of toxicity. Two rabbits died on Day −1, the day after grafting, after 22 days of continuous treatment and another one died on Day 8 with an intact graft, giving an overall mortality of three out of the original group of eight. The five survivors recovered completely and are still alive and well at the time of writing, five months after cessation of therapy.

DISCUSSION

The immunosuppressant effect of procarbazine was best demonstrated in mice. It was as effective as ALS in suppressing cell mediated immunity as judged by skin allograft survival even across the strong H_2 histocompatibility barrier. It was much more effective in suppressing humoral antibody to sheep erythrocytes than ALS. In rats, ALS appeared slightly more effective in suppressing humoral antibody than procarbazine but the difference was not marked.

It was much harder to make a comparison between the two agents in rabbits. With ALS, there was a striking dichotomy in its effectiveness in suppressing cell mediated immune response and its total inability to suppress the other immunological parameter, the humoral antibody response. These results in rabbits confirmed the suspicions raised by the results in mice on the humoral antibody response, and voiced by Lance and Batchelor (13) that ALS suppresses selectively the cell mediated response. Although procarbazine suppressed both parameters in rabbits, it did so in a narrow dosage range and in an amount much lower than that which could be given to mice and rats. This low dosage (which is in all probability due to a different metabolic handling of the drug) limited the immunosuppressant effect that could be obtained.

Certainly, neither agent led to tolerance in rabbits. Procarbazine delayed the primary response to BSA and reduced the amount of antibody produced in the secondary response. The inability of ALS to prevent the humoral antibody production would seem to limit its effectiveness in prolonging skin grafts in rabbits, as it is fair to assume that the rabbits would also be making antibody against the heterologous protein components of the horse anti rabbit ALS.

These results show that procarbazine is a potent new immunosuppressant. These findings confirm the original observations of Floersheim (8 and 9) and enlarge on the results of a preliminary comparative study with ALS (20).

There are difficulties in comparing a biological agent like ALS, whose potency varies from batch to batch, with a pure chemical like procarbazine. It is theoretically possible to produce an even more potent ALS than was used in this study. Allowing for these problems, it seems reasonable to conclude from the results presented here, that procarbazine approaches the potency of ALS as an immunosuppressant in mice, rats and rabbits, making allowances for some species differences.

Procarbazine is a methylhydrazine derivative synthesized by Bollag in 1963 (3) and introduced for the treatment of neoplasms in man, especially those affecting lymphoid tissue and in Hodgkin's disease (7, 15 and 18). Although there has been several years experience with it, its immunosuppressant activity in man has never been tested. The recommended dosage in man is of the order of 300 to 1000 mg per day, and in this regard man seems to handle the drug more like the mouse than the rabbit.

The mode of action of procarbazine is not understood, but the unaltered form is not thought to be active. Dost and Reed (5) suggested that the transitory formation of methyl free radicals may be involved. Weitzel *et al.* (22) showed that procarbazine and two of its metabolites inhibited the transport of nucleosides into lymphatic leukemic cells, and suggest this may be its mode of action.

Procarbazine is similar to ALS in that the immune response is more effectively suppressed if it is given before exposure to antigen. However, why it should be more effective when given three weeks before grafting or immunization remains unexplained.

Procarbazine has the advantage over 6-mercaptopurine, at least in mice, in that it suppresses both the cell mediated and humoral antibody responses, whereas 6-mercaptopurine affects only the humoral antibody response (19). For this reason (and also because it is available) it is suggested that a cautious trial be started in man to test its effect on the immune response.

One serious drawback with procarbazine has been the finding by Kelly *et al.* (12) that procarbazine and some of its metabolites induced pulmonary tumours in mice. This raises the question, reviewed by Penn *et al.* (16), of the carcinogenicity of immunosuppressants. There may be a correlation between the potency of the immunosuppressant and its ability to induce neoplasms, as ALS has induced malignant lymphomas in man. Interference with the immune response may explain why procarbazine, like ALS and azathioprine, have been found to be capable of inducing tumours. In this regard, it should be remembered that known carcinogens such as benzanthracene have the ability to produce long lasting suppression of the immune response (1 and 14). Although this study was not designed to detect the onset of cancer, no animal treated with ALS or procarbazine which died during this study nor any survivor which has been kept for several weeks or months, has been found to have neoplastic disease; but this does not deny that these agents could possibly do this.

SUMMARY

Procarbazine has been shown to be a potent immunosuppressant, capable of suppressing both cell mediated immunity and humoral antibody response in mice and rabbits. It also suppressed the humoral antibody response in rats to bovine serum albumin, and profoundly depressed the level of circulating hemagglutinins to sheep red blood cells in rats. Its potency was approximately equal to that of antilymphocyte serum, making allowances for species diffrences and the fact that in rabbits, antilymphocyte serum suppressed only the cell mediated response.

It is capable of prolonging the survival of skin allografts in mice across strong histocompatibility barriers, and in this regard it is as potent as antilymphocyte serum.

Since it is available for use in man as an anticancer agent, it should be cautiously tried to see if the potent immunosuppressant activity present in animals can be confirmed in man.

ACKNOWLEDGEMENTS

We wish to thank Miss H. Baier and Mr. M. Holeczek for their excellent technical help.

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UN CAS D'HOMOTRANSPLANTATION CARDIAQUE. ANALYSE DES PHÉNOMÈNES DE REJET * †

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Un homme de 55 ans souffrant d'une artériosclérose sévère des trois principaux troncs coronariens a présenté trois infarctus du myocarde. Ultérieurement apparaissent chez lui une insuffisance ventriculaire gauche, puis une insuffisance cardiaque globale, qui ne répond pas au traitement médical. Le cœur d'une femme de 51 ans, victime d'un traumatisme crânien, lui est transplanté. Il existe entre donneur et receveur une compatibilité du type C, selon la nomenclature de Dausset. Le traitement immunosuppresseur de base comprend l'Azathioprine, la Prednisolone et le sérum antilymphocytaire. Les suites opératoires immédiates sont fort simples. Plus tard, surviennent deux épisodes de rejet. Le premier s'étend du sixième au treizième jour, le second apparaît le trente-quatrième jour,

La transplantation cardiaque a déjà acquis, en moins d'un an, droit de cité dans le traitement des cardiopathies classiquement incurables.

Si la réalisation technique d'une telle opération paraît résolue, en revanche, les échecs secondaires ou tardifs, liés à des manifestations de rejet, restent nombreux.

L'analyse de notre observation aura donc essentiellement pour but de préciser les conditions de survenue des deux poussées de rejet (la deuxième ayant entraîné le décès au quarantième jour).

OBSERVATION

Monsieur H. Jacques est âgé de 55 ans. De 1960 à 1965, il a présenté trois infarctus du myocarde. Le dernier a laissé comme séquelle un bloc de branche gauche. En 1967 apparaissent des signes d'insuffisance cardiaque gauche (œdème pulmonaire), puis globale.

En 1968, le malade est confiné au lit depuis le début de l'année. Il présente quatre à cinq crises douloureuses précordiales par jour, ainsi que des épisodes dyspnéiques intenses, malgré la prise de dix à douze trinitrines quotidiennes. La situation n'est pas améliorée malgré le repos strict et un traitement médical bien conduit (anticoagulants, vasodilatateurs, digitaliques, procaïnamide et diurétiques).

À l'examen, le poids de 70 kg pour une taille de 1,71 m (surface corporelle 1,82 m^2). En dehors des crises, la PA est à 100/80, la fréquence cardiaque entre 80 et 100. Les bruits du cœur sont sourds, avec un petit souffle systolique apexien (1/10).

L'E.C.G. montre un axe de QRS à −40 et un axe de T à + 140. Le PR est allongé (0,24 sec) avec un bloc de branche gauche complet (QRS à 0,16 sec). Il existe une onde Q en V1. De nombreuses extrasystoles ventriculaires polymorphes sont retrouvées sur plusieurs tracés (figure 1). Sur la radiographie, le cœur est modérément augmenté de

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† Travail effectué dans le service du docteur Delamare (Hôpital Foch, Suresnes) avec la collaboration des docteurs Durand et Chartrain.

et conduit au décès du patient le quarantième jour après la transplantation. Au cours de ces deux épisodes, on observe de l'asthénie, une accélération du pouls, une baisse de la tension artérielle et des modifications électrocardiographiques (microvoltage, rotation de l'axe vers la droite). C'est seulement au cours de la deuxième crise de rejet qu'apparaît une sous-dénivellation du segment ST. D'autres manifestations accompagnent ce tableau, mais elles ne se retrouvent pas d'un épisode à l'autre. Toute anomalie doit donner l'éveil, les signes retrouvés au cours de ces deux crises de rejet doivent entraîner un accroissement massif et immédiat du traitement immunosuppresseur. Il est regretté que le deuxième épisode de rejet n'ait pas été traité adéquatement dès son début, mais seulement le lendemain, à cause d'un diagnostic malencontreux d'épanchement péricardique.

Au cours de l'évolution sont apparus des épisodes de flutter et de fibrillation auriculaire, en dehors de toute manifestation de rejet. Ils ont cédé aux digitaliques.

volume et l'aorte un peu élargie. Les examens biologiques usuels sont normaux. La perturbation des clearances rénales et hépatiques témoigne de la gravité de l'insuffisance cardiaque (clearance à la créatinine : 80 ml ; rétention de BSP à 17 pour cent à 45′).

L'éventualité d'une transplantation cardiaque est alors évoquée.

Auparavant sont pratiqués trois examens : un débit cardiaque, un cathétérisme gauche et une coronarographie.

a) Le débit cardiaque est très abaissé : 3,45 l/mn avec un index à 1,90 l/m²/mn et un index systolique à 22 ml/m². La pression artérielle est à 83 mm de Hg et les résistances périphériques sont élevées à 4 300 unités arbitraires.

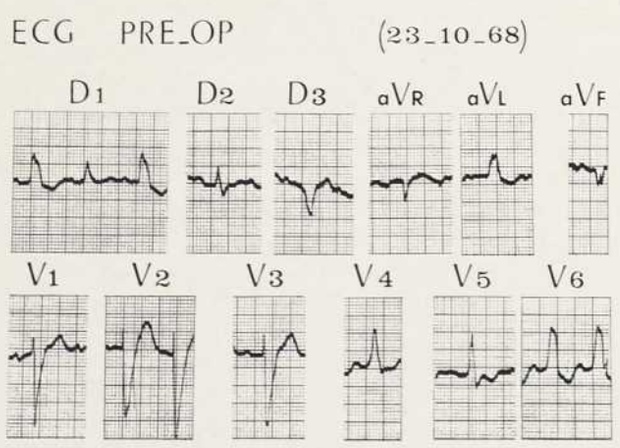

Figure 1 — Electrocardiogramme préopératoire

b) Dans le ventricule gauche, les pressions diastoliques, et surtout télédiastoliques, sont très élevées : 100/20/30.

c) La coronarographie pratiquée par injection dans la racine de l'aorte montre : une interruption de la coronaire droite à deux cm de son origine, après avoir donné au préalable une branche relativement importante, et une coronaire gauche qui se bifurque à environ un cm du bord aortique. L'interventriculaire antérieure est de calibre subnormal (3 à 4 mm), mais s'interrompt après un trajet de 2,5 cm. La circonflexe s'interrompt également au bout de 2 à 3 cm, et le reste de son trajet est invisible.

Ces données seront ultérieurement confirmées par l'injection de la pièce (figure 2).

Au total, devant l'importance des signes fonctionnels, la gravité de l'insuffisance cardiaque et l'importance des lésions artériographiques, est adoptée la décision de pratiquer une transplantation cardiaque.

Le bilan est complété par des épreuves fonctionnelles respiratoires et un transit gastroduodénal. Ce dernier élimine une éventuelle lésion ulcéreuse qui s'opposerait à la poursuite d'un traitement corticoïde.

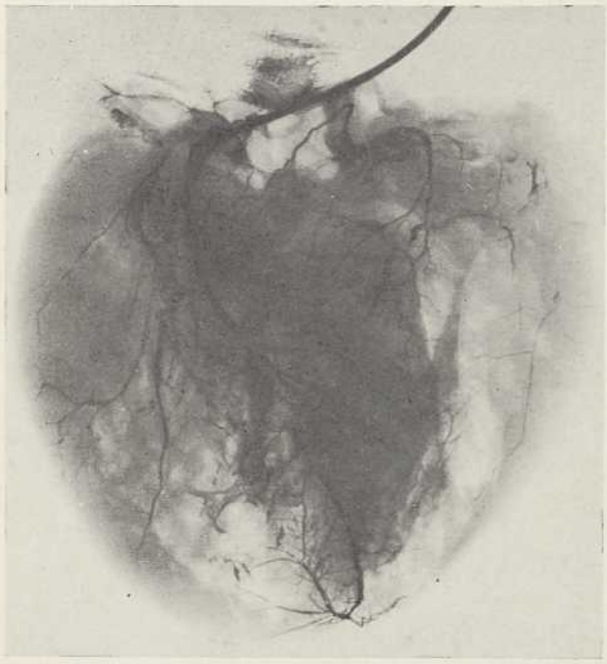

Figure 2 — Radiographie de la pièce après injection des artères coronaires : l'artère circonflexe est thrombosée ; l'artère interventriculaire antérieure présente plusieurs sténoses étagées, puis une thrombose. L'artère coronaire droite est interrompue par une thrombose à trois centimètres de son origine.

CHOIX DU DONNEUR

L'état du malade s'altère rapidement, et on décide de retenir un donneur dont la compatibilité, bien qu'incomplète, semble acceptable en regard de la gravité de l'évolution spontanée (deux donneurs recrutés au cours des trois semaines précédentes ont déjà été récusés pour incompatibilité notoire).

Donneur et receveur ont le même groupe érythrocytaire A +. Les antigènes leucocytaires établis selon la nomenclature de J. Dausset sont présentés dans le tableau I. Il s'agit donc d'une compatibilité du type C.

Le donneur est une femme de 51 ans, décédée d'un traumatisme crânien. L'absence de tout antécédent pathologique notable, une ménopause relativement récente (cinq ans), une auscultation cardiaque normale et des artères périphériques souples, un cœur de volume radiologiquement normal et un E.C.G. normal nous ont décidé à tenter la transplantation malgré l'âge de la malade.

PRÉPARATION DU MALADE

Elle n'a pu commencer que le jour même de l'intervention. Après identification de la flore bactériologique du receveur au niveau de la peau et des orifices naturels, le malade est rasé, lavé avec une solution d'hexachlorophène. La cavité buccale est désinfectée à l'Hextril.

Le malade reçoit :

32 mg de Dexaméthasone (environ 0,5 mg/kg de poids) ;

20 mg de Melphalan ;

Deux injections de cinq ml de globuline antilymphocytaire à une heure d'intervalle. Teneur en protéines : deux pour cent. Lymphocytotoxicité : 1/1024 ;

350 mg d'Azathioprine (5 mg/kg de poids).

TRANSPLANTATION
(17 novembre 1969)

Des précautions d'asepsie rigoureuses, tant au point de vue du personnel que du matériel, ont été prises selon des modalités déjà usitées.

TABLEAU I

*Groupes leucocytaires du receveur et du donneur (*cross match *négatif)*

DAUSSET	1	2	3	4	16	5	6	7	8	11	12	10	15	17
Receveur	—	—	+	+	—	—	+	—	—	—	+	—	—	+
Donneur	—	+	+	+	—	+	+	—	—	+	—	—	—	+
HLA	2					5			8	1	3	7		

A. *Prélèvement du cœur du donneur:*

Une circulation extracorporelle avait été prévue, au cas où serait survenue une défaillance cardiaque brutale chez le donneur, mais elle n'a pas été utilisée.

Le thorax est d'abord rapidement incisé par sternotomie médiane. Le cœur est de petit volume et très tonique. La palpation des orifices aortique et pulmonaire ne révèle pas de calcification. L'aorte est souple et l'artère pulmonaire non tendue. Les coronaires sont normales à l'inspection et à la palpation. La transplantation est alors décidée et l'induction anesthésique du receveur est entreprise.

Pendant la préparation du receveur, les deux reins sont prélevés, un seul se révélant apte à la transplantation, l'autre présentant une anomalie vasculaire.

Le cœur est finalement prélevé à l'instant où la circulation extracorporelle est démarrée chez le receveur:

- contrôle des caves,
- clampage et section des caves à distances de l'oreillette droite,
- clampage à distance de leur origine de l'aorte et de l'artère pulmonaire,
- pédiculisation du cœur sur les veines pulmonaires, permettant de les sectionner au ras de l'oreillette gauche.

La durée de ce prélèvement à cœur battant est de 3′36″. Le cœur est placé dans un cristallisoir contenant du Ringer-lactate à 4°, et amené dans la salle du receveur. L'aorte est libérée de l'artère pulmonaire à son origine, l'oreillette gauche est ouverte à partir des orifices des veines pulmonaires. L'oreillette droite est ouverte d'un orifice cave à l'autre.

B. *Préparation du receveur:*

Après sternotomie médiane et ouverture du péricarde, on découvre un cœur notablement augmenté de volume dont les cavités droites se contractent mal. Il existe plusieurs plaques blanchâtres fibreuses sur l'épicarde.

La circulation extracorporelle est installée entre les deux veines caves canulées assez en arrière sur l'oreillette droite et l'artère fémorale droite.

La circulation extracorporelle mise en route, la cardiectomie est commencée par l'oreillette droite le long du sillon auriculo-ventriculaire, puis artère pulmonaire et aorte sont sectionnées dans le sinus de Theile au ras des orifices valvulaires. L'oreillette gauche est découpée le long du sillon auriculo-ventriculaire, sauf au niveau de l'auricule gauche qui est réséquée.

Ce temps de cardiectomie a duré 4′.

C. *Transplantation:*

Il n'existe pas de problème majeur d'incongruité au niveau des zones de suture entre receveur et donneur. Seule une très petite zone de septum du donneur est réséquée de manière à n'avoir qu'un seul plan de suture à ce niveau.

La suture est commencée par le bord gauche de l'oreillette gauche au voisinage de la partie supérieure du septum. La suture est menée par en dedans et poursuivie sur le septum, puis sur l'oreillette droite par un surjet simple de Tevdek 4–0.

L'artère pulmonaire est ensuite suturée, puis l'aorte par un surjet de Tevdek 5–0.

Après une purge soigneuse des cavités gauches, l'aorte est déclampée. Le temps total d'ischémie cardiaque, compté à partir du prélèvement, est de 47′.

Le cœur se met rapidement en fibrillation active et défibrille spontanément, puis refibrille et défibrille à nouveau spontanément. Le rythme est assez lent à 60 avec une dissociation auriculo-ventriculaire complète, puis 2/1. La circulation extracorporelle est arrêtée après 55′ de perfusion. Le cœur assure d'emblée une pression artérielle efficace à 120/80.

Quelques gouttes d'isuprel (une ampoule dans 250 ml), perfusées pour lutter contre la bradycardie relative, entraînent alors une forte tachycardie. Toute drogue est alors arrêtée. Le rythme

cardiaque redevient d'ailleurs sinusal 20′ après l'arrêt de la circulation extracorporelle, et la pression artérielle se maintient spontanément à 130/80.

La fermeture se fait selon la technique habituelle. Le malade se réveille sur table mais est laissé intubé en vue d'une assistance ventilatoire postopératoire. La durée totale de l'acte opératoire est de quatre heures et 15′.

SURVEILLANCE ET TRAITEMENT POSTOPÉRATOIRE

A. *La surveillance:*

Assurée par la présence permanente d'un médecin et d'une infirmière, la surveillance comprend plusieurs chapitres:

1. *Étude clinique* (figure 3):

L'étude clinique comprend l'appréciation de la vigueur physique et psychique du patient; la prise régulière du pouls, de la pression artérielle, de la pression veineuse et de la température rectale, la surveillance de la diurèse et du poids sont combinés à un examen clinique complet, au moins quotidien, et notamment l'auscultation cardiaque et pulmonaire et la recherche de signes périphériques d'insuffisance cardiaque.

2. *Électrocardiogramme* (figure 4):

Il est surveillé en permanence sur cardioscope; un électrocardiogramme quotidien complet, dérivations périphériques et précordiales, à vitesse et amplitude normales et doubles, permet de calculer les différentes constantes.

Des vectocardiogrammes quotidiens, puis bihebdomadaires ont également été pratiqués[1].

3. *Radiographie:*

Une radiographie thoracique de face a été prise, debout, dès que possible, tous les jours jusqu'au quinzième jour, puis deux fois par semaine.

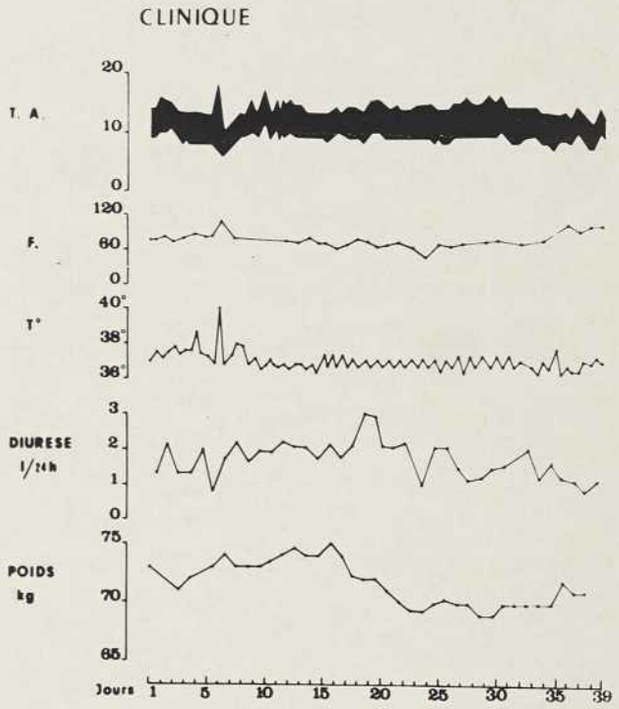

Figure 3 — Courbes des différents paramètres de surveillance clinique : pression artérielle, fréquence cardiaque, température, diurèse de 24 heures, poids.

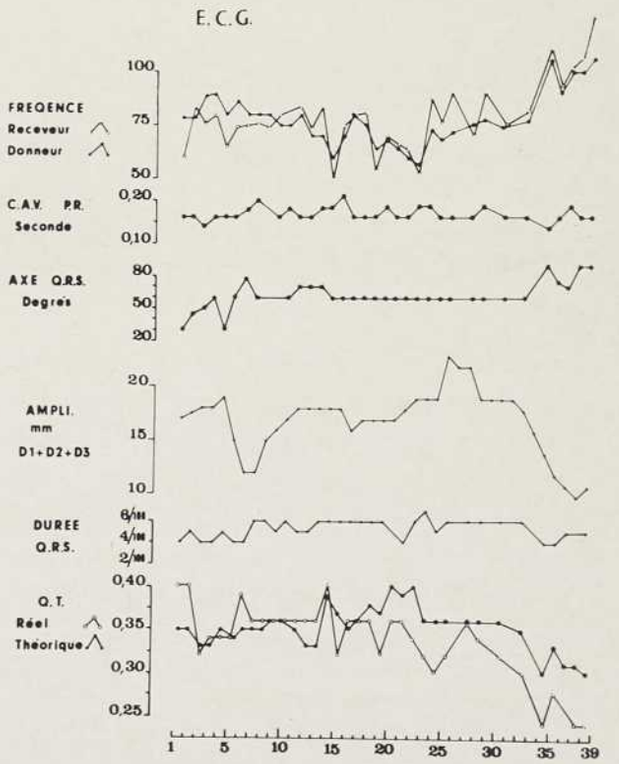

Figure 4 — Courbes des principales constantes électrocardiographiques : fréquence du receveur et du donneur, durée de PR, axe de QRS, voltage, durée de QRS, durée de QT théorique et réelle.

1. Les examens pratiqués par les docteurs Chartrain, Durand et M. Hourdet seront rapportés ultérieurement.

2. Examens pratiqués par les docteurs Mendoca et Schwartz.

4. *Biologie* (figures 5 et 6) :

L'ensemble des examens pratiqués est résumé sur les différentes courbes.

Les enzymes ont été dosés tous les jours durant les dix premiers jours, puis tous les deux jours (figure 7)[2].

B. *Le traitement* (figure 8) :

1. *Traitement anti-infectieux:*

Jusqu'au 21e jour postopératoire, le malade est resté en unité stérile. Des prélèvements bactériologiques répétés ont confirmé la stérilité de l'unité et l'innocuité de la flore saprophyte du patient.

Des antibiotiques (pénicilline: 10 millons U; streptomycine: 1 g par jour) n'ont été administrés que durant les quatre premiers jours postopératoires.

2. *Traitement anticoagulant:*

À partir du sixième jour postopératoire, un traitement anticoagulant, héparine relayée par le pindione, a été institué. Le taux de prothrombine a dès lors été maintenu entre 20 et 30 pour cent.

3. *Traitement immunosuppresseur:*

Le traitement de base est un traitement classique associant azathioprine, corticoïdes, globuline antilymphocytaire et comportant également une courte cure de Melphalan (figure 8).

4. *Diététique:*

La teneur en sodium, la richesse en calories et en protides des apports ont été mesurées.

