EXPERIMENTAL ORTHOTOPIC AND HETEROTOPIC CANINE HEART ALLOGRAFTS

THE PROBLEM OF THE HEART TRANSPLANTATION MODEL

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE
AAN DE ERASMUS UNIVERSITEIT TE ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS PROF. DR. B. LEYNSE
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATS VINDEN OP
WOENSDAG 5 OKTOBER 1977 DES NAMIDDAGS TE 4.15 UUR PRECIES

DOOR

CORNELIS KERST JONGSMA

GEBOREN TE OOSTERBEEK
(GEM. RENKUM)

1977
BRONDER-OFFSET B.V. – ROTTERDAM

PROMOTOR	:	PROF. DR. M.J. DE VRIES
CO-REFERENTEN	:	PROF. DR. J. NAUTA
		PROF. DR. D.W. VAN BEKKUM

Aan de nagedachtenis van
mijn vader

ACKNOWLEDGEMENTS

This dissertation is dedicated to all those who helped me to accomplish the investigations it describes.

The author is especially indebted to:

Prof. M.J. de Vries, M.D., who "promoted" this work. He not only introduced the author to the histopathology of experimental cardiac allograft rejection but also helped to evaluate all of the histological data presented here.

Prof. E. Bos, M.D., the founder of experimental orthotopic and heterotopic canine heart transplantation in The Netherlands, who was the great initiator and supporter of this project. Owing to his efforts, the experimental material described here could be compiled. His unfailing help, friendship, and initiative were indispensable. The credit for this study goes mainly to him.

Prof. J. Nauta, M.D., for being the patron of this project in its initial phase and its final realization. His support and ingenuity were one of the pillars of this work.

Prof. D.W. van Bekkum, M.D., for his enthusiastic interest in this investigation and his critical encouragement.

Dr. D.L. Westbroek, head of the Laboratory for Experimental Surgery of Erasmus University in Rotterdam, who accompanied the project with his personal efforts and experienced comments and placed at the author's disposal the entire know-how of his laboratory.

Mrs. F.H. Lubbe, who performed the immunofluorescence investigations, the evaluation of the results, and helped to put these data on paper.

Dr. K. Laird-Meeter, for supervising the cardiological aspects of this study (catheterization and electrocardiography), and helping in the

evaluation of these data and their presentation in the manuscript.
Dr. G.T. Meester, for his critical comments on the cardiological aspects of this study.
Prof. J.D. Laird, Ph.D., for his practical and theoretical advice concerning the coronary circulation of the grafts.
Prof. P.G. Hugenholtz, M.D., for his interest in the project and for placing the necessary cardiological equipment at the author's disposal.
Mr. R. van Strik, head of the Department of Biomedical Statistics of the Medical Faculty of Erasmus University, who performed the statistical analysis of the data.
Mrs. M.J. Lagerman, Mrs. A.M. Westerveld-Vogelaar, Mrs. M.O. van Seters-van Essen, Mrs. J. Dortland-de Kam, and *H.K.J. Bruynen, M.D.*, who contributed greatly by assisting during the experiments and by maintaining the experimental records.
Messrs. A. Kok and *J. Poortman*, for running the extracorporeal circuit and also for the administration of anesthesia, *Mr. A. Colly* for administering anesthesia, *Mr. D.S. de Jong* for his supervision and advice concerning extracorporeal circulation.
Messrs. J. Kasbergen, R. Naessens, E. Ridderhof, P. Kreeft, J. van Dongen, and *R. Meyer*, for the skilful pre- and post-operative care.
Miss T.P. Visser, Mrs. M. Mosterd-Hettinga, and *Mrs. C. van Reen-de Boer*, who carried out the biochemical analyses.
Messrs. M. Vermey, R. Meyer, and *Miss M. Sunesku*, for preparing the histological sections.
Mrs. F.H. Lubbe and *Mrs. P.C. Delflos*, for making the photographs.
Mrs. H.F. Spijker and *Mr. D.M. Simons* of the Graphics Section of the Audiovisual Service for preparing the Figures.
Mrs. I. Seeger for editing the manuscript, *Mr. J. van der Plas*, who initially corrected the English text, *Mrs. R. Oosterhout-Bos*, for secretarial work, and last but not least *Marry Jongsma-Pruyt*, the author's wife, who gave the most essential immaterial support but also made a material contribution by preparing the final version of the manuscript for the press.

Substantial grants for these investigations and financial support for the publication of this report were received from the Health Organization TNO/Fungo and the Netherlands Heart Foundation.

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CHAPTER I
THE PROBLEM OF THE HEART TRANSPLANT MODEL

1.1. Introduction

Two heart transplantation models have been developed, one orthotopic and the other heterotopic, but the respective value of these models for human heart transplantation is still unknown despite the explosive growth in the field of experimental and clinical transplantation since 1960. Although great advances have been made, the rejection phenomenon continues to challenge all transplantation research workers. Over the past years and in many widely separated countries, almost 277 human hearts have been implanted. The experience gained from these operations has not only confirmed the surgical feasibility of the procedure, but has also shown that transplanted hearts can function adequately until rejection occurs. Since this rejection precludes complete success of the procedure in man, cardiac transplantation research must proceed in animals until the problem of rejection is solved. The oldest of the two research models we have at our disposal today was originated by Carrel and Guthrie in 1905 (Carrel 1907). This is the *heterotopic model*. The experimental animal receives an accessory heart. Survival remains independent of the functioning of the graft. Many investigators have employed a technique in which the aorta of the graft is anastomosed to a systemic artery and the pulmonary artery to a systemic vein of the recipient, use being made of the neck vessels, the main abdominal vessels, and the inguinal vessels. The adjective heterotopic (Gr. *heteros topos* = other place) was chosen to distinguish this heart transplantation research model from the orthotopic model, in which a graft is substituted for the recipient's heart.

In spite of the difference in the position of the transplant, these models have basically the same hemodynamics: the blood flows from the recipient's artery into the aorta of the graft. When the aortic valves function well, the blood can only enter the coronary arteries. It returns through the coronary veins and reaches the recipient's systemic vein via the pulmonary artery. The pulmonary veins and the superior and inferior caval veins are ligated. The only activity of the heterotopic graft is to maintain its own coronary circulation, which means that there is no workload on the left ventricle and only 10 per cent of the normal workload on the right ventricle (Rushmer 1970).

The *orthotopic model* became feasible with the advent and improvement of the technique of extracorporeal circulation and after the solution of many technical and physiological problems. Only one version of this model, originally developed by Lower *et al.* (1960, 1961), is in use. The graft replacing the recipient's heart occupies the position and takes over the entire function of the excised heart. Hence the adjective orthotopic, which means the right and original place, i.e., where the heart belongs. The recipient is completely dependent upon the function of this graft.

Both the heterotopic and orthotopic heart transplantation models are currently used for research on such problems as the following:

Detection of rejection by diagnostic procedures. This includes the close observation and description of clinical symptoms, such as malaise and diminution of exercise tolerance, which are important signs of rejection after orthotopic transplantation. Studies in this field concern ECG changes, alterations of serum enzyme levels, radiographic and contractility changes, ultrasonic analysis of the configuration of the heart chambers, immunological phenomena, and histopathological investigation of sequential biopsy material.

Prevention of rejection by histocompatibility testing, immunosuppressive treatment, and the enhancement or induction of tolerance.

The behavior of the graft after transplantation, which raises many questions. What is the hemodynamic behavior of a heart graft after transplantation? Does rejection influence the hemodynamics and/or

do the altered hemodynamics influence rejection? (This point is of special importance for the heterotopic heart graft in which, as already mentioned, the hemodynamics are completely different from those in the normal heart or an orthotopic graft.) What is the effect of the interruption of nerves and lymphatics, and what effect does tissue anoxia due to postoperative coronary insufficiency have on the graft? How is the graft damaged by immunopathological processes?

For the investigation of these problems the heterotopic model is favored by many investigators, as shown by the review of cardiac transplantation research given in section A.3. But is this model really appropriate for these purposes? This is still a matter of conjecture. The influence of the specific features of the orthotopic and the heterotopic heart transplantation models on the research results is unknown and has never been thoroughly investigated. This question aroused my curiosity and led me to make a search of the literature for indications of specific differences between the models. The results, which are given in the next section, led in turn to the experimental study reported here.

1.2. Practical and theoretical differences between orthotopic and heterotopic cardiac allografts*

After the orthotopic heart transplantation technique came into use, certain differences between the heterotopic and orthotopic cardiac transplantation research models became apparent. Obvious practical and theoretical differences and differences indicated by the literature are briefly and schematically outlines and discussed here, and more details are given in section A.3. In the following, plus signs are used to give a rough idea of the quantitative importance of certain characteristics.

* Except where another species is explicitly mentioned, all data presented here apply to dogs.

HETEROTOPIC	ORTHOTOPIC

EXPERIMENTAL ANIMALS

Dogs, monkeys pigs	Dogs, or animals of equivalent size (> 10 kg; pigs, calves, monkeys)
Small rodents (e.g. rats, rabbits, mice)	Not yet technically possible in small animals
2 animals needed (a donor and a recipient)	3 to 4 dogs or other animals of equivalent size needed (donor, recipient, and blood donors to prime the heart-lung bypass)

(The advantages of small rodents are obvious: well-defined immunological and immunogenetic properties; low costs; larger series within shorter time; and simple procreation, procurement, and maintenance)

TECHNICAL FACTORS

Supporting techniques

Not necessary	Extracorporeal circulation and assisting technicians Profound hypothermia Anticoagulation

Monitoring of atrial and arterial pressures; ECG, etc.

Not obligatory	Indispensable

Operation room facilities

Minimal requirements For small animals, microsurgical instruments and skilled assistants are required	Complicated equipment

Number of surgical techniques

Many, with many variations and locations of the graft	Basically one technique (Lower *et al.* 1961)

Time required to perform the operation

++	+++

Number of vascular anastomoses

2	4, larger (atrial suture lines)

Bleeding tendency

+	+++ (longer suture lines, anticoagulation required)

Technical skill required

++	++++

Per-operative mortality

++	++++

Post-operative care

++	++++

Post-operative mortality

+	+++

Approach for diagnostic procedures, biopsies, flow studies, etc.

Transcutaneous, transvenous: Recipient's life not endangered	Open, Transvenous, Transcutaneous: Recipient's life at risk (Penn *et al.* 1975, 1976)

HETEROTOPIC ORTHOTOPIC

SITE OF THE GRAFT

Cervical
Abdominal

Intrathoracic

Evaluation:

Cervical position: poor protection, since only a thin layer of tissue protects the graft and it is therefore liable to compression and injury when the recipient turns or bends the head. Accumulation of serous fluid around the graft is invariably observed (Rowlands *et al.* 1968; Semb and Tveten 1971).

Abdominal position: probably gives a better protection of the heterotopic graft than the cervical site, especially when it is located in the pelvic region and is protected dorsally by the pelvic bones. Fluid collecting around the graft may be able to escape to the abdominal cavity. There is less chance of discrepancies between the space available for the graft and its size. Wound repair is uncomplicated. The protection and the physiological conditions in the abdominal cavity seemed better to us than those of the cervical pouch, and our grafts were therefore placed in the abdominal cavity, as will be described under *Experiments*. Apart from species-related differences, our canine cardiac allografts are comparable to other authors' rat cardiac allografts with respect to position, protection, and physiological circumstances.

The thoracic cage offers adequate protection for an orthotopic graft, and is the heart's natural environment.

Effects of pressure exerted by the surrounding environment during respiration

Positive (inspiration)
The venous output of the graft is interfered with

Negative (inspiration)
The venous return to the graft is enhanced

Vascular connections

One vascular pedicle, liable to twisting and kinking

Secure fixation to the recipient's atrial walls, twisting or kinking of the vascular connections impossible

Thrombosis

Thrombotic occlusion of the venous connection has been described by Sayegh *et al.* (1957), Jin (1960), and Bacham and Lagardier (1970)	Not to our knowledge described

FUNCTIONING OF THE GRAFT

Survival time

Survival and physical wellbeing of recipient

Unrelated to graft function	Recipient fully dependent on graft function

Unmodified animals

7 days (mean) (e.g. Furuse 1967; Reed *et al.* 1969; Rowlands *et al.* 1968; Semb 1971)	7 days (mean) (Lower *et al.* 1965, 1969)

Modified animals (azathioprine, prednisone)

35.2 days (mean) (Crosby, Reed *et al.* 1969) 6.4 days (mean) (Leedham *et al.* 1971)	17 days (mean) (Lower *et al.* 1965)

However, comparison of modified orthotopic and heterotopic canine cardiac allografts on the basis of the data in the literature is impossible, due to the different dosage schemes for the immunosuppressive drugs applied.

ECG studies

The published data concerning ECG studies during rejection of cardiac allografts do not permit conclusions about qualitative or quantitative differences between orthotopic and heterotopic canine cardiac allografts (Fernandes *et al.* 1972; Lacassagne *et al.* 1969; Lower *et al.* 1966; Sewell *et al.* 1969; Dear *et al.* 1973; DePasquale *et al.* 1965; Semb *et al.* 1971; Vanderbeek *et al.* 1968). These publications give only the classical descriptions of the electro-

cardiographic signs of rejection, without quantitative or qualitative statistical analysis of these signs.

Hemodynamic functions

Function of left ventricle

No workload	Entire workload

Function of right ventricle

Pumps only the blood of the coronary circulation	Entire workload

Valvular functions

Progressive aortic incompetence (Semb and Enge 1971)	No angiographic data available. Left ventricular end-diastolic pressures within normal limits until rejection (Chartrand *et al.* 1968, 1969, 1972; Stinson *et. al.* 1972, 1974)

Data concerning the functions of the other heart valves could not be found in the literature.

Adverse effects of aortic valve incompetence on heterotopic cardiac allografts

Semb and Enge (1971) found aortic valve incompetence to be present as early as one hour after the operation in three cases. All grafts (n = 7) showed aortic insufficiency 96 hours after the operation. This phenomenon is related to the position of the graft and probably to rejection as well. In 2 other experiments moderate aortic insufficiency could be reversed by immunosuppression (azathioprine and predisone) in one case; in the other, progressive incompetence of the aortic valves developed. This might indicate that rejection produces aortic insufficiency.

It is well known from cardiac surgery that aortic valves can easily be rendered incompetent during, for instance, mitral valve replacement or closure of a ventricular septal defect, by the application of slight compression and rotation of the aortic root by means of an atrial retractor. This simulates twisting or kinking of the vascular pedicle of a heterotopic graft.

Aortic insufficiency leads to left ventricular enlargement, as demonstrated by Semb and Enge. This ventricular enlargement sets off a vicious pathophysiological chain of events, damaging the left ventricular myocardium. (See page 16 for the consequences of an increase in the radius of the chamber.)

The coronary circulation

The coronary flow in a heart is determined by the *perfusion time, the pressure difference* between the sinus valsalvae and right atrium, and the *coronary resistance.*

Perfusion time: under normal conditions, coronary perfusion occurs mainly during diastole, accounting for 80 to 90 per cent of the total coronary flow (Rushmer 1970; Cornhill *et al.* 1974).

In an orthotopic cardiac graft the mechanism of coronary circulation is identical to that of a normal heart, except for the denervation factor, the interruption of lymphatics, and the influence of the rejection process.

In a heterotopic graft provided with a circulation according to the standard technique there is, apart from the afore-mentioned factors, no relationship between the systolic diastolic cycle of the recipient's heart and the graft; the systole of the graft can coincide with the systole of the host heart, which is disadvantageous for the graft (high afterload and high coronary resistance in the graft during systole).

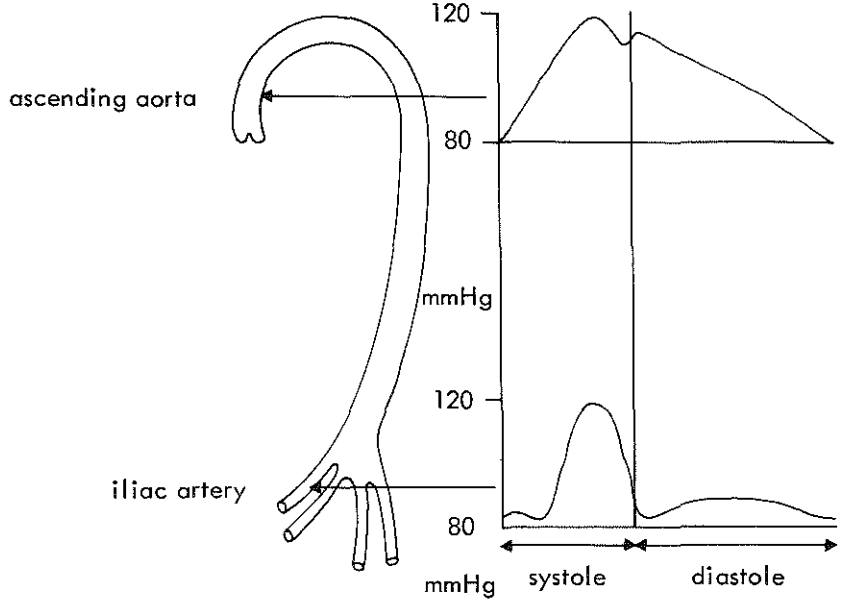


Fig. I.1: Pulse curve characteristics of ascending aorta and peripheral artery in mammalians, showing the differences in systolic and diastolic pressure curves.

Pressure difference: The pressure profiles of peripheral arteries to which the graft's aorta is connected, are disadvantageous as compared with those of the ascending aorta, since in a peripheral artery a systolic peak of 30-40 mm Hg occurs for only 20 per cent of the time, which is short compared with the normal diastolic filling time of coronary arteries. In the ascending aorta, this peak is maintained during 60 per cent of the systolic diastolic cycle (Fig. 1.1.).

Another disadvantageous factor for the coronary flow in the heterotopic graft is the ensuing aortic insufficiency. Cornhill *et al.* (1974) demonstrated that when aortic valves are rendered incompetent, 90 per cent of coronary flow occurs during systole. Finally, an increase in the right ventricular end-diastolic pressure can result from venous thrombosis at the outlet and will considerably hinder coronary venous return by decreasing the coronary perfusion difference.

The resistance of the coronary vascular bed is determined by the intramyocardial pressure, which is directly related to the ventricular geometry. Ventricular-wall tension increases proportionally with enlargement of the chamber according to Laplace's law ($P = \frac{2T}{r}$, in which P = pressure, T = wall tension, r = radius of the chamber), causing the intramyocardial pressure to rise. The regional myocardial flow, which is mainly or entirely diastolic, will diminish when the radius of the chamber increases. Myocardial oxygen demands are estimated from the time tension index. An increase in the radius of the chamber also increases the myocardial oxygen demand (Buckberg *et al.* 1972, 1975).

Owing to the aortic insufficiency, coronary perfusion occurs mainly during the systole of a heterotopic graft, and systolic coronary flow is distributed mainly to the outer and subepicardial layers, as demonstrated by Buckberg *et al.* (in Archie *et al.* 1973, 1974). These phenomena, together with the ventricular enlargement and the subsequent high diastolic left-ventricular pressures, increase the regional myocardial pressure, diminish the regional myocardial flow, and increase the myocardial oxygen demand, all of which may lead to ischemia of the myocardium.

Heterotopic isografts cannot be applied to dogs yet. In other species with the same circulation (e.g. rats), heterotopic cardiac isografts showed scattered areas of fibrosis in the myocardium at autopsy (van Bekkum *et al.* 1969; Heron 1972; Ono 1967), supporting the supposition of ischemic injury to the myocardium of heterotopically allografted hearts.

The poor coronary-flow characteristics of heterotopic canine heart allografts were demonstrated by Folts and Boake (1969). Stinson *et al.* (1972) found much better coronary-flow characteristics in the orthotopic canine cardiac allograft. The former authors' measurements in 12 unmodified heterotopic grafts showed a 50 per cent reduction of the coronary flow on day 3, followed by a progressive decrease, and Stinson found normal values as compared with the controls within the period of normal cardiac function of unmodified orthotopic grafts. Twenty-four hours before death occurred there was a sharp decline in the coronary flow, on average on day 6. Semb and Enge (1971) demonstrated progressive narrowing of coronary arteries in heterotopic canine cardiac allografts during rejection.

Myocardial contractility and pressure-volume-flow conditions in canine cardiac allografts

The performance of a normal heart can be expressed in terms of the force-velocity-length relationships and in pressure-volume-flow relationships. The myocardial rhythmicity (sinoauricular node and other elements of the conduction system) is influenced by preload and afterload conditions and by the sympathetic drive (Skelton and Sonnenblick 1976).

Orthotopic and heterotopic grafts are both deprived of the extrinsic neural control. All other factors are operational in the orthotopic graft, and its performance is controlled by normal mechanisms (Braunwald 1969). In heterotopic cardiac allografts with the usual vascular anastomoses, there is no preload on the left ventricle, the extra initial stretch of myocardial fibers being excluded, when the aortic valve is competent. The contractility relationships which render orthotopic grafts capable of performing the basic cardiac function and allow the recipient to perform moderate physical exercise, do not completely apply to heterotopic grafts.

To the best of our knowledge, no contractility studies have been performed in heterotopic canine cardiac allografts. On the basis of the above theoretical considerations, however, the contractility performance of the ventricle of a heterotopic cardiac graft can be expected to be decreased. The published data on pressure measurements made in the ventricles of orthotopic canine allografts in an unmodified unrelated random combination, show that the ventricular pressure-derived contractility parameters start to deteriorate 24 hours before death occurs on day 5 or 6 (on the average). The final stage of deterioration precedes death by 6 to 12 hours (Chartrand *et al.* 1972; Stinson *et al.* 1972, 1974).

The afterload conditions are also more unfavorable for a heterotopic cardiac allograft than for orthotopic grafts. The afterload is increased by the higher peripheral resistance of a peripheral artery and its limited distal run-off, the *windkessel* function of the ascending aorta being absent. Furthermore, systole of the graft can coincide with systole of the host heart (Fig. I.1.).

The ultimate test for evaluation of the function of a heterotopic graft is orthotopic reimplantation after a period of intermediate host residence. Angell *et al.* (1966, 1967, 1968) described experiments in which a canine heart resided heterotopically in an intermediate host prior to reimplantation into a final recipient. Survival was obtained only under vigorous immunosuppressive treatment (azathioprine and prednisone) and when the graft was reimplanted into the definitive recipient within no more than 4 days of intermediate host residency.

HETEROTOPIC	ORTHOTOPIC
Cardiac output	
Cannot be measured (no left ventricular output)	Normal until 48 hours before death (Chartrand *et al.* 1972)

PATHOLOGICAL DATA
for unmodified dogs

Rowlands *et al.* (1968) Semb *et al.* (1971)	Kosek *et al.* (1968), 1969)
Macroscopic findings	
Myocardial necrosis	
++++	++
Thrombi in heart cavities and vascular anastomoses	
+++	±

Petechial bleeding in the myocardium

±	+++

Microscopical findings

The histological features of cardiac allograft rejection will be defined and discussed in Chapters II and III. Comparison of the published data is difficult, because different definitions are used by the various investigators for the various lesions, and the quantitative and qualitative assessment of the lesions tends to be subjective.

mononuclear cell infiltration (MNI)

commences on day 2	commences on day 2

rupture of small interstitial vessels (RIV)

insignificant lesions	present on day 2

myocytolysis (M)

on day 3 or 4	on day 3 or 4

arteritis (Art)

on day 3 to 8	on day 4

infarction necrosis (IN)

onset on day 3 to 8 on day 8, all grafts 80 per cent necrotic	not obvious; only small scattered areas, starting to appear on day 4

interstitial edema

on day 1	on day 3 or 4

The published pathological findings on modified experimental canine cardiac allografts are even more difficult to compare than those on unmodified grafts, because of the divergent regimens and

dosage schemes used for the immunosuppressive drugs. Both azathioprine and prednisone reverse the signs of acute rejection in orthotopic and heterotopic grafts and alter the acute features, giving more chronic patterns. The degree and quality of these changes cannot be estimated from the available data.

The extrinsic denervation factor and the interruption of lymphatic vessels are rather similar for orthotopic and heterotopic transplanted hearts, and therefore will not be discussed here.

Differences related to non-canine species

The foregoing review of the practical and theoretical aspects of the problem refers only to studies done in unrelated random dogs. There may, however, be species-related factors which would explain some of the divergent results in the literature. These differences are supposed to be predominantly genetic or environmental in nature, and could possible affect physiological and pathophysiological events to a certain extent. To evaluate these factors, studies done in species other than the dog were analyzed (pages 67, 101, 103). The data obtained in unrelated random combinations of rats do not differ appreciably from those originating from heterotopic canine cardiac allografts. The survival time and rejection patterns seemed to be not very divergent from those in dogs, especially when the susceptibility of the heterotopic model to thrombotic complications and the factors or arteritis and infarction necrosis were taken into account.

The work done by Marquet *et al.* (1971, 1972) and by Hollander and Zurcher (1972, 1973) in rhesus monkeys did not disclose any significant divergence from the findings in dogs. Grafts in unmodified monkeys reached survival times comparable to those in dogs. The rejection patterns of heterotopic grafts showed considerably more infarction necrosis and arteritis. In modified monkeys there were no essential differences in survival times, but there was more arteritis and infarction necrosis in the heterotopic grafts. (For pigs, too few data are available to warrant discussion of this species.) *Nevertheless one should be careful not to extrapolate the findings in the dog model to other species in an uncritical way.*

I.3. The present experiments

The fact that the nature of the differences described between orthotopic and heterotopic models in the foregoing had never been investigated and that the heterotopic graft is still preferred as a model for heart transplantation research solely on the basis of its practical advantages, led us to undertake the present study.

Some "heterotopists" have even postulated that during the rejection process heterotopic hearts undergo changes similar to those occurring in orthotopic hearts (Leedham *et al.* 1971) and have also argued that the histological similarities after orthotopic transplantation indicate that the process of rejection is similar in heterotopic and orthotopic grafts. Cullum *et al.* (1971), Crosby *et al.* (1969), and Seki *et al.* (1970) have expressed similar opinions.

The discrepancies between these statements and the results of the analysis of the data in the literature raised doubts concerning the validity of the suppositions made by these authors. If their assumptions were true, results obtained from experimental heterotopic cardiac transplantations could be applied to man without reserve. If wrong, such inadequately founded judgements could lead to faulty and dangerous application of results from heterotopic research. The "unphysiological properties" of the heterotopic heart transplantation model led us to continue our investigations on the basis of the following questions:

1) Can the differences identified here be confirmed?
2) What factors determine the nature of these differences? The answer to this question had to be sought in differences in position of the graft, but even more so in the differences in the hemodynamic properties of the two models.
3) Can these differences be detected adequately in dogs by estimation of: a) graft survival time, b) electrocardiographic signs of rejection, and c) histological signs of rejection?
4) Are differences in the effect of immunosuppression between the 2 models detectable and if so, what is the significance of these findings?

We also considered such fundamental questions as: Can the heterotopic model be maintained as a tool for heart transplantation research? How should it be applied? What is the value of the orthotopic model and how can it be used?

The investigations and the results obtained are described in the following chapters.

CHAPTER II

MATERIAL AND METHODS

II.1. The animals and the composition of the series

For the orthotopic and heterotopic heart transplantations, we chose unrelated mongrel dogs matched as to weight and size to prevent discrepancies between recipient and graft.

The animals were divided into three groups. *Series I* served as an unmodified control group. The animals of *series II* all received the same regimen of immunosuppression for the investigation of the effect of the modification on rejection of both types of grafts.

Although the pre-, per-, and post-operative conditions were otherwise the same in the orthotopic and heterotopic experiments, the use of extracorporeal circulation in the former introduced a difference. The influence of this factor was investigated by the performance of heterotopic experiments in which the per-operative conditions and the amount of blood administered were the same as those in the unmodified orthotopic experiments (see page 93). The animals used for this group of 7 experiments formed *series III.* (See Table II.1.) Controls were not available. The literature provides sufficient information on this point (see Dong *et al.* 1964, 1965; Hurley *et al.* 1962; Kosek *et al.* 1968, 1969; Léandri-Césari *et al.* 1969, 1970; Willman *et al.* 1962, 1963, 1964, 1966, 1967).

The heterotopic operations in series I and III were almost always performed independently of the orthotopic. The operations of series II were carried out alternately. There were no controls for the heterotopic operations, since autografting is impossible and isografts are as yet not available in dogs.

Table II.1.

Numbers of animals and treatment in the various experiments

	SERIES I	SERIES II	SERIES III
Hetorotopic experiments	10	12	7
Orthotopic experiments	14	13	0
Immunosuppressive treatment	–	+	–

The surgical procedures we used were adaptations of standard techniques (Lower *et al.* 1961; Folts and Boake 1969; Legrain *et al.* 1969), and the pre-, per-, and post-operative treatments applied to the experimental animals were kept as similar as possible. Details are given on page 89 etc.

Immunosuppressive treatment

Series II received immunosuppressive treatment in the form of methylprednisone, azathioprine, and prednisone. Methylprednisone was injected intramuscularly; the other drugs were given orally (mixed into minced meat) as follows:

Day	Methylprednisone (mg/kg)	Azathioprine (mg/kg)	Prednisone (mg/kg)
0	10		
1	10	10	
2	8	5	
3	4	3	
4	2	3	
5	0	3	1
6	0	3	1
7	10	10	
8	8	5	

and so on up to the maintenance dose as of day 6, which was reached on day 12 and was continued until the end of the experiment.

Thus, the initial regimen was re-started on day 7, in view of the observation (Lower *et al.* 1965) that in unmodified dogs peak rejec-

tion occurred on day 7 and in human heart transplantation between days 6 and 10 (Dong *et al.* 1972).

Dogs that survived less than two days or showed the signs of graft failure within that period, were excluded from the investigation. In the heterotopic experiments, 30 per cent of the animals failed to satisfy these criteria, in the orthotopic 40 per cent. The main causes of failure in the orthotopic grafts were bleeding from the aortic suture line and rhythm disturbances in association with fatal decompensation of the graft.

All determinations were carried out in all animals.

II.2. The studies performed

Graft survival time

Graft survival time was defined as the interval between the end of the operation and arrest of the graft. In the orthotopic operations the graft survival time was the same as the survival time of the animal. Since the approach of death was usually evident from the animal's clinical condition and the ECG recordings, the exact time of death could be established by one of us or a member of the laboratory staff. For the heterotopic operations, the graft survival time was established by daily palpation of the graft. When contraction decreased to almost nil, the animal was killed or the graft removed.

Graft survival time is given in whole days. A graft survival time of for instance 5 days and at least 12 hours, was rounded off to 6 days, one of 5 days and less than 12 hours to 5 days.

ECG studies

A standardized position of the animal is mandatory during recording, since the dog's mediastinal structures tend to move easily. The heart is suspended free in the thoracic cage, and the electrical axis of the organ changes with the slightest change of posture. The dog was placed on an isolated table in right lateral recumbency, and was kept in this position by an attendant. The forelimbs were fixed by the attendant's right hand, the hindquarters by his left arm and

hand. The animals usually became accustomed to this position within a short time, and sedation was never required.

ECG recorder

We used a Hewlett Packard ECG recorder no. 1511 A (monochannel), with a recorder speed of 25 mm/sec. (voltage: 1 mV = 10 mm). Calibrations were made prior to recording.

Frequency of recording

Recordings were made pre-operatively in the donor and recipient and in the post-operative period daily at about the same hour until arrest of the graft.

ECG leads

In the orthotopic preparations leads I, II, III, AVR, AVL, and AVF were recorded and in the heterotopic ones leads I, II, III, AVR, AVL, AVF, and CF, using an epicardial electrode (see also page 91 under *Heterotopic transplantation technique*).

The electrodes were always placed on the same spots, which were prepared by shaving and the application of an electrode paste. It was essential to keep the limbs separated and perpendicular to the body. The recorder was operated by another assistant.

ECG parameters

Decrease in voltage and change in heart rate seemed to be suitable parameters for the assessment of graft rejection, since they could be quantitated. Other electrocardiographic signs of rejection, such as a shift of the electrical axis, arrythmia, signs of ischemia, and conduction disturbances, could not be quantitated to our satisfaction.

In the heterotopic model the measurements were made via the

epicardial wire (lead CF), because in this lead the disturbing influence of the simultaneous recording of the host heart ECG was minimal. In the orthotopic model, lead II was chosen for the quantitative measurements in view of the findings of Lower *et al.* (1966) and Sewell *et al.* (1969), who recommended this lead for these purposes. The voltage of the daily recordings is given in mm. The heart rate was measured by means of an ECG calculator, and is given in beats per minute.

Controls

The characteristics of the "normal dog" ECG were obtained from Ettinger and Suter's *Canine cardiology (1970).* To exclude the influence of the operation (anasthesia, surgical procedures, etc.) and of the "transplantation factor", 9 dogs which had undergone a small-bowel exchange operation and 9 dogs which had undergone tuba reimplantation were used as ECG controls. In all heterotopic experiments the recipient's own heart (host heart) was also used as control. In the controls, daily recordings were made over a period of at least a week.

Histopathology

Autopsy procedure

All animals (from series I, II, and III) were autopsied immediately after arrest of the graft, according to a standard protocol. Specimens were taken routinely from all organs except the brain and bone marrow. In organs showing macroscopic abnormalities, additional samples were taken from the relevant areas. Small pieces measuring approximately 5 mm^3 were cut from the graft in a standardized way.

The areas sampled are shown in Fig. II.1.

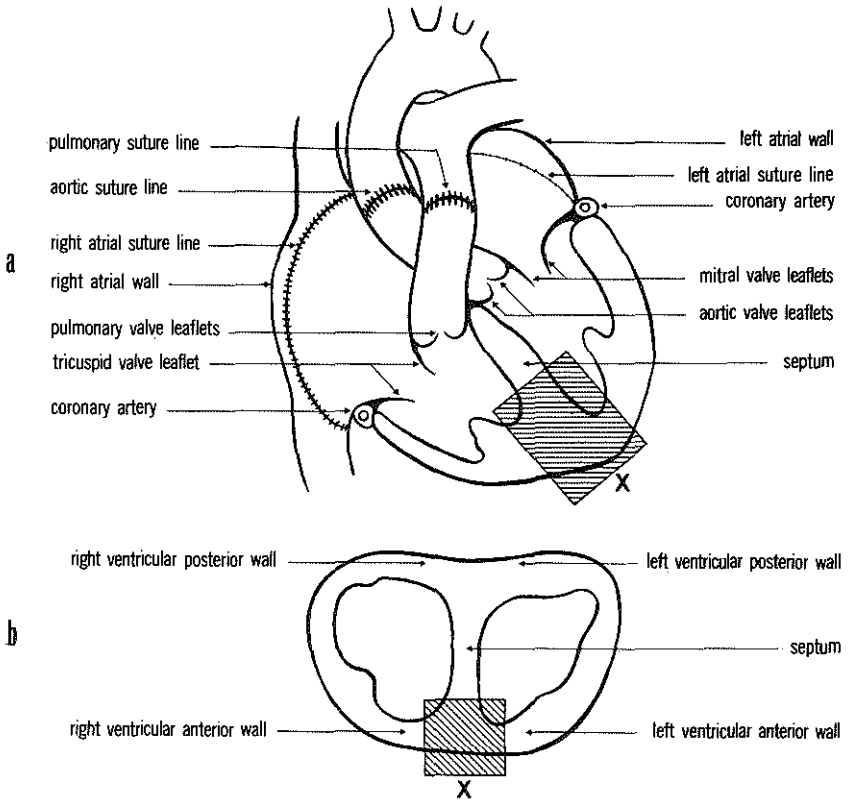


Fig. II.1: a. A frontal section of the graft, and b. a cross-section indicating the sampled areas. X is a cubical specimen (5 mm^3) taken from the right and left ventricular anterior walls and septum for immunofluorescence studies (see Appendix 1b).

Histology

The specimens were fixed in 4 per cent phosphate-buffered formalin (pH 7.0) and imbedded in a paraffin-paraplast-beewax mixture. Sections were cut 5-7μ thick and stained with hematoxylin-azophloxin-saffron (HAS).

Histopathological assessment

Since this assessment is based on what is known about the microscopical histopathology of the rejection process, it will be use-

ful to mention the main characteristics of this process and some theoretical considerations involved.

The histopathological characteristics of the rejection process in an allografted organ can be divided into two main groups (de Vries *et al.* 1968). 1) *Changes in parenchymal cells* (for the myocardium these cells are the myocardial fibers). These changes, which are called myocytolysis (M), are always associated with an involvement of cappillaries and venules defined as rupture of small interstitial vessels (RIV) and with infiltration of lymphoid cells, called mononuclear cell infiltration (MNI). 2) *Changes in larger blood vessels*, consisting of a necrotizing arteritis of larger arteries (Art) with subsequent infarction necrosis (IN) of the myocardium. These changes are accompanied by infiltration of the vascular wall by granulocytes and lymphoid cells, endothelial proliferation, thrombotic occlusion of the vascular lumen and fibrin depositions in and around the vessel.

The changes referred to under point 1 are called parenchymal rejection and are considered to be a cell-mediated process; those under point 2 constitute vascular rejection and are postulated to be antibody-mediated.

Two theories have been put forward to explain the necrotizing arterisis: a) Deposition of antigen-antibody complexes in the vascular wall, followed by phagocytosis of these complexes by granulocytes; lysosomal enzymes of disintegrating granulocytes then cause the vascular damage (a mechanism similar to that occuring in serum sickness arteritis). O'Connel and Mowbray (1973) postulated that leakage of these immuno-complexes provokes platelet aggregation on the intima, leading to platelet thrombi and fibrinoid necrosis in the media. These authors succeeded in producing such lesions experimentally. b) Kosek *et al.* (1971) thought that perivascular mononuclear cell infiltration compresses the vasa vasorum of the arteries, leading to hypoxia of myo-intimal cells and medial destruction.

In the present study the histological slides were examined without knowledge of the group or the particular animal from which the samples were obtained. An over-all qualitative review was carried out first, and on this basis five main histological rejection phenomena were selected: mononuclear cell infiltration, rupture of small interstitial vessels, myocytolysis, arteritis, and infarction necrosis. Attention was of course also paid to other pathological features such as thrombi in heart cavities and on suture lines.

Scoring of pathological findings

All slides were re-examined, and semi-quantitative scores were assigned to each of the items under study. Grading ranged from 0 (minimal change) to 4 (extensive change) as follows.

Cellular infiltration, rupture of small interstitial vessels, myocytolysis, and infarction necrosis were each scored according to the percentage of the surface of the section involved, i.e. from 1 = 25% to 4 = 100%.

Arteritis was scored as:

1 = Cellular infiltration (histiocytes, granulocytes (polymorphs), and lymphocytes in the vessel wall.
2 = Infiltration combined with medial necrosis.
3 = Infiltration and medial necrosis plus intimal proliferation.
4 = Infiltration, medial necrosis, and intimal proliferation plus occlusion of the vessel lumen by platelet thrombi.

Per animal, the scores of one type of change were added, multiplied by 100, divided by the number of slides examined, and rounded off (e.g. 37 to 40, 34 to 30, 35 to 30, etc.). In all cases 8 selections were minutely inspected. The following example shows the result for animal 9, Series 1, orthotopic model:

	MNI	RIV	M	Art	IN
Right atrium	2	2	3	1	3
Right ventricle, anterior	1	2	2	2	0
Right ventricle, posterior	2	3	3	0	0
Left atrium	1	2	1	0	2
Left ventricle, anterior	1	2	1	0	0
Left ventricle, posterior	1	1	0	0	0
Septum	1	3	1	0	0
Coronary artery	1	1	2	0	0
	(10	16	13	3	5) x 100 : 8
	120	200	160	40	60

Because the vascular anatomoses showed signs of an inflammatory reaction due to surgical trauma, they were not scored. Valvular changes differed from those seen in the myocardium and blood vessels, and were therefore also excluded.

Thrombi (T) were classified according to their frequency distribution. Five locations were examined for the presence of thrombi: the aortic suture line, the pulmonary-artery suture line, the right and left atria, the valves, and the ventricular cavities. One positive location was scored as 20, two were scored as 40, and so on, five being 100. For the other organs the microscopical examination was restricted to qualitative evaluation.

Controls

The controls consisted of 10 normal dog hearts from blood donors and all host hearts taken from the heterotopic recipients. This material was examined and scored in the same way.