ÉVOLUTION

A. *Les cinq premiers jours postopératoires:*

Le malade est extubé à la sixième heure postopératoire après contrôle des gaz du sang. Il est très tonique, tant sur le plan psychique que physique, se lève au deuxième jour après ablation des

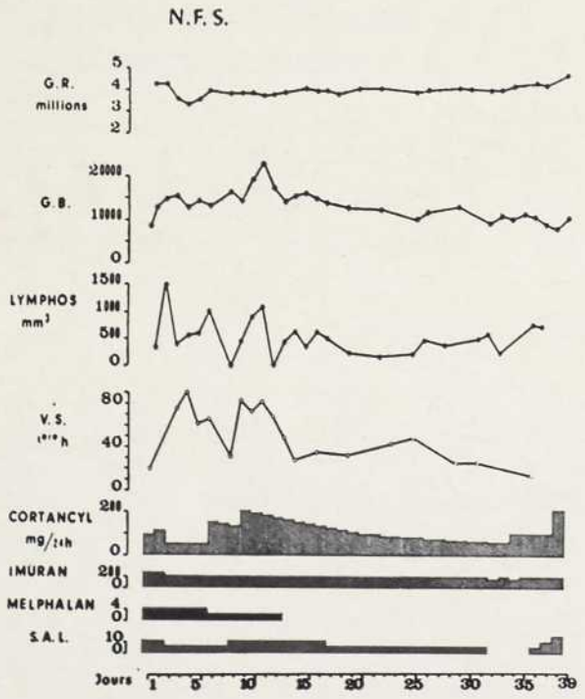

Figure 5 — Courbes de différents examens postopératoires : numération globulaire, numération des leucocytes, numération des lymphocytes, vitesse de sédimentation. Schéma thérapeutique au-dessous.

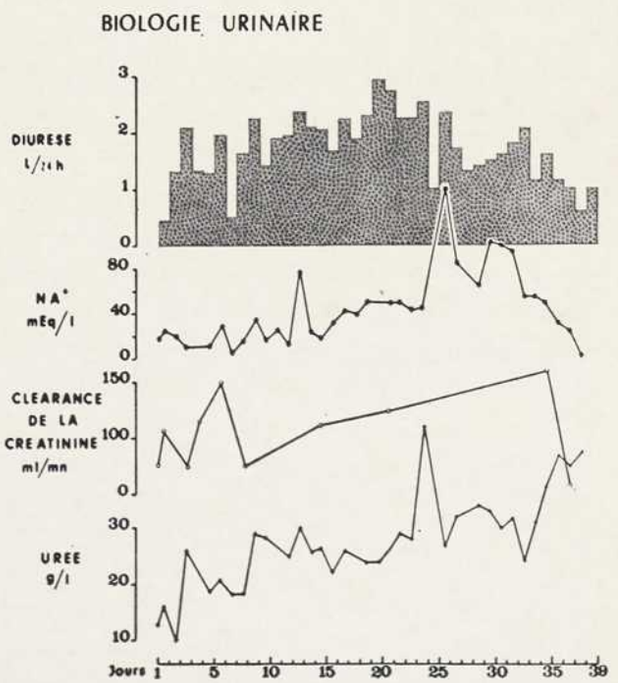

Figure 6 — Courbes de différents examens postopératoires : diurèse de 24 heures, natrurie, clearance à la créatinine, urée urinaire.

drains et cathéters, et s'alimente normalement à partir du troisième jour.

Une diplopie et quelques fourmillements dans le medius et l'annulaire droits sont notés dès le deuxième jour, puis s'atténuent rapidement.

Sur le plan hémodynamique, la pression artérielle se maintient sans aucune drogue entre 130 et 150 de maxima et 80–90 de minima, le pouls entre 80 et 90. La diurèse est abondante (1 300 à 2 000 ml par jour).

L'E.C.G. montre un rythme régulier, sinusal, avec commande par le nœud du donneur, les ondes P du receveur restant visibles (figure 9).

Le traitement immunosuppresseur est bien supporté en dehors d'une vive douleur survenant au point de piqûre, une heure après l'injection de GAL.

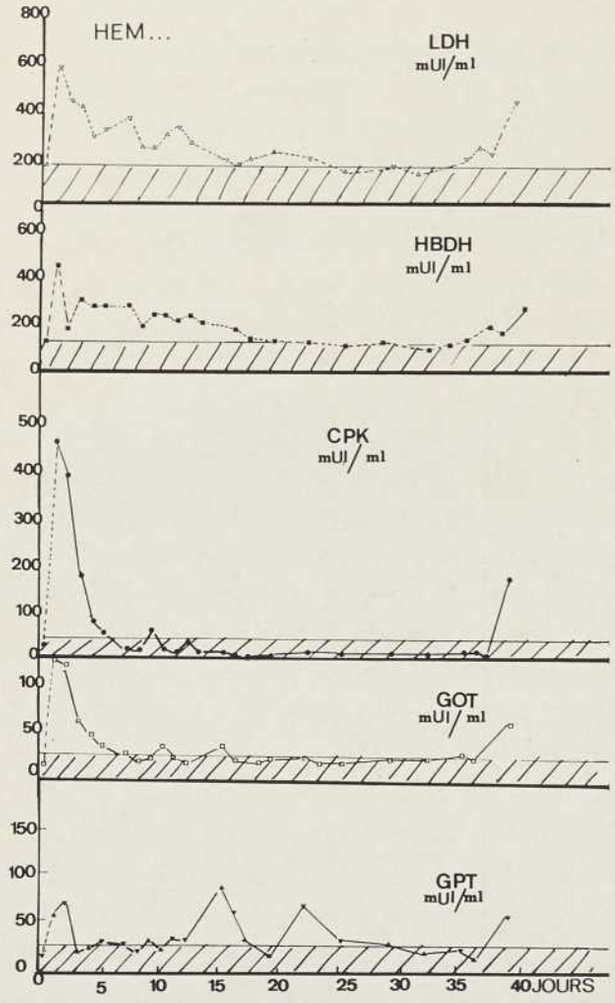

Figure 7 — Courbes des enzymes : LDH, HBDH, CPK, et des transaminases SGOT et SGPT.

B. *Premier épisode de rejet (6e–13e jours postopératoires) :*

Dans la nuit du cinquième au sixième jour postopératoire surviennent une baisse momentanée de la pression systolique à 100 et plusieurs extrasystoles ventriculaires monomorphes isolées.

Le sixième jour, à 15 h., une brutale hémiplégie droite avec aphasie, mais sans perte de conscience, apparaît. Elle régresse en une quinzaine de minutes et un examen neurologique complet pratiqué trois heures plus tard est pratiquement négatif.

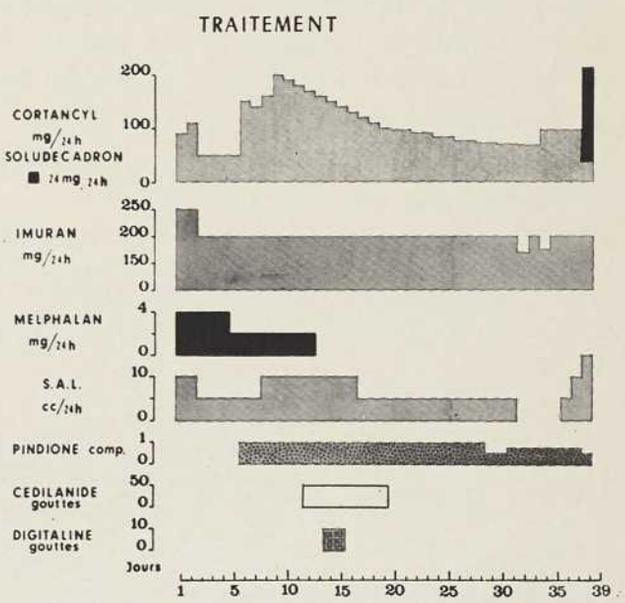

Figure 8 — Schéma thérapeutique

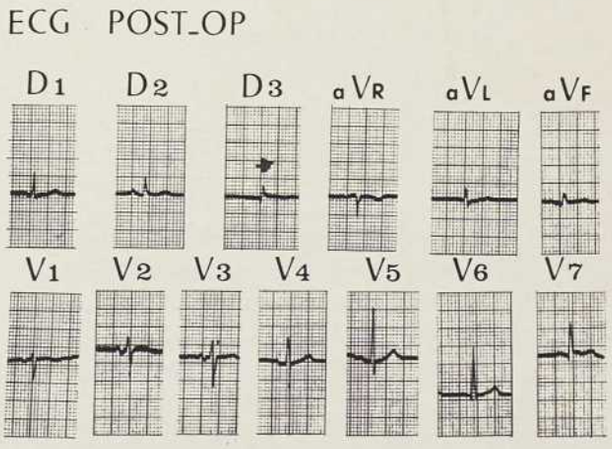

Figure 9 — Electrocardiogramme immédiatement postopératoire (48e heure).

Cet incident s'accompagne d'une poussée tensionnelle également transitoire à 180 de maxima et est suivi d'une montée fébrile atteignant 39°7 à vingt heures, avec frissons et malaise général (impression de « grippe »).

L'E.C.G. montre un microvoltage (figure 10).

Le lendemain (septième jour postopératoire), le malade est asthénique, a peu uriné (750 ml) et pris du poids (1 kg).

La température est revenue à la normale. La pression artérielle est un peu basse et pincée (90 à 100 sur 70–80). Les bruits du cœur sont sourds et l'électrocardiogramme s'est modifié: rotation de l'axe électrique à + 80 (axe antérieur entre + 30 et + 60), baisse de l'indice de Barnard de 19 à 12 mm.

Malgré l'absence de modification de la silhouette radiologique, le diagnostic de rejet semble certain: prednisone et GAL sont donc augmentés (250 mg de cortancyl et 10 ml de GAL par jour). D'autre part, un traitement anticoagulant est entrepris sur les conseils des neurologues.

Sous l'influence du traitement, l'amélioration est très rapide. En effet, dès le huitième jour, l'asthénie disparaît, la diurèse s'élève, le malade restant apyrétique.

La pression artérielle remonte à ses chiffres antérieurs. L'indice de Barnard revient à 19 mm, mais le rythme, bien que sinusal, est plutôt lent (70–80) et descend même au-dessous de 70 durant les périodes de sommeil.

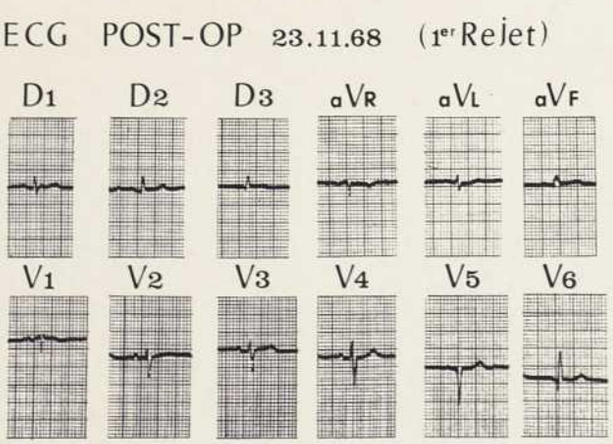

Figure 10 — Electrocardiogramme enregistré au moment de la première poussée de rejet.

À partir du neuvième jour postopératoire apparaissent des troubles du rythme à type de flutter 4/1 ou de fibrillation auriculaire, de durée variable (15′ à 2 h.). Le rythme ventriculaire ne dépasse pas 95, et le retour en rythme sinusal se fait spontanément après un repos compensateur de quelques secondes, ou, dans un cas, après injection de lanatoside C.

Parrallèlement à ces troubles du rythme, un frottement péricardique est noté à l'auscultation cardiaque.

Sur le plan biologique, une rétention sodée urinaire a accompagné la prise de poids. Les dosages enzymatiques ont montré une élévation transitoire entre le septième et le dixième jour postopératoire.

C. Période du 14e au 20e jour postopératoire:

Au 14e jour postopératoire, la poussée de rejet semble jugulée. Tant sur le plan général qu'hémodynamique, l'état du patient est satisfaisant. Le frottement péricardique disparaît au 16e jour; la rétention sodée s'estompe et le malade perd du poids.

Les indices électrocardiographiques sont stables et les enzymes sont à des taux comparables à ceux de la période pré-opératoire.

Cependant, les troubles du rythme persistent: accès de fibrillation auriculaire d'une durée de dix heures le 14e jour, de cinq heures le 15e jour, puis flutter 4/1 (rythme ventriculaire à 80) du 16e au 20e jour postopératoire. Ces troubles n'ont aucun retentissement hémodynamique, mais un traitement digitalique est néanmoins entrepris et poursuivi jusqu'au 21e jour postopératoire.

D. *Période du 21e au 33e jour postopératoire:*

L'état général du malade est parfait. Le poids est stable et autorise la reprise du régime salé. La pression artérielle est à 130/80. Le rythme est lent (60–80), mais se stabilise vers 80 à partir du 25e jour. Jusqu'au 29e jour surviennent encore quelques extrasystoles auriculaires, puis le rythme reste en permanence sinusal (figure 11).

Le traitement immunodépresseur est progressivement diminué. La globuline antilymphocytaire doit être arrêtée le 33e jour du fait d'une thrombopénie (60 000 plaquettes/mm³).

Durant cette période ont été pratiqués :

1. Un mécanogramme et un piézogramme : la courbe artérielle est très nettement améliorée.

2. Un débit cardiaque (27e jour postopératoire) : débit à 4,38 l/mn avec index cardiaque à 2,43 l/mn/m² et un index systolique à 32 ml/m². La pression moyenne est à 110, la fréquence cardiaque étant à 77. Les résistances périphériques restent élevées : 4 400 unités arbitraires.

E. *Deuxième rejet du 34e au 40e jour postopératoire :*

À partir du 34e jour postopératoire, la situation se dégrade progressivement :

1. *Sur le plan clinique :*

Le patient est asthénique et anxieux ; il se plaint d'une gêne précordiale, sensation assez vague, continue, couvrant toute l'aire précordiale et n'irradiant pas. Plus tard surviennent des épisodes dyspnéiques intenses et de courte durée (3 à 5'), sans sueurs et sans cyanose, sans anomalie auscultatoire ou radiologique.

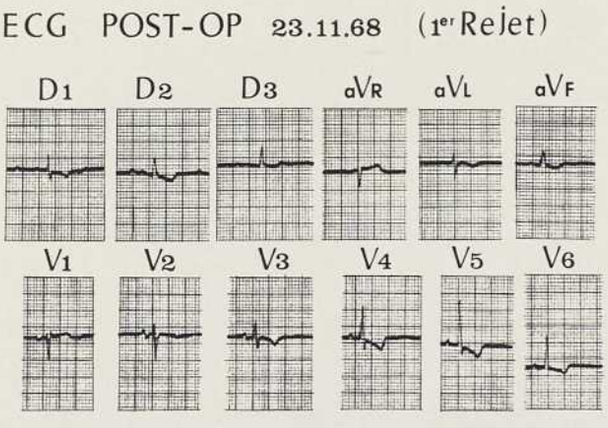

Figure 11 — Tracé électrocardiographique enregistré entre les deux rejets : rythme, voltage et axes sont normaux, mais il existe des troubles de la repolarisation dans toutes les dérivations.

En dehors d'une montée fébrile à 37°6 le 34e jour, il reste apyrétique ; mais sa diurèse baisse (600 à 1 000 ml/24 h), avec des natruries faibles et il tend à prendre du poids.

À l'examen les bruits du cœur sont sourds, et un frottement précardique est perçu par intermittence. La PA s'abaisse à 100, puis 90 de maxima et le rythme s'accélère vers 90, puis 100.

2. *Sur le plan électrocardiographique* (figure 12) :

Si la radiographie thoracique est inchangée, l'E.C.G. se modifie avec rotation de l'axe de QRS vers la droite, chute progressive de l'indice de Barnard à 14, puis 10 mm.

Au 37e jour, on note un sous-décalage de ST retrouvé en D_1, VL, V_3, V_4, V_5 (figure 13).

L'élévation des doses de prednisone à 100 mg par jour le 35e jour postopératoire n'entraîne qu'une amélioration fugace. La reprise du GAL au 37e jour, avec un nouveau lot et après remontée des plaquettes, n'évite pas une évolution défavorable, et, le 39e jour, la situation est grave.

Le malade est très asthénique ; la PA est à 80 de maxima et l'auscultation retrouve un bruit de galop présystolique. L'élévation des enzymes est franche.

Dans la matinée survient un épisode de ralentissement cardiaque avec syncope. Le rythme est alors nodal. Sous perfusion d'Isuprel (10 g/mn d'une solution contenant trois ampoules : 0,6 mg dans

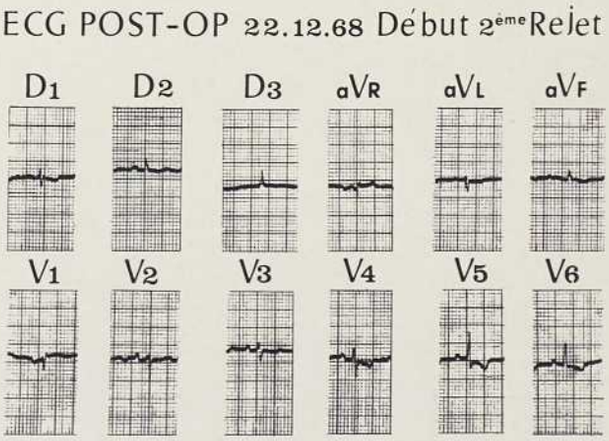

Figure 12 — Electrocardiogramme enregistré au début du deuxième rejet.

250 ml), la PA remonte à 110 et le rythme cardiaque redevient sinusal avec une fréquence entre 110 et 120.

Quatre mg de dexaméthasone sont alors injectés par voie intraveineuse toutes les trois heures. On passe à 15 ml de GAL par 24 heures et, dans la soirée, sera pratiquée une perfusion de GAL purifiée (deux fois 5 ml dans 100 ml de soluté salé à raison de 10 $g^{tt.}$/mn). Une irradiation locale de 200 rads par bombe au cobalt et 200 gammas d'actinomycine C complètent la thérapeutique.

Cependant, les doses d'Isuprel doivent être progressivement augmentées; deux inefficacités cardiaques vont survenir: la première dans la soirée du 39e jour récupérée par massage cardiaque externe, la seconde dans la matinée du 40e jour rebelle à toute tentative de réanimation.

AUTOPSIE

Au niveau du péricarde, les feuillets viscéral et pariétal adhèrent de façon intime. Il existe une péricardite fibrineuse des deux feuillets.

Le cœur est très augmenté de volume; il pèse 500 grammes. Extérieurement, il existe des zones apoplectiques et des zones infarcies particulièrement visibles au niveau de la face antérieure. L'ensemble de la paroi myocardique est très épaissie de façon diffuse. Le myocarde présente une teinte rouge sombre avec, par place, des petites zones marron foncé de nécrose récente. Sa consistance est molle.

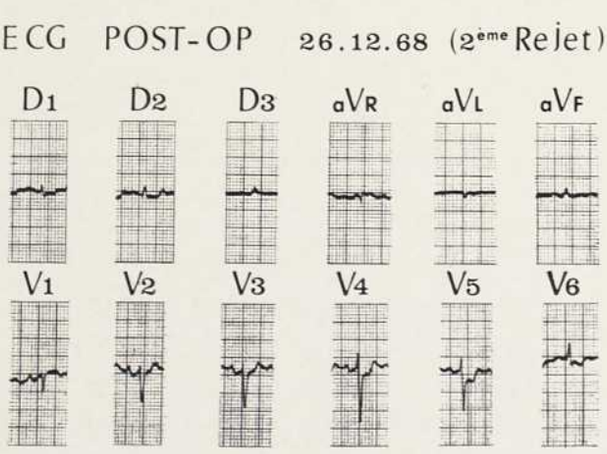

Figure 13 — Rejet confirmé. Electrocardiogramme enregistré 24 heures avant l'exitus.

À la coupe, on ne retrouve pas d'aspect même minime d'infarctus ancien.

La grande valve mitrale apparaît épaissie et dure, à la fois sur son bord externe et sur sa zone d'insertion.

Les zones de suture apparaissent normales, sans thrombose, avec une transition brutale entre l'aspect apoplectique du cœur transplanté et la paroi normale du receveur.

La dissection soigneuse des artères coronaires montre que celles-ci sont perméables jusqu'à leur extrémité. Aucune anomalie de la paroi et de l'endothélium à leur niveau.

Il faut enfin signaler un épanchement citrin de volume modéré dans la plèvre gauche et un foie légèrement congestif.

Le reste de l'examen autopsique ne révèle rien; l'athéromatose artérielle est très modérée.

Histologie:

Les prélèvements pratiqués au niveau du myocarde (ventricule gauche à la pointe et dans sa partie moyenne, le ventricule droit à la pointe et dans sa partie moyenne et cloison interventriculaire) révèlent d'une part des lésions diffuses et d'autre part des lésions focales.

Les lésions diffuses sont constituées par un œdème très important, dissociant les faisceaux ou les fibres myocardiques contenant un nombre variable de cellules, parfois modéré mais parfois beaucoup plus important. Ces cellules sont le plus souvent groupées autour des capillaires ou des petites artérioles terminales, pénétrant parfois dans la paroi et très nombreuses dans la lumière où on les retrouve souvent au niveau de l'endothélium. Ces cellules sont toujours mononuclées, constituées par des lymphocytes, des plasmocytes en nombre relativement minime et en nombre relativement modéré de cellules de type pyroninophile. Les lésions artériolaires sont variables, segmentaires au niveau des artères de moyen calibre, pratiquement constantes sur les petites artérioles et les capillaires.

Il existe soit une dédifférentiation de la média qui est congestive et où on ne retrouve plus de fibres

musculaires, soit un amenuisement ou une disparition totale de la limitante élastique ; enfin, fréquemment, on retrouve de petits foyers de nécrose fibrinoïde pariétale. Toujours dans les lésions diffuses, il existe une disparition pratiquement totale de la charge glycogénique musculaire, mais pas de disparition des fibrilles myocardiques.

En plusieurs foyers, soit relativement minimes et épars au niveau du ventricule droit, soit beaucoup plus étendus en particulier au niveau de la cloison interventriculaire, existent des zones de nécrose myocardique avec soit clarification et aspect vacuolaire complet de la fibre myocardique qui ne contient plus qu'un noyau picnotique, plus ou moins envahi par des cellules de type rejet, soit nécrose granuleuse avec disparition de la fibrillation et picnose nucléaire.

Enfin, au niveau du ventricule gauche et prédominant dans la partie sous-épicardique du myocarde existent de très abondants infarcissements hémorragiques dissociant les faisceaux ou les fibres musculaires qui présentent à ce niveau soit des altérations dégénératives, soit des petits îlots de nécrose.

L'examen de la grande valve mitrale montre que celle-ci est épaissie de façon régulière par une substance de type fibrine, le plus souvent en voie de hyalinose plus ou moins complète, contenant des foyers de sclérose élastigène et ne contenant pas de cellules de rejet. Ces lésions semblent relativement plus anciennes que les précédentes, mais il apparaît difficile d'en préciser l'origine.

Un fragment d'aorte du donneur montre que l'endothélium et la média sont normaux. Au niveau de l'adventice et dans la zone périadventitielle, on notera la présence de quelques rares cellules de type rejet autour des *vasa vasorum* en particulier. Un fragment de l'aorte du receveur ne montre pas d'anomalies particulières.

COMMENTAIRES

A. *Modalités techniques:*

En ce qui concerne le prélèvement et la réimplantation du cœur, ces modalités n'ont rien de très particulier ; il s'agit de la technique de Shumway (11) qui laisse en place la paroi postérieure des oreillettes. Le cœur a été prélevé sans le recours à une perfusion extracorporelle ; le temps de réimplantation de 57′ n'a pas nécessité de perfusion coronarienne. Au total, la technique chirurgicale proprement dite apparaît relativement simple puisqu'il s'agit d'affronter deux berges de tissu sain. La difficulté concerne la purge gazeuse à la fin du temps de réimplantation, avant l'arrêt de la circulation extracorporelle.

Pour ce faire, nous pensons qu'il est utile de mettre en place une ligne de dérivation, par le sillon de Sondergaard allant jusqu'à la pointe du ventricule gauche (8). D'autre part, il convient d'assurer une purge très soigneuse et prolongée, en freinant le retour cave, avant la défibrillation du cœur. Cette défibrillation doit, enfin, survenir sur un cœur dont l'aorte est clampée, avec un trocard implanté dans la racine de cette dernière.

Un dernier point mérite d'être souligné : il concerne le prélèvement simultané de plusieurs organes sur le même donneur. Dans notre cas, le prélèvement des reins fait préalablement au prélèvement du cœur n'a posé aucun problème. Au contraire, ce temps de prélèvement rénal a été utilisé pour préparer le receveur, après avoir vu et palpé le cœur du donneur, ce qui semble être une précaution utile quand il s'agit de donneurs polytraumatisés ou d'âge limite.

B. *Résultats fonctionnels et hémodynamiques:*

Le résultat immédiat a été excellent, le cœur est reparti spontanément et a assuré d'emblée une pression artérielle très stable, sans recours à aucune drogue vaso-active. À certains moments, cette pression était même plutôt élevée, comme si le malade « retrouvait » l'hypertension artérielle préexistante à la survenue de ses infarctus.

La faible durée d'ischémie vraie, l'absence de perfusion extracorporelle prolongée, des manœuvres de purge soigneuse à l'arrêt de la circulation extracorporelle, évitant toute embolie gazeuse coronaire, c'est-à-dire au total l'excellent état du transplant, nous semblent directement en cause.

La fréquence cardiaque, elle, est restée, dans l'ensemble, relativement lente entre 60 et 80 par minute, surtout si l'on se réfère à d'autres observations (1, 4, 6 et 13) où il est habituel de constater une tachycardie vers 100.

La mesure de débit cardiaque, faite entre les deux poussées de rejet, a montré une amélioration très importante, mais sans retour à des chiffres physiologiques. Ce débit inférieur à la normale peut être expliqué soit par l'insuffisance « fonctionnelle » du cœur transplanté, soit par le fait que le donneur avait une surface corporelle de 1,50 m^2 bien inférieure à celle du receveur (1,82 m^2) (13).

C. *Les troubles du rythme:*

Le bloc auriculo-ventriculaire constaté au redémarrage du cœur était, à l'évidence, lié à l'ischémie myocardique; et, de fait, cet épisode a disparu en 15 à 20′ pour ne plus réapparaître.

Plus intéressants à analyser sont les troubles du rythme survenus à partir du 9e jour et maximum entre le 14e et le 20e jour postopératoire, période où aucune manifestation clinique ou biologique de rejet ne peut être mise en évidence.