CHAPTER III

RESULTS

III.1. Results of measurements in models

Graft survival time

Series 1 (unmodified animals, 10 heterotopic and 14 orthotopic)

The survival of the heterotopic grafts ranged from 4 to 11 days with a mean of 6.9 days. The orthotopic grafts survived from 3 to 15 days with a mean survival time of 9.1 days. (See Fig. III.1.) The differences between the distributions of graft survival times in our unmodified material are not statistically significant ($p > 0.10$; Wilcoxon's two-sample test).

Series II (modified animals, heterotopic 12 and orthotopic 13)

The heterotopic grafts survived from 3 to 22 days with a mean survival time of 10.6 days. The orthotopic grafts survived from 7 to 282 days with a mean survival time of 64.0 days. (See Fig. III.2)

The differences between the distributions of graft survival time in our modified material are highly significant ($p < 0.002$; Wilcoxon's two-sample test). In Series III (unmodified animals, heterotopic ($n = 7$), for investigation of the effect of blood transfusion and extracorporeal circulation) the graft survival time ranged from 3 to 19 days with a mean of 9.5 days. The cumulative frequency distribu-

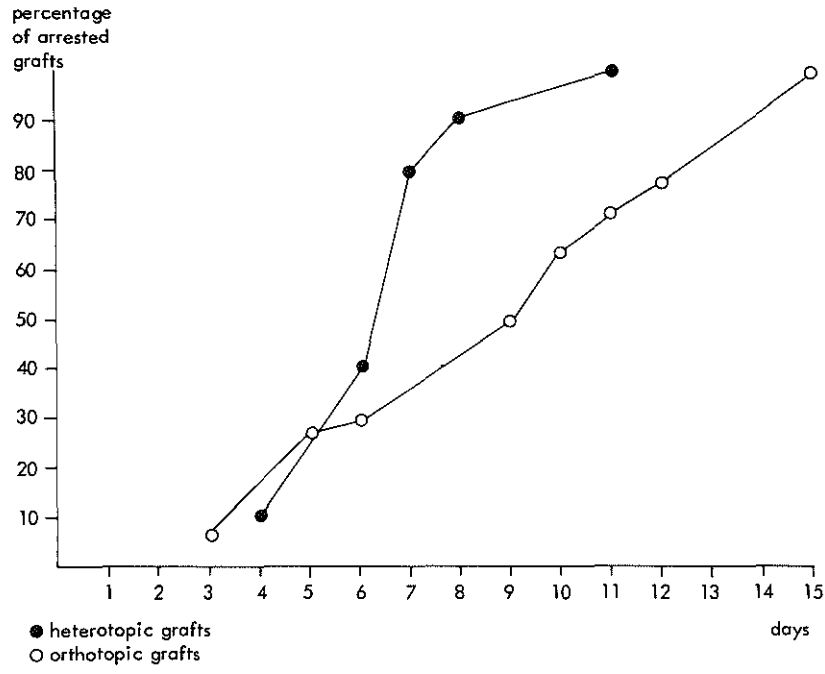


Fig. III.1: Cumulative frequency distribution of the graft survival time in Series I (unmodified animals).

tion did not differ from that of Series I (heterotopic grafts, Fig. III.1) (Wilcoxon's two-sample test: $p > 0.10$). Blood transfusion and extracorporeal circulation did not influence the survival time of unmodified heterotopic grafts.

Statistical analysis of the effect of immunosuppression on graft survival showed that this effect is not significant for heterotopic grafts ($p > 0.05$) but is distinctly significant for the orthotopic grafts ($p < 0.01$; Wilcoxon's two-sample test). Complications associated with immunosuppressive treatment were not taken into consideration in this analysis.

Conclusions

Immunosuppressive treatment on the basis described has no significant influence on the survival of heterotopic grafts, whereas it

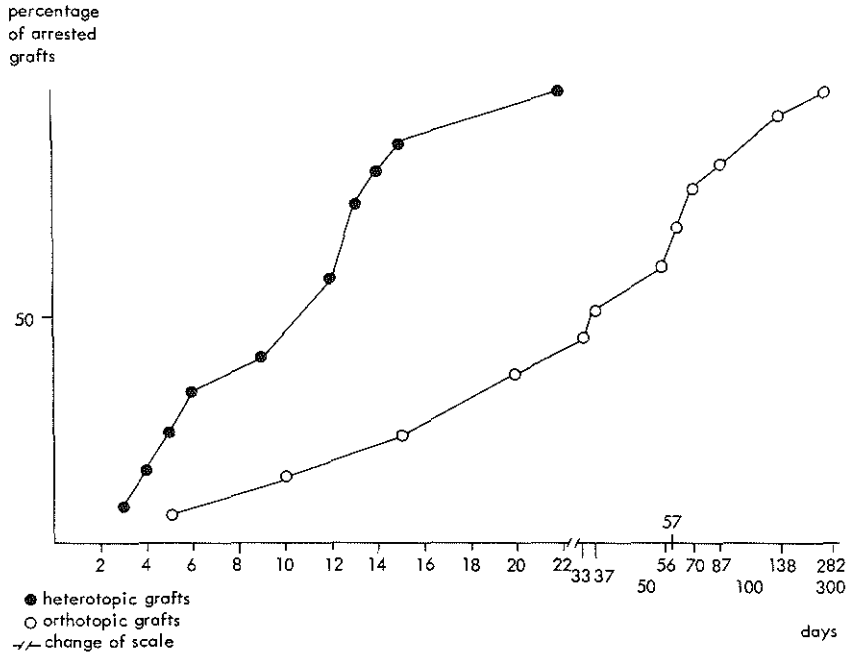


Fig. III.2: Cummulative frequency distribution of the graft survival time in Series II (modified animals).

has an unequivocal influence on orthotopic grafts. This is one of the most important differences between these two types of transplants in this experimental design.

ECG

Voltage measurements

Trends in the individual voltage change per animal were estimated and compared for the three series. The slope values obtained by the least squares method for each animal were compared for orthotopic and heterotopic recipients with Wilcoxon's two-sample test. The voltage decline is significantly more progressive in unmodified heterotopically transplanted animals than in orthotopically transplanted recipients ($p < 0.05$). The differences between the volt-

age decline in animals with modified orthotopic and heterotopic grafts is not significant ($p > 0.5$) (Fig. III.3a, c and III. 4a,b).

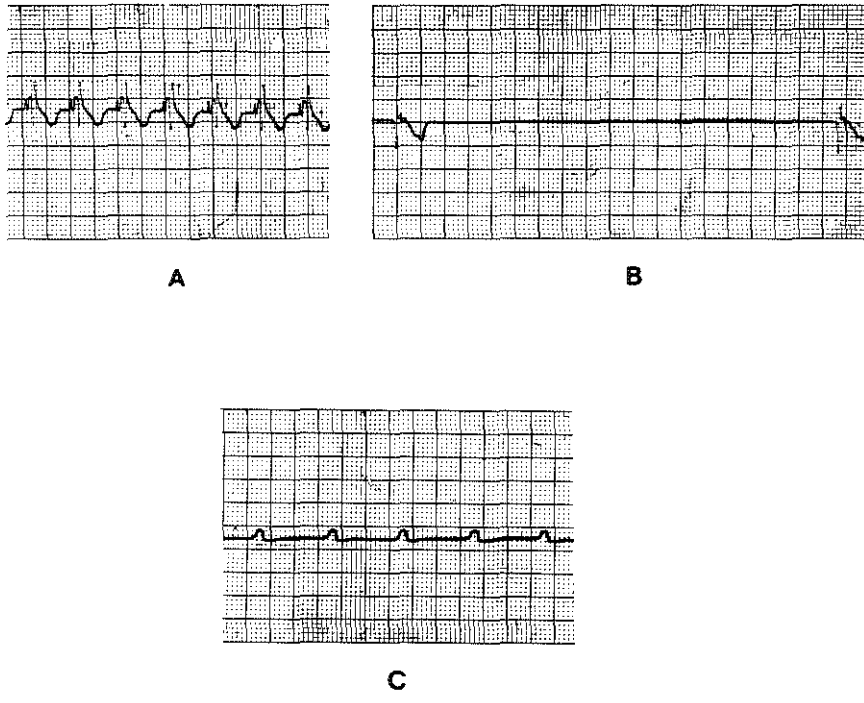


Fig. III.3: ECG recording (lead CF) on day 4, of graft for unmodified heterotopic recipient no. 5, taken in right lateral recumbency according to the protocol.
A: Heart rate 150 per minute, voltage 9 mm.
B: The same recording after the animal was turned on its back. The heart rate decreased to less than 30 beats per minute, the voltage remained unaltered.
C: Recording on day 7 in right lateral recumbency, showing the voltage decline during rejection. Heart rate 100 per minute, voltage 2.5 mm.

Heart-rate measurements

Trends in the individual heart rate changes per animal were estimated and compared in the three series. The statistical analysis was performed in the same way as for the voltage. The changes in heart rate from normal toward bradycardia do not significantly differ between orthotopic and heterotopic canine cardiac allografts in un-

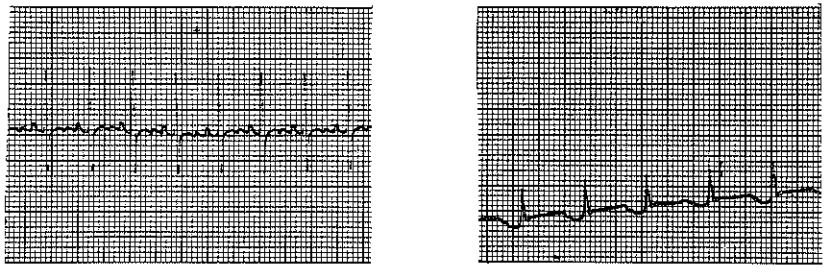


Fig. III.4: ECG recording according to the protocol (lead II) for an unmodified orthotopic graft. Recipient no. 5.
A: Recording on day 0. Heart rate 200 per minute, voltage 19 mm.
B: Recording on day 8, showing voltage decrease during rejection. Heart rate 130 per minute, voltage 7 mm.

modified dogs, but do differ significantly in modified animals (series I : $p > 0.10$; series II: $p < 0.05$). The results in series III were the same as those in series I (heterotopic grafts).

Other observations

Bradycardia could be produced easily in heterotopic recipients by turning the animal on its back, as can be seen from the ECG recordings shown in Fig. III.3a,b. This phenomenon is explained by interference in the blood supply to the graft, due to twisting or kinking of the vascular pedicle caused by change of posture.

Control ECG studies

The control ECG in normal dogs which had undergone other operations (small-bowel exchange, tuba reimplantation, and strangulation of the small bowel), did not show either voltage decline or bradycardia.

The control ECGs of the recipient's own heart (host heart) in the heterotopically transplanted dogs, revealed sinus arrythmias, T-wave changes, and changes in the QRS configuration. These observations led to new investigations, which are discussed on page 81 etc.

Conclusions

The ECG studies with respect to voltage and heart rate revealed differences between orthotopic and heterotopic canine cardiac allografts. In Series I (unmodified grafts) the course of the voltage decline was distinctly more pronounced in the heterotopic than in the orthotopic grafts. In Series II (modified grafts) the voltage decline did not differ significantly between orthotopic and heterotopic. The course of the heart rate did not differ between unmodified grafts, but did in modified ones in that the heart rate of modified heterotopic grafts decreased more progressively than those of the orthotopics.

Immunosuppressive treatment seems to equalize the development of voltage decline between orthotopic and heterotopic grafts during rejection. An influence of immunosuppression on the host heart ECG could not be established.

The influence of extracorporeal circulation and blood transfusion (Series III) on the electrocardiographic signs of unmodified rejection is minor and does not explain the electrocardiographic differences between unmodified heterotopic and orthotopic grafts.

III.2. Histopathology

a. Series I and III (unmodified animals)

a.1. General autopsy findings

In the *heterotopic* experiments none of the general autopsy findings were related to the graft (Table III.1). One case of nephritis and one of a small-bowel invagination were found accidentally in nos. 2 and 6 (small-bowel invagination is known to occur in dogs after all kinds of abdominal operations). No other lesions were observed in the recipients' organs.

In the *orthotopic* experiments the organ systems of all the animals showed features associated with cardiac failure (Table III.1). The graft findings indicated acute rejection as the cause of death in all but no. 12, where cardiac failure was due to rhythm disturbances in the graft. Animals 2 and 6 developed bronchopneumonia probably

Table III.1.

General autopsy findings in Series I and III* (unmodified animals)

Heterotopic recipients				Orthotopic recipients					
N	GST	Infections	Other signs	N	GST	Infections	Organ congestion	Pleural effusion (in ml)	Other signs
1	6	0	0	1	6	0	++	–	–
2	7	nephritis R	0	2	5	Br.Pn.R	++	–	–
3	6	0	0	3	11	0	++	–	Renal infarcts
4	4	0	0	4	9	0	++	500	–
5	7	8	0	5	10	0	++	100	–
6	6	0	invagination Ileum	6	9	Br.Pn.L	+++	800	–
7	7	0	0	7	15	Pyel.neph.R&L	+++	800	Renal infarcts
8	11	0	0						Thrombosis
9	8	0	0						mesenteric artery
10	7	0	0	8	10	0	++++	1,000	–
				9	5	0	++	100	Renal infarcts
				10	15	Pyel.nephr.R&L	++	600	Renal infarcts
				11	5	0	++	200	Renal infarcts
				12	3	0	0	50	Renal infarcts
				13	15	0	+++	200	Ascites (2,000 ml)
				14	10	0	+++	280	–

Abbreviations:

N = number of animal
GST = graft survival time in days
R = right
L = left
Br.Pn. = bronchopneumonia
Pyel.nephr. = pyelonephritis
+ = the amount of congestion graded as + = minimal to ++++ = maximal change
0 = no pathology
– = no data available

The cause of death in the heterotopic recipients was selective sacrifice in all cases. In the orthotopic recipients death was due to rejection in all cases except no. 12 (attributed to fatal arrhythmia).

* The general autopsy findings in Series III were without pathology .

related to pulmonary congestion. The amount of pleural effusion appeared to be proportional to the degree of congestion of the various organ systems, and was on average 500 ml. Renal infarctions can be explained by arterial emboli originating from the surgical field, since small thrombi were found on the left atrial suture lines in nos. 9, 10, and 11. The cause of the pyelonephritis observed in nos. 7 and 10 remains unexplained.

a.2. Findings in grafts

Macroscopical features

All cardiac grafts showed extensive adhesions to surrounding

tissues, e.g. of the omentum majus, bowel, pericardium, and lungs. The transplant was blueish-red, the epicardium being covered by layers of organized fibrin.

The vascular anastomoses in the heterotopic grafts frequently showed thrombosis on the suture lines, especially at the venous outlet. The heart cavities contained black-stained clots, mostly adhering to the endocardium. The ventricular walls were edematous, stiff, and thickened. On cross-section, the ventricular wall showed a yellow mottling. The endocardium sometimes showed small petechial hemorrhages. The valvular leaflets seemed predominantly normal.

In the orthotopic grafts the vascular anastomoses were usually intact. The heart cavities were filled with post-mortem clots not adhering to the myocardial walls. On cross-section, the myocardium showed features resembling those seen in the heterotopic grafts. The severity of the lesions seemed to be lower.

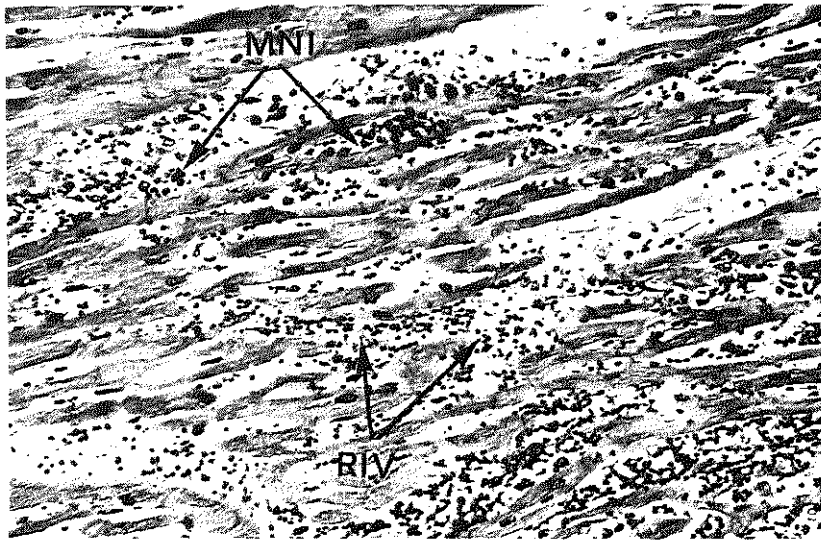


Fig. III.5a: Right ventricular anterior wall of the unmodified orthotopic graft of recipient no. 9 (Series I); graft survival time 5 days. Mononuclear cell infiltration is only present around myocardial fibers and occupies approximately 25 per cent of the slide; scored as 1. Rupture of small interstitial vessels is a more striking feature, scored as 2. Signs of myocytolysis, arteritis, and infarction necrosis are absent. Hematoxylin-azophloxin-saffron (HAS) staining. x300.

Microscopical features

Microscopically, the acutely rejected unmodified canine cardiac allografts showed widespread interstitial edema.

Mononuclear cell infiltration (MNI) was present to a striking degree in grafts surviving more than 4 days (see Fig. III.5a,b). The mononuclear cells were mainly histiocytes and lymphoid cells; blast-like mononuclear cells were sometimes found. The mononuclear cell infiltrations had accumulated mainly around small arteries and myocardial fibers. Polymorphonuclear cells were occasionally found in heterotopic grafts but hardly ever in orthotopic grafts, this difference possibly being attributable to the necrosis in the heterotopic grafts.

Rupture of small interstitial vessels (RIV). The first stage of this lesion appears to be the apposition of lymphoid cells against the endothelium of small capillaries and venules. Widespread congestion of these blood vessels – which contained cellular debris, accumulations of erythrocytes, and small thrombi composed of fibrin, plate-

Fig. III.5b: Left ventricular anterior wall of the unmodified heterotopic graft of recipient no. 1 (Series I), graft survival time 6 days. Mononuclear cell infiltration around myocardial fibers, scored as 2; rupture of small interstitial vessels is insignificant. Myocytolysis scored as 2, and infarction necrosis of the myocardial fibers scored as 2. HAS. x300.

lets, and red cells – was common and was often associated with rupture of the basal membrane, extravasation of the capillary contents into the surrounding tissues, and local hemorrhages. This feature was more pronounced in orthotopic than in heterotopic grafts (compare Fig. III.5c with Fig. III.5b).

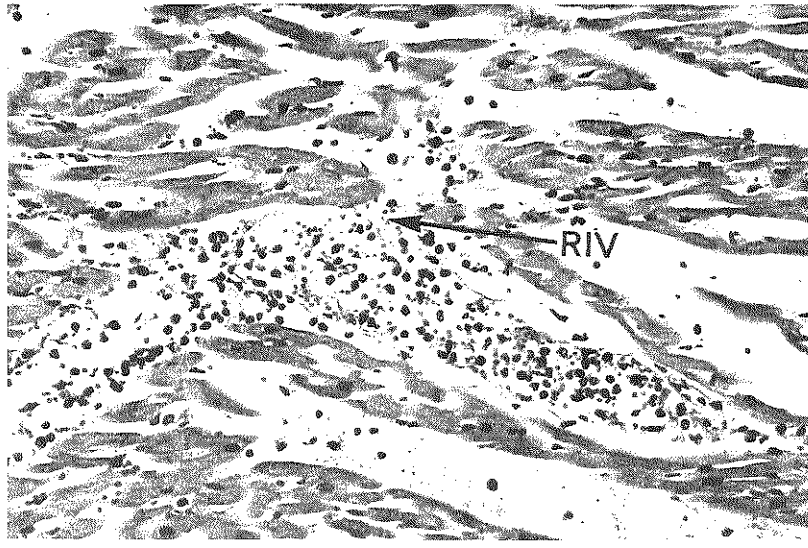


Fig. III.5c: Right ventricular anterior wall of the unmodified orthotopic graft of recipient no. 9 (Series I); graft survival time 5 days. Rupture of the basal membrane of a small interstitial capillary. HAS. x300.

Myocytolysis (M) was characterized by the shrinkage, dissolution, fragmentation, phagocytosis, and disappearance of myocardial fibers in areas infiltrated by mononuclear cells. The myocytes were swollen, and lipid droplets were occasionally present (see Fig. III.5d). Myocytolysis was rather marked in orthotopic grafts, but in the heterotopic grafts was often obscured by the presence of infarction necrosis (Fig. III.5b).

Arteritis (art) (Fig. III.5e). The wall of small arteries showed invasion by mononuclear cells and polymorphonuclear granulocytes, apparently leading to alteration of the media. Medial myocytes had lost their normal structure and appeared to be necrotic, as evidenced by dark nuclear staining with hematoxylin, disappearance of nuclei, increased eosinophilia, and smudging of the intimal cells. The cyto-

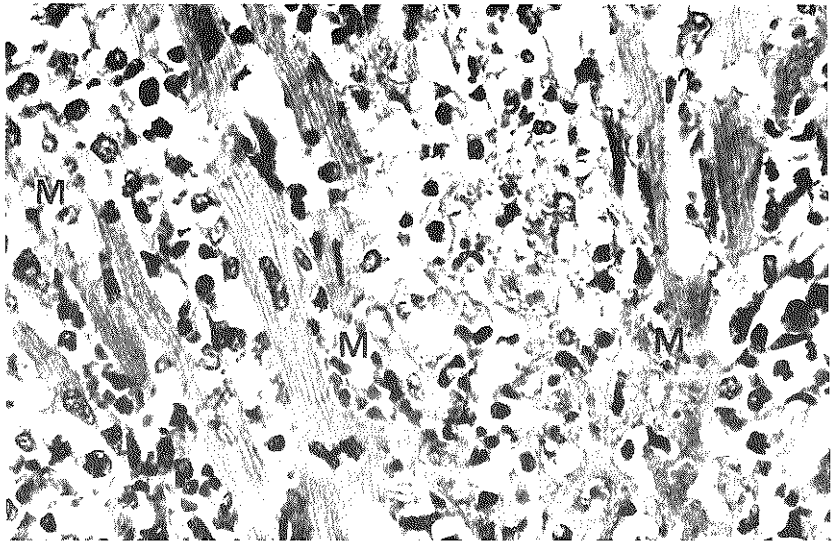


Fig. III.5d: Left ventricular posterior wall of the unmodified heterotopic graft of recipient no. 5 (Series I); graft survival time 7 days, showing myocytolysis. HAS. x900.

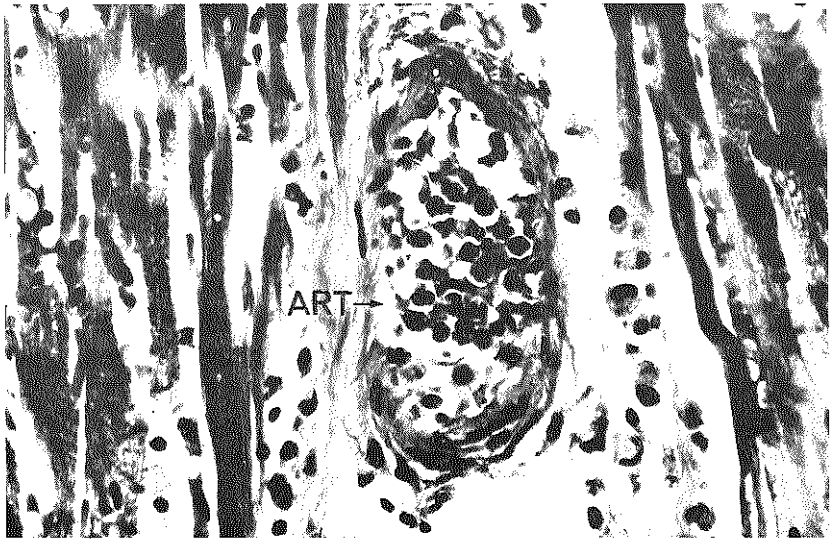


Fig. III.5e: Small artery in left ventricular anterior wall of the unmodified orthotopic graft of recipient no. 6 (Series I); graft survival time 9 days. Mononuclear cells have invaded the media, whose normal structure is severely altered. Medial necrosis and smudging and destruction of intimal cells are present. HAS. x900.

plasm of the intimal cells was swollen, and histiocytes or lymphoid cells closely lined intimal cells. Fusion of cytoplasm of mononuclear and intimal cells was sometimes seen. The vessel wall and the adventitia often showed deposition of fibrin. In severe arteritis the intimal cells were necrotic and the endothelial layer was absent. This was often associated with accumulation of platelet and fibrin thrombi partially or totally occluding the lumen.

Severe arteritis was rarely seen in unmodified orthotopic canine cardiac allografts, but was a prominent feature in heterotopic grafts that survived more than 5 days.

Infarction necrosis (IN). This feature was related to the amount of arteritis present in the grafts. Scattered areas of necrotic myocardial fibers were only found occasionally in the orthotopic grafts; in the heterotopic grafts such lesions were already present on day 4. The distribution and size of these areas were larger in grafts that survived for longer periods. Grafts surviving more than 8 days showed hardly any viable myocardial tissue (Fig. III.5f).

The valvular leaflets only showed mononuclear cell infiltration and edema, the degree being proportional to the signs of rejection observed in the myocardium of the graft in question.

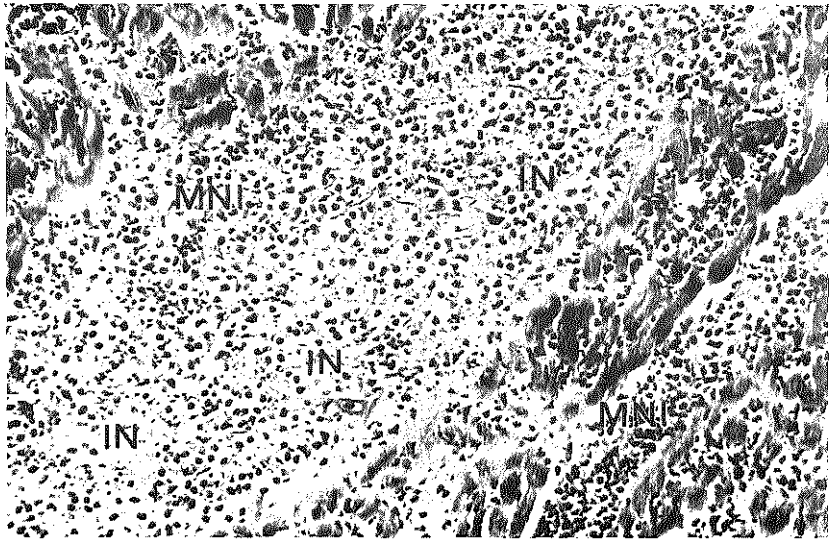


Fig. III.5f: Infarction necrosis in the right ventricular anterior wall of the unmodified heterotopic graft of recipient no. 5 (Series I); graft survival time 7 days. HAS. x300.

Thrombi (T). Distinctly more thrombi were present in heterotopic than in orthotopic grafts. In the heterotopic grafts the preferred localization was the pulmonary artery suture line. Increased embolization originating from the sites of thrombi was not observed in the heterotopic grafts but sometimes occurred in orthotopic recipients, as described under *General autopsy findings* (see page 37).

Controls

Microscopical examination of the 10 blood-donor hearts showed normal myocardial structures. In the heterotopic experiments significant changes were not seen in the host heart either.

Quantitation of histological characteristics of rejection

Quantitation of the histological characteristics of rejection was carried out as described in Chapter II. The results are given in Table III.2.

Table III.2.

Results of quantitation of histological characteristics of rejection in Series I and III. All grafts showed acute rejection except the orthotopic graft in no. 12.

Series I (unmodified grafts)																Series III (extra corporeal circulation and blood transfusion							
Heterotopic grafts								Orthotopic grafts								Heterotopic grafts							
N	GST	MNI	RIV	M	Art	IN	T	N	GST	MNI	RIV	M	Art	IN	T	N	GST	MNI	RIV	M	Art	IN	T
1	6	270	100	200	270	270	20	1	6	250	220	200	0	0	0	1	6	320	270	230	300	390	20
2	7	100	0	30	130	30	60	2	5	100	100	90	80	0	0	2	19	255	40	175	215	105	0
3	6	100	0	0	200	200	0	3	11	160	40	90	10	10	0	3	7	245	30	100	215	220	0
4	4	300	0	170	130	130	100	4	9	150	140	100	200	60	0	4	3	75	65	40	115	125	40
5	7	320	120	150	250	370	60	5	10	300	120	300	200	40	0	5	8	215	150	75	230	300	0
6	6	240	100	160	240	200	20	6	9	200	200	150	160	50	0	6	9	240	235	15	140	285	0
7	7	340	0	180	300	360	40	7	15	200	200	70	30	20	0	7	15	255	15	160	245	225	20
8	11	210	40	70	300	210	20	8	10	200	200	170	50	40	0								
9	8	270	70	150	300	300	20	9	5	120	200	160	40	60	20								
10	7	270	180	130	230	260	20	10	15	260	120	300	150	110	20								
								11	5	170	140	50	60	12	20								
								12	3	20	10	0	0	50	0								
								13	15	260	80	250	280	230	0								
								14	10	100	110	100	60	80	0								

Abbreviations:

N = number of animal
GST = graft survival time in days
For abbreviations of histological signs of rejection, see page 28, 29 and 30.

a.3. Semi-quantitative comparison of histological characteristics of rejection in unmodified orthotopic and heterotopic canine heart allografts

The semi-quantified values were plotted, giving five graphs for each item, with the histological values on the X axis, the survival time of the individual dogs on the Y axis. The results are shown in Fig. III.6.

Statistical analysis of these data was carried out according to Wilcoxon's two-sample test. The significance of the differences in rejection signs between unmodified orthotopic and heterotopic cardiac allografts in series I was, for each of the characteristics, as follows: MNI: $p \sim 0.05$, RIV: $p \sim 0.01$, M: $p > 0.10$, Art: $p < 0.01$, IN: $p < 0.01$. The differences between the incidence of thrombi in orthotopic and heterotopic unmodified grafts of series 1 was 3 score points with a maximum of 20 in the orthotopics and 9 in the heterotopic; scores of 20 occurred five times, 40 one time, 60 two times, and 100 one time ($p < 0.001$).

As can be seen from Table III.2 and Fig. III.7a,b, there is no significant difference between the heterotopic grafts of series I and III for any of the histological items ($p > 0.1$; Wilcoxon's two-sample test).

a.4. Conclusions

Mononuclear cel infiltration: Prominent in unmodified orthotopic and heterotopic canine heart allografts. Possibly slightly more intense in heterotopic grafts.
Rupture of small interstitial vessels: Prominent in orthotopic grafts, rather insignificant in heterotopic grafts.
Myocytolysis: Probably of similar degree in heterotopic and orthotopic grafts; the assessment of this lesion in heterotopic grafts was hampered by the concurrent development of infarction necrosis.
Arteritis: Prevalent in heterotopic grafts, insignificant in orthotopic grafts up to about day 10.
Infarction necrosis: High levels reached already at day 7 in heterotopic grafts. Trivial in orthotopic grafts before day 10.

The unmodified rejection of the orthotopic graft is mainly

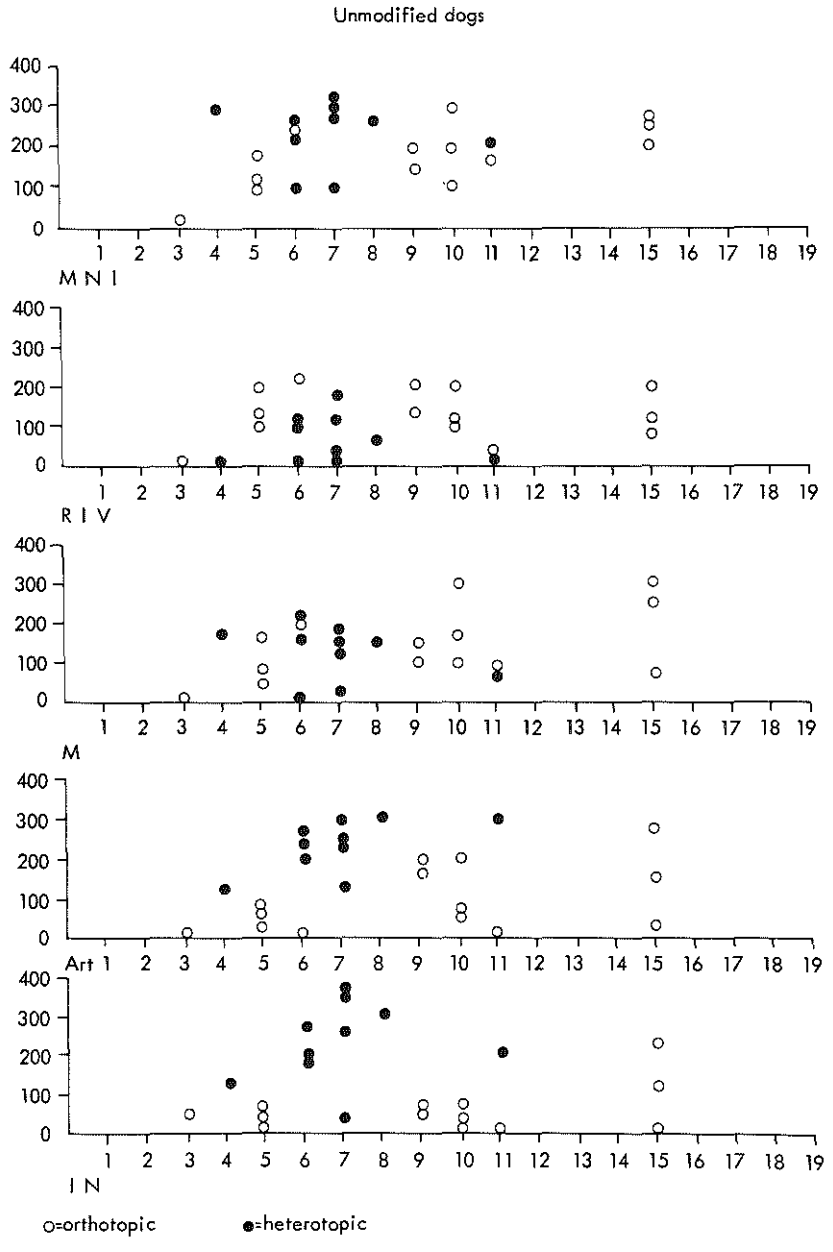
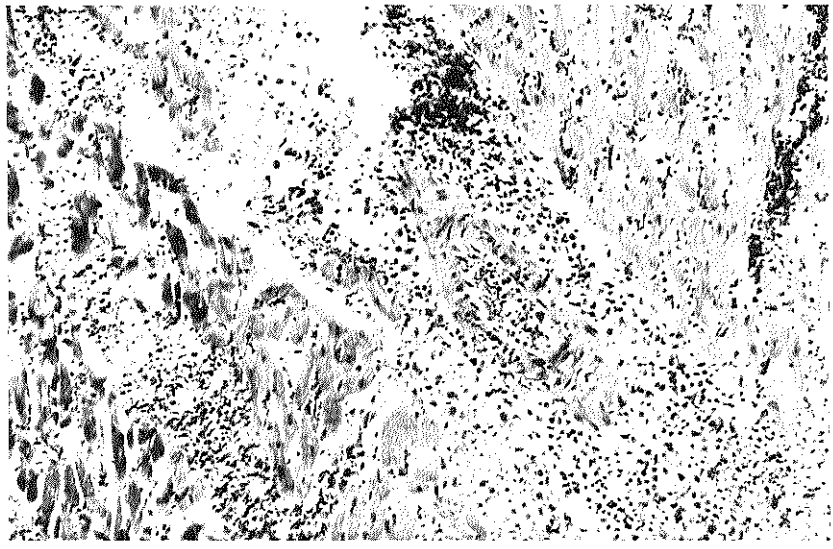
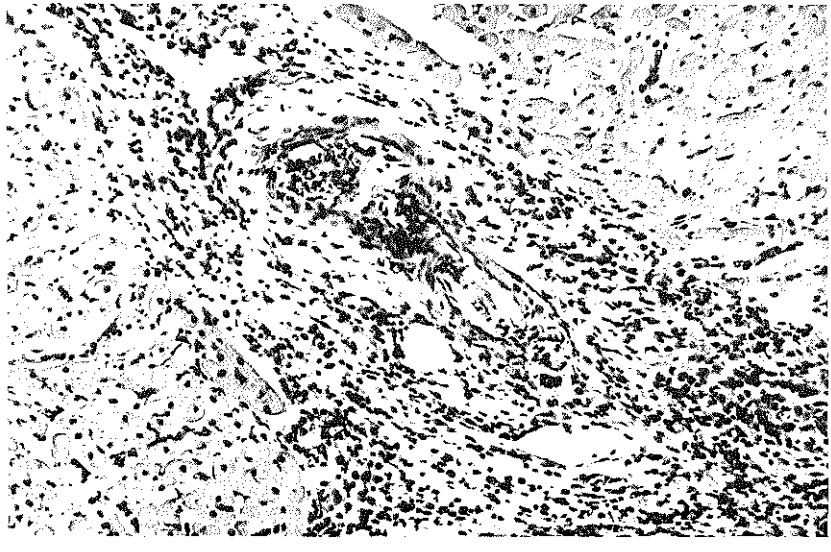


Fig. III.6: Semi-quantitative histological rejection signs in Series I. For abbreviations of the histological signs of rejection, see page 28.



a. Series 1, no. 5, graft survival time: 7 days.



b. Series III, no. 3, graft survival time: 7 days.

Fig. III.7: Representative sections showing the histology of the rejection of heterotopic grafts from Series I and Series III. The sections were taken through the anterior wall of the left ventricle. Both sections show mononuclear cell infiltration, myocytolysis, arteritis, and infarction necrosis in approximately the same degree. HAS. x300.

associated with mononuclear cell infiltration, rupture of small interstitial vessels, and myocytolysis (=parenchymal rejection), whereas that of the heterotopic graft is mainly associated with arteritis and infarction necrosis (vascular rejection), the former process presumably being cell mediated, the latter antibody mediated (see page 28). All the lesions in question are more extensive in the heterotopic grafts at the moment of cardiac arrest than in the orthotopic graft.

Thrombi: The occurrence and extent of thrombotic lesions, especially at the venous outlet anastomosis, is significantly more pronounced in the heterotopic than in the orthotopic grafts. Partial thrombotic occlusion of the venous outlet of the heterotopic graft (6 cases) must result in considerable deterioration of the hemodynamic performance.

The use of blood transfusion and extracorporeal circulation in unmodified heterotopic transplantation did not lead to histological signs of rejection differing significantly from those seen in transplantations performed without these supporting techniques.

b. Series II (modified animals)

b.1. Immunosuppressive treatment

The reports dealing with the principles, practice, and side effects of immunosuppressive agents applied to modify allograft rejection are too numerous to list here exhaustively. Relevant publications in the present context include those by Berenbaum (1967), Kaplan *et al.* (1973), Mannack (1970), Pierce *et al.* (1972), Hardy (1974), and Kjellstrand (1975), as well as special papers related to cardiac transplantation by Lower *et al.* (1965) and Stinson *et al.* (1969). In our experiments we employed methylprednisolone, prednisone, and azathioprine (for regimen, see page 23). The precise action of these drugs is not completely known yet. The steroids have a preferential influence on thymus-dependent lymphocytes and reduce the cell-mediated rejection processes; they also have strong anti-inflammatory properties, prevent rupture of lysosomes and release of their hydrolytic enzymes, and stabilize cell membranes. These compounds suppress allograft rejection more effectively when combined with other immunosuppressive agents. Azathioprine, the

agent most widely employed in transplantation at present, impairs the nucleic-acid synthesis of the immunologically active cells, especially during the early stages of antigen recognition and subsequent lymphocyte stimulation, mainly via its metabolites, which are "produced" by the liver. The formation of humoral antibodies is suppressed. Hence, the influence of azathioprine mainly concerns antibody-mediated rejection (see page 28).