Ces troubles du rythme ont essentiellement été constitués par des accès de flutter ou de fibrillation auriculaire. Quelques tracés heureusement enregistrés au moment du passage de rythme sinusal en arythmie complète semblent montrer (docteur Slama) que cette fibrillation auriculaire a été déclenchée par la survenue d'une onde P du receveur dans la période vulnérable de l'oreillette du donneur.

Pour ce faire, il faut donc envisager le passage de l'influx de l'oreillette du receveur vers celle du donneur à travers la cicatrice d'auriculotomie. Or, sur certains tracés, on constate l'apparition de systoles couplées: la première est déclenchée par l'oreillette du donneur, mais la seconde activation ventriculaire qui suit semble bien déclenchée par une onde P du receveur (figure 14).

Tout ceci constitue une présomption en faveur du passage de l'influx à travers la cicatrice d'auriculotomie; encore ne peut-on éliminer le rôle mécanique de la contraction du moignon auriculaire du receveur, qui, à elle seule, peut aussi déclencher, sans doute, une extrasystole auriculaire. Cette dernière hypothèse plaiderait en faveur d'une technique laissant en place le moins possible d'oreillette chez le receveur.

TROUBLES DU RYTHME

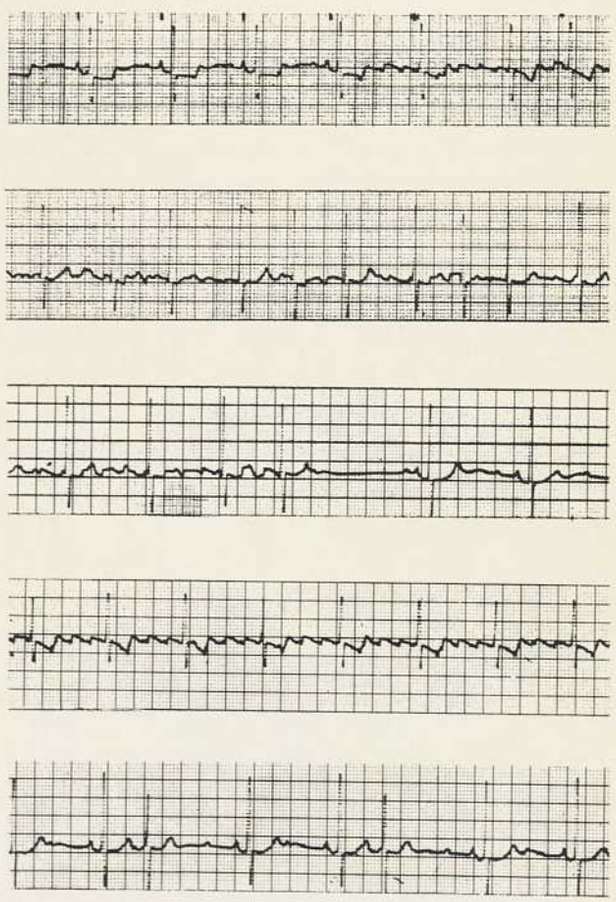

Figure 14 — Troubles du rythme survenus entre le 15e et le 25e jour. Les trois tracés du haut montrent l'apparition, puis la disparition d'une arythmie complète par fibrillation auriculaire. L'analyse de la première ligne semble montrer que la survenue d'une onde P du receveur en période vulnérable de l'oreillette du donneur déclenche la fibrillation auriculaire. Le quatrième tracé en partant du haut montre un aspect du flutter 4/1.Le tracé du bas montre l'apparition de systoles couplées. Le premier QRS est bien déclenché par l'oreillette du donneur, mais le deuxième QRS par contre semble déclenché par l'onde P du receveur quand elle tombe dans la branche descendante de l'onde T. Ceci représente un argument en faveur du passage de l'influx à travers la cicatrice d'auriculotomie.

Ces troubles du rythme rendent compte également d'un certain degré d'hyperexcitabilité de l'oreillette greffée. Dans notre observation, le rôle propre du rejet est difficile à mettre en évidence, car les troubles du rythme sont survenus même à distance du premier épisode de rejet. Les électrolytes sanguins, potassium et calcium en particulier, sont restés à des taux normaux. Par contre, l'importance de l'« atriotomie », en particulier lorsqu'il s'agit, comme dans notre observation, d'un transplant relativement âgé (51 ans), est un facteur nettement favorisant.

Sur le plan thérapeutique, le recours aux digitaliques a été efficace.

Au total, il importe de souligner que la survenue de troubles du rythme à type de flutter ou de fibrillation auriculaire, si elle témoigne d'une certaine irritabilité auriculaire, ne permet en rien, au moins dans la phase postopératoire précoce, d'affirmer l'existence d'une crise de rejet. Tous ces troubles du rythme ont atteint leur acmé dans notre observation, en dehors des deux poussées de rejet, dans une période de parfaite stabilité clinique, électrocardiographique (axe du QRS, indice de Barnard) et enzymatique.

C. *Analyse des phénomènes de rejet:*

1. *Cliniquement,* ces deux poussées de rejet ont été bien différentes.

Le premier épisode de rejet a été annoncé par un accident neurologique rapidement régressif. Cette hémiplégie droite a suscité deux interprétations sur le plan étiologique: la première est qu'il s'agissait d'une embolie ayant son point de départ sur une zone de suture; dans la seconde, il était conclu à un accident vasculaire cérébral survenu chez un artériel. Dans cette dernière hypothèse, le rôle d'une chute momentanée du débit cardiaque en rapport avec le rejet ne peut être exclu.

Les conséquences possibles d'un tel accident montrent l'intérêt qu'il faut accorder:

a) d'une part, à la vérification radiologique des troncs artériels à destinée cervicale dans le bilan pré-opératoire pour une transplantation chez un artériel;

b) d'autre part, à l'indication d'un traitement anticoagulant systématique au décours de la transplantation.

En dehors de cet épisode neurologique, le premier rejet a surtout été essentiellement marqué par des phénomènes généraux: malaise, frissons, ascension thermique a 39°7, précédant l'injection quotidienne de globuline antilymphocytaire, c'est-à-dire un tableau qui rappelle fort celui observé au cours des rejets aigus en transplantation rénale.

La deuxième crise de rejet, survenue au 35e jour, a été moins franche du point de vue général, essentiellement marquée au début par une légère asthénie, une fébricule à 37°6, quelques vertiges en position debout, mais, au total, rien de très alarmant en soi.

Par contre, à ce syndrome général atténué s'associait une gêne précordiale qui a subsisté pendant toute la durée de ce rejet jusqu'à la mort. Cette gêne était assez proche de celle d'une péricardite, malgré l'absence de variations avec la position ou la respiration. Elle ne présentait, en tout cas, aucun caractère angineux. Associée à un assourdissement net des bruits du cœur, elle a été d'abord abusivement mise sur le compte d'un épanchement péricardique qui n'existait pas.

2. *Le retentissement hémodynamique* a été net au cours des deux poussées de rejet.

Au cours du premier épisode, le pouls s'est modérément accéléré de 70 à 80–90 et la pression artérielle maxima a chuté de 140 à 100–110.

Au cours du deuxième épisode, la tachycardie a été nette, le pouls passant de 70 à 90, puis à 100, et la baisse de la pression artérielle nette de 140 à 100, puis 90.

Ce retentissement hémodynamique a également été marqué dans les deux cas par une oligurie et une baisse notable des natruries, s'accompagnant de prise de poids.

Les épisodes de dyspnée paroxystique survenus au troisième jour du second épisode de rejet témoignent sans doute de chutes importantes mais très transitoires du débit cardiaque. Ils surviennent en

effet brutalement, sans cause déclenchante, et ne s'accompagnent d'aucun autre signe, en particulier d'œdème pulmonaire. Ils ont donc, ainsi que le bruit de galop perçu la veille de la mort, une signification extrêmement péjorative.

À une phase ultime sont enfin apparues des syncopes par inefficacité cardiaque complète, le cœur conservant une activité électrique sous forme d'un rythme nodal.

3. *L'électrocardiogramme* a fourni au cours des deux « accidents » de rejet des données fondamentales. Certaines de ces données sont communes aux deux accidents; ce sont:

a) Le microvoltage, toujours net, calculé sur l'indice de Barnard (2), c'est-à-dire la somme arithmétique de l'amplitude du vecteur ventriculaire en D_1, D_2, D_3. Cet indice est passé dans le premier rejet de 19 à 12 mm, et au cours du deuxième rejet de 19 à 14, puis 10 mm;

b) La rotation de l'axe électrique vers la droite avec verticalisation presque complète.

Les troubles de la repolarisation, par contre, ont été différents au cours des deux épisodes de rejet. Le premier rejet n'a été marqué par aucune modification du segment ST, ni de l'onde T. À l'inverse, le deuxième rejet a vu apparaître une modification du segment ST, sous forme d'une sous-dénivellation particulièrement nette dans les précordiales gauches, au 37e jour postopératoire, c'est-à-dire au troisième jour de la crise. Il faut noter que l'onde T était inversée depuis le dixième jour postopératoire, ce qui n'est vraisemblablement qu'une conséquence banale de la péricardotomie.

Si l'on excepte la période terminale où est apparu un rythme nodal au moment des syncopes, aucun trouble portant sur l'espace PR, la durée de QRS ou l'espace QT n'est apparu.

4. *La radiographie thoracique* n'a montré aucune variation de l'ombre d'un cœur noyé, au début, il est vrai, dans un sac péricardique trop large. La pose peropératoire de deux clips métalliques sur le ventricule (2) devrait, dans ces cas, permettre de déceler des modifications même peu importantes.

5. *Les dosages enzymathiques* ont montré des élévations nettes en ce qui concerne les CPK et les HBDH, mais ces élévations sont encore difficiles à interpréter (2, 4, 5, 13 et 16) et parviennent avec un certain retard pour permettre de poser le diagnostic de « rejet ». L'intérêt principal de ces dosages, au moins dans les premières semaines, semble plutôt de vérifier le contrôle des crises par le traitement (16). En tout cas, les premiers résultats obtenus justifient la poursuite de cette étude.

Au total, nous retiendrons de cette analyse qu'il faut tenir compte de toute anomalie et ne pas se laisser entraîner à mettre abusivement les modifications constatées sur le compte d'un épiphénomène, épanchement péricardique postopératoire par exemple, ce qui a été notre attitude au début du deuxième rejet.

E. *Prévention et traitement des phénomènes de rejet:*

L'étude préalable à toute transplantation des groupes leucocytaires est maintenant admise par tous les auteurs. Celle-ci est loin, toutefois, de permettre des conclusions définitives en matière de compatibilité tissulaire.

Cette étude dans notre observation a montré l'existence de deux incompatibilités dans le système Hla, ce qui classe notre transplantation dans le groupe C d'échelle de compatibilité.

On soulignera, d'autre part, que la transplantation rénale faite dans le même temps (9) a également abouti à un échec chez un receveur présentant pourtant des groupes leucocytaires différents de notre patient. Ceci pourrait conduire à envisager l'antigénicité particulière de certains donneurs.

Sur un plan général, dans la mesure où le patient candidat à une transplantation cardiaque peut attendre un donneur parfaitement accouplé, la situation est idéale; mais bien souvent, ces malades sont à un stade où la transplantation se situe dans un climat de semi-urgence. Et les circonstances peuvent alors obliger au choix d'un donneur de compatibilité plus ou moins satisfaisante. C'est dire

l'importance du traitement immunodépresseur en matière de transplantation cardiaque.

Le traitement immunodépresseur de base doit être particulièrement énergique en matière de transplantation cardiaque pour prévenir, dans la mesure du possible, toute poussée de rejet qui risque d'avoir rapidement un retentissement hémodynamique inquiétant.

Le traitement des crises de rejet doit être extrêmement précoce et massif pour les mêmes raisons. C'est redire tout l'intérêt qui s'attache au dépistage précoce de ces crises.

Le traitement de notre patient a été conduit d'après l'expérience acquise par l'un de nous en matière de transplantation rénale, avec notamment vingt transplantations rénales consécutives sans échec.

L'azathioprine a été donnée au dosage maximum. Au cours du premier épisode de rejet, les doses de prednisone ont été fortement augmentées, puis très lentement diminuées avec deux inconvénients : d'une part, le bilan azoté, en dépit de forts apports protidiques, est longtemps resté négatif ; surtout la survenue du deuxième rejet a été accueillie avec un certain doute, en raison de son caractère clinique plus flou et de l'intensité du traitement corticoïde encore en cours, ces deux phénomènes étant peut-être liés. L'usage fait par certains (16) de très fortes doses, mais très rapidement dégressives, de corticoïdes en perfusion dans le contrôle des rejets nous semble donc assez séduisante.

La GAL n'a entraîné aucune réaction aiguë en dehors de la douleur en cas d'injection intramusculaire, la voie intraveineuse étant par contre parfaitement supportée. Une thrombopénie progressive, en rapport avec le lot utilisé, a nécessité l'arrêt de cette thérapeutique au 33e jour postopératoire. La concordance entre cet arrêt et la survenue de la deuxième crise de rejet au 34e jour est nette ; mais le déclenchement de la crise par cet arrêt de la GAL reste à discuter (10). Peut-être aurions-nous dû changer de lot de GAL sans l'arrêter.

L'héparinothérapie que certains utilisent systématiquement en cas de crise de rejet (16) n'a été employée par nous, et pour d'autres raisons, que lors de la première crise. Les phénomènes vasculaires du rejet aigu d'une part, la baisse parallèle du débit cardiaque d'autre part nous semblent deux arguments très favorables à ce traitement.

CONCLUSION

Notre observation montre la gravité des poussées de rejet survenant dans les deux premiers mois qui suivent la transplantation. Ces poussées de rejet ont rapidement un retentissement hémodynamique important, engageant le pronostic vital. Cependant, essentiellement œdemateuses, elles peuvent régresser avec peu de séquelles au prix d'un traitement particulièrement énergique et précoce.

L'amélioration du pronostic de la transplantation cardiaque, au moins dans les premiers mois, nous semble donc fonction de deux facteurs :

Le premier facteur est une excellente compatibilité leucocytaire entre donneur et receveur, tout en sachant que même l'identité absolue dans les systèmes actuellement connus ne met pas à l'abri des crises et que rien n'est encore fixé définitivement dans cette science en plein essor (15). D'autre part, actuellement, les receveurs éventuels ont une espérance de vie très courte (14) et la recherche d'un donneur éventuel est donc limitée dans le temps. Cela conduit soit à récuser des donneurs de compatibilité jugée insuffisante, et donc à laisser le receveur mourir entre-temps, soit à tenter une transplantation avec un donneur de compatibilité aléatoire. Peut-être dans l'avenir sera-t-on autorisé à pratiquer la transplantation dans des cas moins évolués, permettant une recherche prolongée d'un donneur parfaitement compatible.

Le deuxième facteur consiste en l'amélioration du traitement immunodépresseur. Le traitement immunodépresseur est d'abord préventif et doit, pour nous, comporter des doses relativement élevées en GAL, azathioprine et corticoïdes, auxquels nous associerons volontiers l'héparinothérapie, par voie intraveineuse et sous-cutanée. Le traitement des crises est un traitement d'intensité « maximum ». Des doses considérables de corticoïdes (16) et de

globulines antilymphocytaires, données par voie intraveineuse, nous semblent licites. Actinomycine C et irradiation locale complètent éventuellement l'arsenal thérapeutique. La précocité de ce traitement est capitale, car, appliqué avec quelques jours de retard, comme dans notre observation, il risque d'être inefficace. Cela implique de faire très tôt le diagnostic de poussée de rejet.

Le diagnostic précoce de crise du transplant représente ainsi, de manière indirecte, un facteur capital d'amélioration du pronostic.

L'aspect clinique de ces sujets est assez polymorphe et ainsi plusieurs tableaux peuvent être individualisés.

Toutefois, il existe des signes communs à toute poussée de rejet qui méritent d'être regroupés :

a) Asthénie toujours nette si on la recherche par l'interrogatoire ou si on la met en évidence par un test d'effort ;

b) Retentissement hémodynamique sous forme d'une baisse de la pression artérielle et d'une élévation de la pression veineuse avec tachycardie, prise de poids et rétention sodée ;

c) Altérations électrocardiographiques à type de rotation de l'axe (vers la droite le plus souvent) et de microvoltage.

D'autres signes, par contre, nous ont semblé fonction de la date de survenue du rejet.

Ainsi, le rejet précoce du sixième jour a été essentiellement marqué par des signes généraux intenses : fièvre, frissons, sensation de grippe.

À l'inverse, le rejet du quarantième jour a été marqué par la discrétion des signes généraux et, au contraire, l'importance des signes locaux :

a) Sensation de gêne précordiale, sans irradiation brachiale, très suggestive d'une irritation péricardique ;

b) Assourdissement des bruits du cœur en l'absence de tout épanchement péricardique.

Certains signes plus tardifs ont une signification très péjorative :

a) Épisodes de dyspnée paroxystique ;

b) Bruit de galop ;

c) Sous dénivellation du segment ST ;

d) Épisode d'inefficacité cardiaque.

À ces rejets précoces et secondaires, il faut enfin opposer les manifestations de rejet plus tardives observées par d'autres auteurs, essentiellement marquées par des troubles du rythme : allongement de PR, bloc auriculo-ventriculaire, rythme nodal.

Un certain nombre de ces troubles du rythme permettent d'expliquer des cas de mort subite. C'est dire l'intérêt d'une surveillance électrocardiographique journalière au long cours.

Au total, la transplantation cardiaque constitue un acte thérapeutique réservé à un petit nombre de cardiopathies condamnées à brève échéance, mais les exigences de la compatibilité tissulaire impliquent une décision relativement précoce, de manière à trouver un donneur de compatibilité suffisante. Une meilleure connaissance des phénomènes de rejet et de leur traitement doit permettre d'améliorer de façon importante les résultats actuels ; cette connaissance ne peut naître que de l'expérience acquise progressivement auprès de chaque cas de transplantation cardiaque. Malgré le nombre actuellement élevé des échecs, la transplantation cardiaque nous semble donc pleinement justifiée.

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PHÉNOMÈNE DE REJET ET COAGULATION INTRAVASCULAIRE DISSÉMINÉE. ÉTUDE D'UNE OBSERVATION DE TRANSPLANTATION CARDIAQUE HUMAINE * †

Y. BAILLE, T. BLANC, J.-R. MONTIES, A. GOUDARD et E. HENRY.

Il existe au cours de la chirurgie de transplantation un phénomène de coagulation se traduisant :

— Anatomiquement, par des lésions thrombohémorragiques siégeant au niveau du greffon, et dont la lésion anatomopathologique est la coexistence de microthromboses et d'hémorragies parenchymateuses ;
— Biologiquement, par une thrombopénie ;
— Cliniquement, par des accidents thrombotiques et thrombohémorragiques.

Ce problème sera, ici, étudié à la lumière d'une expérience personnelle de transplantation cardiaque sous l'angle : rejet et coagulopathie de consommation.

(suite du résumé en page suivante)

Pour poser d'emblée le problème, nous rappellerons quelques notions acquises et non discutées.

1. *Notions anatomiques:*

a) Le rejet d'un organe transplanté relève d'un processus immunologique complexe dont la traduction anatomique est, entre autres lésions, une lésion thrombohémorragique le plus souvent localisée au niveau du transplant.

b) La lésion anatomopathologique caractéristique du phénomène de coagulation intravasculairè disséminée est la coexistence au niveau de certains parenchymes de microthromboses et d'hémorragies.

2. *Notions biologiques:*

a) Au cours du rejet, les thrombopénies ne sont pas rares.

b) L'élément biologique le plus constant au cours du phénomène de coagulation intravasculaire disséminée est une thrombopénie.

3. *Notions cliniques:*

Au décours des transplantations d'organes, les accidents thrombotiques, et même thrombohémorragiques, sont signalés avec une fréquence non négligeable.

À la lumière de ces constatations que volontairement nous ne détaillerons pas, on peut dire qu'il existe un réel problème « coagulation » au cours de la chirurgie de transplantation.

Le problème coagulation et transplantation peut être étudié sous deux rubriques :

a) Transplantation et thrombose ;

b) Rejet et coagulopathie de consommation.

En ce qui concerne ce second point, sur lequel nous insisterons, une expérience personnelle de transplantation cardiaque nous permet d'affirmer que rejet et coagulopathie de consommation sont étroitement liés. Il restera à préciser la nature exacte des rapports.

Nous nous proposons de verser notre observation au dossier « coagulation et transplantation », dossier qui prendra une place de plus en plus grande, nous en sommes certains, dans l'avenir.

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† Travail effectué par le laboratoire d'expérimentation. Centre Jules Cantini (professeur Henry), Marseille.

I – *Un cas de transplantation cardiaque étudié sous l'angle de la coagulation :*

Le 28 novembre 1968, on procède à une transplantation cardiaque chez un malade qui, depuis deux mois était sous héparine sous-cutanée. À la fin de la circulation extracorporelle, la neutralisation est réalisée de manière progressive et incomplète.

Dès la huitième heure après l'opération, une hypercoagulabilité apparaît, nécessitant des doses croissantes d'héparine allant jusqu'à 415 mg pour placer le malade en légère hypocoagulabilité.

À partir du quatrième jour après l'intervention, la dose d'héparine sera délivrée par voie sous-cutanée, et sera diminuée progressivement.

Dès le 22 décembre 1968, on interrompt l'héparine.

Le 31 décembre 1968, le malade qui allait très bien présente des signes de rejet avec thrombopénie à 70 000 par mm³.

On reprend alors l'héparinothérapie de manière à placer le malade entre 20 et 30 de temps de coagulation. Sept heures après, le nombre des plaquettes avait doublé, indiquant ainsi que la thrombopénie était bien due à un syndrome de coagulation intravasculaire disséminée.

I. Un cas de transplantation cardiaque étudié sous l'angle coagulation

M. V. est porteur d'un anévrisme du ventricule gauche, thrombosé, pour lequel il est sous traitement anticoagulant depuis plusieurs mois, et plus précisément sous héparine sous-cutanée depuis deux mois.

a) *La période opératoire:*

Le bilan préopératoire est normal :

Thrombelastogramme ST :

r : 15 k : 15 r + k : 30 a : 52

IPT : 7,2

Plaquettes : 295 000

TQ : 100 pour cent

Temps de thrombine : 27′ malade

27′ témoin

Fibrine : 3,5 g

La transplantation s'effectue le 28 novembre 1968 sans problèmes et la neutralisation en fin de circulation extracorporelle est réalisée selon la technique de routine dans le service, c'est-à-dire de manière progressive et incomplète. Le malade ne reçoit aucun enzyme antifibrinolytique.

À la huitième heure postopératoire apparaît une hypercoagulabilité relative (TC à sept minutes) ; les plaquettes sont à 190 000 éléments/mm³.

On place le malade sous héparine, selon la technique de «coagulation assistée » (rappelons que le but recherché est de placer l'opéré en légère hypocoagulabilité).

Au cours des trois premières heures, il faut sept mg d'héparine par heure pour placer ce malade entre 10 et 20 minutes de temps de coagulation.

Dans les heures qui suivent, le temps de coagulation est maintenu à ce même niveau avec des doses d'héparine qui seront progressivement croissantes (figure 1).

C'est ainsi que le troisième jour postopératoire nous serons amenés à délivrer 415 mg pour maintenir ce malade au niveau de coagulabilité que nous avions choisi.

Le quatrième jour, la perfusion est supprimée et l'héparine est délivrée par voie sous-cutanée.

Au cours des jours suivants, la dose d'héparine pourra être diminuée progressivement tout en conservant une efficacité identique. La « crise thrombogène postopératoire » est alors terminée.

II – *Commentaire :*

Les accidents thromboemboliques au décours des transplantations représentent un risque vital non négligeable. Il y a lieu de souligner la dose de 415 mg qui fut nécessaire au troisième jour après l'opération pour obtenir une légère hypocoagulabilité.

III – *Coagulopathie de consommation et rejet :*

L'étude attentive des troubles de coagulation après transplantation semble intéressante à deux points de vue :

a) en permettant le diagnostic précoce de rejet ;

b) en empêchant les phénomènes thrombo-hémorragiques.

Conclusion :

La transplantation d'organes perturbe l'équilibre coagulation – hémostase de l'opéré dans les périodes postopératoires précoce et éloignée.

b) *La période postopératoire éloignée:*

Depuis le jour de l'intervention, l'opéré est soumis au traitement classique (corticoïdes, azathioprine, immunoglobulines antilymphocytaires (Choay) auquel nous avons ajouté l'héparine).

Le 10 décembre 1968, l'état du malade est satisfaisant à tout point de vue. Il reçoit: 10 ml de globulines antilymphocytaires, 200 mg d'Immurel et 75 mg de prednisone.

L'héparinothérapie est faite par voie sous-cutanée en trois injections par 24 heures, à la dose de 175 mg.

Le bilan de la coagulation effectué huit heures après l'injection montre :

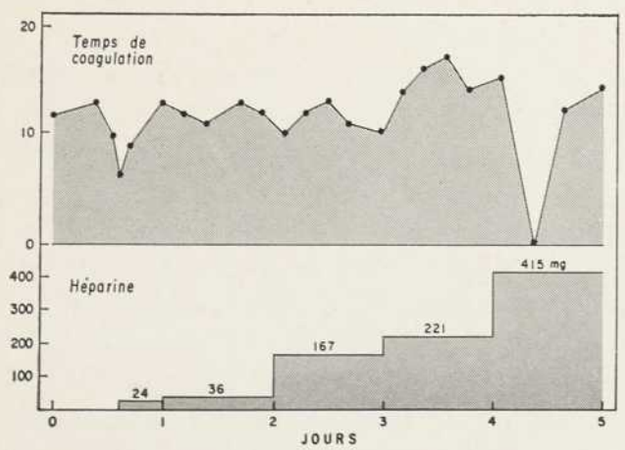

Figure 1 — Doses croissantes d'héparine pour maintenir le temps de coagulation entre 10 et 20 minutes.

Thrombelastogramme ST :

r : 18 k : 10 r + k : 28 à 53 IPT : 11,2

Plaquettes : 110 000

Temps de Quick : 100 pour cent

Fibrinémie : 5,9 g/l

Le 22 décembre, on décide d'interrompre l'héparinothérapie sous-cutanée continue après une courte période de transition ; on espère pouvoir faire au malade un traitement discontinu (deux ou trois injections d'héparine sous-cutanée par semaine).

De la même manière, le 28 décembre, on espace les injections de globulines antilymphocytaires, qui sont de 10 ml, un jour sur deux.

Mais nous allions très vite être amenés à reconsidérer ce schéma thérapeutique.

En effet, le 31 décembre surviennent des éléments nouveaux, cliniques et biologiques, qui font porter le diagnostic de rejet.

Le malade qui, jusque-là, était en parfait état, accuse de l'asthénie et de l'inappétence ; il est inquiet, se plaint de douleurs précordiales diffuses, et un épistaxis devient vite important (soulignons que depuis cinq jours le malade n'a plus reçu d'héparine).

Biologiquement, on note une thrombopénie à 70 000 éléments par mm³. Le temps de Quick est à 80 pour cent (alors que depuis l'intervention, il était à 100 pour cent) et la fibrine à 4 g.

Le tracé thrombodynamographique sur sang total est typique d'une coagulopathie de consommation.

Nous décidons de considérer le malade comme faisant un rejet au début avec coagulopathie de consommation, et de le traiter comme tel.

On renforce le traitement par globulines antilymphocytaires et corticoïdes, et on poursuit le traitement à l'azathioprine (il n'y a pas de leucopénie associée à la thrombopénie).

La thrombopénie et l'épistaxis nous semblent une indication formelle à la reprise d'une héparinothérapie continue.

En surveillant toutes les heures le temps de coagulation et le nombre des plaquettes, on règle le débit de l'héparinothérapie de manière à placer le malade entre 20 et 30 mn de temps de coagulation.

Sept heures après le début de l'héparinothérapie le nombre des plaquettes a doublé, et nous avons la preuve que la thrombopénie était bien due à un syndrome de coagulation intravasculaire disséminée (figure 2).

Le traitement héparinique est continué, mais au cours de la nuit, un défaut de surveillance ramène le malade en hypercoagulabilité et la thrombopénie à 75 000 éléments réapparaît avec récidive de l'épistaxis. Le traitement héparinique est alors réajusté et tout rentre dans l'ordre. Quarante-huit heures plus tard, on passe à la voie sous-cutanée, et depuis le malade n'a plus présenté d'incidents, ni cliniques, ni biologiques.

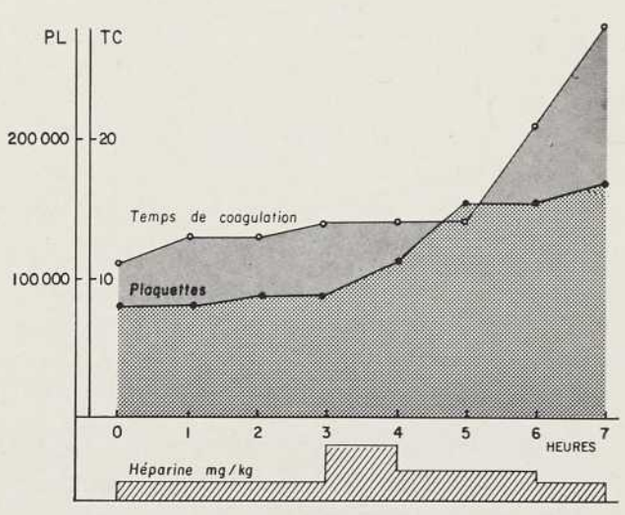

Figure 2 — Le nombre des plaquettes double après sept heures d'héparinothérapie.

Nous avons pu réduire les doses de globulines antilymphocytaires, mais l'héparinothérapie sous-cutanée est toujours poursuivie.

II. COMMENTAIRES

a) *Concernant la période opératoire:*

Les accidents thromboemboliques au décours des transplantations sont bien connus et représentent un risque vital non négligeable.

Il est difficile d'en établir exactement la fréquence; Starzl rapporte neuf thrombophlébites sur ses 42 premières transplantations rénales avec six embolies pulmonaires. Il souligne également le risque thromboembolique, en insistant sur la fréquence de ceux-ci au cours des transplantations ABO incompatibles. On peut voir dans ce rapprochement une preuve supplémentaire des rapports coagulation-immunologie.

Le transplantation cardiaque que nous avons effectuée a été suivie et traitée, au point de vue « coagulation », comme toutes les autres interventions sous circulation extracorporelle réalisées dans le service.

Nous devons souligner que nous n'avions jamais dû, sur près de 200 malades ainsi traités, délivrer au troisième jour postopératoire une dose d'héparine aussi importante pour obtenir une hypocoagulabilité légère.

Le fait d'implanter un organe étranger semble créer des conditions un peu différentes de celles que l'on a coutume d'observer en chirurgie cardiaque.

Si on interprète la dose d'héparine nécessaire pour rendre à un malade un temps de coagulation entre 10 et 15 minutes comme le reflet de la tendance thrombogène, on concevra qu'au troisième jour postopératoire notre malade a développé une tendance thrombogène grave.

En pratique, l'héparinothérapie postopératoire précoce par la « neutralisation progressive incomplète » d'une part, et par la reprise de l'héparine

dès que le malade devient hypercoagulable, d'autre part, n'est pas plus délicate que pour n'importe quel autre acte de chirurgie cardiaque sous circulation extracorporelle.

b) *Commentaires concernant la période postopératoire à distance:*

Les foyers de nécrose hémorragique, les infarcissements, les thromboses des microvaisseaux sont des lésions que l'on peut rencontrer aussi bien sur le greffon victime de rejet que sur les viscères d'un malade décédé par hémorragie grave due au phénomène de coagulation intravasculaire disséminée. La thrombopénie, on le sait, est un des éléments les plus caractéristiques du syndrome biologique de la coagulation intravasculaire disséminée.

La thrombopénie est également observée, dans certains cas, dans les suites de transplantation d'organe.

Mais on peut l'interpréter de différentes manières:

– L'azathioprine a une cytotoxicité qui peut atteindre les plaquettes.

– Les globulines antilymphocytaires selon l'origine et le degré de purification peuvent contenir un certain taux de globulines antiplaquettaires qui expliqueraient la thrombopénie. Sans nier ces interprétations, nous voudrions introduire une troisième cause possible de thrombopénie post-transplantation.

Dans notre observation, nous avons rattaché la thrombopénie à une coagulopathie de consommation, et ceci parce que le syndrome biologique était hautement significatif.

Mais, de plus, il était difficile de rendre responsable l'azathioprine ou les globulines antilymphocytaires.

Quant aux globulines antilymphocytaires, une expérimentation animale préalable nous avait permis de constater leur absence d'action sur le système de coagulation.

La remontée du chiffre des plaquettes sous héparine est une preuve absolue qu'il s'agissait bien d'une coagulopathie de consommation. Aucune autre thrombopénie n'est curable par l'héparine.

Certes, nous avons augmenté la corticothérapie en même temps que nous intensifions l'héparinothérapie; mais dans les heures qui ont suivi, une erreur de conduite du traitement héparinique nous a permis de vérifier l'action bénéfique de l'héparine.

III. COAGULOPATHIE DE CONSOMMATION ET REJET

L'observation que nous avons présentée, étant isolée, n'est peut-être qu'une exception et ne saurait suffire à établir une théorie. Mais, en lisant les travaux des autres équipes de transplantations, on peut suspecter des observations sensiblement analogues.

Mowbray signale la thrombopénie comme signe précurseur de rejet mais n'en tire pas des conclusions thérapeutiques.

Starzl rapporte des thrombopénies au cours de crise de rejet « comme si le malade avait un subit changement de sensibilité à l'azathioprine ».

Pour nous, l'étude attentive des troubles de la coagulabilité post-transplantation nous semble intéressante à deux points de vue.

a) *Le diagnostic précoce de rejet:*

Certaines formes de rejet s'accompagnent de thrombopénies dues à des coagulopathies de consommation. Ceci nous semble maintenant évident.

Certes, la chute des plaquettes est un signe caractéristique et c'est ce signe qui, le premier, a attiré notre attention.

Cependant, nous avons observé que le thrombelastogramme sur sang total s'était modifié six jours avant que la chute des plaquettes ne soit manifeste.

On note, le 20 décembre, un effondrement de l'I.P.T. et, *a posteriori*, nous pensons que nous aurions dû reprendre plus précocement l'héparinothérapie (figure 3).

La courbe d'I.P.T. (dérivée de constantes thrombodynamographiques) doit permettre un diagnostic précoce de coagulopathie de consommation et, partant, de rejet.

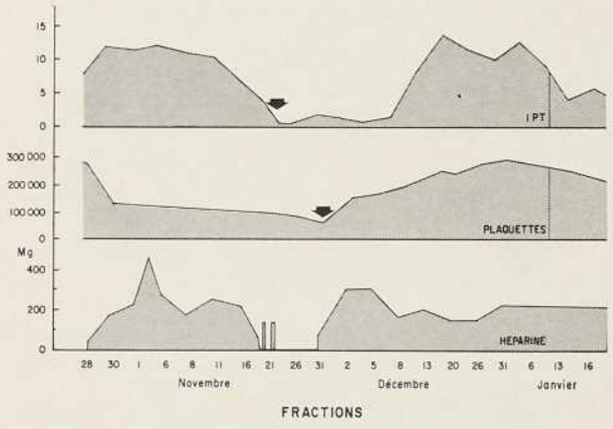

Figure 3 — La courbe de l'IPT (dérivée de constantes thrombodynamographiques) s'affaise le 20 décembre.

En matière de signes de rejet, au cours des transplantations cardiaques, tous les auteurs sont d'accord pour dire que les signes classiques sont très tardifs.

En pratique, dans la surveillance actuelle de notre opéré, nous donnons un rôle très important au thrombelastogramme et à la numération plaquettaire.

b) *Intérêt de la thérapeutique anticoagulante après la transplantation:*

Il semble logique de penser qu'une thérapeutique anticoagulante bien conduite évitera l'apparition des phénomènes thrombohémorragiques au cours du rejet.

Cela ne veut pas dire que l'héparine est une substance antirejet. Au mieux, on peut espérer que, limitant les lésions thrombohémorragiques irréversibles, elle permettra au traitement spécifique (immunosuppresseur, globulines antilymphocytaires, corticoïdes) d'intervenir et de régler le conflit immunologique au niveau greffon.

CONCLUSION

La transplantation d'organe perturbe l'équilibre coagulation-hémostase de l'opéré, et ce à différentes étapes:

a) Dans la période postopératoire précoce, en induisant une hypercoagulabilité profonde qui est une cause possible d'échec;

b) Dans la période postopératoire éloignée, en déclenchant, dans l'observation rapportée, une coagulopathie de consommation dont on ne peut s'empêcher de penser qu'elle a des rapports étroits avec le phénomène de rejet.

SUMMARY

A case of human heart transplantation is reported in which coagulation disorders were noted. On the third postoperative day, a very marked hypercoagulability status required 415 mg of heparin to render the patient's blood slightly hypocoagulable.

On the 34th day, a further coagulation disorder developed with clinical signs of graft rejection. The signs again disappeared with heparin therapy.

Disseminated intravascular coagulation may be observed in acute rejection. With heparin, irreversible damage (coronary occlusion or infarction) is perhaps less extensive and this treatment permits to wait the action of immunosuppressive and steroid therapy. Heparin given for a long period of time (several months) may help to prevent chronic rejection.

ADDENDUM

Depuis la rédaction de ce travail notre opéré évolue favorablement et demeure placé sous la protection de l'héparine (janvier 1970).

COMMENTS ON PATHOGENESIS OF THE REJECTION PHENOMENON *

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L'expérience a démontré qu'un bon typage tissulaire n'est pas nécessairement associé à une plus longue survie. Le problème fondamental réside dans le mécanisme du rejet qui n'a pu encore être complètement défini.

L'immunité cellulaire est le facteur critique dans le rejet. Le rôle des facteurs humoraux est encore spéculatif, et on se demande si les anticorps anticœurs se développent au cours de la transplantation cardiaque et s'il en est ainsi, quand et quel rôle ils jouent dans le rejet ? D'autre part, quel rôle jouent les globules antilymphocytaires dans le rejet. Les
(suite du résumé en page suivante)

Although histocompatibility typing appears to have some value in predicting long-term survivors from cardiac transplantation there have been numerous exceptions to the rule that good tissue matches survive for longer periods than poor ones. The fundamental problem of course remains that the precise mechanisms of rejection are not defined.

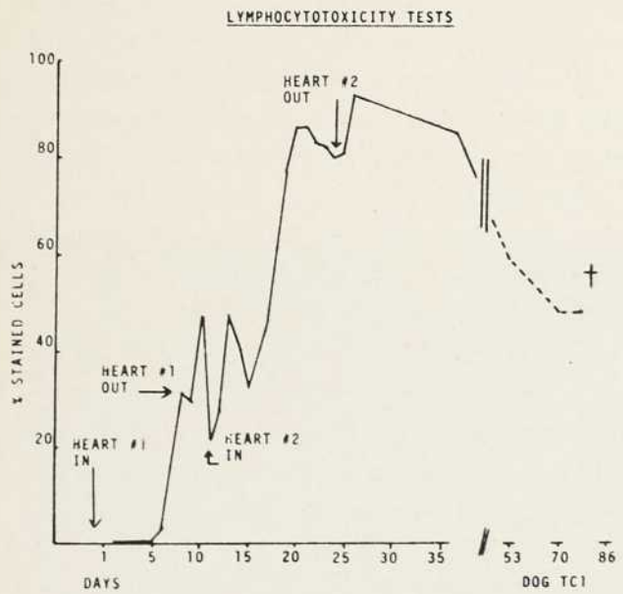

Figure 1 — Demonstration of cytotoxic antibodies in recipient sera before, during, and after rejection of the heterotopic allografted canine heart with a subsequent rise after insertion of a second cardiac allograft.

It is generally accepted that cellular immunity is the critical factor in the rejection of organ transplants. However, there is speculation concerning the role of humoral factors; one wonders if circulating anti-heart antibodies do develop in cardiac transplant recipients and if so, when, and what role do they play in rejection. Furthermore does treatment with antilymphocyte globulin have any affect on this response?

We are exploring these questions with an experimental model using unrelated mongrel dogs receiving heterotopic abdominal cardiac allotransplants.

Figure 2 — The classic histologic picture of acute, unmodified cardiac allograft rejection. The clinical, pathologic and immunofluorescent studies all correlated in both timing and degree.

* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6-8, 1969.

auteurs rapportent leurs observations, suite aux expériences faites sur des chiens recevant des allotransplantations cardiaques intra-abdominales. Ces observations sont :

1. des anticorps cytotoxiques ont été démontrés dans la séreuse d'un pourcentage assez élevé de receveurs, soit juste avant le rejet, soit après le retrait du cœur transplanté ;
2. on observe le tableau clinique typique du rejet aigu ;
3. des études immunofluorescentes de cœurs rejetés ont démontré la présence de gamma globulines G dans le sarcolemme ;
4. chez les chiens traités avec du sérum antilymphocytaire, le rejet est retardé, les modifications pathologiques du rejet sont nettement minimisées et le dépôt de gamma globulines G le long du sarcolemme est réduit ou même évité.

Les auteurs soulignent que les gamma globulines G circulantes se développent de façon précoce et se fixent sur le cœur, et que ce phénomène peut être aboli ou modifié en utilisant le sérum antilymphocytaire.

Several interesting preliminary observations have been made:

1. Cytotoxic antibodies have been demonstrated in the sera of a significant percentage of the recipients either just before rejection of, or after removal of, the transplanted heart (Figure 1).
2. With the heterotopic model, the classic pathologic picture of acute rejection has developed (Figure 2).
3. Immunofluorescent studies of the rejected hearts have revealed gamma G globulin deposited along the sarcolemma (Figure 3).
4. In those dogs treated with ALS, rejection has been delayed, the pathologic changes of rejection have been greatly minimized but the deposits of gamma G globulin along the sarcolemma were not reduced or prevented (Figure 4).

These observations emphasize the usefulness of this experimental model in studying the rejection phenomenon in cardiac transplants without the complications of circulatory failure and early death of the animal. Most importantly, they suggest that circulating gamma G antibody does develop early and fix to the heart and that this response is not suppressed or modified by the use of an antilymphocytic serum.

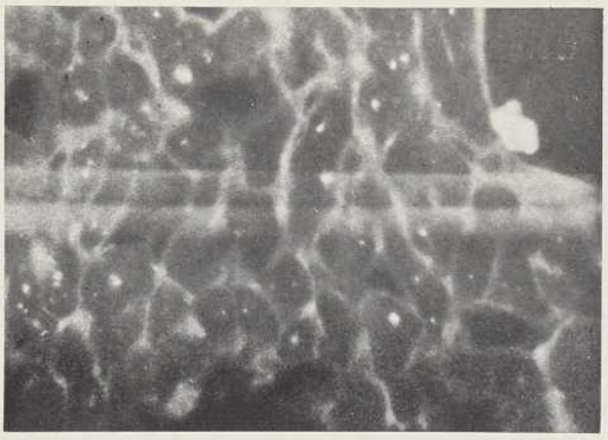

Figure 3 — Immunofluorescent studies have revealed deposits of gamma G globulin deposited upon the sarcolemma of the myocardial fibres, seen here in cross section.

Figure 4 — Photomicrograph of a canine cardiac allograft after treatment with antilymphocytic serum. Except for a few small foci of mononuclear cells, the histologic picture of rejection is considerably modified.

ACUTE CARDIAC ALLOGRAFT REJECTION — MORPHOLOGICAL FINDINGS * †

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Les auteurs ont étudié les données morphologiques observées chez deux malades morts de rejet aigu d'une allogreffe cardiaque, 6 et 13 jours après la transplantation. Dans les deux cas, le typage tissulaire a été du groupe D, et après l'intervention, la thérapie immunosuppressive consistait en Prednisolone, globules antilymphocytaires et Azathioprine.

Chez le premier malade, l'autopsie révèle des poumons indurés attribuables à une défaillance cardiaque latente précédant la chirurgie. Dans le deuxième cas, l'examen a révélé la présence d'un œdème pulmonaire minime avec des surfaces multifocales de nécrose graisseuse du pancréas et des granulomes microfocals non caséeux, des poumons et du foie. L'étiologie de ces granulomes reste obscure.

(suite du résumé en page suivante)

At St. Luke's Episcopal Hospital, Houston, Texas, cardiac allografts have been performed in a total of 16 patients. Fourteen of the 16 cases have expired at periods from 2 to 267 days.

Table I summarizes the cause of death and lists the mean survival times. Surgical techniques (3), clinical details (2 and 7), and morphological findings (6) in the cardiac allografts, as well as, autopsy findings of the cases have been previously presented. Other papers have dealt primarily with morphological findings in canine (5) and human allografts (8 and 10). The primary objective of this paper is to present the morphological findings in two cases who expired at 6 and 13 days due to acute cardiac allograft rejection.

Case 1:

This 50 year old male experienced his first episode of heart failure five years prior to surgery. One year later a second episode of heart failure occurred and a clinical diagnosis of cardiomyopathy was made. He was admitted to St. Luke's Episcopal

TABLE I

Cardiac allografts. 16 patients

	MEAN SURVIVAL TIME
14 Patients expired	90 (days)
7 Cardiac allograft rejection :	130
2 Acute	10
5 Chronic	178
6 Infection :	31
Pneumonia due to Klebsiella-Enterobacter and Escherichia Pneumonia due to Pseudomonas (2 patients) Pulmonary abscesses due to Pseudomonas Pneumonia due to Serratia Septicemia due to Serratia	
1 Combination infection (Listeria monocytogenes septicemia and meningitis) and chronic cardiac allograft rejection	170
2 Patients living	154 *

* June 7, 1969.

* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6–8, 1969.

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L'examen de l'allogreffe révèle la présence d'une hémorragie endocardique étendue portant sur toutes les cavités de l'allogreffe, tandis que l'on note très peu d'hémorragie endocardique des oreillettes droite et gauche du receveur. On observe également un épaississement mural de toutes les cavités et le myocarde est rouge foncé, de consistance ferme.

L'examen microscopique révèle un infiltrat interstitiel diffus composé de larges lymphocytes, de leucocytes polymorphonucléaires, d'histiocytes et d'eosinophiles. Les larges lymphocytes contiennent un grand noyau hyperchromatique occupant la majeure partie de la cellule, avec un très petit anneau de cytoplasme. Certains leucocytes polymorphonucléaires présentent des modifications dégénératives. Un dépôt de fibrine est associé à l'infiltrat cellulaire. Les artères coronaires présentent au niveau de leur intima un épaississement et cette partie de l'intima adjacente à la lumière présente un infiltrat cellulaire semblable à celui rencontré au niveau du myocarde. En général, la média est intacte, mais l'on rencontre quelquefois de la nécrose. Les artères intra-murales présentent de la dégénération acidophile, une vacuolisation de la média, de même qu'une perte de substance au niveau de l'endothélium. L'infiltrat cellulaire de l'adventice est semblable à celui rencontré au niveau du myocarde. Aucune de

Hospital on 9.15.68 because of a progressive deterioration in his condition despite good medical therapy. A donor became available on 11.5.68 and cardiac transplantation was carried out. Surgical pathology examination revealed a cardiomyopathy. Tissue matching revealed a D tissue match [Terasaki (9)]. Immunosuppressive therapy consisting of prednisolone, antilymphocyte globulin, and azathioprine was started on the day of transplantation. Postoperatively no difficulties were experienced until the fifth day when fever, leukocytosis and development of a pericardial friction rub were noted.

Death occurred on the sixth postoperative day despite massive doses of corticosteroids, cytoxan, antilymphocyte globulin and drugs for cardiac support.

Autopsy exhibited brown induration of the lungs which was attributed to the presence of long standing severe congestive heart failure prior to surgery. The cardiac allograft findings will be described with those of Case 2.

Case 2:

This 54 year old male had his first myocardial infarction eight years previously and had episodes of congestive heart failure for seven years prior to surgery. The diagnosis of severe coronary artery disease with myocardial failure was confirmed by cardiac catheterization at St. Luke's Episcopal Hospital approximately one month prior to surgery. Cardiac transplantation was carried out on 11.29.68. Tissue typing revealed a D tissue match [Terasaki (9)]. Pathological examination of the surgically excised heart revealed severe coronary arteriosclerosis with associated old myocardial infarcts. Immunosuppression therapy consisting of prednisolone, antilymphocyte globulin and azathioprine was initiated on the day of transplantation. A pericardial friction rub was detected on 12.6.68 and four days later leukocytosis and increase in size of the cardiac silhouette developed. The blood pressure dropped on 12.11.68 with two episodes of respiratory arrest on the same date. The patient expired thirteen days (12.12.68) following surgery, due to acute cardiac allograft rejection despite the addition of cytoxan and numerous drugs for cardiac support.

Necropsy revealed pleural effusion, minimal ascites, minimal pulmonary edema, multifocal areas of fat necrosis in the pancreas, and rare microfocal noncaseating granulomata involving the lungs and

ces modifications ne sont rencontrées au niveau des oreillettes droite et gauche du receveur.

Chez les malades qui décèdent assez rapidement après la transplantation, l'on note un œdème interstitiel qui peut être attribué à l'état même du cœur, aux manipulations chirurgicales, ou à une souffrance des lymphatiques cardiaques avec obstruction. Chez les malades dont la mort est attribuée à une infection, on observe des foyers de cellules mononucléaires au niveau-même du myocarde et des zones péri-vasculaires. De tels foyers mononucléaires sont considérés comme une réponse immunologique, mais leur étendue n'est pas suffisante pour expliquer un malfonctionnement de l'allogreffe.

Notons que le cœur du donneur est souvent celui d'un malade présentant des lésions intra-crâniennes et l'on rencontre dans ces cas des zones d'œdème myocardique ou de myotolyse.

liver (rare noncaseating microfocal granulomata were also found in the surgically resected heart but not in the allograft specimen). The etiology of the granulomata remains obscure. Special stains (acid fast stains, periodic acid-Schiff reaction, and methenamine silver stains) failed to reveal the presence of organisms. Clinical findings including serum protein electrophoresis were not suggestive of sarcoidosis. Complement fixation test for histoplasmosis, blastomycosis and coccidioidomycosis were anticomplementary.

Hearts (Allografts of Cases 1 and 2): The allograft specimens on Cases 1 and 2 weighed 535 and 430 grams respectively at necropsy. The suture lines between the donor and recipient portions of the atria were intact and uncomplicated. There

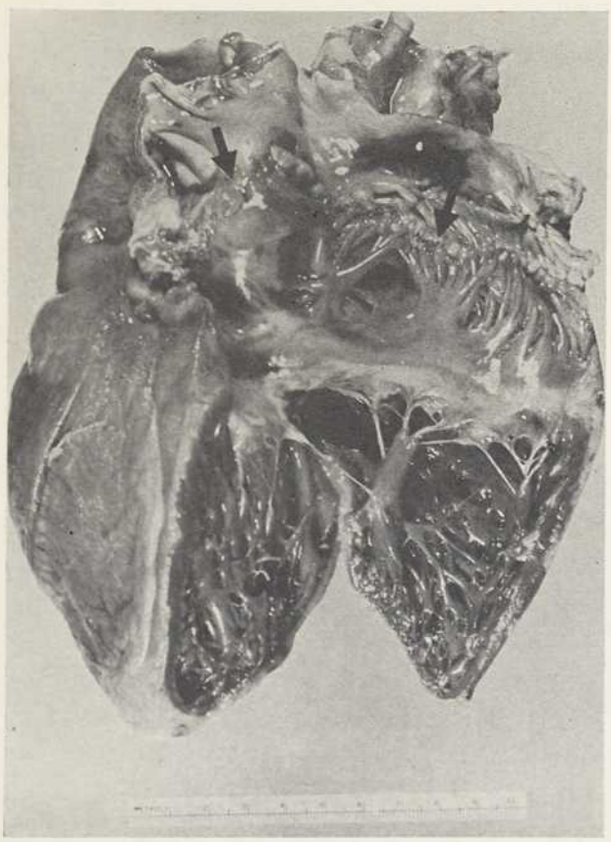

Figure 1 — Cardiac allograft 13 days following surgery. Patient died due to acute cardiac allograft rejection. Arrows point to intact suture lines between donor and recipient portions of right atria. There is extensive endocardial hemorrhage in the donor right atrial endocardium. This case was a D tissue match (Terasaki).