Significant prolongation of the graft survival time was obtained by the indicated authors but not without the inevitable complications. Among the numerous side effects of corticosteroids we may mention steroid diabetes, elevated catabolism, potassium loss, increased sensitivity to infections, gastro-intestinal ulcers and bleeding, and hypercoagulability. Azathioprine on the other hand reduces the cellular metabolism in normal proliferating tissues such as bone marrow, gastro-intestinal mucosa, reproductive tissues, and hormone-producing tissues (adrenal glands), which, together with bone marrow depletion, usually leads to overwhelming infection. Azathioprine is also toxic to the liver, which can result in damage of the liver parenchyme, leading to necrosis. Long-term immunosuppression is also known to enhance malignancy. The combination of lesions attributable to immunosuppressive side effects is called drug intoxication here.

b.2. General autopsy findings

Since the immunosuppressive regimens were identical for the heterotopic and orthotopic groups in series II, the same side effects of this treatment could be expected in these recipients (Table III.3).

Massive bronchopneumonia developed in 10 dogs (Fig. III.8a.) Atrophy of intestinal mucosa and adrenal glands was observed in 16 dogs. Necrosis of liver parenchyme was present in 13 cases (Fig. III. 8b).

Study of bone marrow samples was not regularly performed.

Table III.3.

General autopsy findings in series II (modified animals)

Heterotopic recipients							Orthotopic recipients							
N	GST	Infections	Liver necrosis	Atrophy	Other signs	Cause of death	N	GST	Infections	Liver necrosis	Atrophy	Other signs	organ congestion	Cause of death
1	9	Br.Pn.R&L	++	+	0	DI	1	20	Br.Pn.R&L	0	0	0	++	Re.DI
2	12	Br.Pn.R&L	+++	++	0	DI	2	87	Br.Pn.R&L	++	++	Inf.R.Lung	++	Re.DI
3	3	0	0	0	0	?	3	56	0	0	+	0	0	Re.DI
4	15	0	0	0	0	SS	4	33	0	0	+	Renal Inf.R	++	Re.DI
5	13	Br.Pn.R&L	+++	++	0	DI	5	282	Br.Pn.R&L	++	++	0	0	Re.DI
6	14	Br.Pn.R&L	+	+	0	SS	6	37	0	0	0	Inf.L.Lung	++	Re
7	5	Br.Pn.R&L	0	0	0	SS	7	20	0	0	0	0	0	Re
8	4	–	–	–	–	SS	8	57	0	+	++	0	+++	Re.DI
9	22	Br.Pn.R&L	0	+	Renal Inf.R&L	SS	9	10	0	++++	++	Thromb.V.C.J.	0	Re.DI
10	6	Br.Pn.R&L	++	++	0	DI	10	7	0	++	++	Renal Inf.L	0	Re.DI
11	13	0	+	+	Renal Inf.R&L	SS	11	70	Pyel.nephr.	+	+	Inf.L lung	+	Lung.Inf.
12	12	0	0	0	0	SS	12	15	0	0	0	0	++	Re
							13	138	0	++	++	Inf.Spleen	0	DI

Abbreviations:

N = number of animal
GST = graft survival time in days
R = right
L = left
Br.Pn. = bronchal pneumonia
Pyel.nephr. = pyelonephritis
+ = grading of lesion: + = minimal change, ++++ = maximal change
0 = no pathology
– = no data available
Inf. = infarction
Thromb.V.C.I. = thrombosis of vena cava inferior
Re. = rejection
DI = drug intoxication
Atrophy = of intestinal mucosa and adrenal glands
SS = selective sacrifice
? = no. 3, heterotopic recipient – cause of death not clear from the autopsy findings

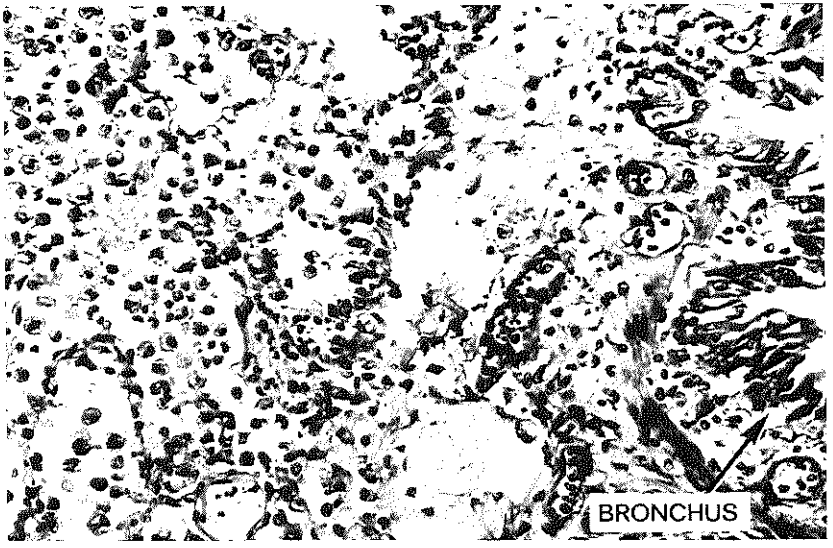


Fig. III.8a: Lung of modified heterotopic recipient no. 5, showing massive bronchopneumonia. HAS. x300.

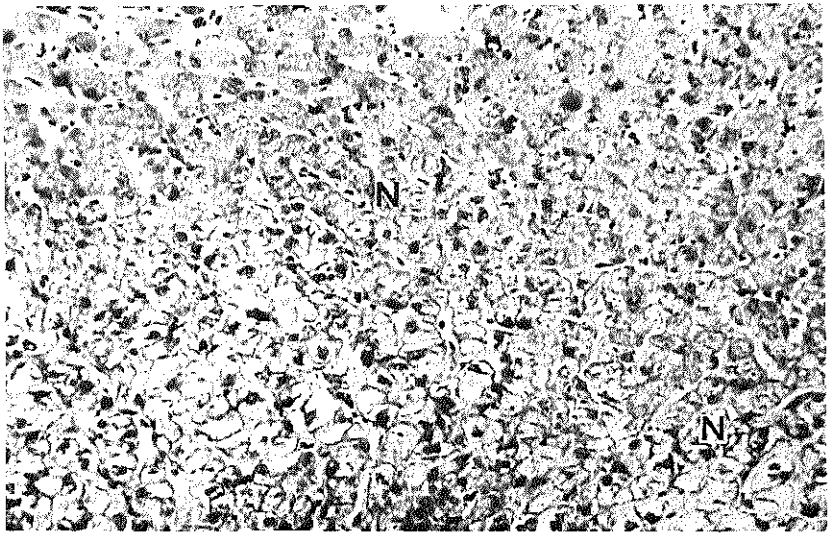


Fig. III.8b: Liver of modified orthotopic recipient no. 9, showing extensive liver necrosis (N). HAS. x300.

Other findings

In the heterotopic group renal infarctions were found in two cases, in one of these associated with thrombi in the graft.

In the orthotopic recipients renal infarctions were found in two dogs, in one explained by small thrombi along the left atrial suture line. Lung infarctions were observed in three animals, an infarction of the spleen in one, and a thrombus in the inferior vena cava in one. Two of the lung infarctions were explained by small thrombi on the right atrial suture line.

The cause of death could be determined easily in the heterotopic recipients, where survival was not dependent on the function of the graft. In nos. 1, 2, 5, and 10, death occurred before graft failure and was attributed to drug intoxication. In no. 3 the cause of death was not explained by the autopsy findings. The remaining 7 dogs were killed when the graft ceased to function. Signs of drug intoxication were found in four of these animals.

In the orthotopic recipients the cause of death could be cardiac failure, drug intoxication, or both. In nos. 6, 7, and 12, death was attributed to rejection. In no. 11 death was due to complete left-lung infarction without signs of rejection or drug intoxication. In no. 13 only drug intoxication could have been responsible for the recipient's death. The remaining cases showed signs of both rejection and drug intoxication, which explained the recipient's death. Rejection of the graft was usually combined with congestion of the recipient's organ systems, giving pictures similar to those of unmodified orthotopic recipients.

b.3. Graft findings

Macroscopically, apart from adhesions attributable to the operation, the picture was rather normal in the unrejected grafts (nos. 2, 3, 9, 10, and 12 of the heterotopic[1] and nos. 11 and 13 of the orthotopic grafts). According to the degree of rejection, the rejected hearts generally showed the same macroscopic appearance as described for unmodified grafts.

[1] Cardiac arrest occurred in nos. 9 and 12 due to catheterization (Table A.2, page 79).

Microscopically, the modified grafts showed:

Mononuclear cell infiltration (MNI): The influence of the immunosuppressive drugs on this lesion was striking except in no. 4 of the heterotopic and no. 7 of the orthotopic group. (See Fig. III.9a,b.)

Rupture of small interstitial vessels (RIV): Insignificant except in no. 4 heterotopic.

Myocytolysis (M): Distinctly less marked than in the unmodified grafts. Orthotopic grafts surviving more than 50 days occasionally showed small scattered areas of fibrosis reflecting previous damage to myocardial fibers.

Arteritis (Art): The incidence of arteritis was also clearly lower than in the unmodified series. In addition, arteritis showed a different character under modification, especially in the older grafts showing fibrinoid degeneration and necrosis of the media as well as areas with moderate endothelial swelling and proliferation of intimal cells. Grafts surviving more than 14 days showed transmural lymphoid cell infiltration, enlargement of endothelial cells, and sometimes desquamation of the intima. (See Fig. 9c.)

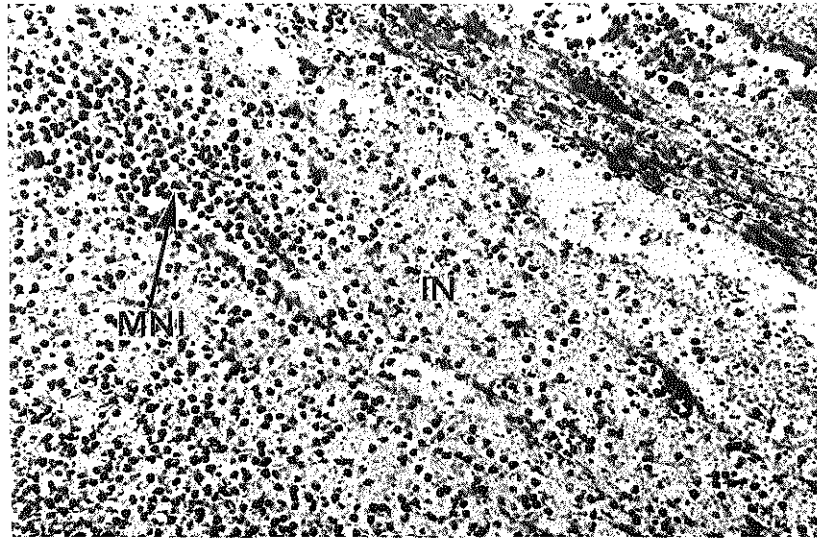


Fig. III.9a: Right ventricular anterior wall of the modified heterotopic graft of recipient no. 4; graft survival time 15 days. Mononuclear cell infiltration is scored as 2, infarction necrosis as 3; no signs of myocytolysis or rupture of small interstitial vessels are present. HAS. x300.

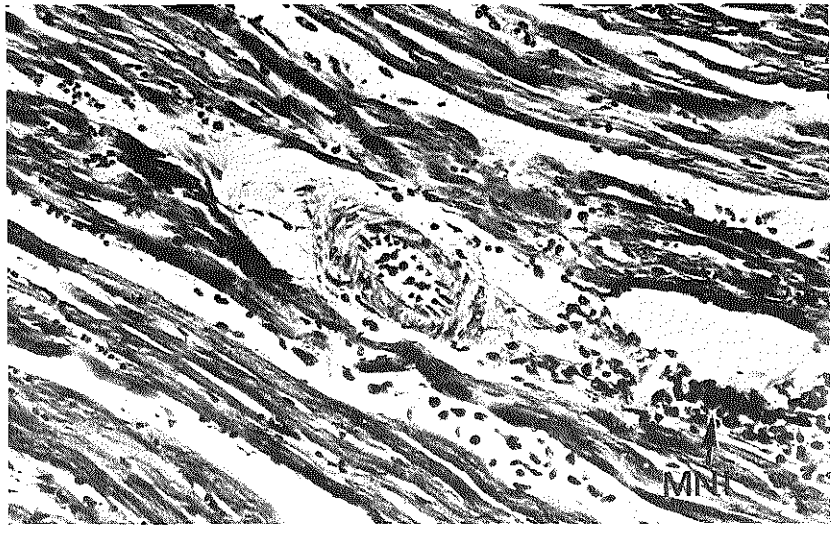


Fig. III.9b: Left ventricular anterior wall of the modified orthotopic graft of recipient no. 7; graft survival time 20 days. Mononuclear cell infiltration scored as 1, myocytolysis as 1, arteritis as 0; no signs of rupture of small interstitial vessels or infarction. HAS. x300.

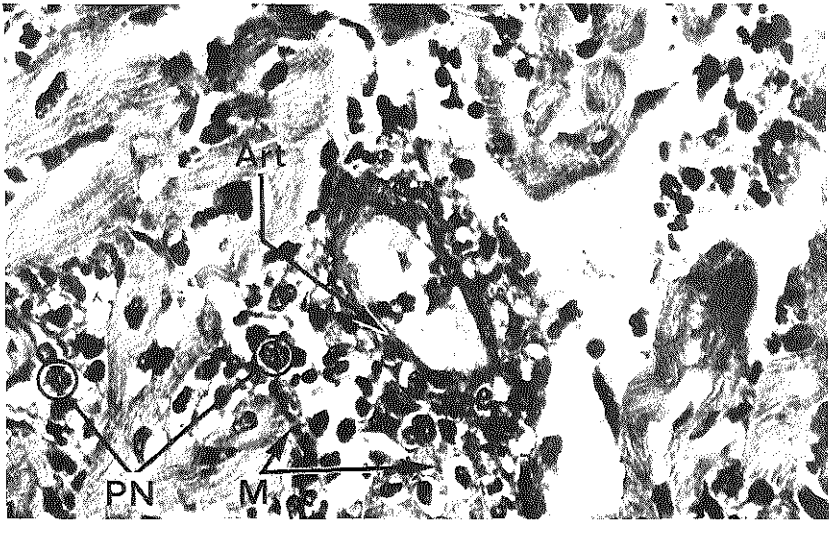


Fig. III.9c: Small artery from the septum of the modified heterotopic graft of recipient no. 9; graft survival time 8 days. The vessel is surrounded by mononuclear and polymorphonuclear cells (PN); myocytolysis (M) is also present. The arteritis (Art) is characterized by cellular infiltration of the media, media necrosis and smudging and destruction of intimal cells. HAS. x900.

The vessel wall showed exudation of red blood cells and fibrin thrombi on the intimal side. In some cases the entire vascular wall became involved in this process. The lesions in the dogs with a longer survival time (more than 60 days) were sometimes gradually replaced by fibrous tissue causing thickening of the vascular walls and subsequent narrowing of the lumen. This feature is defined by Kosek *et al.* (1971) as graft arteriosclerosis or chronic rejection (Lower *et al.* 1976) (see page 72). It was hardly ever seen in our material.

Infarction necrosis (IN). The incidence of necrosis parallelled that of the arteritis. The main features have been described above (page 42) for the unmodified grafts.

The valvular leaflets showed edema, infiltration of mononuclear cells, and exudation of fibrinous material and red blood cells. In the grafts with a long survival time this material was sometimes replaced by dense fibrous scar tissue.

Thrombi (T): In the heterotopic grafts thrombi were seen in 8 cases. The incidence and localization were the same as in the unmodified heterotopic grafts, which means that the immunosuppressive drugs

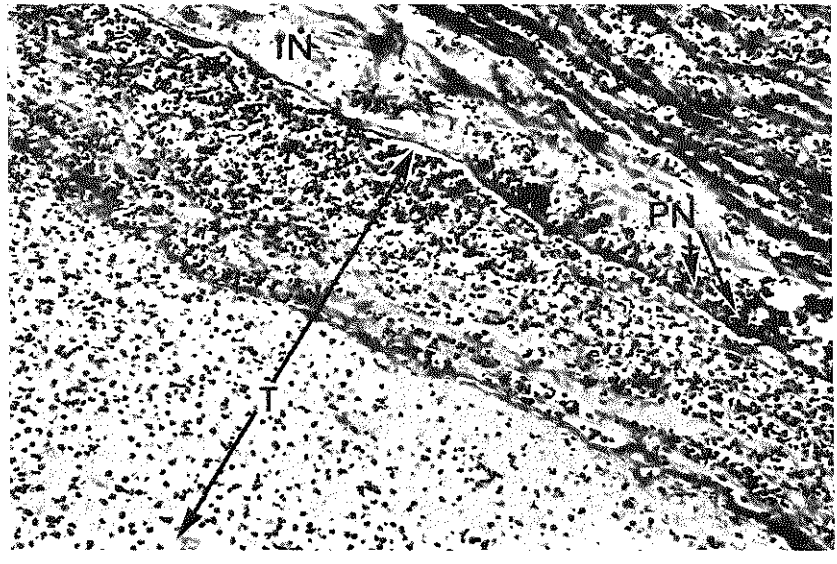


Fig. III.9d: Left ventricular anterior wall of the modified heterotopic graft of recipient no. 7; graft survival time 5 days. A large thrombus (T) is seen on the endocardial side; infarction necrosis (IN) and infiltration with lymphoid and polymorphonuclear cells (PN) is also present. HAS. x300.

did not alter the frequency and distribution of thrombi in heterotopic grafts (Fig. III.9d). No significant correlation was found between vascular complications and thrombi.

Only three orthotopic grafts showed small thrombi, these occurring in association with infarctions in other organs. The incidence of thrombi was the same as in the unmodified orthotopic grafts, which excluded an influence of the immunosuppression.

Controls

The recipient's own heart served as control tissue for the immunosuppression series. No abnormalities were observed. (Cardiac autografts in dogs given immunosuppression to provide controls for the orthotopic grafts were not available.)

Quantitation of histological characteristics of modified rejection

Quantitation of the histological characteristics of rejection was carried out as described in Chapter II. The results are shown in Table III.4.

b.4. Semi-quantitative comparison of histological characteristics of rejection in modified orthotopic and heterotopic canine heart allografts

The semiquantified values were plotted, giving five graphs for each item, with the histological value on the X axis and the survival time of the individual dogs on the Y axis. The results are shown in Fig. III.10.

Statistical analysis of these data was carried out according to Wilcoxon's two-sample test. The significance of the differences between modified orthotopic and heterotopic canine cardiac allografts with respect to the signs of rejection under study was as follows: MNI: $p > 0.10$, RIV: $p > 0.10$, M: $p > 0.10$, Art: $p \sim 0.10$, IN: $p > 0.10$. The difference between the incidence of thrombi in orthotopic and heterotopic modified grafts was 3 scores, with a maximum of 20 in the orthotopics and 8 in the heterotopics, with scores of 20 (four times), 40 (two times), and 80 two times): $p < 0.05$.

Table III.4.

Results of quantitation of histological characteristics of rejection in Series II
Series II (modified grafts)

Heterotopic grafts									Orthotopic grafts								
N	GST	MNI	RIV	M	Art	IN	T	Cause of death	N	GST	MNI	RIV	M	Art	IN	T	Cause of death
1	9	80	20	30	130	120	40	DI	1	20	80	0	50	100	50	0	R + DI
2	12	20	10	20	50	50	20	DI	2	87	90	0	20	160	140	20	R + DI
3	3	20	0	10	0	20	20	unexplained	3	56	50	0	0	190	40	0	R + DI
4	15	310	200	0	300	360	80	SS	4	33	200	100	100	200	120	20	R + DI
5	13	30	10	30	120	70	80	DI	5	282	20	0	0	110	0	0	R + DI
6	14	80	0	30	100	100	0	SS	6	37	40	70	40	70	140	20	R
7	5	90	100	20	100	90	20	SS	7	20	290	40	60	170	170	0	R
8	4	110	0	0	110	100	0	SS	8	57	40	0	20	70	100	0	R + DI
9	22	130	0	100	50	0	20	SS	9	10	30	0	30	100	20	0	R + DI
10	6	20	0	20	50	40	0	DI	10	7	180	0	140	180	40	0	R + DI
11	13	100	0	50	130	150	0	SS	11	70	10	0	10	70	10	0	Lung infarction L
12	12	40	0	40	30	10	40	SS	12	15	150	80	50	170	130	0	R
									13	138	10	0	10	50	20	0	DI

Abbreviations:

N = number of animal
GST = graft survival time in days
DI = drug intoxication
SS = selective sacrafice
R = rejection
For abbreviations for the histological signs of rejection, see page 28, 29 and 30.