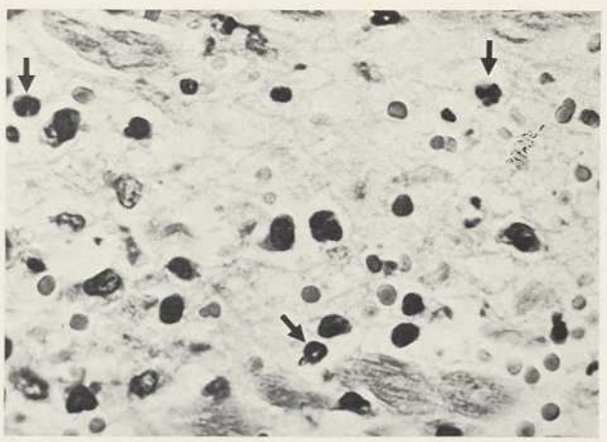

Figure 2 — 1125X. Acute cardiac allograft rejection at 6 days. Sections of myocardium reveals dehiscence of myocardial fibers, and infiltration of plump lymphocytes with large hyperchromatic nuclei, polymorphonuclear leukocytes (arrows), histiocytes, and extravasated erythrocytes. Fibrin deposition is evident. Case was a D tissue match (Terasaki).

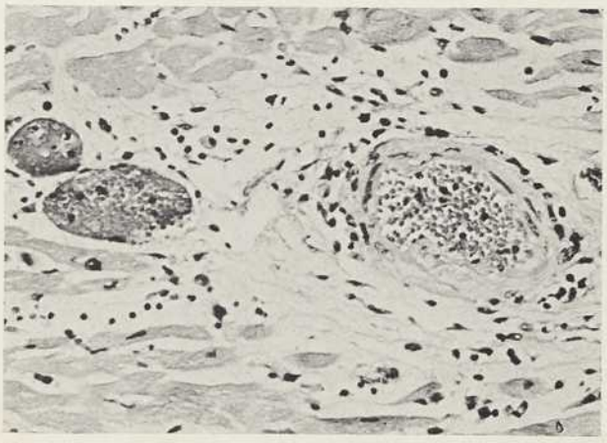

Figure 3 — 350X. Myocardium of same specimen in Figure 1. Section shows small intramural coronary artery exhibiting medial degeneration, and vacuolization. Also vascular congestion and perivascular cellular infiltrate present.

was extensive endocardial hemorrhage involving all chambers of the allografts and there was little endocardial hemorrhage involving the recipient portion of the right and left atria. Mural thickening of all chambers was present. The thickness of the left ventricular wall of Case 1 measured up to 2.2 cm. The myocardia of the cardiac allografts were dark red, mottled in appearance and firm in consistency. The chambers were not notably dilated.

Multiple representative microscopic sections were prepared throughout the myocardium of the allografts. There was a diffuse interstitial infiltrate consisting of large lymphocytes, varying numbers of polymorphonuclear leukocytes, histiocytes and eosinophils. The large lymphocytes tended to contain a large hyperchromatic nucleus which occupied most of the cell and often only a thin rim of cytoplasm was often apparent. Some polymorphonuclear leukocytes exhibited degenerative changes. Fibrin deposition was often associated with the cellular infiltrate. Myocardial fiber dehiscence and fragmentation was apparent and the cellular infiltrate and fibrin was abundant in the separated areas as well as in perivascular areas. The extramural coronary arteries exhibited slight to moderate intimal thickening. (The ages of donor patient for Cases 1 and 2 were 15 and 40 years respectively and each was a male). The portion of intima adjacent to the lumen was often loosely arranged and a cellular infiltrate similar to that of the myocardium was present within the more central portions of the intima. There was disruption of endothelial cells but some that were intact were conspicuously enlarged. Generally the tunica media of the extramural coronary arteries were intact but necrosis of the tunica media was sometimes present. The intramural arteries often exhibited degeneration, acidophilia, and vacuolization of the tunica media along with disruption of the endothelial lining and plump endothelial cells. An adventitial cellular infiltrate was similar to that of the myocardium. The recipient portion of the right and left atria was spared of the dense cellular infiltrate, myocardial fiber disruption and vascular changes which were observed in the allograft. Each of the two cases was a D tissue match [Terasaki (9)] and the histocompatibility tests were performed locally and in collaboration with Terasaki. The survival time of the two cases was considerably shorter than that of the patients with chronic cardiac allograft rejection (Table 1).

The cardiac allograft morphology of the patients who expired in relatively short periods of time due to causes other than rejection deserves some emphasis. Interstitial edema is observed and is attributed to the status of the donor heart, effects of surgical manipulation, and severance of cardiac lymphatics with lymphatic obstruction. In some patients whose cause of death is clearly related to infection and who expired in relatively short periods of time, rare foci of mononuclear cells were present within myocardium and perivascular areas and this was regarded as an immunological response, but the magnitude was not great enough to contribute significantly to dysfunction of the allograft.

In heart transplantation procedures donor patients have generally been those with intracranial lesions. Focal areas of myocardial edema (4) or myocytolysis (1) may be found in patients with intracranial lesions, and these changes may be related to suitability of hearts for transplantation

procedures. Also the changes may be morphologically reflected in the examination of the allograft, especially those who expire in relatively short periods following surgery.

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NON CARDIAC AUTOPSY FINDINGS AFTER HEART TRANSPLANTATION *

Pierre SIMARD, M.D.†

Les principales modifications sont de plusieurs ordres :

1. *Les complications infectieuses*

Les injections les plus fréquentes se présentent sous forme de pneumonies ou de broncho-pneumonies, associées ou non à des abcès. Dans un cas particulier, on a noté la présence de nombreux abcès mycotiques disséminés dans tous les parenchymes.

Les autres complications sont notamment des trachéobronchites hémorragiques, des pyélonéphrites chroniques bilatérales, du zona thoracique gauche, des œsophagites ulcératives, des pharyngites ulcératives aiguës et des orchites chroniques bilatérales non spécifiques.

2. *Pathologie vasculaire*

Celle-ci se présente sous forme d'athérosclérose généralisée, éventuellement associée à des anévrismes et thromboses ou infractus récents. Notons également les phlébo-thromboses fémorales.

Nine patients have sustained a heart transplant at the Montreal Heart Institute between May 30, 1968 and November 29, 1968. All transplanted patients were males, their age ranging between 35 to 59 years, and were suffering from coronary atherosclerosis with old extended myocardial infarctions. Out of these nine transplanted patients, one presented with his coronary disease a chronic rheumatic mitral disease.

The first of the heart transplants must be excluded from this lecture; he is a 59 year old man (the oldest) who survived only two days after the transplantation: the transplanted heart could not regain its normal function, and had to be assisted by an intermittent cardio-pulmonary "bypass". In that case, the autopsy had only demonstrated secondary changes of cardiac insufficiency, and multiples hemorrhages caused very likely by a terminal defibrillation.

The eight remaining cases were ranging from 35 to 58 years of age and the transplanted hearts were from donors whose ages ranged from 15 to 35 years (Table I). That Table also indicates the survival days and the type of rejection seen histologically. The principal non-cardiac modifications found at autopsy can be classified as follows (Table II):

1. Infectious complications;
2. Vascular findings;
3. Changes due to cardiac failure;
4. Secondary modifications to treatment;
5. Others.

1. INFECTIOUS COMPLICATIONS

a) The more frequent infectious complications in transplanted patients were pneumonia and bronchopneumonia (cases No. 4, 5, 6 and 7). In No. 4, there was a massive extended pneumonia and in patients No. 5, 6 and 7, this pneumonia was complicated by necrosis and pulmonary abscesses.

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* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6-8, 1969.

† Pathologist, Montreal Heart Institute.

3. *Modifications secondaires à la défaillance cardiaque*

Ces modifications se présentent sous forme de défaillance cardiaque aiguë ou chronique survenant soit après la transplantation, soit qu'elle est déjà présente au moment de l'intervention.

4. *Modifications secondaires au traitement*

a) les corticostéroïdes.

On observe des atrophies de la corticale surrénalienne, des ulcérations rectales hémorragiques, des ulcères gastriques aigus avec hémorragie gastro-intestinale et des myopathies.

b) Globulines antilymphocytaires.

Avec ce traitement, on note une atrophie des tissus lymphoïdes. L'examen microscopique des ganglions lymphoïdes démontre une pulpe ap-

(suite du résumé en page suivante)

Patient No. 5 showed some small colonies of aspergillus in the lungs and a small area of cerebral aspergillosis.

Patient No. 6 is of particular interest: it consists of a bilateral massive pneumonia with pulmonary abscess due to aspergillus. A bacteriological specimen showed the presence of aspergillus fumigatus, and the histology showed an important proliferation of aspergillus in pulmonary parenchyma. This patient also had a massive necrotic and hemorrhagic encephalitis with aspergillus and we also found some mycotic abscesses identical in the kidney, liver, epicardium, myocardium, endocardium and thyroid. It is interesting to note that this patient did not present any sign of cardiac rejection and was in coma five days prior to death.

In patients No. 6 and 7 pleuritis was found in addition to pneumonia.

b) The other infectious complications were found as follows:

TABLE I

Age of the receivers and of the donors, survival time and histologic caracteristics of rejection

RECEIVER N°	AGE OF THE RECEIVERS	AGE OF THE DONORS	SURVIVAL TIME (days)	REJECTION (Histology)
1	58	35	11	Acute, fulminating
2	43	15	38	Acute
3	45	20	47	Acute
4	35	25	64	Chronic and acute
5	51	19	68	Chronic, discrete
6	53	23	106	None
7	58	16	120	Chronic
8	49	23	156	Chronic

pauvrie, une diminution du nombre des lymphocytes, de petits follicules avec très peu de cellules et une absence de centre germinatif. Les vaisseaux, les sinusoïdes et les cellules réticulaires ainsi que les histiocytes sont proportionnellement abondants. Macroscopiquement, la pulpe blanche de la rate n'était pas visible et du point de vue histiologique elle semble montrer une diminution marquée des follicules péri-artériolaires.

c) L'azathioprine (Imuran).

Un examen de la moelle osseuse n'a pu démontrer une diminution d'aucune des cellules, et les séries cellulaires ne semblent pas être atteintes.

5. *Autres*

Parmi les autres complications, la plus fréquemment rencontrée est l'hémorragie de la muqueuse gastro-intestinale.

1. Hemorrhagic tracheobronchitis (No. 3) ;
2. Bilateral chronic pyelonephritis (No. 5) ;
3. Left thoracic zona (No. 5) ;
4. Ulcerative œsophagitis (No. 5) ;
5. Acute ulcerative pharyngitis (No. 7) ;
6. Non specific chronic bilateral orchitis (No. 8).

2. VASCULAR FINDINGS

A moderate generalized atherosclerosis was present in No. 4, 6, 7 and 8. In patients No. 1, 2, 3 and 5 an important generalized atherosclerosis was found. Patient No. 1 of the second group had aortic and iliac aneurysms with thrombosis, for which a bilateral aorto-femoral graft was performed two days following heart transplantation. The same patient also showed aneurysms of both internal carotids with complete thrombosis of the left internal carotid artery, partial thrombosis of the right and a small region of recent hemorrhagic cerebral infarction. Multiple recent infarctions of the spleen were also present.

Nevertheless of all these complications, we believe the principal cause of the death in this patient was due to acute fulminating rejection of the grafted heart.

Patients No. 4 and 8 showed some old infarctions of the left kidney. No. 5 showed a left femoral phlebothrombosis. Finally some small areas of old cerebral cortical hemorrhagic infarctions were found in No. 8.

3. CHANGES DUE TO CARDIAC FAILURE

Rapidly let us mention that most of the cases showed some modifications due to cardiac failure either chronic or acute, such as cardiac lungs (No. 2, 4, 6, 7 and 8), pulmonary œdema (No. 3, 4 and 7), and hydrothorax (No. 1 and 3), stasis of the liver and spleen (No. 2, 3, 4, 7 and 8).

I should mention that No. 6 did not present any histological signs of rejection. Cardiac lungs were already present due to his heart condition prior to transplantation.

(*Note.* Survival ranging from 11 to 64 days seen in the first four cases showed some histological and clinical signs of acute rejection. Histological signs of progressive chronic cardiac rejection were found in the last five patients where survival ranged from 64 to 156 days.)

4. SECONDARY MODIFICATIONS DUE TO TREATMENT

a) *Corticosteroids:*

A moderate degree of cortical adrenal atrophy was noted in a few instances. No. 5 showed superficial slightly hemorrhagic rectal ulcerations. Two

acute gastric ulcers with gastro-intestinal hemorrhage were seen in patient No. 6. Finally two cases showed some histological signs compatible with steroid myopathy. Some clinical signs of this muscular lesion were also seen in No. 8.

It is noticeable that survival time was much longer in these two patients.

b) *A.L.G.:*

An important atrophy of the lymphoid tissues attributed to antilymphocytic globulin was present in most patients. All the lymph nodes were atrophic and it was rather difficult to find them at autopsy. Microscopical examination of the lymph nodes showed a depleted pulp, the number of lymphocytes was decreased, the follicles were very small and less cellular, with the germinative centers absent. The vessels, sinusoids, reticular cells and the histiocytes appeared proportionally abundant.

Macroscopically the white pulp of spleen was not visible. Histology showed a marked diminution of peri-arteriolar follicles.

Often these arterioles appeared isolated, surrounded by rare lymphocytes.

TABLE II

Non cardiac autopsy findings

Receiver N°.	Infectious	Generalized atherosclerosis	Vascular complications	Cardiac failure	Others
1		Marked	Thrombosed aorto-iliac aneurysms. Internal carotid aneurysms.	Hydrothorax	
2		Marked		Cardiac lungs Cardiac liver	
3	Acute tracheobronchitis	Marked		Hydrothorax Acute pulm. œd. Cardiac liver	
4	Pneumonia	Light		Cardiac lungs Acute pulm. œd. Cardiac liver	
5	Pneumonia with abscesses Chronic pyelonephritis Herpes Zoster Oesophagitis	Marked	Left femoral phlebothrombosis		
6	Aspergillus pneumonia Aspergillus encephalitis	Light		Cardiac lungs	Pyloric ulcers (steroid)
7	Pneumonia with abscesses Acute ulcerative pharyngitis	Moderate		Cardiac lungs Acute pulm. œd. Cardiac liver	Steroid myopathy
8	Chronic orchitis	Moderate		Cardiac lungs Cardiac liver	Steroid myopathy

c) *Imuran:*

For the patients treated with azathioprin (Imuran), a summary examination of the bone marrow did not show any cellular depletion and the cellular series also did not seem affected.

5. OTHERS

Among other complications, the more frequently present were, diffuse hemorrhage of the gastro-intestinal tract mucosa in three patients: one of these (No. 3) showed massive diffuse hemorrhage of gastric mucosa and two others some discrete gastric suffusions (No. 4), and more important of colon (No. 5).

In No. 1 and 7, gall-bladder stones were seen. A pancreatic cytosteatonecrosis was present in No. 7 and 8.

THE ROLE OF PLATELETS IN CHRONIC REJECTION *

Geoffrey EVANS, M.B., **and J. F. MUSTARD**, M.D.†

Au cours des épisodes de rejet survenant plus de 11 jours après une transplantation rénale, des biopsies rénales ont démontré une obstruction des capillaires glomérulaires par des agrégats plaquettaires. Ces agrégats ont également été rencontrés dans les cas de cœurs et foies transplantés.

L'organisation de ces thrombi riches en plaquettes dans les artères conduisent à un épaississement de l'intima formé de cellules musculaires lisses, collagène, fibres élastiques et tissu endothélial. Dans les vaisseaux des organes transplantés, un tel épaississement de l'intima apparaît et semble être en relation avec l'organisation de ces thrombi plaquettaires.

On a démontré qu'au cours des épisodes de rejet on notait une diminution assez marquée du taux des plaquettes circulantes au moyen de plaquettes marquées au chrome 51, qui s'accumulaient dans le rein transplanté.

(suite du résumé en page suivante)

There is good evidence in the rejection episodes occurring more than eleven days after renal allograft transplantation in man that obstruction of the circulation by platelet aggregates is an important mechanism (4 and 9). Biopsies of kidneys taken during these episodes have shown the glomerular capillaries to contain aggregated platelets which in some cases completely obstruct the vessel lumen. Aggregated platelets have also been found in some of the transplanted livers and hearts (5).

Organization of platelet rich thrombi in arteries leads to intimal thickening consisting of smooth muscle cells, collagen, and elastic fibers and endothelium tissue (3). In the vessels of transplanted organs such intimal thickening also occurs and it seems likely that this could in part be related to the organization of platelet thrombi. In the heart lesions these areas of intimal thickening are focal and sometimes produce considerable narrowing of the lumen of major coronary arteries (5).

It has been shown that during the rejection episode a fall in the level of circulating platelets occurs and by labelling the platelets with chromium^{51} it was demonstrated that the labelled platelets accumulated in the transplanted kidney (6).

The mechanisms by which the platelet aggregates could be initiated have not been defined. However, several can be considered. It is known that *in vitro*, the interaction of platelets with collagen, antigen antibody complexes and gamma globulin coated surfaces leads to the release of platelets constituents including adenosine diphosphate (ADP) which is believed to be the main stimulus for platelet aggregation.

The stimulus or stimuli leading to platelet aggregation *in vivo* in transplanted organs could be due to damage to the endothelium with exposure of the subendothelial tissues such as collagen and the basement membrane. In addition the interaction of antibody with the endothelial cells or the basement membrane creating an antigen antibody complex could provide the stimulus for platelet aggregation (8).

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Le mécanisme par lequel ces agrégats plaquettaires se forment n'a pu être expliqué. Cependant, on sait que *in vitro*, l'inter-action des plaquettes avec le collagène, avec le complexe anticorps — antigène et avec les gamma globulines, s'accompagne de la libération des constituants des plaquettes incluant l'adénosine diphosphate, (ADP), qui semble être le facteur principal de l'agrégation plaquettaire. *In vivo*, et au niveau des organes transplantés, il semble que les lésions endothéliales avec mise à nu des tissus sub-endothéliaux tels que collagène et membrane basale, soient le facteur responsable de l'agrégation plaquettaire. L'interaction entre les anticorps et les cellules endothéliales ou la membrane basale créant un complexe anticorps — antigène, peut être un stimulant de l'agrégation plaquettaire.

In vitro, l'agrégation plaquettaire peut être inhibée par des médicaments anti-inflammatoires tels le Phénylbutazone, Sulfinpyrazone et acide acétylsalicylique. Il en est de même pour l'hydrocortisone et la 6-mercaptopurine.

Du point de vue clinique, on a noté qu'au cours des épisodes de rejet l'administration de Phénylbutazone pouvait contrôler l'accumulation plaquettaire et la diminution des plaquettes circulantes. Notons que si les agrégats plaquettaires persistent depuis trop longtemps, ce processus ne sera plus inversé par la thérapie.

In vitro platelet aggregation induced by collagen, antigen antibody complexes and gamma globulin coated surfaced can be inhibited by anti-inflammatory drugs such as phenylbutazone, sulfinpyrazone (7) and acetylsalicylic acid (2). In addition, drugs which are used to control rejection (hydrocortisone and 6-mercapto-purine) inhibit collagen induced platelet aggregation (6). In experiments with rabbits using extracorporeal shunts the anti-inflammatory drugs reduced the amount of deposit which formed on the gamma globulin coated surfaces of the shunts (1).

During rejection episodes in human renal allograft transplants the administration of phenylbutazone has been shown to control the process. The fall in the circulating platelet count and the accumulation of platelets in the transplanted kidney was reversed by this treatment and renal function was restored (6).

This evidence indicates that the most likely stimulus for the formation of platelet aggregates is platelet surface interaction involving either the collagen and basement membrane or antigen antibody complexes since both of these interactions are blocked by the anti-inflammatory compounds. The fact that platelet thrombi which have been established for at least an hour can be reversed by these drugs must mean that formation and breaking down of the platelet mass is occurring and that if one interferes with the process the balance can be shifted so that no longer does the platelet mass form in such a way that it is maintained. However, it seems likely that if the platelet aggregates are allowed to persist long enough for some degree of transformation with fibrin formation to occur that this process cannot be reversed by such therapy.

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MICROBIOLOGICAL ASPECTS OF HUMAN HEART ALLOGRAFT RECIPIENTS *

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Outre le rejet aigu, les complications infectieuses constituent la cause de mort la plus fréquente au cours des transplantations.

Notons que tous les transplantés cardiaques recevaient de l'Azathioprine, des corticostéroïdes, des globulines antilymphocytaires et, comme antibiotique, de la Méthiciline ou Céphalotin. En vue de prévenir les infections, de nombreux spécimens en provenance du nasopharynx, peau, sang, expectorations, urine et selles, furent prélevés pour examen bactériologique. Dans le cas de décès, l'examen microbiologique portait sur la recherche de bactéries, mycoses et virus du cœur et des poumons, et autres spécimens suspects. Sur les 14 transplantations, 6 malades décédèrent sans évidence de rejet, mais avec évidence d'infection bactérienne comme cause principale menant à la mort. Les micro-organismes qui paraissent avoir contribué de façon significative à l'infection sont surtout le Pseudomonas et le Serratia marcesens. Parmi les autres micro-organismes, notons

Other than rejection, increased susceptibility to infection is clearly recognized as the major problem in transplant recipients under immunosuppressive therapy. Death of the heart transplant recipients at St. Luke's Episcopal Hospital has been attributed to immune rejection of tissue in eight cases while six cases terminated primarily due to complications involving severe infections, mostly of a bacterial nature. The purpose of this report was to identify the types of infections and the organisms most prominent in the infections which occurred in the 16 heart transplant cases at St. Luke's Hospital.

MATERIALS AND METHODS

The heart transplantation techniques used in 16 cases at St. Luke's Episcopal Hospital have been described (1) as well as other aspects of the transplantations (2). Morphologic findings in 13 of the 16 cases also have been reported (3). All of the heart recipients received azothioprine, corticosteroids and antilymphocyte globulin for immunosuppressive therapy (2). Methicillin or cephalothin was routinely administered as prophylaxis for the control of staphylococci.

Bacteriological analyses were conducted preoperatively as part of the routine laboratory workup on each recipient. Specimens from the throat, nasopharynx, skin (axillary area), blood, sputum, urine and feces were generally received by the laboratory. Throat, nasopharyngeal and skin cultures were taken with swabs which were used for inoculation of plates of blood agar, phenylethyl alcohol (PEA) blood agar, eosin methylene blue (EMB) agar, chocolate agar and thioglycollate broth. For blood culture 5 ml specimens of blood were obtained by aseptic venipuncture and introduced into blood culture bottles containing culture broth under vacuum and with CO_2 (Thiol, Difco). Sputum specimens were inoculated onto plates of blood agar, PEA agar, chocolate agar, EMB agar and into tubes

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Le listeria monocytogenes, le Streptocoque bêta-hémolytique, le Proteus, l'Escherichia, le Klebsiella et l'Entérobacter. Le virus de l'Herpès était le plus souvent responsable des infections de la bouche, des lèvres et de la face. Dans la plupart des cas, on a également isolé du Candida albicans à partir de cultures de la bouche, mais également à partir d'urine et de selles.

De nombreux facteurs contribuent à ces infections ; ce sont notamment l'état du malade au moment de l'envahissement par les micro-organismes, les facteurs environnants, la susceptibilité de l'organisme aux drogues, l'intensité et le type de thérapie immunosuppressive et la nature de la flore normale. Il faut noter également que la plupart de ces malades présentent un état de décompensation cardiaque latente au moment de la transplantation, ceci étant une cause prédisposante aux infections pulmonaires sous forme de pneumonie ou bronchopneumonie.

of thioglycollate broth. Urine cultures were prepared by the inoculation of blood agar, PEA agar, EMB agar and thioglycollate broth. The bacterial content of urine was estimated by the colony count method using a standard loop inoculum (0.001 ml) on Trypticase Soy (BBL) agar plates. Stool specimens were cultured on EMB agar, PEA agar, mannitol salt agar, *Salmonella-Shigella* (SS) agar, XLD (Difco) agar, and into tetrathionate and Hajna GN broth (Difco). Broth cultures were subcultured to SS agar after 18 to 24 hours of incubation. All cultures were incubated at 37° C and examined after 18 to 24 hours and at daily intervals thereafter when necessary. Blood cultures were incubated for 14 days before discarding as negative.

The antibiotic susceptibility of bacterial isolates was routinely determined by the paper disc method using high concentration discs only. Columbia blood agar was used for Gram-positive isolates and Sensitivity test agar (BBL) or EMB agar was used for Gram-negative isolates. The laboratory ordinarily employed separate agar plates for each group of discs containing antibiotics effective primarily against Gram-positive organisms, Gram-negative organisms and those discs containing antibiotics with broad spectra. The tube dilution method was also employed for quantitative antibiotic susceptibility determinations in selected cases.

Identification was made of all bacteria isolated except those from stool cultures which were screened for enteric pathogens and *Staphylococcus aureus*. After the initial work-up subsequent specimens were received by the laboratory periodically and upon evidence of infection. All postmortem examinations included bacteriological, mycological and virological studies of the heart and lungs, and bacteriological analysis of the heart blood and any other specimens as indicated.