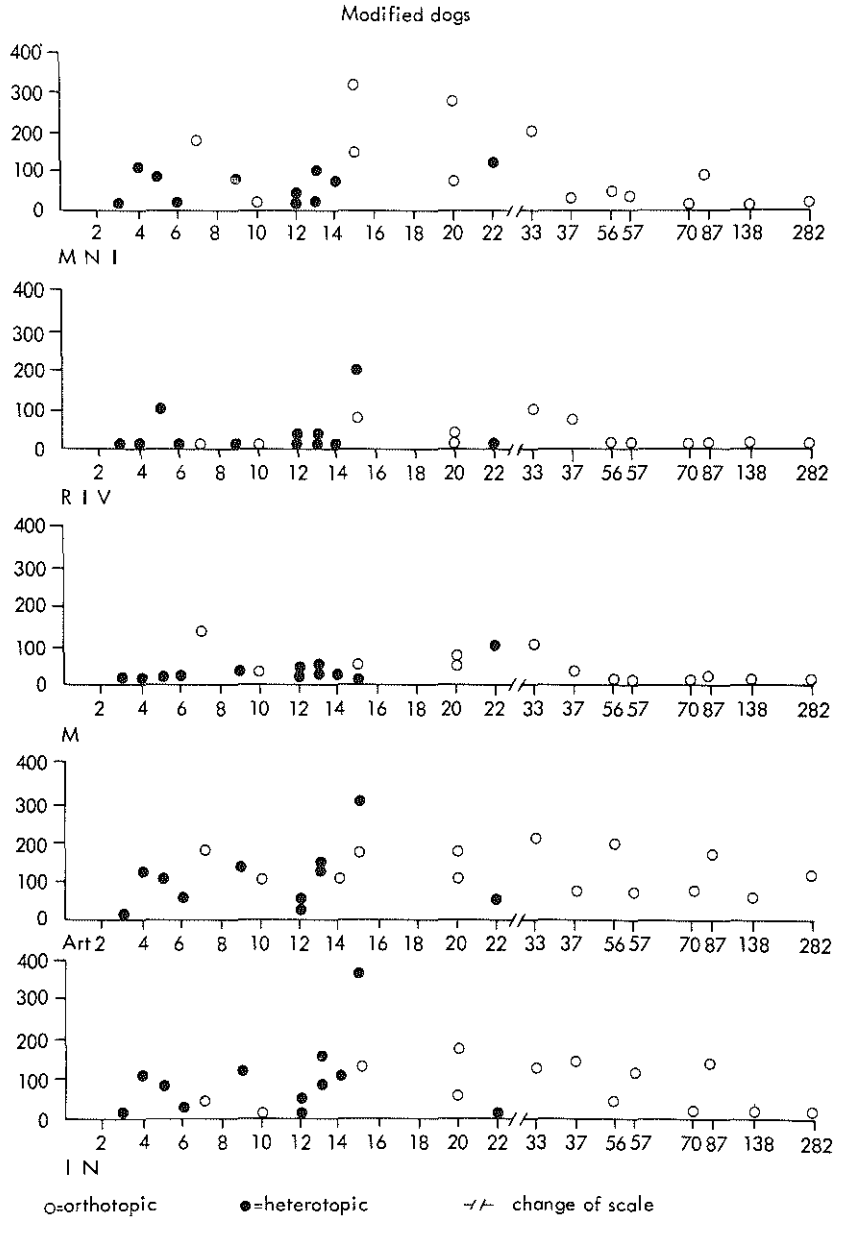


Fig. III.10: Semi-quantitative histological rejection signs in Series II. For abbreviations of the histological signs of rejection, see page 28.

The results of the statistical analysis (Wilcoxon's two-sample test) of the data on the effect of the immunosuppression on the parameters of histological rejection were as follows. For the heterotopic grafts the effect on MNI was significant ($p < 0.01$), on RIV non-significant ($p > 0.05$), on M significant ($p < 0.01$), on Art significant ($p < 0.01$), on IN significant ($p < 0.01$, and on T non-significant ($p > 0.05$). For the orthotopic grafts the effect was significant for MNI ($p < 0.05$), RIV ($p < 0.01$), and M ($p < 0.01$) and non-significant for Art, IN, and T ($p > 0.05$ for all three).

b.5. Conclusions[1]

Immunosuppressive treatment (azathioprine and prednisone) modifies the histological rejection patterns of orthotopic and heterotopic canine heart allografts. It also leads to damage in other organs of the recipient. Under this therapy, infiltration of mononuclear cells, rupture of small interstitial vessels, and myocytolysis are reduced to a low level in both types of graft. Arteritis and infarction necrosis are not prevented, but their occurrence is postponed, as the results of the graft survival time studies indicate. Infarction necrosis is distinctly prominent in both types of graft.

As a result of immunosuppressive treatment, the histological rejection pattern in orthotopic grafts comes to resemble that in heterotopic grafts more closely. The scores of the histological signs of rejection thus become similar for the two types of graft. Azathioprine and prednisone influenced parenchymal rejection (presumably cell mediated) in both types of grafts. Arteritis (presumably antibody mediated) persisted, but was postponed significantly longer in the orthotopic than in the heterotopic grafts (results of the graft survival time studies), where the prolongation only amounted to a few days, as compared with unmodified dogs. Thus, the effectiveness of azathioprine, which is considered to suppress antibody production, was insignificant in heterotopic grafts, and in orthotopic grafts the drug became less effective after day 12, probably due to the rela-

[1] The effect of immunosuppressive treatment in heterotopic grafts was only assessed in dogs without blood transfusion (Series II); all conclusions should be considered in the light of this restriction.

tively low maintenance dose given after day 12. The very limited influence on the heterotopic grafts is attributed to the adverse features of this heart transplantation model in dogs, as discussed below (page 65 to 71).

Immunosuppressive treatment had no influence on the occurrence of thrombosis. The incidence of thrombotic lesions was significantly higher in the heterotopic than in the orthotopic grafts and did not differ from those found in the unmodified heterotopic grafts. The statements made on the unfavorable hemodynamic effects of such thrombi are therefore similar to those made for the unmodified heterotopic grafts.

CHAPTER IV

DISCUSSION*

IV.1. Similarities and differences between orthotopic and heterotopic canine cardiac allografts

In Chapter I the question of why there are two heart transplantation models was posed. It is shown that the heterotopic model was maintained largely because of its practical and economic advantages. In Chapter II, the practical and theoretical differences between the two models were discussed in detail on the basis of the literature. The animal species and technical factors leading to differences favoring the heterotopic model were not taken into consideration. Here, the other subjects of Chapter II are discussed in the same sequence, starting with *Site of the graft.* These subjects are dealt with in relation to the results of the present study.

Site of the graft

We found no differences in wound repair between the abdominal incision used to implant heterotopic grafts and the thoracic incisions used for the orthotopic grafts. Accumulation of serous fluid was observed around both the heterotopic and the orthotopic grafts and in approximately the same amounts. Measurement of the press-

* Except where another species is explicitly mentioned, all data presented here apply to dogs.

ure exerted by the surrounding environment could not be performed, and therefore the influence of this factor on the function of the graft could not be determined. The negative pressure in the thorax during inspiration must certainly have a favorable influence on the venous return to the orthotopic graft. Positive pressure (10-15 mm Hg) in the abdomen restricts movement of the heterotopic graft and impedes the right-atrial diastolic filling.

We observed twisting or kinking of the vascular connections of the heterotopic graft when the animal was turned on its back, resulting in bradycardia (Fig. III.3a,b). This phenomenon was reproducible (see also page 77), and could not be provoked in orthotopic recipients.

Thrombotic manifestations, especially in the venous connections, occurred significantly more often in the heterotopic than in the orthotopic grafts ($p < 0.001$). This phenomenon, which has also been described by others (see page 13), is possibly to be explained by the low output of the right ventricle of the heterotopic graft (10 per cent of the normal value) and the well-known tendency of experimental venous vascular anastomoses to thrombosis, especially those involving the inferior caval vein, as described by Scherck *et al.* (1974). Thrombosis of the venous outlet could also have been promoted by twisting of the vessel as well as by the positive pressure of the surrounding tissues, and might have been reversed by anticoagulant therapy (to avoid introducing another variable, we deliberately refrained from the use of anticoagulant agents).

Functioning of the graft

Graft survival time

The survival times of unmodified grafts did not differ significantly between heterotopic and orthotopic grafts. The findings are similar to those reported in the literature. In modified grafts a distinct difference was found between heterotopics and orthotopics: orthotopic grafts survived considerably longer ($p < 0.002$). (Mean survival time – heterotopic grafts: 10.6 days, orthotopic grafts: 64 days.)

The published reports on immunosuppression in canine cardiac

allografting are confirmed by our findings indicating extreme ranges of graft survival times for the same immunosuppressive drugs. This divergence described for modified cardiac allografts must be attributed to dissimilarities between the dosage schemes and regimens of administration.

Our results suggest that orthotopic grafts are more susceptible to the effects of the immunosuppressive drugs in question, which means that the rejection process was influenced more favorably than in the identically treated heterotopic grafts. The latter possibility is supported by the results of our histological studies, which will be discussed below.

The unfavorable characteristics of the coronary circulation of the heterotopic graft, owing to its position and hemodynamics, lead to a diminished blood supply and to stasis of blood in its myocardial tissue. This feature might explain most of the differences in the rejection process in the two types of graft as well as the more limited effect of immunosuppressive therapy in the heterotopic type.

ECG studies

To the best of our knowledge, data from rejecting cardiac allografts have never been analysed quantitatively. According to the literature, qualitative ECG signs of rejection seem to be the same for unmodified and modified orthotopic and heterotopic cardiac allografts (page 13). Many investigators have accepted a voltage decline in a cardiac allograft as a reliable electrocardiographic sign of rejection (page 13, 26). Our data show that the progression of the voltage decrease is significantly faster in unmodified heterotopic than in orthotopic grafts ($p < 0.05$).

These findings suggest that the process of rejection is more strongly progressive in heterotopic grafts. ECG studies in allografted animals treated with immunosuppressive drugs showed a reduction of the damage to the myocardium. This effect of immunosuppressive drugs was described by Graham *et al.* (1969) for orthotopic grafts and by Semb *et al.* (1971) for heterotopic canine cardiac allografts. Under modification of the rejection process, the significant differences in voltage decline between the two types of unmodified graft became non-significant ($p > 0.5$). This suggests that modification

leads to changes in the quality of the rejection process in both types of graft, making the processes occurring in them more similar. The histological rejection patterns of modified grafts support this view.

The difference in the effect of the rejection process on the heart rate was not significant for unmodified grafts ($p > 0.10$), the decrease being similar in the two types of graft. The opposite was the case for the modified grafts, in which the decline in the heart rate was more progressive in heterotopic than in orthotopic grafts ($p < 0.05$). In a denervated heart the rate is determined only by its intrinsic rhythmicity, which is a property of the graft's conductive tissue. The integrity and function of the conduction system of the heart is dependent on an adequate blood supply. This suggests that in unmodified rejection the effect of the altered blood supply to the conduction system is not reflected in heart-rate differences in the two types of graft, but that in modified rejection the blood supply is preserved better for the orthotopic than for the heterotopic graft. The results show a correlation between graft survival time and the changes in heart rate. Development of bradycardia indicates that cardiac arrest can be expected "soon". Because of the short graft survival time in untreated animals, these changes did not reach clear expression in them.

Hemodynamic functions

This section concerns the results of catheterization studies (see Appendix 1.a). Since our material was too small to allow statistical analysis, the conclusions drawn are tentative.

The results of our catheterization investigations are similar to and confirm the findings of Semb and Enge (1971). They also contribute to our knowledge about pressure development in the ventricles of heterotopic grafts in rejection. The data support our assumptions regarding the deleterious effects of aortic incompetence on the function and fate of heterotopic cardiac allografts (page 16). The occurrence in heterotopics of ventricular enlargement and the subsequent deterioration of the myocardial blood supply were demonstrated by our contractility studies, which show extremely bad values, much worse than those reported for orthotopic grafts (Chartrand *et al.* 1968, 1972; Stinson *et al.* 1972, 1974). The un-

physiological effect of non-synchronization between the systolic-diastolic cycles of the host heart and the graft was made probable for the heterotopic heart (page 17). The unphysiological conditions of the coronary circulation of the graft, as well as its aortic insufficiency, subsequent ventricular enlargement, and obstruction of the venous outlet by thrombosis, were also demonstrated (see page 77 to 81).

The adverse effect of depriving the heterotopic graft of the normal preload and afterload conditions needs no further explanation (page 16), especially where the unfavorable influence on the left ventricular function and the coronary circulation is concerned. The consequences of these phenomena are discussed in detail in relation to the rejection process on page 66 etc.

Pathological findings

There are very few descriptions of general autopsy findings in the literature on experimental cardiac transplantation. Under unmodified conditions, acute rejection in orthotopic recipients was characterized by extensive signs of the effects of heart failure expressed in all organ systems, whereas in heterotopic recipients no changes related to acute rejection were found. This again underlines the difference between the two models with respect to the systemic effects of graft rejection, the animal's clinical condition, and survival. The organic lesions in the orthotopic grafts limited the extent of cardiac damage compatible with survival. Disparity between graft function and survival is strikingly demonstrated by the heterotopic recipients.

Graft findings in unmodified dogs

There are numerous reports concerning the pathology of heterotopic canine cardiac allografts, but the descriptions vary so widely that it is difficult to select findings characteristic for unmodified rejection of a heterotopic canine cardiac allograft. The lesions we found in the heterotopic grafts were similar to those described by Rowlands *et al.* (1968) and Semb *et al.* (1971). Other authors, including Leedham *et al.* (1971) and Shumak *et al.* (1970), also con-

cluded that arteritis and infarction necrosis are obvious features of heterotopic grafts, clearly expressed by day 6. Few pathologists have described the features of orthotopic canine cardiac allograft rejection (Kosek *et al.* 1968, 1969, 1971; Léandri Césari 1967, 1969, 1970). Our orthotopic material did not diverge from their findings, rupture of small interstitial vessels followed by wide-spread petechial hemorrhages being the characteristic lesions.. Our semi-quantitative assessment of the different types of lesions may indicate that decrease of function of the orthotopic graft is mainly determined by the rupture of small interstitial vessels. In contrast, the viability of the heterotopic graft is restricted by the occurrence of arteritis and infarction necrosis. All "graft-incapacitating" injuries caused by rejection are more severe and more advanced in the heterotopic grafts than in the orthotopic ones. This again points to the absence of physiological limits to cardiac damage in the heterotopic recipient. Removal of the graft did not affect the recipient at all (see page 82).

Modified rejection

Reports on human organ transplantation have shown the toxic side effects of the immunosuppressive agents in current use, but this subject is neglected in the literature concerning cardiac transplantation in dogs, which concentrates on the pathology of the graft. Our results demonstrate the side effects of azathioprine and prednisone (see pages 48 to 51). In our experiments the number of complications was unacceptably high. These findings underline once again the unfavorable effects of immunosuppressive therapy.

The graft findings in modified recipients were generally similar to the descriptions given in the relevant literature. As already mentioned (page 28), a rejection process has two main components: *parenchymal rejection*, characterized by infiltration of lymphoid cells, rupture of small interstitial blood vessels, and myocytolysis, and *vascular rejection*, comprising changes in large blood vessels characterized by vasculitis, medial necrosis, and such intimal alterations as endothelial proliferation, thrombocyte aggregation, and occlusion of the lumen by thrombi, leading to infarction necrosis in the surrounding muscle tissue. The first of these components is considered to be mainly cell-mediated, the second to be the result of

humoral factors (O'Connel and Mowbray 1973; MacSween *et al.* 1971; Sinclair *et al.* 1972). The results of our immunofluorescence studies support this hypothesis (see pages 86 and 87). As mentioned above, the corticosteroids used in the present study act mainly on the lymphoid cells (cell-mediated immune response) whereas azathioprine predominantly affects the production of humoral antibodies.

The results obtained with immunosuppression show a distinct influence on the first component (cell-mediated rejection), whereas the effect on the second component (humoral rejection) was not strong enough to completely eliminate arteritis. There was no significant influence on the survival of heterotopic heart transplants ($p > 0.05$), but the effect on the orthotopic grafts was distinctly significant ($p < 0.01$). This means that the sensitivity of the heterotopic model for immunosuppressive therapy is lower than that of the orthotopic model. In animals which survived longer, the arteritis acquired a more "chronic" character, partially due to the immunosuppression regimen. The maintenance dose of 3 mg azathioprine and 1 mg prednisone per kg body weight starting on day 12 is relatively low. The phenomenon of "chronic" rejection is also known from the pathology of human heart transplants, the most prominent feature being "graft arteriosclerosis". A comparison between the histological characteristics in human and canine material is given on page 73.

IV.2. A physiological explanation of the differences between orthotopic and heterotopic cardiac allografts

a) Factors related to hemodynamics

The results of our research demonstrate the unphysiological hemodynamics of the heterotopic graft resulting from its position and vascular connections, the tendency of its vascular pedicle to kink and twist, and the thrombotic occlusion of its venous and arterial outlets. The adverse conditions prevailing for the coronary circulation due to aortic insufficiency, left ventricular enlargement, and the abolition of the normal preload and afterload conditions were shown to result in myocardial ischemia. The susceptibility of heterotopic cardiac allograft to ischemia has also been proved by the

finding of myocardial infarctions in rat cardiac isografts five days after transplantation (Ono *et al.* 1967) and by the descriptions given by van Bekkum *et al.* (1969) and Heron (1972) of disseminated areas of fibrosis in the myocardium of their long-surviving rat isografts, as evidence of previous myocardial infarctions.

The patterns of unmodified histological and electrocardiographical rejection differed clearly between heterotopic and orthotopic grafts. *It may be tentatively concluded that this difference is caused by ischemia of the heterotopic graft.* Just how ischemia and rejection together destroy the heterotopic graft is still an open question. Modification of the rejection process reduced the divergence between these patterns in the two types of graft, but was significantly less effective in enhancing survival of heterotopic grafts. The experiments done by Angell *et al.* (1966, 1967, 1968) showed that the heterotopic graft is only suitable for orthotopic studies if reimplantation occurs within four days of intermediate host residence, the graft being kept under vigorous immunosuppressive treatment during this period. On the other hand, orthotopic grafts remained viable for at least seven days without the use of immunosuppression in the primary recipient.

Although we have put forward arguments and facts supporting our thesis that the heterotopic graft suffers from ischemia and therefore shows a different rejection process, other phenomena remain to be explained. These are dealt with in the following sections.

b) Difference in species, immunosuppressive treatment, and supportive techniques during surgery

Marquet, Heystek, Hollander and van Bekkum (1971) compared the results obtained in allogeneic orthotopic and heterotopic heart allografts in rhesus monkeys with respect to survival time and the histopathology of the rejected grafts. In unmodified recipients the mean survival time of heterotopic grafts was 7 days, that of orthotopic grafts 13 days. In monkeys modified with a combination of anti-lymphocyte serum and azathioprine, the mean survival time of heterotopic grafts (10) was 48 days and that of orthotopic grafts (11) 45 days. These differences are minimal, as the authors state, but no mention is made of the pathology of the unmodified grafts. In the

modified animals, the incidence of arteritis amounted to 100 per cent in the heterotopic and 60 per cent in the orthotopic grafts; for infarction necrosis these percentages were 90 and nil, respectively. The only distinct divergence between these results and those obtained in our dogs is the survival time in the modified grafts, which did not differ significantly between the orthotopic and heterotopic grafts in the monkeys.

This dissimilarity might be related to species differences or to differences in the immunosuppressive treatment or other techniques applied. As already mentioned, the literature on immunosuppressive therapy showed wide variations in the results obtained with one regimen in one model, with various regimens in one model, and with various regimens in different models (compare the results of Crosby *et al.* (1969), Leedham *et al.* (1971), Lower *et al.* (1965), and Graham *et al.* (1969).

The orthotopic operations on the rhesus monkeys were performed without extracorporeal circulation but under profound hypothermia, which may be one source of divergence. The importance of this factor cannot be estimated yet. The discrepancies possibly resulting from the priming volume used in the extracorporeal circuit and from the amount of blood used in the required post-operative transfusion as compared with the relatively small amount of blood used during profound hypothermia, will be discussed below.

c) Preservation of the graft before transplantation

Gonzalez Lavin *et al.* and O'Connel *et al.* (1974) discussed the importance of the "clearance of passenger blood cells as an adjunct in decreasing rejection in canine heterotopic heart transplants" and reported improved survival of heterotopic canine cardiac allografts treated in this way. The passenger blood cells in the prospective unmodified grafts were removed by perfusing the transplant with a specially constituted perfusate. The reduction of passenger blood cells amounted to 98 per cent and the mean survival time increased from 7 days in non-perfused to 11 days in the perfused grafts. These findings might suggest that reduction of the immunogenic load can prolong survival.

We did not use these methods in our experiments. In the ortho-

topic situation there is rapid clearance of passenger cells from the graft by the recipient's circulation and the immunogeneic load is diluted in the extracorporeal circuit, partially removed by the post-operative blood loss, and further diluted by the post-operative blood transfusion given to compensate for this loss.

In the above-mentioned study immunosuppressive treatment (azathioprine 3.5-7 mg/kg) did not result in divergence between the survival times of perfused and non-perfused animals. Histological investigation of unmodified perfused grafts showed "some" reduction of cellular infiltration as compared with unmodified non-perfused grafts. No reference is made to a statistical analysis of their results.

Clearance of passenger cells was performed in the experiments of Marquet *et al.* referred to in connection with species differences. This procedure may have contributed to the divergence between their results and ours. Although passenger-cell clearance is supposed to occur in the orthotopic experiments, in our opinion this factor does not play a role of any importance. The results of series III, in which this supposed clearance was present in the same way as in the unmodified orthotopic experiments, did not differ from those obtained in the unmodified heterotopic grafts of series I.

d) Administration of blood during experiments

The administration of large aliquots of blood via extra-corporeal circulation can have immunological sequelae. Shumway obtained longer graft survival times in human cardiac transplant recipients who had previously been on extracorporeal circulation (Cachera *et al.* 1974). In rats receiving kidney allografts, Tinbergen (1971) demonstrated the tolerance-inducing effect of pretreatment of recipients with donor blood.

The influence of this factor was investigated in series III, and the results show that there were no significant differences when unmodified heterotopic grafts were treated with blood transfusion as in the orthotopic experiments. The induction of tolerance probably only occurs when donor blood is used and a certain interval (1 to 2 weeks) must elapse to permit this mechanism to reach peak activity.

IV.3. Conclusions as to the value of the two heart transplantation models

The present study yielded indirect evidence concerning susceptibility of the heterotopic graft to ischemia. Direct evidence can only be obtained from comparative studies on oxygen tension in myocardial tissues of heterotopic and orthotopic isografts. The chance that such experiments will be performed in the near future seems rather remote, however, since an animal in which isologous orthotopic heart transplantation can be carried out with satisfactory results is not yet available. Consequently, for the present the evaluation of the two heart transplantation models must be based on the findings reported here.

The differences we found between the heterotopic and orthotopic models are in all probability determined by the hemodynamic properties of the heterotopic transplant, as shown by the results of catheterization studies, the occurrence of thrombi, and the observed bradycardia, all of which may be considered to alter the histological rejection process. It is our conclusion that, owing to hemodynamic differences leading to ischemia, the heterotopic cardiac allograft develops a specific rejection process characterized by the predominance of arteritis and infarction necrosis, as judged on the basis of histological criteria. This rejection pattern is mainly determined by vascular factors (see page 47, 58 and 59), and is the result of humoral rejection mechanisms on which immunosuppressive drugs in the dosages used in this study have a very limited influence.

The divergent physiological behavior, the histological rejection pattern, and the weak response to immunosuppressive therapy, all mean that the heterotopic model is not always recommendable for fundamental research in the field of heart transplantation, at least not in dogs. This model may, however, have some value for the investigation of rejection arteritis and the suppression of this phenomenon.

With respect to the value of the orthotopic heart transplantation model, it may be said that this type of graft offers a more satisfactory model for prospective human transplantation. It also serves for the training of cardiac surgeons in the techniques applied in this surgical procedure. Further investigations are needed to overcome the problem of rejection. The shortcomings of the ortho-

topic model with respect to the problems of rejection are clearly illustrated by the results of heart transplantation in man. (See also next section.)

IV.4. Some aspects of human cardiac transplantation

The increasing success of orthotopic experimental cardiac transplantation led Hardy and his associates to apply this procedure in man. In 1964, a chimpanzee heart was implanted in a 68-year-old patient about to die from myocardial ischemic disease. The graft was unable to handle the patient's circulatory workload after implantation, and the attempt was abandoned about an hour after removal of the bypass catheters (Hardy *et al.* 1964, 1969).

The first human cardiac allotransplantation was performed by Barnard (1967) and had a snowball effect in many parts of the world, the number of operations increasing rapidly in the period between 1968 and 1970. The interested reader is referred to the numerous reports from Capetown (Barnard *et al.*), Stanford-Richmond (Shumway and Lower *et al.*), and Houston (Cooley *et al.* and de Bakey *et al.*) published between 1968 and 1973 and to the review on heart transplantation in man by Haller *et al.* (1969).

Dong *et al.* (1972), Clark *et al.* (1973), and Griepp *et al.* (1973), all of the Stanford group, described the state of and the perspectives for human cardiac transplantation at that time. Their experience includes the follow-up of 45 patients, of whom 46 per cent survived up to 1 year, 36 per cent up to 2 years, and 18 per cent up to 3 years. Initially, their one-year survival rate was 22 per cent, but in 1972 this percentage increased to 50.

Up to August 1975, 90 heart transplantations were performed by the Stanford team. Thirty of these patients are still alive. Survival was as follows: 1 year, 47 per cent; 2 years, 37 per cent; 3 years, 27 per cent; 4 years, 24 per cent; and 5 years 20 per cent (Schroeder *et al.* 1976). In this series the main indication for transplantation was end-stage myocardial disease due primarily to ischemia; other indications included rheumatic multivalvular disease and irreversible myocarditis or cardiomyopathy.

Up to August of 1975, 277 human heart transplantations had been carried out all over the world by 64 transplantation teams. At

that time, 49 recipients were alive. The longest survival at that time was more than 6.8 years (Bergan 1975).

In addition to logistic problems (selection of donors and recipients, tissue typing) and medical problems of an ethical nature (determination of death of the donor, the need for life-long immunosuppressive therapy in the recipient, the uncertainty of the long-term results, and the extreme dependence of the recipient on his transplantation center), the most important impediment to success is *rejection*. However, the autopsy findings in deceased patients show also the importance of the many complications associated with immunosuppressive therapy. In the 90 patients described by Schroeder *et al.* (1976), there were 75 bacterial infections, 33 fungal infections, and 21 cases of other forms of lung infection. These data are mentioned under "Other aspects of the early postoperative period".

Chronic rejection

The major cause of late death after human cardiac transplantation is the development of occlusive lesions in the coronary arteries, a process which has been called chronic rejection (Lower *et al.* 1976) and graft arteriosclerosis (Kosek *et al.* 1971). This process also occurs in renal and liver grafts that survive longer than 3 to 6 months after transplantation, but it has a distinctly higher incidence in the long survivors of cardiac transplantation. The changes in the epicardial vessels of these patients are more severe than those in the intramyocardial vessels (Lower *et al.* 1976). Accelerated coronary arteriosclerosis has been reported to develop in the transplanted heart from 18 months to several years after cardiac transplantation. The etiology of this process is poorly understood, but it is thought to result from acute rejection episodes (Schroeder *et al.* 1976).

Chronic rejection has been ascribed to the action of circulating immune complexes. O'Connell and Mowbray (1973) and many others adhere to this theory, whereas Kosek *et al.* (1971) put forward a myointimal hypoxemia theory (see page 28). Both mechanisms probably participate in the production of vessel-wall injuries in chronic rejection. The lesions begin as a proliferation of fibro-cellular elements in the intimal layer, possibly caused by chronic immunological injury to the endothelium.

Kosek *et al.* (1971) compared the histological patterns of chronic rejection in 150 canine heart allografts with those in human recipients. Since the arterial lesions proved to be quite similar in man and the dog, a unifying hypothesis might explain these similarities.

The morphological descriptions concerning clinical heart transplantations performed in the Stanford University Medical Center (Bieber *et al.* 1969, 1970) closely resemble the observations made in dogs, as do the morphological findings in human cardiac allografts reported by other cardiac transplantation centers, e.g. in Houston (Milam and Cooley *et al.* 1970) and Capetown (Barnard, reported by Thompson 1969). The same vascular lesions also develop in patients given a graft because of cardiomyopathy without the special risk factors for coronary artery disease, which provides further evidence supporting the view that the underlying basis is primarily immunologic. Neither immunosuppression nor such measures as anticoagulation, "anti-platelet" therapy, lipid-lowering drugs, and special diets significantly reduce the incidence and severity of these lesions (Lower *et al.* 1976).

When the problem of rejection is finally solved, this treatment for heart diseases in a terminal stage will deserve renewed interest.

The title page of this volume represents our evaluation of the two experimental heart transplantation models. An ace of hearts is superior to a two of hearts, but only when hearts are trump. In heart transplantation surgery, rejection, symbolized by the red background here, is still trump.

APPENDIX

A.1. Supplementary studies in heterotopic experiments

a. Catheterization of the host heart and graft

These investigations were performed to supplement the sparse information in the literature, which is restricted to the hemodynamic performance of orthotopically transplanted canine cardiac allografts (Chartrand *et al.* 1968, 1972; Stinson *et al.* 1972, 1974). The measurements in question are extremely difficult, and require not only technical skill but also a very well equipped laboratory.

Material

The material was derived from the heterotopic experiments of *Series I* and *Series II* (see Table II.1, page 23).

Unfortunately, not all of the animals in these series could be catheterized due to inadequate facilities and technical equipment. In series I, 4 animals were catheterized on the 4th or 5th post-operative day; in series II, 3 animals were cathetarized on the 6th, 12th, 13th, and 22nd days (animal no. 11 was subjected to the procedure twice (days 6 and 13)).

Procedure

Nembutal was administered in a dose of 30 mg/kg body weight,

and after endotracheal intubation and artificial ventilation had been applied, fluid-filled angiocatheters (e.g. no. 7 or 8F NIH) (NIH = National Institutes of Health) were introduced into the right femoral artery and vein and connected to pressure transducers (Statham P 23 dB and Statham SF 1). Occasionally, a Telco micromanometer catheter (MMC No. 7 or 8) was used.

Measurements

The right ventricular pressure (RVP) and the left ventricular pressure (LVP) of the graft and host heart were measured, as well as the pressure in the aorta and pulmonary artery of the graft before and after intravenous administration of isoproterenol (3 mg in 500 ml 0.9 NaCl) given at a rate of 0.006 mg/min over a 5-min period, which was used to determine the effect on the graft of an inotorpic stimulus. The zero base-line was taken at the mid-chest for the host heart and the mid-abdomen for the transplant. The pressures are given in mm Hg, dp/dt values in mm Hg/sec, and peak Vce (K = 1) in sec^{-1}.

Oxygen saturation was assessed in the ventricles, the anastomosing recipient's vessels, and in the host's ascending aorta. Angiocardiography was performed by injecting 76 per cent Urografine® with a Reynolds injection device, 10 to 15 ml of the contrast medium being injected into the anastomosing vessels and the heart cavities of the graft. For non-selective coronary angiography, contrast medium was injected into the sinus valsavae. Cineangiography was performed with an Arriflex camera on Eastman-Kodak RAR 2498-35 mm film, rate: 32 frames/sec. The film was developed according to the standard method, and a Philips (9/5 inch) image intensifier was utilized.

Controls

The values obtained in the host hearts and those of two normal dogs catheterized in the same manner served as controls. During the catheterization, ECG lead II was recorded.

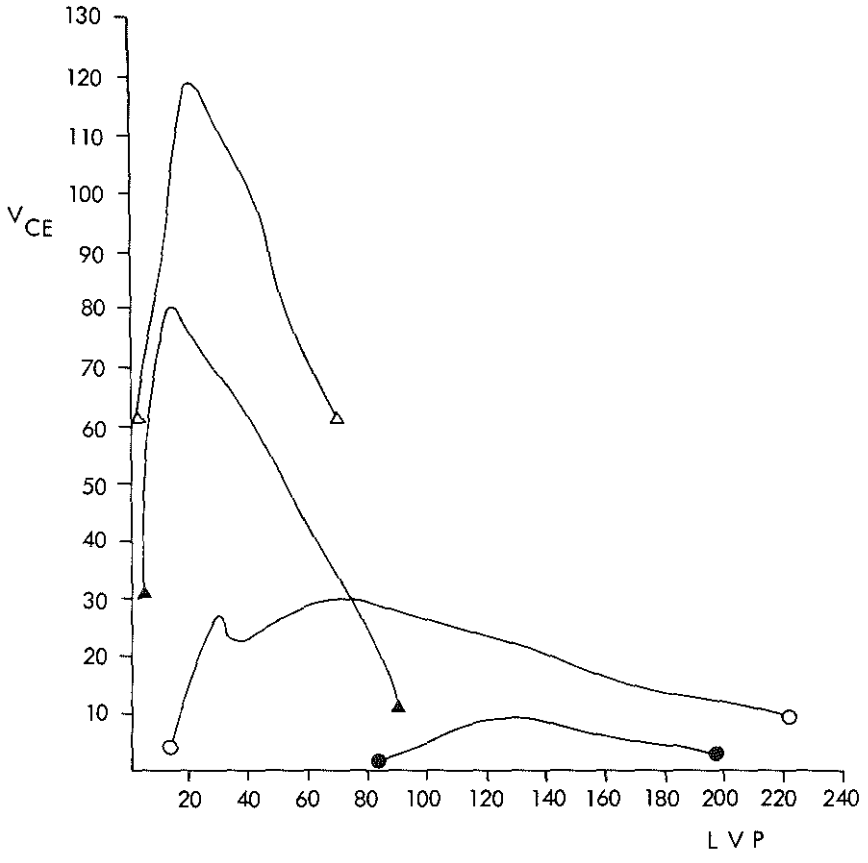


Fig. A.1. Example of the contractility measurements (Animal no. 5, see Table A.1).
● and ○: Graft values before and after isoproterenol administration, respectively.
▲ and △: Values of control dogs before and after isoproterenol administration, respectively.
LVP: left ventricular pressure (in mm Hg).
V_{CE}: $dp/dt \times \frac{1}{p}$ (in sec^{-1}).
The velocity of the contractile elements of the graft is clearly diminished compared with the control values.

Results

Series I

Pressure measurements, contractility studies, and angiograms of the catheterized unmodified heterotopically transplanted animals are shown in Table A.1.

Series II

Pressure measurements, contractility studies, and angiograms of the cathetarized modified heterotopically transplanted animals are shown in Table A.2.

The pressures measured in the host hearts were the same as those of the unmodified host hearts and the controls, and are thereafter not given.

Blood-gas measurements made in all catheterized animals showed on all occasions 95 per cent arterial oxygen saturation in the graft's left ventricle, 98 per cent in the sinus valsavae and left iliac artery, 45 per cent in the right ventricle, and 45-50 per cent in the pulmonary artery and iliac vein.

The graft always developed bradycardia when the animal was placed on its back, and it was invariably necessary to change its position to lateral recumbency to re-establish the previous heart rate.

Conclusions

The paucity of this material and of the data collected, as well as the experimental design used, do not permit statistical analysis. Nevertheless, the following *tentative conclusions* may be drawn:

1) On day 4, "pathological" pressures developed in both ventricles.
2) The velocity of contractile elements of the graft's ventricles was clearly diminished on and after day 4.
3) Under modified conditions, the onset of both forms of deterioration seems to be more gradual.
4) The angiographic picture is characterized by minimal myocardial excursions, a high incidence of aortic insufficiency (Fig. A.

Table A.1.

Catheterization data for Series 1

N	GST	CD	HR	Pressures RVP	LVP	dp/dt	Peak Vce	SV	Angiograms	ME	HC	V	CA
Unmodified heterotopic grafts													
2	7	4	120	25/0-25	110/0-25	–	–	–	LV RV	min min	T O	AI O	O
3	6	5	60	60/48	145/55	500	9.5	140/85	LV RV	min min	O O	O O	O
4	4	4	VF	–	–	–	–	–	–	–	–	–	–
			54	–	220/60-80	1200	9.0	180/150	LV	min	O	AI	–
5	7	5	90		240/12.5	3000	30.0						
Host Hearts													
2			150	25/0	115/0-10			115/75					
3			160	–	140/0			135/80					
5			140	–	190/0			190/160					
Normal control dogs													
			140		140/0	3100	60						
1			180			4800	125						
			120		90/0	2750	80						
2			180		100/0	4400	120						

Abbreviations

N	=	rank number of experimental animal in Series I
GST	=	graft sutvival time (in days)
CD	=	catheterization day
HR	=	heart rate (in beats per minute)
RVP	=	right ventricular pressure (in mm Hg)
LVP	=	left ventricular pressue (in mm Hg)
dp/dt	=	pressure change (in mm Hg per second)
peak Vce	=	$dp/dt \times \frac{1}{p}$ (in sec^{-1})
SV	=	sinus valsalvae
ME	=	myocardial excursions
HC	=	heart cavities (size and configuration)
V	=	valve qualities and function
CA	=	coronary arteries
LV	=	left ventricle
RV	=	right ventricle
Min	=	minimal excursion compared with the normal excursion of the host and control hearts.
T	=	thrombus present
AI	=	aortic insufficiency
O	=	normal findings compared with the controls
–	=	no data obtained
VF	=	ventricular fibrillation
SM	=	slow motion of contrast medium in coronary arteries related to controls.

For animal no. 5 and the normal control dogs, two values are given, the upper one obtained before and the lower one after administration of isoproterenol. The pressures measured in the host hearts and control dogs are the same as those given by Ettinger and Suter (1970) for normal dogs.

Table A.2.

Catheterization data for Series II

Modified heterotopic grafts										
				Pressures			Angiograms			
N	GST	CD	HR	RVP	LVP	SV	ME	HC	V	CA
9	22	22	50	VF	–	–	min	0	–	–
11	13	6	120	–	170/0-5	180	min	0	AI	SM
		13	VF	–	–	–	min	–	–	–
12	12	12	60	100/50	120/0-8	–	Min	0	0	SM

Abbreviations:

N = number of animals in Series II
GST = graft survival time (in days)
CD = catheterization day
RVP = richt ventricular pressure (in mm Hg)
LVP = left ventricular pressure (in mm Hg)
SV = pressure measurements in sinus valsavae
ME = myocardial excursion
HC = heart cavities (size and configuration)
V = valve function
CA = coronary arteries
– = no data obtained
VF = ventricular fibrillation
min = minimal excursion compared with the excursions of the normal heart (see Table A.1: normal control dogs)
0 = normal findings as compared with controls
AI = aortic insufficiency
SM = slow motion of contrast medium in the coronary arteries relative to controls

2), and occasionally thrombus or clot formation in the heart cavities.

5) The graft has a tendency to develop ventricular fibrillation when angiocatheters are introduced into the lumen.

6) The susceptibility of the graft to bradycardia was confirmed (see Fig. III.3, a and b).

Discussion

The main points have already been discussed (see page 63, 64).