RESULTS AND DISCUSSION

Examination of survival times of the heart recipients (Table I) shows part of the effect of infection upon the survival of the recipients. The mean survival time of 14 heart recipients which have expired was 90 days while five recipients have survived for 95 and 213 days at the time of this presentation. There was evidence of rejection in eight recipients whose mean survival time was 135 days. The other six recipients which have expired, survived a mean 31 days and expired without significant evidence of rejection but with evidence of bacterial infection as a principal cause of conditions leading to death. Infections of recipients whose demise was not directly related to rejection (3) are not reflected by the information in Table I.

On the basis of repeated isolation by culture and by association with clinical evidence of infection,

certain microorganisms were identified as predominating agents of infection in most of the transplant recipients. These organisms are listed according to case No. in Table II. In 12 of the recipients Gram-negative bacilli were the cause of severe or fatal infections and *Pseudomonas sp.* were the organisms responsible in seven of these cases. *Serratia marcescens,* although not always predominant, was a persistent isolate. This organism has considerable capability to develop resistance to antibiotics. Both pigmented and non-pigmented forms were isolated.

TABLE I

Survival time (days) of heart recipients at St. Luke's Episcopal Hospital

RECIPIENT (Case N°.)	SURVIVAL TIME OF CASES TERMINATING WITH REJECTION AS A PRIMARY CAUSE OF DEATH	SURVIVAL TIME OF CASE TERMINATING WITH INFECTION AS A PRIMARY CAUSE OF DEATH
1	204	
2		2
3		7
4	146	
5	149	
6	267	
7	170	
8		56
9		68
10	126	
11	6	
12		47
13	(living)	
14	13	
15	(living)	
16		4

In Case 2 there was consolidation of lungs due to pneumonia *Klebsiella Enterobacter* and *Escherichia* were cultured from tracheal secretions and from specimens taken at autopsy.

Case 3 had pneumonia and *Pseudomonas* was isolated from lung tissue at autopsy.

Case 9 had *Pseudomonas* septicemia two days before death and the same organism was isolated from postmortem heart blood and from abscesses in the right lung. Morphologically cytomegalovirus was evident in each lung and the pancreas and the patient had *Herpesvirus* infection of the mouth and face.

Case 16 initially had an orthotopic cardiac prosthesis. Approximately two and one-half days later a cardiac allograft was substituted for the device and the patient expired about 36 hours later. The patient produced sputum during life from which a pure culture of *Pseudomonas* was isolated. After death from bilateral pneumonia and renal failure, *Pseudomonas* was the only organism isolated at autopsy and was recovered from cultures of both lungs.

Case 8 died from septicemia, bronchopneumonia and gastrointestinal hemorrhage. The septicemia and bronchopneumonia evidently were caused by *Serratia marcescens.* The organism was predominant in numerous bacterial cultures during life and was isolated from specimens of postmortem blood, trachea, kidney and liver. The organism apparently made it's entrance into the body through

TABLE II

Predominanting bacteria isolated from infections in heart recipients at St. Luke's Episcopal Hospital

ORGANISM	RECIPIENT (Case N°.)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Pseudomonas sp.	+		+			+			+	+				+		+
Serratia marcescens								+				+				
Escherichia coli		+														
Proteus sp.						+			+							
Streptococcus sp.				+	+							+				
Listeria monocytogenes								+								
Klebsiella-Enterobacter		+														

an inguinal wound which resulted from a cut-down. *Serratia* was cultured on several occasions from this site. *Herpesvirus* infection of the lips and face was also observed. *Herpesvirus* was not detected elsewhere in the body.

Serratia marcescens was predominant in the sputum of Case 12 prior to death from pneumonia but no microorganisms were isolated from postmortem examination.

In the cases with evidence of cardiac allograft rejection, severe infections were also seen. Case 7 developed septicemia due to *Listeria monocytogenes* and a β-hemolytic *Streptococcus*. The former organism was also cultured from the meninges at autopsy and evidence of meningo-encephalitis was observed.

Case 14 had *Pseudomonas* bacteremia and the organism was isolated from postmortem heart blood and from the right lung. Four heart recipients developed bacteremia and in each case the condition developed shortly before death (Table III).

Other bacterial infections were observed on numerous occasions but their courses were either stopped or subdued by therapeutic measures. Table IV contains a list of all the microorganisms which appeared to contribute significantly to infection of

TABLE III

Incidence of bacteremia in heart recipients at St. Luke's Episcopal Hospital, causative organism and time developed *

RECIPIENT (Case N°.)	*Pseudomonas*	*Serratia*	*Streptococcus*	*Proteus*	*Listeria*
7			168/170		166/170
8		46/56			
9	66/68		68/68		
14	11/63				

* Number (11/13 etc.) indicate day in patients life after heart transplantation.

TABLE IV

Organisms appearing to contribute to infection in heart transplant patients at St. Luke's Episcopal Hospital and source

SPUTUM	URINE	BLOOD	WOUNDS
Streptococcus faecalis	*Proteus sp.*	*Clostridium perfringens*	*Pseudomonas sp.*
Pseudomonas sp.	*Pseudomonas sp.*	*Staphylococcus aureus*	*Serratia marcescens*
Proteus sp.	*Enterobacter sp.*	Beta-hemolytic *Streptococcus*	*Proteus sp.*
Serratia marcescens		*Listeria monocytogenes*	*Straphylococcus epidermidis*
Escherichia gr.		*Proteus sp.*	
Klebsiella-Enterobacter gr.		*Pseudomonas sp.*	

Cytomegalovirus * — lung and pancreas (Case 9).
Herpesvirus hominis * — penis (Case 5) ; face and buccal cavity (Cases 8 and 9).

* Virus cultures were done by the Department of Virology and Epidemiology, Baylor University College of Medicine.

heart recipients after surgery, and the source of the specimen from which the organism was isolated. The bacterial isolates are skewed somewhat toward the Gram-negative side and this probably reflects the prophylactic use of therapy geared toward the control of resistant Gram-positive organisms, viz., *Staphylococcus aureus.*

Candida sp. was isolated from nearly all of the transplant recipients at sometime, usually from the mouth but also on two or three occasions from urine and stool specimens. Only minor oral lesions were attributed to this organisms, and no other fungi were cultured from the patients nor observed in pathology specimens.

In retrospect, certain microorganisms have assumed a prominent role in infection of the immunosuppressed heart transplant recipients observed at this hospital. It is apparent that certain of the accepted "normal flora" may assume strong roles as opportunists under conditions likely encountered in these patients. Many factors contributed to the nature and circumstances of each individual infection reported. Those factors probably included (1) condition of the patient at the time of tissue invasion by a microorganism, (2) various environmental factors, (3) drug susceptibility of the organisms, (4) intensity and type of immunosuppressive therapy, and (5) nature of pathogenic and opportunistic microflora present at a given time. It should also be pointed out that many of these patients were in a state of severe congestive heart failure prior to transplantation. The increased susceptibility to pneumonia which exists is also carried over as a problem after surgery and bacterial pneumonia constituted a major factor in the survival of these patients after transplantation.

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COMPTE RENDU DE LA TROISIÈME RÉUNION PLÉNIÈRE — PROCEEDINGS OF THE THIRD GENERAL SESSION :

— Etat clinique des survivants à court et à long terme ;
— Le rejet : pathogenèse, prévention et traitement ;
— Anatomie pathologique de la transplantation du cœur

Président : Charles DUBOST, Paris.
Rapporteurs : Charles DUBOST, Paris, et Lucien CAMPEAU, Montréal ;
James MOWBRAY, Londres, et Gilles LAMOUREUX, Montréal
Richard LOWER, Richmond, U.S.A., et Pierre SIMARD, Montréal.
Rédacteur : Gilles LEPAGE.

Docteur Dubost:

Successivement dans cette session, des remarques ont été faites sur trois points particuliers. D'abord l'état clinique des survivants à long terme que je résumerai, ensuite la pathogénie, la prévention et le traitement du rejet qui seront rassemblés par le docteur Mowbray, et enfin l'anatomopathologie de la transplantation du cœur qui sera rapportée par le docteur Lower.

Je crois que nous pouvons dire actuellement qu'il y a environ une vingtaine de survivants à long terme. Je dis bien environ, parce que nous n'avons pas pu obtenir le chiffre exact des survivants de plus de six mois. Parmi ces vingt survivants à plus de six mois, seulement dix d'entre eux approximativement ont été présentés à la session de cet après-midi. Néanmoins, il y a dans ces dix années une unité suffisante dans de nombreux domaines pour que l'on puisse faire le point de la question et envisager successivement les différentes rubriques qui sont intéressantes à plus d'un titre tant pour reconnaître un rejet incipiens que pour le traiter et, partant, pour prolonger la survie à long terme de ces opérés, qui demeure, en fin de compte, le but principal de nos efforts.

Nous envisagerons donc successivement et rapidement devant vous, en les résumant, les différentes données fournies par les orateurs qui ont pris la parole cet après-midi. D'abord, sur le plan de l'état général, on peut dire que dans l'ensemble, malgré les incidents de parcours qui ont été présentés, l'état général de ces opérés est satisfaisant. Nous connaissons ces malades transplantés qui peuvent actuellement mener une vie normale ou voisine de la normale, qui sont actifs soit sur le plan physique pur, soit sur le plan intellectuel et physique, soit sur le plan intellectuel seulement. Il y a donc là dès maintenant une certitude pour tous que la survie à long terme est un gain considérable pour ces malades, et je cite comme exemple l'évolution de Blaiberg, du Père Boulogne et de bien d'autres présentés cet après-midi. Sur le plan psycho-émotionnel, et c'est un point qui n'a pas été débattu, il y a eu l'affirmation que certains de ces transplantés présentaient au début des troubles psychiques qui pouvaient interférer plus ou moins gravement sur la qualité du résultat général obtenu. Il n'a été fait mention cet après-midi d'aucun trouble psychique tardif chez aucun des survivants à long terme.

Par contre, et c'est une notion extrêmement importante qui a dominé le débat de cet après-midi, la notion de trouble du rythme doit servir de substratum au diagnostic du rejet et à son traitement rapide. Il y a lieu de dissocier, croyons-nous, deux catégories de troubles: ceux qui concernent le rythme cardiaque lui-même et la conduction, et, d'autre part, ceux qui sont reliés à la repolarisation. Les deux premiers sont, à notre avis, des signes absolument formels de rejet incipiens et doivent être traités comme tels. Dans notre expérience avec Cachera, nous avons au moins une fois interprété une modeste et transitoire bradycardie sinusale comme un signe possible de rejet et traité le malade comme s'il faisait réellement un rejet manifeste.

Nous nous en sommes bien trouvés et par la suite, à deux reprises, nous avons interprété des troubles plus importants du rythme et de la conduction comme étant des signes de rejet, et nous pensons que, actuellement, nous pouvons affirmer ce point avec certitude. Par contre, l'interprétation de troubles de la repolarisation peut être plus délicate, car nous avons entendu cet après-midi plusieurs orateurs faire état de modifications de l'onde T qui, négative au début, se repositivait ensuite. Dans notre expérience personnelle, nous avons un cas où l'onde T est demeurée négative depuis le jour même de la transplantation jusqu'après six mois sans qu'aucune altération d'aucune sorte sur le plan clinique, sur le plan enzymatique, biologique ou autre n'apparaisse. Ce malade, comme l'a signalé Cachera, n'a montré aucun signe évident de rejet. Il y a donc là, croyons-nous, une différence importante à formuler entre ces troubles du rythme et ces troubles de la conduction signant en quelque sorte le rejet et les troubles de la repolarisation, d'interprétation plus difficile.

Il y a un autre signe qui a été mentionné à deux reprises cet après-midi, c'est l'apparition d'un souffle systolique. Ce souffle systolique de la pointe est apparu chez deux malades dans des conditions diverses. Dans un de nos malades, il est apparu très précocement après la transplantation cardiaque, et nous l'avons interprété comme un souffle d'insuffisance mitrale vraisemblable, encore que ce diagnostic n'ait pu être longtemps retenu, car le souffle a secondairement disparu. Néanmoins, relié à l'existence sur l'électrocardiogramme d'ondes T inversées, peut-être y a-t-il quelque chose à chercher dans ce domaine et à préciser pour l'avenir. Dans une autre observation, ce souffle systolique est apparu tardivement au 17e ou 18e mois et il est alors, pensons-nous, la signature d'une insuffisance mitrale fonctionnelle qui peut être la traduction d'une grande insuffisance cardiaque.

En ce qui concerne l'insuffisance cardiaque susceptible d'être présentée par les transplantés après six mois, il y a eu, jusqu'à présent, relativement peu de cas qui se soient prêtés à l'administration de drogues tonicardiaques ou diurétiques. À part une ou deux observations, nous n'avons pas noté qu'il y ait dans l'évolution à long terme des opérés de phénomènes importants d'insuffisance cardiaque. Il faut bien reconnaître, cependant, que cette insuffisance cardiaque tardive apparaît à la suite de plusieurs poussées de rejet et n'est en quelque sorte que la traduction de lésions myocardiaques successives.

Un autre problème envisagé est celui de l'altération du système vasculaire coronarien, et il est surprenant de voir, à la lumière des pièces qui ont été présentées, illustrant des oblitérations coronariennes importantes, que les signes habituels d'insuffisance coronarienne soient absents.

La capacité fonctionnelle des malades transplantés est, dans l'ensemble, pour ceux qui vont bien, tout à fait excellente, non seulement la capacité intellectuelle mais aussi la capacité physique, et il y a là un élément de satisfaction tout particulier. Les études hémodynamiques qui ont été présentées par de nombreux orateurs confirment ce fait. Cependant, le débit cardiaque et les autres données du cathétérisme se sont détériorés chaque fois que la condition cardiaque s'était aggravée. Il y a un nombre important d'investigations qui ont été proposées, les unes relativement compliquées, peut-être même dangereuses à certains égards, tels l'angiocardiographie et le cathétérisme cardiaque répétés. Ces techniques peuvent être une source d'infection chez ces malades soumis à un traitement immunodépressif important. C'est la raison pour laquelle nous pensons, quant à nous, que pour commencer tout au moins, des méthodes simples doivent être utilisées, je veux dire par là le carotidogramme par exemple, reflet tout à fait satisfaisant de la fonction du ventricule gauche. La mesure de la pression veineuse périphérique est le reflet très précis et fidèle de la capacité du cœur droit. Il y a intérêt, croyons-nous, à utiliser ces méthodes simples avant les autres, quitte à recommander des méthodes plus complexes lorsqu'une altération même modeste de ces éléments simples apparaîtrait. Je retiens la proposition de Dobell et de sa mesure simple du débit cardiaque, technique, qu'il pratique deux ou trois fois par semaine sans difficulté et sans risque, et qui

donnera certainement des précisions importantes pour l'avenir. Nous recommandons, quant à nous, l'utilisation du cathéter flottant qui est utilisé très largement en Europe et qui permet, au lit du malade, de donner des précisions très importantes sur la qualité du débit du cœur droit. Il est bien évident que l'augmentation de la pression veineuse et que l'augmentation de la pression télédiastolique du ventricule droit doivent constituer des éléments de pronostic très fâcheux. Un détail très intéressant nous a été fourni par le docteur Leachman qui a noté chez un de ses malades que la pression pulmonaire qui était élevée avant la transplantation est demeurée élevée pendant plusieurs mois sans que l'on ait jamais observé de baisse.

Il a été question de réinnervation du cœur transplanté sans qu'aucune des observations publiées n'ait pu apporter la preuve d'une telle réinnervation. L'expérience animale de Lower, de Shumway, de Cachera et d'autres ont montré que dans certains cas, rares il est vrai, le cœur transplanté était réinnervé secondairement. Mais jusqu'à présent, nous n'avons pas, dans l'expérience clinique, enregistré de réinnervation de la masse cardiaque.

Quel est l'effet du temps sur l'hémodynamique et la capacité fonctionnelle? Eh bien! il est vrai qu'il est évident que dans la mesure où aucun phénomène de rejet ne se fait jour, la capacité fonctionnelle et l'hémodynamique demeurent absolument parfaites. Nous avons pour preuve, quant à nous, les mesures isotopiques du débit cardiaque que nous avons pratiquées sur le malade qui n'a pas présenté de phénomène de rejet et qui est en tout point satisfaisant. D'un autre côté, il va sans dire que les atteintes successives du myocarde, à la suite de phénomènes de rejet répétés, constituent autant de raisons pour que cliniquement et hémodynamiquement les possibilités d'éjection des ventricules diminuent. Il ne semble pas nécessaire d'administrer aux malades une médication de support, digitaline ou diurétique, lorsque le malade va bien, lorsqu'il n'a pas présenté de séquelle de rejet. En tout cas, c'est l'expérience que nous avons dans notre équipe, où nos deux survivants à long terme n'ont jamais reçu ni digitaline ni diurétique.

Il y a une question importante qui a été soulevée par le docteur Fontan et par d'autres orateurs, c'est celle de l'intérêt d'utiliser les anticoagulants, qui ont semble-t-il, du moins dans le cas présenté par Fontan, apporté une modification radicale et dramatique des troubles du rythme présentés par ces opérés. Il y a là une notion intéressante à reconnaître qui peut être mise en pratique sur une plus grande échelle si l'on veut se rappeler la fréquence des oblitérations coronariennes qui ont été présentées. Il est intéressant enfin de s'interroger sur le fonctionnement des différents organes annexes, pourrait-on dire, à la transplantation cardiaque, je veux dire du foie, des poumons, des reins, et à cet égard plusieurs précisions intéressantes ont été apportées. Le foie, après la transplantation, reprend son volume normal. Mais il peut être altéré par l'administration de doses importantes d'azathioprine, et l'on sait que la plupart des équipes ont été amenées à diminuer, à un moment ou un autre de l'évolution, l'administration de cette drogue toxique. Les fonctions respiratoires sont également intéressantes à connaître dans les suites opératoires et, à cet égard, le docteur Wilson nous a apporté des précisions très importantes sur ce point particulièrement intéressant. Les fonctions respiratoires de l'opéré qu'il nous a présenté sont, en effet, presque en tout point absolument normales.

Sur la durée et la nature de la dépendance hospitalière, il ne faut pas se dissimuler, et je crois que c'est la conclusion générale de tous les orateurs, que les transplantés du cœur demeurent dépendants de l'équipe chirurgicale et de l'équipe médicale qui les a entrepris. Il y a en effet comme un cordon ombilical, comme un fil téléphonique qui rejoint l'opéré et son équipe, et nous ne pensons pas que cette dépendance très étroite puisse être jamais abandonnée. Il y a peut-être une nécessité de simplifier les choses, mais aussi de les continuer envers et contre tout pendant une très longue période sinon pendant toute la durée de la survie du malade. À cet égard, on peut imaginer des moyens électroniques, par exemple la transmission des électrocardiogrammes tous les matins, voire un enregistrement cardiographique continu portatif qui pourrait souligner l'éventuelle

modification du tracé au fil des heures ou au fil des jours. En tout cas, nous pensons qu'il est très important que, au moins une fois tous les deux jours, un ensemble d'examens soit pratiqué sur le malade transplanté, si l'on veut saisir à temps un rejet incipiens et le traiter comme il convient de le faire.

Pour terminer, quelques mots sur les complications au long cours qui accompagnent le traitement et qui ont été longuement évoquées au cours de cette session. Les complications du sérum sont connues. Par contre, l'hépatotoxicité de l'Imuran doit être retenue. Il faut noter également les menaces d'infection, mais surtout les dangers de la cortisone, qui par l'ostéoporose qu'elle entraîne peut constituer une véritable maladie évolutive dont il convient de connaître la gravité et les conséquences.

Voici ce que nous avions très rapidement à résumer devant vous concernant la survie à long terme, à plus de six mois, d'une dizaine d'opérés. Nous n'avons pas lieu d'être entièrement satisfaits, mais si nous considérons que nous sommes seulement au début de la route et que ce que nous observons maintenant était à peine pensable il y a un an et demi, nous avons lieu de nous déclarer satisfaits d'avoir appris, dans cette session, un nombre très considérable de choses qui vont sans aucun doute nous permettre d'aller de l'avant, de simplifier les choses et, qui sait, de multiplier les succès.

Je vais donner la parole maintenant au docteur James Mowbray, qui va parler de la pathogénie, de la prévention et du traitement du rejet.

Doctor Mowbray:

I think we had quite a good afternoon, really, discussing the problems of rejection.

The major problems that really were not (and I do not think one could expect them to be) completely defined were the respective roles of cell-mediated immunity and cytotoxic antibodies in the induction of graft rejection. We also discussed the problem of transmission of disease and the role of anti-heart or other cross-reacting antibodies in producing lesions in cardiac transplants. Finally we have discussed the way for producing a better protection against immunization.

As much of our work this afternoon was to study the prevention of lesions, we obviously were concerned more with pathology than with long survivors, and for this reason much animal work was inevitably discussed. Doctor Lamoureux covered the field in terms of the methods in which sensitization of individuals with an allograft may occur. He presented the process by which the sensitizing antigens of the graft induce in the recipient cell-mediated and humoral immunity; two forms of immunity that might damage the organ. I introduced a concept of direct cytotoxic destruction of myocardial cells as a phenomenon in cardiac transplantation, and made the point which, I think, is clinically important that histological changes frequently occur before impairment of the function and that in the case of the classical vascular rejection episode, there may be changes before we detect them, by functional criteria or by any other sort of diagnostic criterias as we are only too well aware. This also applies to the cell-mediated rejection occurring usually in the first few days after transplantation.

Doctor Rappaport then presented a paper showing the relationship between the antigenecity of some cell membrane components and those of group "A" streptococci. I think this is of much importance in terms of transmission of disease, that is the original disease of a patient into a transplant. This could be as important in the heart with rheumatic carditis as it is in post-streptococcal glomerulo-nephritis. He demonstrated that antibodies to streptococcal antigens may produce severe lesions in organs but not in the organs of all individuals. He preferred, in some ways, to say that there was no real species specificity for this and that some individuals of many species can have their tissues damaged by perfusion into an organ of antibody directed against streptococcal components. Perhaps, this could be best represented in operational terms as saying that some people get glomerulo-nephritis and some do not; some get rheumatic carditis and some do not.

Doctor DeVeber covered the clinical management of rejection. What agents one could use, what

agents one does use that have grown out of an experience of treating rejection; not specifically the agents used to damp down all specific immune responses such as Azathioprine, Prednisone or anti-lymphocyte serum, but what to do with the rejection episode. I think probably the most important statement in this regard was that if you are doubtful of the diagnosis, treat it rather than wait. I think it is obvious to many of us that there have been occasions to treat as long as the treatment which is being used is not too poisonous; as long as it does not produce too big a risk as a result of treating something that is not a rejection episode, it is obviously safer to treat it than to get to the stage where you cannot treat an episode which almost invariably would kill the patient. He recommended giving the dosage as one dose a day rather than divided doses and I must say I agree. I think there is a lot to be said for putting all your dose in once in the day, if for no other reason than that you cannot change your mind later in the day. It also insures that the patients do get all of their dose. He discussed briefly the use of hydrocortisone as used by some groups rather than Prednisone or Prednisolone because of a feeling that, mole for mole, it was a little more effective. He recommended using the maximum tolerated dose of Azathioprine during rejection, in order that when the patients come out of the episode, they are well under control in terms of their baseline immunosuppression. He also suggested that Actynomycin might be used for the treatment of rejection episodes where other things were not terribly effective. He pointed out that the effects obtainable from Actynomycin often occur only three to four days after its administration. This may be a little late in the case of some of the more acute cardiac rejection episodes. He also discussed something very close to my heart, the use of other agents which might interfere with the consequences of immunity in rejection episodes; the use of other agents which might interfere with platelet agents such as Aspirin, Persantine, Phenylbutazone. He also mentioned Heparin, which was discussed considerably later as a possible prophylaxis against intravascular coagulation and platelet deposition. Admittedly, it is difficult to use it as a chronic medication if you require large doses for a long time. However, in the episode itself, it might conceivably be used, but so far as we were aware, nobody had tried a consistent study of this until the information that we heard today from one center. He also showed one example of what I think has become a fairly classical demonstration of the failure of cardiac transplantation; the dosage of Azathioprine had to be reduced in this patient and from that time on, things went from bad to worse, down the slippery slope which ultimately resulted in the demise of the patient who died of the complications of pneumonic infection from pneumocystis carinii and cytomgalo virus.

Doctor Stuart introduced a different note by comparing another relatively new immunosuppressive agent, Procarbozine, an Isoniazid analogue, which had originally been used as an anti-tumor agent. He showed that it was capable of producing considerably more depression of humoral antibody than was anti-lymphocyte serum in his hands, and yet both were capable of prolonging the survival of rabbit skin-grafts. I think this demonstrates a problem that we all have, and we would like to overcome, to knock-out the circulating antibody if we could, and Procarbozine is quite a good way of knocking it out. But it does have a lethal limit as do nearly all the cytotoxic immunosuppressive drugs.

Doctor Hans Selye produced a beautifully illustrated talk, as he always does, of the completely non-immunological mechanisms which may prevent damage to heart muscle in a pre-conditioned animal: these animals were pre-conditioned with the use of steroids and a high sodium intake and then stressed by restraint on a board for some hours. He was perhaps a little apologetic about putting this into an immunologically orientated session but I think the beautiful demonstration of the protective effects which he showed might perhaps be more widely undertaken as a study in other areas of cardiac surgery where myocardial damage can occur. Of course this sort of approach has not been used to see if you can interfere with the myocardial damage produced by immunity itself.

Doctor Goldman has shown, by immunofluorescence on the muscle itself, that a circulating anti-heart antibody, which maybe is cytotoxic and either a transplantation antibody or an anti-heart antiboby, was present very rapidly after transplantation. This is a very agreable confirmation of the phenomenon which has been so widely described with other sorts of organ transplants.

Another impressive piece of information came from Doctor Biorgilli who presented briefly the results of a relatively large series of dogs in which local irradiation was used over a period of a few weeks after transplantation. Seventy-five per cent of his dogs with cardiac allografts survived 90 days or more. The control animals showed complete destruction of the allograft in twelve days. I think this is something which might be close to the heart of Christiaan Barnard who is probably the only person to have tried local irradiation in cardiac transplantation. In the light of the experiments described today, he may have abandoned local irradiation a little too quickly.

We concluded that there might soon be better ways of interfering with the consequences of antibody, in particular by the use of anti-inflammatory agents; here again, the use of Heparin was discussed. Doctor Rappaport closed with a very nice demonstration of the possible reasons why tissue typing was not quite as efficient as some people would hope it to be. His demonstration suggested that not all patients get immunized quite as readily with the same antigens all the time, and hence, that to have a D match might not always be a bad thing if the patient did not make any antibody to the array of antigens represented by a D match. And on that note we concluded, having gotten much more agreement, I think, than it could have been said to be true at the end of the first meeting of this kind last year. Thank you.

Doctor Dubost:

Thank you, Doctor Mowbray. Doctor Richard Lower will now tell you what happened during the session on pathology of heart transplantation.

Doctor Lower:

Quite obviously, there was a good deal of overlap between the sessions, since our group not only reviewed the pathologic changes in acute and in chronic rejection, but also grappled somewhat with the problems of pathogenesis as well.

Doctor Milam presented observations made in seven of their patients, two of whom died of acute rejection, and five, of chronic rejection. He reviewed the typical findings of acute rejection which we have come to recognize quite well, including the extensive cellular infiltrate, not only in a perivascular location, but also in the interstitium. In addition, there is always extensive interstitial edema and hemorrhage secondary to the vascular destruction which occurs with acute rejection. From the standpoint of pathogenesis, it would appear that the endothelium is again the primary target for the immunologic process and other pathologic changes are secondary to the vascular damage.

It is not only the capillary and venular endothelium which is damaged, but the endothelium of the arterial vessels as well. This can be seen in acute rejection as a sub-intimal infiltration of mononuclear cells wherever the damage occurs. Where there is extensive small vessel necrosis, one may also see micro-infarcts and focal necrosis of the myocardium as well. Doctor Milam reminded us of Doctor Heggtveit's observations yesterday that all lesions which one sees in the transplanted donor heart may not necessarily be due to rejection but some may indeed be residual damage suffered before the transplant as the result of brain damage.

Doctor Simard presented the non-cardiac observations in a series of autopsies and emphasized the frequency of infections with uncommon organisms, and also the incidence of steroid ulcers and steroid myopathy. Again the general concensus is that the non-cardiac pathologic findings are in general related to the hazards of high dose steroid therapy. In addition he described three patients with rather extensive submucosal hemorrhage throughout the GI tract, the pathogenesis of which is not entirely clear.

Then we came to what is, in my opinion, the major problem, that of chronic rejection. When one reviews the sections of many patient dying beyond three months after transplantation, one is struck right between the eyes with the magnitude of this problem. We of course reviewed the observations that were made a couple of years ago in dogs in which we saw an alarming incidence of vascular lesions represented by a marked intimal proliferation, narrowing or even occluding the lumens of the arterial vessels. At that time we hoped that this was either a species problem occurring more often in dogs or that immunosuppression in humans might be more efficient, particularly with the use of ALG, and that we could avoid this complications. I think it's apparent with the very high incidence of this chronic rejection lesion seen in patients dying within a few months and sometimes within a few weeks after transplant, that this is still a tremendously important problem. The pathogenesis of both acute and chronic rejection was reviewed by Doctor Hume and by Doctor Harris. There seems to be some agreement that the sequence begins with injury to endothelium as the primary target organ, followed by platelet aggregation as a response to the injury, then formation of thrombus with organization and gradual encroachment on the lumen of the vessel. How to prevent this process is certainly not at all clear. Obviously one could stop it at the outset if we had perfect histocompatibility matching, thereby avoiding the endothelial injury to begin with. Since this is now rarely achieved, other approaches were suggested, some of which were just mentioned by Doctor Mowbray, including of all things Aspirin. Certainly this is the area which requires a great deal of our attention and needs some kind of solution if we are to achieve long term function of the cardiac allograft.

Docteur Dubost:

Merci, docteur Lower.

Nous avons vu cet après-midi tous les risques auxquels étaient soumis les transplantés du cœur, risques non seulement inhérents à la transplantation elle-même, mais également secondaires à la thérapeutique qui est instituée. Nous avons entendu les désordres coronariens objectivés sur l'animal mais qu'on retrouve déjà avec une fréquence inquiétante sur les spécimens d'autopsie des hommes. Nous avons entendu également que l'histocompatibilité n'était sans doute pas tout et qu'un très bon *tissue-typing* n'était peut-être pas la réponse totale à nos problèmes. Cela revient à dire que nous sommes encore dans l'ignorance d'un nombre très important de facteurs mais que ceux-ci, sans aucun doute, apparaîtront plus clairement dans les années qui viennent. On ne peut que souhaiter qu'un jour prochain une méthode biologique, chimique ou autre intervienne, qui permette soit à tout receveur d'accepter le cœur d'un donneur, soit à tout cœur de donneur d'être toléré par n'importe quel receveur. C'est le souhait sur lequel je vais terminer cette session non sans demander d'ailleurs s'il en est parmi vous qui désireraient obtenir des membres du panel quelques éclaircissements supplémentaires sur le chapitre de l'histocompatibilité, des lésions anatomo-pathologiques ou de l'évaluation clinique des résultats obtenus. Est-ce que quelqu'un demande la parole? Je rends donc la liberté aux membres de cet après-midi en les remerciant de leur assiduité bienveillante.

TWO-STAGED CARDIAC REPLACEMENT USING AN ORTHOTOPIC PROSTHESIS * †

Denton A. COOLEY, M.D., **Domingo LIOTTA**, M.D., **and Grady L. HALLMAN**, M.D.

Le manuscrit porte sur l'emploi d'une prothèse cardiaque orthotopique comme traitement palliatif temporaire, dans le cas où une allogreffe n'est pas disponible.

L'auteur donne une description détaillée de cette prothèse cardiaque consistant en deux ventricules et quatre valves, ceci donnant un système de pompe avec des caractéristiques similaires à celles du cœur humain. La pression dans le système est maintenue à 100 mm Hg pour le côté gauche, et à 50 mm Hg pour le côté droit, la pression négative pour les deux côtés variant entre — 10 et — 20 mm Hg. Le rythme optimal est de 80/minute. Ceci assure une pression artérielle constante d'environ 110/60 mm Hg, et une pression dans l'artère pulmonaire d'environ 50 mm Hg. Les pressions auriculaires sont maintenues entre 10 et 20 mm Hg.

L'auteur décrit l'histoire d'un malade dont le cœur n'a pu être ressuscité après résection myocardique et ventriculoplastie. Étant donné l'absence d'allogreffe immédiate, une prothèse cardiaque fut installée. Au

The sudden death of a potential recipient which results when an allograft is not available represents a major obstacle to successful application of cardiac transplantation (2 and 5). To meet this situation a technique of two-staged cardiac replacement was conceived, consisting of the initial insertion of an orthotopic cardiac prosthesis and subsequent replacement of the prosthesis with a cardiac allograft. In December, 1968, plans were begun for the fabrication of the prosthesis, which was first applied clinically on April 4, 1969, in a 47-year-old patient with extensive myocardial damage due to coronary atherosclerosis.

THE CARDIAC PROSTHESIS

A pneumatically controlled device consisting of two "ventricles" and four prosthetic valves was constructed to provide pumping characteristics similar to the human heart (Figure 1). Design and flow details have been reported elsewhere (6). The chambers were diaphragm-type reciprocating pumps made of Dacron polyester[1] embedded in Silastic[R 2] and were fabricated in three parts: body, dome, and diaphragms. The diaphragm was .045 inch thick and modified to resist stress and wear (10). A reticular Dacron fabric[3] designed to promote formation of an autologous blood interface lined the chambers. Four Wada-Cutter hingeless valves were chosen because of the central flow characteristics and their comparatively low incidence of thromboembolism (Figure 2). Woven Dacron arterial grafts 25 mm in diameter were sutured to the pulmonary artery and aorta. Atrial cuffs were fabricated of layers of Dacron fabric bonded with Silastic to prevent aspiration of air. Sealed to the gas chamber in the prosthesis, Silastic tubes (15 mm I.D.) covered with Dacron fabric were tunneled through

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† From the Texas Heart Institute of St. Luke's-Texas Children's Hospitals.

1. United States Catheter and Instrument Corp., Glens Falls, New York.

2. Dow Corning Corp., Midland, Michigan.

3. Developed in conjunction with Philadelphia College of Textile (Prof. T. Edman).

cours de la période postopératoire, le patient reçut de l'Imuran, des globulines antilymphocytaires et de la Prednisolone comme traitement préparatoire à une transplantation cardiaque. 64 heures après, une transplantation cardiaque fut entreprise avec succès. Notons que la leucocytose s'élevait à 1 700 seulement.

Malheureusement, 32 heures après cette deuxième intervention, le patient décéda par insuffisance respiratoire.

L'examen post-mortem révéla la présence de Pseudomonas aeruginosa au niveau du lobe inférieur du poumon droit et une bronchopneumonie bilatérale nécrosante et confluente. Il n'y avait aucune évidence de rejet de l'allogreffe. Les reins étaient grossièrement œdémaciés, avec des zones cicatricielles et de la nécrose tubulaire.

La prothèse cardiaque était libre de toute formation thrombotique, et l'examen microscopique démontrait la présence d'un dépôt de fibrine.

the chest wall of the patient and were connected to the external energizing unit. The prosthesis was activated by pressure and vacuum generated by two pneumatic power units (Figure 3). Carbon dioxide was used as the transmitting gas, and the solenoid valve was controlled by rate, systolic duration and byphasic pressures in the pulse timer unit.

The pressure in the energizing system of this pump was kept around 110 mm Hg for the left side and 50 mm Hg for the right side; the negative pressure for both sides varied between –10 and –20 mm Hg. The optimal pulse rate was 80/min; the systolic duration, 290 milliseconds on the left, and 350 milliseconds on the right. These values made possible a stable arterial pressure (about 110/60 mm Hg) and a pulmonary artery pressure of about 50 mm Hg. Both atrial pressures were maintained at a mean of 10 to 20 mm Hg.

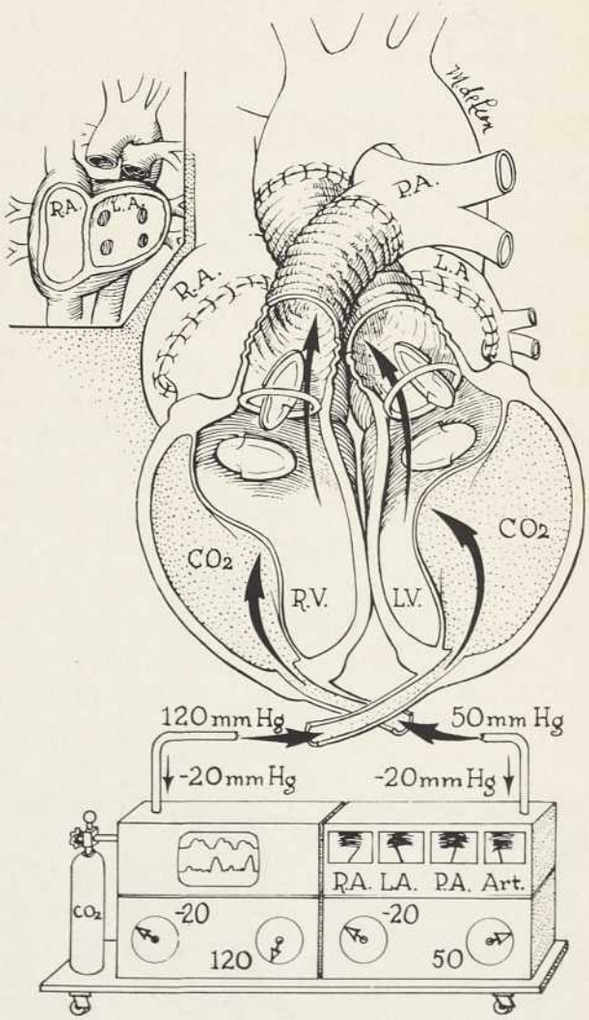

Figure 1 — Diagram of total cardiac prosthesis, showing technique of implantation and method of activating the reciprocating pump units with an extracorporeal pneumatic system.

CASE REPORT

A 47-year-old man was admitted to The Texas Heart Institute on March 5, 1969, with advanced coronary arterial occlusive disease and complete heart block. He had a history of several myocardial infarctions and had been hospitalized frequently for arrhythmias, congestive failure, and acute myocardial ischemia. In May, 1968, a demand pacemaker had been inserted following three episodes of Stokes-Adams syncope.

Le matériel de Dacron utilisé pour la surface interne de la prothèse ne nécessita pas l'emploi d'anticoagulants. Le taux des plaquettes était satisfaisant, et on ne rencontra aucun saignement postopératoire significatif.

L'hémolyse élevée notée après l'intervention était probablement en relation avec l'oxygénateur à bulles, ou peut-être en relation avec la prothèse jusqu'au moment où la surface interne était recouverte d'une couche de fibrine, de plaquettes et de cellules sanguines. Ceci peut être également le facteur responsable du développement de la leucopénie, bien que l'insuffisance de la moelle osseuse démontrée résultait du traitement à l'Azathioprine administrée à doses élevées. Ce taux d'hémoglobine plasmatique élevé, associé à la vasoconstriction générale, est probablement la cause de l'insuffisance rénale. Notons que l'on n'a observé aucun foyer hémorragique ou nécrotique au niveau du cerveau ou autres organes vitaux, suite à l'emploi de cette prothèse.

The patient was considered Functional Class III bordering on IV (New York Heart Association Classification). He had orthopnea and mild dyspnea at rest. Blood pressure was 110/80 mm Hg and pulse was regular at 72 beats per minute synchronous with the pacemaker, which was palpable in the right pectoral region. Heart was moderately enlarged (Figure 4), and crepitant pulmonary rales were noted over the lower lobes of the lungs.

Electrocardiogram showed complete atrioventricular block with activation of the ventricle by the pacemaker impulse. Urinalysis, blood urea nitrogen, and other blood chemistries were normal. Blood type was O Rh positive. On catheterization, left ventricular end-diastolic pressure was elevated to 25 mm Hg, pulmonary artery pressure to 45/19, and the left ventriculogram revealed paradoxic expansion during systole with markedly delayed emptying of the left ventricle. Severe narrowing of the right coronary artery four centimeters beyond its origin and complete occlusion of the anterior descending and left circumflex arteries were revealed on the arteriogram.

Although cardiac transplantation was indicated, it was opposed by the patient who preferred a myocardial resection with ventriculoplasty.

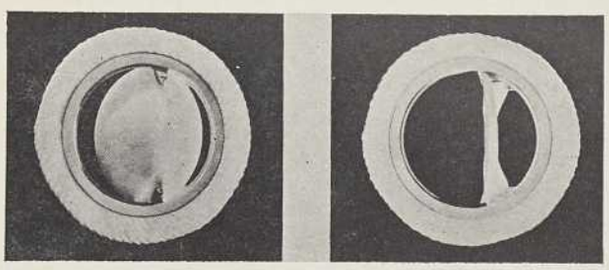

Figure 2 — Photograph of the hingeless Wada-Cutter valve prosthesis. Four such valves were incorporated in the cardiac prosthesis.

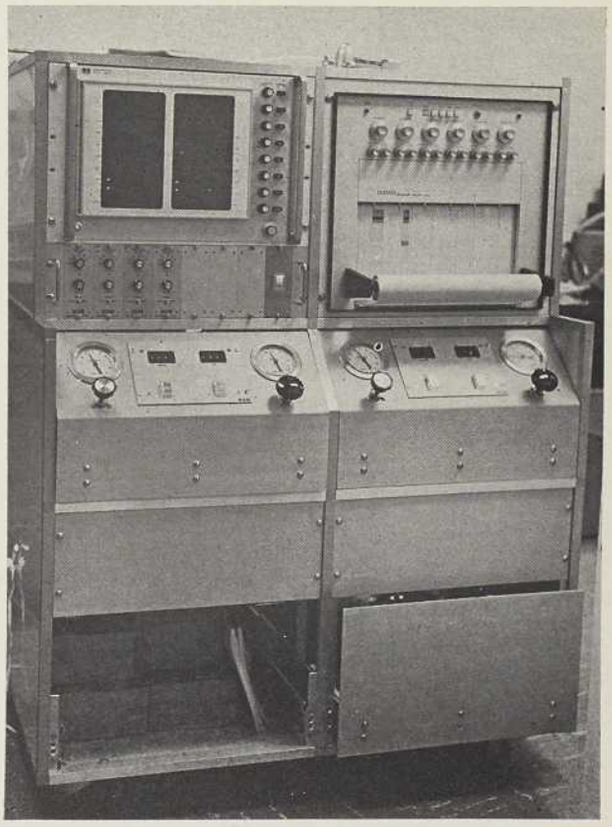

Figure 3 — Photograph of control console and manometers which activated the prosthesis.

First surgical procedure:

On April 4, 1969, a median sternotomy incision was made under general anesthesia, cannulations were made for cardiopulmonary bypass (using a plastic disposable oxygenator primed with five per cent dextrose in water) and the patient was maintained normothermic (5). Heparin 3,0 mg per kilogram body weight was administered. Over two-thirds of the left ventricular myocardium and almost the entire septum were covered by scar tissue; and after excision of the involved tissue and suture approximation of the edges, the heart could not be resuscitated. Cardiopulmonary bypass was continued and the heart removed by the same method used in preparation for cardiac allografting (4 and 5). The prosthesis was inserted with continuous suture anastomoses of the left atrium, right atrium, main pulmonary artery, and ascending aorta in sequential order. The CO_2 energizing tubes were directed through the thoracic cage and connected to the extracorporeal console control. Upon release of the venae cava and ascending aorta, and activation of the prosthesis, cardiopulmonary bypass was discontinued and the caval cannulae removed. Immediately afterwards, protamine sulfate 4.5 mg per kilogram body weight was administered. Before closure of the thoracic incision, a 24 mm electromagnetic flowmeter probe was placed around the Dacron pulmonary artery and measured the output of the right ventricle as 4.5 liters/minute.

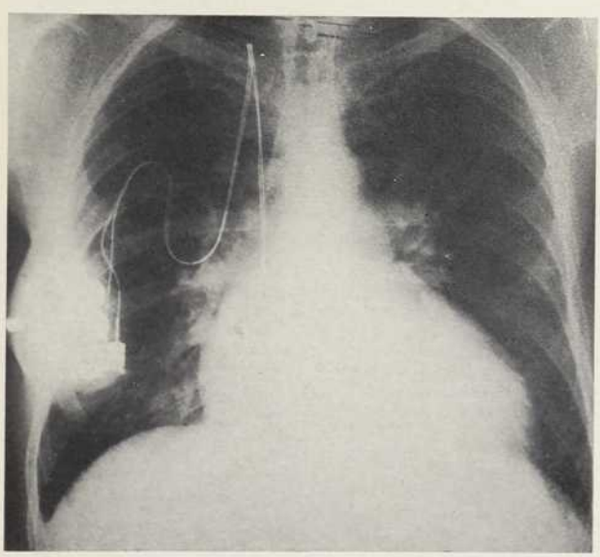

Figure 4 — Roentgenogram of the chest before operation in 47-year-old male, showing cardiomegaly involving mostly the left ventricle and intracardiac pacemaker with battery unit located subcutaneously in the right pectoral fold.

Postoperative course:

At the end of cardiopulmonary bypass the urine was blood-stained. Plasma hemoglobin shortly after the operation was 300 mg per cent, but it decreased during the 64 hours that the prosthesis supported the patient's circulation and was 32 mg per cent just before cardiac allografting (Table I). Within fifteen minutes after operation the patient was mentally alert and able to move all extremities. Urinary output during the postoperative period decreased gradually. During this interim a total of 500 mg of azathioprine (Imuran^R) was administered in conjunction with prednisolone and antilymphocytic globulin, as part of an immunosuppressive program carried out in anticipation of performing cardiac transplantation when a donor heart arrived. Leukopenia and moderate thrombocytopenia appeared on April 6, 1969 (Table I), and on the day before transplantation the leukocyte count was 1,700. There were no signs of pulmonary or surgical infection (Figure 5).

Cardiac transplantation was scheduled on April 7, 1969, following admission of a donor patient with irreversible brain damage. Tissue typing and matching established histocompatibility between donor and recipient before operation.

Second surgical procedure:

The sternotomy incision was reopened and cardiac output measured with electromagnetic flowmeter demonstrated 5.21 liters/minute. Cardiopulmonary bypass was instituted with cannulation of the inferior vena cava through the right common femoral vein and the superior vena cava through the cuff of the prosthetic right atrium. Return of oxygenated blood was through the right common femoral artery. When the energizing console was

turned off and the prosthesis removed, cardiac allografting proceeded in the customary manner (5). Cardiac action resumed in ventricular fibrillation which converted to sinus rhythm by a direct current countershock. With the aid of isoproterenol (Isuprel^{R}), satisfactory function of the allograft was established, and heparin effect was counteracted with protamine sulfate.

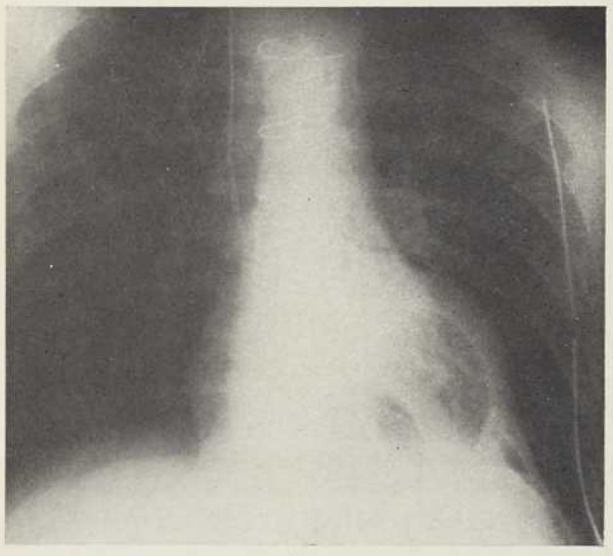

Figure 5 — Roentgenogram of the same patient 36 hours after insertion of prosthesis, revealing unusually normal cardiac contour with double gas shadow indicating that the pumps were in systolic phase.

Postoperative course:

Within one hour of operation the patient regained consciousness. Despite low urinary output, no serious azotemia or hyperkalemia was evident. A roentgenographic finding of condensation in the right lower lobe increased over the ensuing 32 hours until it filled that entire lung field, at which time respiratory failure caused the patient's death.

At autopsy pure culture of *Pseudomonas aeruginosa* was isolated on cut section of the lower lobe of the right lung, and necrotizing and confluent bilateral bronchopneumonia was found on microscopic examination. There was no evidence of allograft rejection. The kidneys were grossly edematous with scattered areas of tubular necrosis.

A smooth glistening neoendocardium was observed in the pump's lumen. Both chambers and all valves of the cardiac prosthesis were free of

TABLE I

Function of orthotopic cardiac prosthesis

DATE/TIME	HCT (percent)	WBC (per mm^3)	PLATELETS (mg%)	PLASMA Hgb (mg%)	BUN (mgm%)	CARDIAC OUTPUT
4-4-69/8:00 a.m.	36			—	26	
4:00 p.m.	Cardiac prosthesis functioning					4.5 L/min. *
6:00 p.m.	35	14 100	79 000	300	32	
4-5-69/6:00 a.m.	35	10 700	122 000	209	32	1.95 L/min. **
6:00 a.m.	35	9 200	77 000	146	35	2.94 L/min. ***
4-6-69/6:00 a.m.	36	9 900	66 000	52	30	
6:00 p.m.	35	2 700	55 000	32	60	
4-7-69/6.00 a.m.	35	3 200	60 000	—		
						5.2 L/min. ***
8:00 a.m.	Cardiac prosthesis discontinued					
	36	5 400		65		
6:00 p.m.	38	2 850	70 000	130	70	
4-8-69/6:00 a.m.	40	4 600		87	65	
2:00 p.m.	Patient expired					

*** Chest open, immediately after implantation of cardiac prosthesis. Determined by electromagnetic flowmeter.**

**** Start Arfonad drip.**

***** Change directly related to the use of Arfonad.**

thrombus formation (Figure 6). Microscopic examination showed fibrin deposition with enmeshed erythrocytes between the Dacron fibers and a uniform, smooth fibrin layer (Figure 7). The complete post mortem examination has been reported previously (6 and 7).

DISCUSSION

Kolff *et al.* (1, 8 and 11) have made extensive experiments in total heart replacement using an orthotopic cardiac prosthesis in dogs, calves, and sheep. The first clinical application of an implantable blood pump was reported by Liotta *et al.* (9) who performed a partial functional replacement of the human left ventricle. Total heart replacement by a cardiac prosthesis in a two-staged technique has been demonstrated to have important clinical value (7).

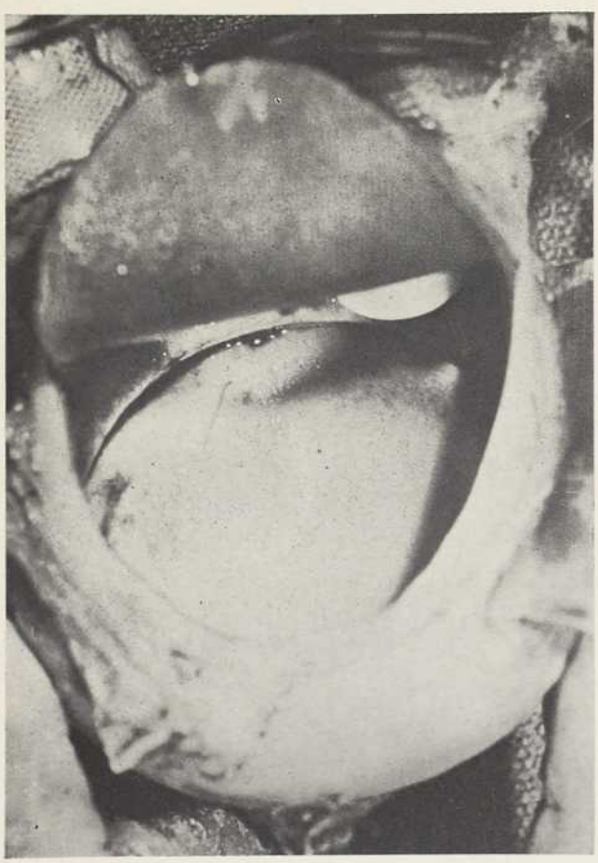

Figure 6 — Macroscopic photograph of prosthesis after removal, showing valve entirely free of thrombus. Both pumping chambers and all valves were free of thrombus.

The reticular Dacron material used for inner lining of the prosthesis was effective in promoting an autologous blood interface, and no anticoagulants were required during function of the prosthesis. Platelet count was satisfactory. No significant postoperative bleeding occurred.

Since the plasma hemoglobin was initially elevated, the hemolysis which developed was probably due to the bubble oxygenator, or perhaps was related to the prosthesis in that it persisted until the inner lining was covered by a layer of fibrin, platelets, and blood cells. This also may have been a factor in the development of leukopenia, although the marrow depression evidenced on biopsy likely resulted from the azathioprine which was administered in more than 7 mg per kilogram body weight during early circulatory support. The originally high plasma hemoglobin in conjunction with severe generalized vasoconstriction caused terminal renal failure, which was related to the tubular necrosis found at autopsy. The successful performance of the prosthesis was further borne out by the absence of hemorrhagic or necrotic foci in the brain or other vital organs.

SUMMARY

The two-staged approach to cardiac transplantation has special significance for the urgently ill cardiac patient who faces death on the operating

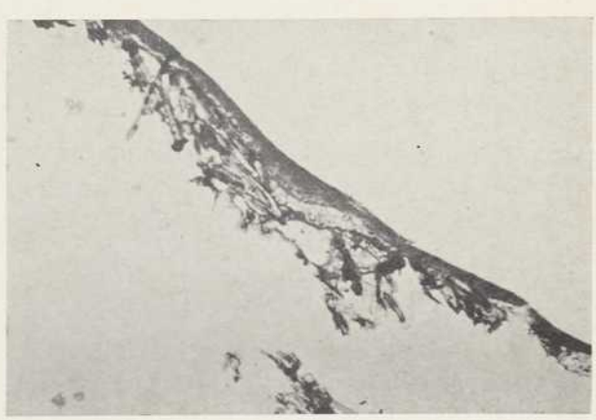

Figure 7 — Microscopic photograph of fabric lining the body of the prosthesic pump, showing a smooth internal lining with fibrin enmeshed in the fibers of Dacron.

table when a donor heart is not available for immediate allografting. An orthotopic cardiac prosthesis, consisting of two reciprocating pumps constructed entirely of synthetic materials, was inserted in a 47-year-old patient and was activated pneumatically by a control console connected by tubes passed through the chest wall. The patient's circulation was supported by the prosthesis for a period of 64 hours when a donor heart was obtained and allografting performed. Thirty-two hours later death occurred from *Pseudomonas pneumonia*.

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THE ROLE OF SOLUBLE TRANSPLANTATION ANTIGENS IN THE INDUCTION OF ORGAN TRANSPLANT TOLERANCE *

Sidney LESKOWITZ, Ph.D.

Dans l'immunologie, le cœur du problème réside dans la recherche de méthodes produisant une tolérance immunologique spécifique de longue durée après un contact initial avec l'antigène.

De nombreux types de protéines antigéniques ont été utilisés pour maintenir une tolérance immunologique chez de nombreux animaux. En général on peut dire que plus une espèce est éloignée de l'autre, plus le nombre de mutations susceptibles de se produire pour une protéine particulière est élevé, et plus cette protéine contiendra des déterminants antigéniques. Sur une base statistique, il sera certes plus difficile de supprimer une réponse pour 4 déterminants antigéniques que pour 2. Ce principe a déjà été appliqué dans le cas de transplantations d'organes. Il est de plus en plus possible, à l'aide de typages tissulaires, de sélectionner un donneur avec le moins de différences antigéniques. Cette même méthode devrait être applicable pour sélectionner l'antigène le moins étranger, avec le plus de chances d'induire une tolérance.

(suite du résumé en page suivante)

Clinical immunology (particularly as applied to human organ transplants) and basic immunology as practiced under ideal laboratory conditions have followed two parallel but separate tracks in their approaches to the general problem of transplantation rejection reactions.

In the case of clinical organ transplants the actual transplantation of organs followed quickly on the heels of the development of technical ability to perform the necessary surgery. With the organ graft now in place it became necessary by a variety of empirical procedures to develop immunosuppression measures ensuring a usefully prolonged life of the graft. The net result in many situations such as kidney grafts has been most encouraging while in other situations (one of which I gather is heart transplantation) the results have been something less than satisfactory. Despite the success with kidney transplants the prospect of keeping patients on life-long immunosuppressive regimens is not an appealing one and is certainly less than ideal.

It is in this situation that basic immunology may have much to offer. As immunologists with no medical or surgical ax to grind our interest in immunity developed around the use of simple, easily purified antigens. Here the heart of the problem always seemed to be the search for methods of producing a specific immunologic tolerance following initial contact with antigen that would last through a reasonable portion of the animals total life span. The lessons learned here seem to me to be directly applicable to the problems of heart transplantation and I wish to consider in detail several that may prove helpful in the future.

In evaluating the many types of protein antigens that have been involved in induction of immunologic tolerance in many animal species a useful generalization emerged quite early. One example will suffice. Tolerance in mice is more readily pro-

* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6–8, 1969.

La forme physique et la structure d'un antigène jouent un rôle important dans la détermination de ses capacités d'immuniser ou d'induire la tolérance. Ainsi, on a démontré que dans une solution de gamma globulines bovines soumise à une vitesse de centrifugation très élevée, la partie contenant les molécules de poids les plus élevées avait une capacité d'immunisation plus grande, tandis que le matériel soluble surnageant pouvait induire une tolérance mais non une immunisation.

Il en résulte donc qu'un antigène présente deux propriétés distinctes, l'une « Immunogenicity » ou capacité d'immuniser, l'autre « Toleragenicity » ou capacité de produire une insensibilité. L'application de ce principe dans la transplantation est utilisée dans de nombreux centres. Avec succès, on a pu solubiliser et fractionner des antigènes de la transplantation à partir de cellules entières. Même avec les préparations actuelles, il est possible de démontrer une prolongation de la greffe de peau après une injection d'antigènes solubles de transplantation. Au fur et à mesure que l'on parvient à purifier et à caractériser ces antigènes, on espère éliminer leur « Immunogenicity » et augmenter leur « Toleragenicity ».

L'administration par voie intraveineuse d'un antigène soluble a moins de chances d'immuniser, tandis qu'elle a plus de chances d'induire la tolérance. Avec un nombre variable d'antigènes, on a pu démontrer que le dosage a un certain effet, et que la tolérance est plus marquée, avec de larges doses et de petites doses, tandis que l'immunité résulte de doses intermédiaires.

duced with human γ-globulin than with turkey γ-globulin. On the other hand, tolerance in chickens is more readily induced with turkey γ-globulin than with human γ-globulin. Phylogenetic relatedness or foreigness of an antigen has much to do then with its ability to induce tolerance. This is most easily explicable in quantitative terms by assuming that the further away one species is from another the more mutations will have occurred in any particular protein and the more antigenic determinants will this protein contain. It seems likely on statistical grounds alone that it will be harder to suppress a response to four antigenic determinants than to two.

This principle is already being applied directly to organ transplantation. It is known that tissue histocompatibility differences in several species are complex and made up presumably of many antigenic determinants. It is becoming increasingly possible by appropriate tissue typing procedures to select a donor graft with fewest antigenic differences. This same procedure should be directly applicable to selecting the least ''foreign'' antigen with the greatest chance to induce tolerance.

The physical form and structure of an antigen have been found to play a major role in determining its ability to immunize or induce tolerance. In one classic example it was found that if a solution of bovine γ-globulin was subjected to high speed centrifugation the pellet of higher molecular weight aggregated material had a greater capacity to immunize. The supernatant soluble material would induce tolerance and not immunity. In another instance the flagellae of certain bacteria have a high immunizing potency. As these structures are separated into protein molecules called flagellin and finally broken down to sub-molecular peptides their ability to immunize declined markedly, but the ability to induce tolerance remains.

We consider therefore that any antigen has two distinct properties: ''immunogenicity'' or the ability to immunize and ''toleragenicity'' or the ability to produce unresponsiveness. The application of this concept to transplantation is obvious and is

Étant donné que l'antigène seul peut immuniser ou induire la tolérance, il est nécessaire d'associer à son administration un antimétabolique tel l'Imuran. On suppose que toutes cellules stimulées par un antigène en vue de prolifération et différenciation sont tuées par l'effet dit de « Trojan horse ». Étant donné que l'antigène n'ouvre les portes de façon spécifique que dans les cellules capables de réponse génétique, il y a un temps de latence spécifique pour ces cellules uniquement.

Un autre moyen d'induire une tolérance est basé sur le fait que le passage de nombreux antigènes, chez un animal, résulte en une filtration apparente du matériel immunisant, tandis que le matériel efficace induisant la tolérance reste dans la circulation. C'est au niveau du système réticulo-endothélial du foie, des poumons et de la rate, que le matériel immunogénique est filtré. Certaines expériences semblent démontrer que l'injection d'antigènes de transplantation ou autres directement dans la circulation hépatique donne un degré plus élevé d'insensibilité que l'injection dans la circulation générale.

En résumé l'on peut dire que le schéma actuel serait celui d'une infusion continue dans la circulation hépatique de très petites quantités de fragments antigéniques de dimension sub-moléculaire provenant d'un donneur le plus proche du point de vue histocompatibilité, et ce, sous le couvert de l'Imuran.

proceeding rapidly at many centers. Already great success has been achieved in solublizing and fractionating transplantation antigens from whole cells. Even with the relatively crude preparations now available some investigators have demonstrated marked prolongation of skin grafts following injections of soluble transplantation antigen. As the ability to purify and characterize these antigens develops it is to be hoped if not expected that ways of eliminating their ''immunogenicity'' and enhancing their ''toleragenicity'' will appear with appropriate chemical manipulation.

The way in which an antigen is given has a lot to do with whether it immunizes or induces unresponsiveness. In general intravenous administration of soluble antigens is least likely to immunize and most likely to induce tolerance. With a number of antigens it has been found that dosage effects are marked and tolerance may be induced with very large or very small doses; immunity resulting from intermediate size doses. If transplantation antigen proves to be in short supply, low-dose tolerance has obvious attractions.

Finally it is necessary to mention at least two potential aides in establishing tolerance other than antigen alone. Since antigen alone immunizes or induces tolerance (sometimes simultaneous) it has often been found useful to couple its administration with an antimetabolite such as Imuran. Here it has been presumed that any cells stimulated by the antigen to proliferate and differentiate are killed by a ''Trojan horse'' effect. Since the antigen ''opens the gates'' specifically in only those cells genetically capable of responding, there is a specific deletion of only these cells.

The last potential aide to tolerance induction I wish to mention represents an intriguing biologic trick. It has been found that passage of several antigens through a live animal results in an apparent filtering out of immunizing material with the more effective tolerance-inducing material remaining in the circulation. It is felt that the more immunogenic material is filtered out by the reticulo-endothelial system in liver, lungs and spleen. Some experimental evidence suggests that injection of transplantation and other antigens directly into

the hepatic circulation gives a greater degree of unresponsiveness than injection into the general circulation.

In summary, the future prospects for induction of organ transplant tolerance with soluble antigens is bright. In fact one might almost construct an eminently rational scheme which (on paper) should be completely successful. It would go something like this: continuous infusion into the hepatic circulation of very low doses of submolecular size antigenic fragments from a closely matched histocompatible donor under cover of Imuran.

How close to this ideal we can or need to approach is our problem for the next five years.

CONTRIBUTIONS OF AN INTERNATIONAL HEART REGISTRY *

John J. BERGMAN, M.D.,[1] **and Donald E. KAYHOE**, M.D.[2]

Le manuscrit souligne l'importance qu'il y a de former un bureau où toutes les informations en relation avec les transplantations cardiaques seraient compilées. Cette centralisation des informations donnerait aux chercheurs qualifiés des réponses à des questions spécifiques en relation avec ces transplantations.

> "Lured by the possibility for success, we are often tempted to venture beyond the limits of our knowledge. We tend to remember our successes and forget our failures. The need to share experiences is obvious."
>
> J. E. MURRAY, 1963 (1)

Consideration of formation of a human heart transplantation registry had been an important idea in many centers throughout 1968. The accelerating pace of clinical transplantation made clear the need to compare experiences and avoid duplication of error. Survival data, which become significant only when large numbers of patients are studied, are best obtained by pooling the results of transplants by all surgeons. Additionally, a need had been demonstrated for a roster of surgeons and institutions participating in transplant programs. Simultaneously, there was an awareness within the membership of the Board of Regents of the American College of Surgeons and at the National Institutes of Health that central registration of data was important and desirable. Since surgeons were a vital part of the transplant effort and since lines of international communication of the American College of Surgeons were well established, it was logical to begin the project within this organization.

In June 1968, the matter of formation of a registry to centralize information reported about human organ transplants was extensively discussed at the Board of Regents meeting of the American College of Surgeons. A decision was made to approve the formation of the registry in cooperation with the Transplantation Research Committee of the National Institutes of Health.

By January 1969, plans for formation and financing of the Transplant Registry were nearing final form and a meeting was held in Chicago to gather together a number of persons concerned with transplantation as well as representatives of interested organizations to crystallize ideas regarding the venture (2). Among the organizations were the American Cancer Society, American College of Chest Physicians, American Heart Association, American Medical Association, American College of Cardiology, and the National Research Council. Individuals representing a considerable experience in transplantation included: Benjamin O. Barnes, Boston; David M. Hume, Richmond; John S. Najarian, Minneapolis; Felix T. Rapaport, New York; George Santos, Baltimore; Thomas E. Starzl, Denver; and Paul Terasaki, Los Angeles. Doctors Shiela Mitchell and Donald Kayhoe represented the National Institutes of Health, while William P. Longmire and C. Rollins Hanlon spoke for the Ad Hoc Committee of the Regents of the American

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* Paper presented at the Second world symposium on heart transplantation, Montreal, June 6–8, 1969.

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College of Surgeons. Doctor Hanlon had chaired this committee which also included J. Englebert Dunphy of San Francisco.

The January meeting was important because it set guidelines for function of the registry. Chief among these was simplicity of reporting. It was generally agreed that the main function of the registry was to record the number of transplants done, their results and the follow-up data on them. Detailed functional information, immunosuppressive regimens, organ preservation and perfusion techniques and intricacies of histocompatibility testing were not to be registered. On the other hand, first line information for each of the various organs was to be pursued by the registry staff. Thus, the registry was to have an active, rather than a passive role.

Other administrative decisions were made at the time of this meeting. For example, an International Advisory Committee was to be formed for periodic review of registry activities. Similarly, individual committees were to be formed for advising on collection of data on specific organ transplants such as heart, bone marrow, and liver. It was agreed that data collected would be kept confidential but that periodic reports would be made.

The Cape Town Symposium, the First International Symposium on Cardiac Transplantation, held in July 1968, gathered 13 transplant teams together and contributed greatly to the accumulation and dissemination of knowledge about heart transplantation. During this meeting, it was agreed that a heart transplant registry would be useful. Doctor Christiaan Barnard, who had organized the meeting, agreed to activate such a registry. Plans were made for data collection and questionnaires were sent to transplant teams. When the American College of Surgeons — National Institutes of Health Organ Transplant Registry became active, Doctor Barnard graciously decided not to proceed with plans for a cardiac registry in Cape Town.

Table I

Actuarial analysis of survival clinical cardiac transplantation *

Time interval	Fraction surviving
24 hours	.85
48 hours	.75
7 days	.58
14 days	.53
28 days	.52
3 months	.31
6 months	.15
9 months	.07

* Includes all cases January 1964 through May 1969.

Design of the ACS/NIH Transplant Registry now facilitates collection of first line information. Thus, the number of transplants done, donor source, recipient disease, and results of grafting are easily retrievable. Such simple information can be analyzed in many ways depending upon the questions which are formulated. For example, actuarial techniques allow accurate estimation of survival. These methods include short lived cases which contribute information at early intervals of follow-up as well as long term cases which define survival for longer periods of time. Table I shows survival fractions for all humans receiving heart transplants from whatever source since Hardy's first case in 1964. These should represent baseline results since all efforts including three xenografts, two second transplants, and one simultaneous bilateral lung and heart allograft are included.

In avoiding detailed reporting of cases, the registry will help transplantors by not imposing a complex questionnaire on them. A morass of data will not be accumulated. However, the registry will serve as a centralized resource guiding qualified investigators with a specific question to transplant teams whose detailed case records may supply appropriate answers.

SUMMARY

A human heart transplant registry has been activated. It is located within the headquarters of the American College of Surgeons at 55 East Erie Street in Chicago and is funded under a contract from the National Institutes of Health. Its

future contributions will be as a central source of basic data and a resource to help in answering specific questions raised by transplant teams.

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IS A SECOND CARDIAC TRANSPLANT POSSIBLE ? *

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Les auteurs résument leur expérience avec le cas d'une seconde transplantation cardiaque.

Notons au préalable que cette deuxième transplantation peut être précoce, c'est-à-dire pour des raisons techniques, notamment un cœur endommagé par une anoxie prolongée ou bien parce qu'il existe un rejet aigu. Dans ce cas, les chances de succès pour une transplantation ultérieure sont réduites de façon significative. Le rejet tardif constitue l'indication d'une deuxième transplantation cardiaque. Ainsi, pour cette dernière raison, un malade fut transplanté à une deuxième occasion. Le typage tissulaire était du groupe C, soit du même groupe que lors de la première transplantation. Il n'y avait aucune évidence d'anticorps préformés entre le donneur et le receveur. D'autre part, il n'y avait aucune évidence que le deuxième donneur possédait des antigènes auxquels le receveur aurait été sensibilisé par le premier donneur. Cependant, après 2 jours, le patient décédait et l'autopsie révéla la présence de nécrose focale et d'œdème du myocarde sans infiltration de mononucléaires ou de polymorphonucléaires. Il faut noter cependant qu'au moment de la deuxième transplantation, trois autres antigènes furent étudiés, alors qu'ils ne le furent point au cours de la première transplantation. Pour l'un de ces antigènes, le

In approaching this question one must first overcome the initial inclination to dispense with the subject with three words: at present, no. Although this answer would be impeccably correct on immunologic and empiric grounds, there is more involved in this topic than a review of an unsuccessful experience in attempting to perform a second cardiac transplant.

First, one must consider the factor of the time the second transplant is contemplated. Is it an immediate replacement of the graft, that is, in a matter of hours, or is it a late replacement of a transplanted heart which has been functioning for months?

If the procedure is immediate replacement, then the reason for the failure of the first graft determines the feasibility of a second attempt. A graft that is failing for technical reasons, for example a heart damaged by prolonged anoxia, may conceivably be replaced in a matter of hours following the first transplant. If, however, the graft is hyperacutely rejected, the chances of another transplant being successful are significantly reduced. In either situation, even at the most active centers, the likelihood of having another compatible donor immediately available is small.

The major potential use of a second heart transplant is the late replacement of a failing graft. We

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deuxième donneur était positif, le receveur négatif. On notait ici que le premier donneur possédait cet antigène. Le fait que des anticorps préformés n'aient pu être démontrés ne fait que réfléter les moyens limités de leur détection.

Il en résulte donc qu'il n'est pas possible de sélectionner de façon certaine un deuxième donneur ne possédant pas des antigènes auxquels le receveur aurait pu être exposé par le premier donneur.

En fait, quelles sont les possibilités d'une seconde transplantation ? L'avenir réside dans l'induction de la tolérance avec des antigènes solubles de transplantation. L'intérêt principal, dans la transplantation d'antigènes, n'est pas celui d'un support pour une deuxième transplantation cardiaque, mais une approche pour une implantation avec succès de la première greffe. Ceci nous donnera un degré de spécificité immunologique qu'il n'est pas possible d'obtenir actuellement dans les allogreffes cardiaques humaines.

L'immunosuppression non spécifique avec les corticostéroïdes cytotoxiques et les globulines antilymphocytaires pourrait être supprimée, et le receveur pourrait être laissé avec l'incapacité de reconnaître le cœur greffé comme non à lui.

Ces méthodes d'induction de tolérance avec possibilité d'outrepasser les défenses immunologiques vis-à-vis d'un deuxième cœur transplanté auront comme véritable application la survie à long terme de la première greffe, sans les effets secondaires de la suppression immunologique non spécifique. Le but de la deuxième transplantation cardiaque serait la spécificité.

have had experience with one such effort. Our first patient who had a cardiac allograft was the eventual subject of an attempt at a second transplant.

This patient was a 47 year old certified public accountant who had multivalvular rheumatic heart disease with extensive calcification into the ventricular septum. His initial histocompatibility match was interpreted by Doctor Terasaki as being grade C (Table I). A first set rejection episode beginning the eighth postoperative day was readily overcome and this patient was restored to an unusually active life from the time he was discharged from the hospital until he was re-admitted on his 168th postoperative day, with evidence that he was undergoing his second episode of cardiac rejection. Towards the end of a month of intensive care it became obvious that his condition was deteriorating. On his 200th postoperative day he was offered the option of a second cardiac transplant. This second donor provided another Terasaki grade C match.

TABLE I

Histocompatibility profile of cardiac transplant recipient and first and second donors

	HLA 1	HLA 2	HLA 3	B 4	HLA 5	HLA 7	HLA 8	B 9	B 11	B 6	B 10	B 12
Recipient	—	—	+	—	—	±	—	—	±	—	—	±
Donor # 1	—	(+)	—	—	—	(+)	—	?	(+)	—	?	?
Donor # 2	—	—	—	—	—	—	—	+	—	—	—	±

There was no evidence of preformed antibodies between the donor and recipient by the microdroplet lymphocyte cytotoxicity test. Further (Table I), there was no evidence that the second donor possessed an antigen to which the recipient had been sensitized by the first donor. The second transplant was performed but as soon as the new heart was in place, its behavior mimicked that of a hyperacutely rejected xenograft. The heart contracted tightly in systolic arrest. Following massive administration of adrenocorticosteroids, normal contractility was restored; however, adequate cardiac output could not be maintained. The patient died two days after surgery without regaining consciousness. Necropsy findings revealed focal necrosis and edema of the myocardium without infiltration of mononuclear or polymorphonuclear cells.

What are the limiting factors in this clinical experience? Although the Table I shows that no preformed antibodies could be detected and that there was no demonstration of an antigen in the second donor to which the recipient had already been exposed, the histocompatibility data are deficient. By the time of the second transplant, three new antigens were being tested for, which were not studied at the time of the first transplant. For one of these antigens (B-9) the second donor was positive, the recipient negative, and we have no knowledge as to whether or not the first donor could have possessed this antigen. The fact that the presence of preformed antibodies was not demonstrated most likely reflects our limited capacity for detection.

Therefore, because it is not possible to select with confidence a second donor not possessing antigens to which the recipient has been exposed by the first donor, and of lesser importance, because mechanical devices are presently not capable of maintaining the circulation for a time comparable to the period afforded by extracorporeal support in renal transplants, it must be concluded that a second heart transplant is not feasible today for the patient who has experienced failure of a long-term first graft.

Will it be possible in the future to perform a second heart transplant? One potential device we are investigating is the use of high-zone induction of tolerance with solubilized transplantation antigen. But the major interest in transplantation antigen is not as an assist in performing second heart transplants, but as an approach to successful implantation of the first grafts, which will permit a degree of immunological specificity not possible in the present series of human cardiac allografts. Hopefully, non-specific immunosuppression with adrenocorticosteroids, cytotoxic chemicals and eventually antilymphocytic globulin may be withdrawn, and recipients of heart transplants left with only one notable immunologic blind-spot, the inability to recognize the grafted heart as non-self.

The first round of heart transplants is now nearing conclusion. The feasibility of the surgical procedure has been established. In the immunologic area, the recipient of the cardiac allograft has been found to be more immune-competent than the uremic recipient of the renal allograft and the human heart has proved to be highly vulnerable to rejection. Perhaps this vulnerability has contributed to our early recognition of the predictable relevance of histocompatibility to survival of the transplanted heart. The wishful hope that the apparent lesser antigenicity of the heart would make it less susceptible to rejection must be abandoned in the face of clinical experience. For the remainder of the first round of cardiac transplants, it would seem appropriate to limit the procedure to the best possible donor-recipient matches obtainable within the serious limitations of present histocompatibility typing techniques, and to exploit the still unrealized potential of antilymphocytic globulin through well-designed studies. Under these circumstances a heart transplant should be a procedure offering significant clinical benefit to the patient while providing a source of needed information on long-term graft survival.

It is the second round of heart transplants that we look forward to. Immunosuppressive techniques handed down from renal transplant experience have not been all that unsatisfactory to many heart transplant investigators; however, from our point

of view, we find present immunosuppressive techniques deficient to the extent that new, more powerful methods are more than provocative possibilities, but are real necessities. Methods such as induction of tolerance, while potentially capable of overriding the immunologic defenses against a second heart transplant will have their real application in producing long-term survival of the first graft (be it heart, kidney or liver) unattended by the side-effects of non-specific immune suppression.

The challenge for the second round of heart transplants is specificity.