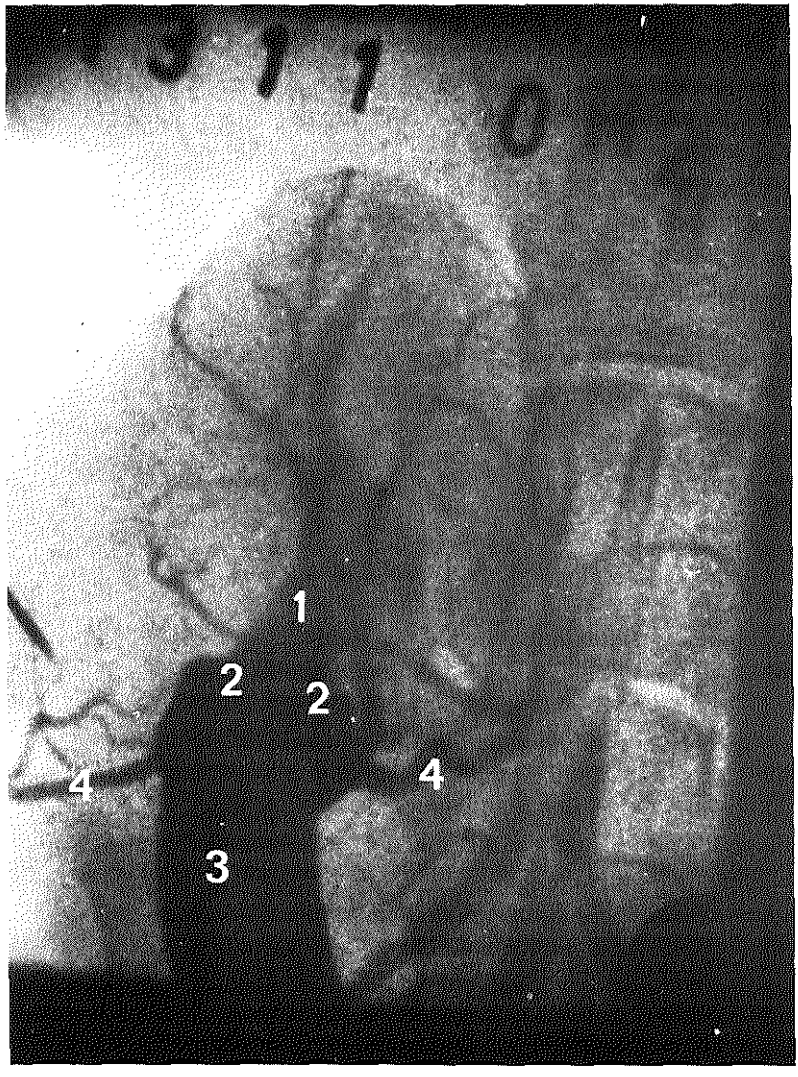


Fig. A.2. Signs of aortic insufficiency in a modified heterotopic graft.
The main branches of the coronary arteries have a normal appearance.
1: regurgitation, 2: aortic valves, 3: aorta, and 4: coronary arteries.

b. Immune myocarditis in the host heart in animals carrying a heterotopic graft

To test this hypothesis, ECG and immunofluorescence studies were performed in unmodified animals. The ECG tracings of the host heart after heterotopic heart transplantation showed changes suggestive of "immune myocarditis". This phenomenon, which was first described by DePasquale *et al.* (1965), comprises sinus arrythmia, T-wave changes, and alterations in the QRS configuration, which these authors attributed to immunological changes in the host heart mediated by organ-specific antibodies produced during the rejection process. Shumak *et al.* (1970) and Goldman *et al.* (1971) demonstrated alterations in both host hearts and grafts on the basis of histological and immunofluorescence investigations.

Our ECG studies done in host hearts also showed some changes after transplantation. To verify the hypothesis concerning host heart immune myocarditis, we performed supplementary ECG studies and immunofluorescence studies in unmodified animals. Modified animals were excluded from the experiments, because of the unpredictable influence of the immunosuppressive treatment.

Material and methods

ECG studies

Daily recordings of the host hearts of all unmodified heterotopic recipients were made, as previously described (page 24). These recordings were compared with those of the controls, which consisted of 9 dogs that had undergone orthotopic small-bowel exchange operations, to rule out the factor of organ transplantation itself, and 9 dogs subjected to bowel strangulation procedures or to tuba reimplantation, to rule out the factor of surgery (abdominal operation, anaesthesia, etc.) (see page 26).

Immunofluorescence studies

The material for these investigations was chosen from among

the unmodified heterotopic animals of series I (nos. 5-10) and from a supplementary unmodified series especially designed for these purposes: *Series IV*[1] (nos. 1-11). These 17 dogs were divided into two groups, group A (9 dogs) and group B (8 dogs) (Table A.3, page 85). The animals of group A were killed when the graft stopped beating. In group B the failing graft was removed, and the recipient was kept alive for a week to eliminate the postulated capacity of the transplant to absorb antibodies as well as to demonstrate any increase in the number of circulating antibodies. At the end of the week the dog was killed and an autopsy was performed. In all cases fresh specimens from the graft and host organs were taken for immunofluorescence investigation. We examined the graft and the host heart for deposits of immunoglobulins and complement, and attempted to determine whether circulating antibodies against the recipient's heart tissue or other tissues could be detected during the week following rejection of the graft. The transverse tissue blocks used for these studies were always taken from the same place in the grafts, host hearts, and controls (Fig. 11.1). The controls were normal heart tissues from 10 blood-donor dogs.

The specificity of the fluorescence was established by a blocking test in which non-conjugated rabbit anti-dog immunoglobulin was added before application of the fluorescent rabbit anti-dog immunoglobulin. The fluorescence was always distinctly diminished. The indirect test was controlled by omitting the unconjugated sera; in these cases only the fluorescent horse anti-rabbit immunoglobulin was applied. These slides were always negative.

The transverse tissue blocks were sealed quickly in small polyethylene bags and snap-frozen in liquid nitrogen prior to storage at -70°C. Control tissues from normal dog and rat hearts were treated similarly. Serial sections (2 μ) were cut on a cryostat at -20°C.

To detect immunoglobulin and complement (B_1C globulin), we used the direct immunofluorescence test. The sections were washed in phosphate-buffered saline (pH 7.2) (0.05 M. NaCl + 0.01 M. phosphate) 3 times for 10 minutes each, incubated with the fluorescent antisera for 30 minutes at room temperature, washed again with phosphate-buffered saline (pH 7.2), mounted in phosphate-buffered

[1] In Series IV the same studies were performed as described in Chapter II.

glycerol (1 part phosphate to 9 parts glycerol; pH 7.8), and sealed with paraffin.

For the detection of albumin, we used the indirect immunofluorescent test, applying first a non-fluorescent rabbit anti-dog albumin antiserum and then a fluorescent horse anti-rabbit immunoglobulin. (The fluorescent rabbit anti-dog B_1C globulin and the rabbit anti-dog albumin were obtained from Nordic Pharmaceuticals and Diagnostics, Tilburg. The Netherlands, and the fluorescent horse anti-rabbit immunoglobulin from the Central Blood Transfusion Service of the Red Cross, Amsterdam).

To determine whether circulating antibodies were present in the recipient's serum during and a week after rejection, sera (5 ml) collected at both times were applied in an indirect test done in normal heart tissue from dogs and rats. These sera were used undiluted and in a 1:4 dilution. The pre-immune sera of the recipients were used as controls to exclude the effect of antibodies occurring naturally in the dog. The intensity of fluorescence was graded as 0, ±, + (i.e., none, moderate, strong).

A Leitz Orthoplan fluorescence microscope was employed. The light source was an Osram HBO 200 W lamp, with epi-illumination and a BG 38-3 mm thick filter, a BG 12-5 mm excitation filter, a K 530 barrier filter, a cardiod dark-field condensor, and a phase-contrast condensor.

Results

ECG studies

The control ECG studies in the control dogs showed the same "changes" (sinus arrythmia, T-wave changes, alterations in the QRS configuration in the same frequency as the host heart ECG after transplantation. The host hearts of group B (Table A.3), in which the graft was removed at arrest and the recipient was kept alive for a week to permit investigation of the effect of a postulated rise of the antibody titers, showed no divergence from the control ECGs. Furthermore, on the basis of the criteria for the normal canine electrocardiogram (Ettinger and Suter 1970), the tracings conformed with the standard canine electrocardiogram.

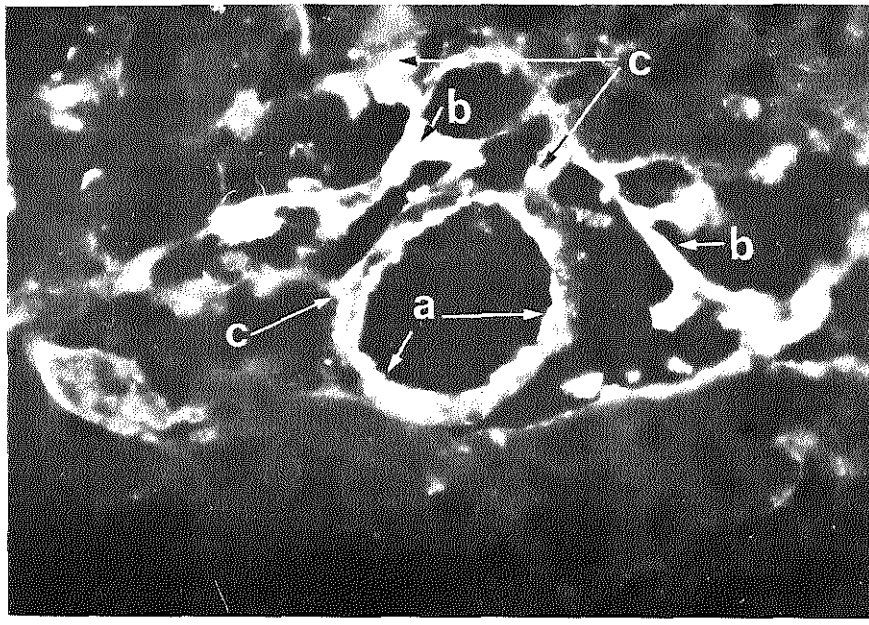


Fig. A.3a. Frozen section from myocardium of heterotopic graft (recipient no. 8, Series I) after incubation with fluorescein-conjugated rabbit anti-dog complement. Deposits of B_1C globulin are present in a small artery (a), an interstitial capillary (b), and in cells (c). x 600.

Conclusion

The electrocardiographic evidence did not support the existence of an immune myocarditis of the recipient's own heart after heterotopic heart transplantation.

Immunofluorescence studies

The results of the immunofluorescence studies and the histological findings are shown in Table A.3. Specimens almost or completely necrotized by infarction necrosis were excluded (nos. I-5, I-9, and III-7), because non-specific immunofluorescence can occur in dead tissue and can hamper the evaluation of arterioles, venules, and capillaries. Special attention was paid to the arteries, i.e., immuno-

Table A.3.

Results obtained in the immunofluorescence and histological studies

		Presence of immunoglobulins and B_1C globulins						Histopathology*						
		GRAFT					HH	GRAFT					HH	
	N	GST	Arter	IN	IC	PC		MNI	RIV	M	Art	IN	Art	MNI
GROUP A**	1-5	7	–	+	–	–	0	320	120	150	250	370	0	0
	1-6	6	0	0	0	0	0	240	100	160	240	200	0	0
	1-7	7	+	0	+	0	0	340	0	180	300	360	0	0
	1-8	11	+	0	+	+	0	210	40	70	300	210	0	0
	1-9	8	–	+	–	–	0	270	70	150	300	300	10	0
	1-10	7	+	0	+	+	0	270	180	130	230	260	0	0
	IV-1	9	0	0	0	+	0	310	150	150	400	400	0	0
	IV-2	9	+	0	±	+	0	250	0	10	370	400	0	0
	IV-3	5	0	0	0	0	0	110	0	0	250	400	0	0
GROUP B***	IV-4	15	+	0	+	±	0	350	70	210	400	370	10	10
	IV-5	9	+	0	+	+	0	400	0	330	370	400	0	0
	IV-6	3	0	0	0	0	0	200	0	150	130	130	10	0
	IV-7	10	–	+	–	–	0	340	0	350	310	320	10	10
	IV-8	3	+	0	0	+	0	100	0	100	110	170	0	10
	IV-9	6	±	0	±	+	0	330	10	200	330	370	0	0
	IV-10	7	+	0	+	+	0	270	20	200	300	300	0	10
	IV-11	8	+	0	±	±	0	300	0	400	300	300	0	10

Abbreviations

N = number of animals in series I and IV (unmodified heterotopic grafts)
GST = graft survival time (in days)
Arter = immunoglobulin deposits in arterial walls
IN = infarction necrosis
IC = interstitial capillaries
PC = positive cells (immunofluorescence of histiocytes and macrophages phagocytosing immunofluorescent immune complexes)
0 = no immunoglobulin or B_1C globulins
± = moderate amount of immunoglobulin or B_1C globulins
+ = large amount of immunoglobulin or B_1C globulins
HH = host hearts (no lesions detectable by the immunofluorescence methods used)
MNI = mononuclear cell infiltration
RIV = rupture of small interstitial vessels
M = myocytolysis
Art = arteritis
IN = infarction necrosis

* graded as described on page 29
** Group A: animals killed at arrest of the graft
*** Group B: animals killed one week after removal of the graft

globulin deposits in arterial walls (Arter), immunofluorescence in interstitial capillaries (IC) and positive cells (PC), i.e., histiocytes and macrophages that exhibited a granular positive immunofluorescence for IgG and complement, which is thought to indicate the presence of phagocytosed immune complexes in the cells. Histological investi-

gation of the same sections showed that these cells contained eosinophilic material. Such positive cells have also been found in the walls of small arteries of other organ allografts during rejection (Lubbe and de Vries 1972).

The immunological origin of the arteritis in the graft was supported by the absence of albumin deposits in the damaged arteries. The small albumin molecules, which are highly soluble, could escape through the damaged vessel wall, the larger immunoglobulin molecules could not pass that easily. In 10 cases immunoglobulin deposits were found in the media and intima of small arteries in the graft and proved to correspond with the histological findings of arteritis (Fig. A.3b). When complement (B_1C globulin) was found, we also found immunoglobulins in the same areas, which indicates that we were dealing with immunocomplexes.

Animals I-6 and III-6 did not show these deposits, although there was significant arteritis in the histological sections. In inter-

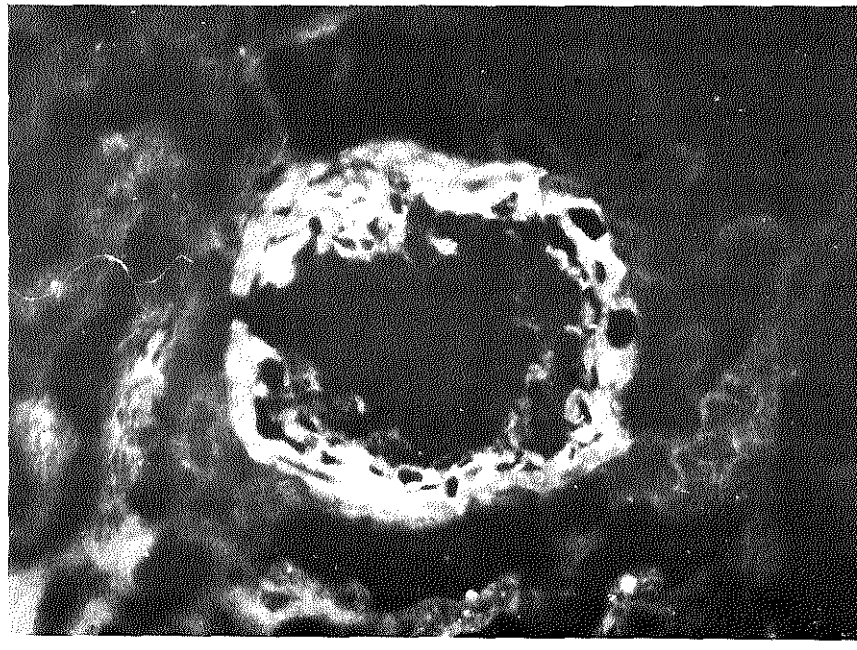


Fig. A.3b. Frozen section from myocardium of heterotopic graft (recipient no. 1, Series III) showing deposits of B_1C globulin in media and intima of a small artery. x 800.

stitial capillaries, deposits were found in 9 out of 14 cases. This feature did not always show correlation with histologically demonstrated rupture of small interstitial vessels. No signs of immunoglobulin and complement deposition were found in any of the host hearts. Some minor histological changes observed in the histological sections probably had no immunological significance.

Since the absence of immunofluorescence in the host heart made it highly unlikely that deposits would be found in other organs, the sections of these organs were left out of further consideration.

Controls

The control heart tissues were invariably negative. When graft tissue was pre-treated with unconjugated rabbit anti-dog complement and then with a fluorescent rabbit anti-dog complement, the fluorescence was always strongly diminished, and the same was observed when graft tissues were pre-treated with unconjugated rabbit anti-dog immunoglobulin followed by a conjugated rabbit anti-dog immunoglobulin (blocking test). When conjugated antisera were absorbed with their specific antigens, no fluorescence was seen. The canine sera sampled during and a week after rejection of the graft did not show any antibodies against dog and rat heart tissues. These findings are consistent with the absence of immunoglobulins and complement deposits in the host heart.

For a positive control, we prepared a rabbit anti-dog heart serum. When normal heart tissues were incubated first with this antiserum and then with fluorescent horse anti-rabbit serum, the sarcolemma showed linear fluorescence.

Conclusions

1) The positive correllation found between the immunofluorescence and the histological findings shows that the rejection process is immunological in nature.
2) The theory concerning immune myocarditis of the host heart could not be confirmed.

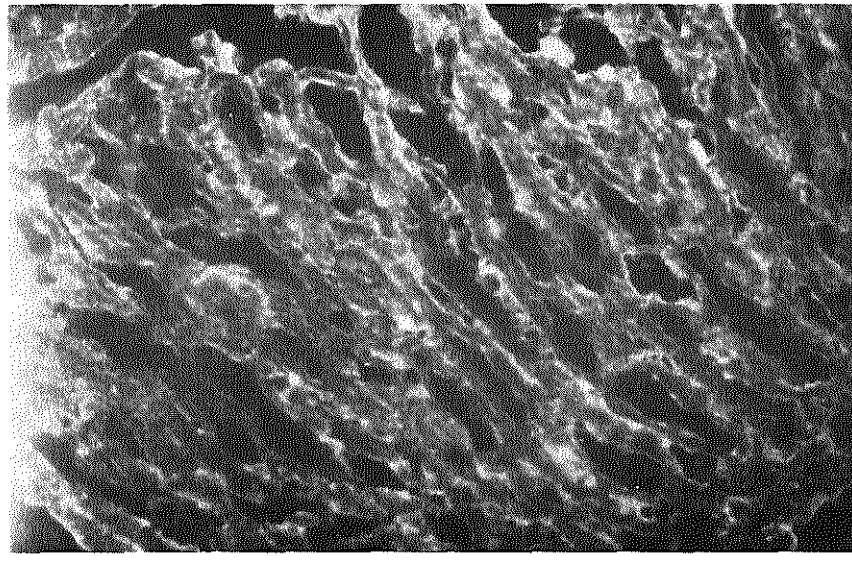


Fig. A.3c. Frozen section from control (myocardium of normal dog, incubated first with rabbit anti-dog heart serum and then with fluorescent horse anti-rabbit serum) showing linear sarcolemnal fluorescence. x 800.

Discussion

ECG studies

The misconception of DePasquale *et al.* concerning the immune myocarditis in the host heart can be explained by the fact that they applied criteria for the human ECG and its pathology to the canine ECG.

Immunofluorescence

To the best of our knowledge, only a few experimental studies have been done in heterotopic cardiac allotransplants to investigate the immunofluorescence features of rejection. Our results are similar to those of MacSween *et al.* (1971) and Sinclair *et al.* (1972), who used immunofluorescent techniques to study heterotopic rat cardiac allografts. Deposits of immunoglobulin and complement were found

on day 5 or later, when signs of arteritis also appeared.

According to Goldman *et al.* (1971), distinct linear sarcolemmal fluorescence demonstrates the deposition of immunoglobulin and complement in small capillaries. We could only obtain similar results by applying a rabbit anti-dog heart muscle fiber serum to normal dog heart tissue (Fig. A.3c), which gives a completely different picture from that of deposits in small arteries (Fig. A.3b) or of complement and immunoglobulin occurring between the myocardial fibers in small capillaries and immunocomplexes phagocytosed by histiocytes and macrophages, i.e. the so-called positive cells (Fig. A.3a). We consider Fig. A.3a and b to represent the immunofluorescent patterns of heterotopic cardiac allograft rejection, in agreement with similar findings made by MacSween *et al.* (1971) and Sinclair *et al.* (1972). We therefore assume the concept of Goldman *et al.* to be incorrect.

Like MacSween, we were unable to demonstrate in the host heart the immunological myocarditis described by Goldman *et al.* (1971) and Shumack *et al.* (1970). One difficulty here is that these authors give no data on the pre-operative immune status of their animals, and in addition the immunosuppressive agent used in their experiments was anti-dog lymphocyte serum, which is known to produce a non-specific myocarditis.

A.2. Surgical procedures used in the present study

Heterotopic transplantation technique

The heterotopic transplantation technique used in the experiments referred to here is the method shown in Fig. A.9. The right iliac fossa of the abdominal cavity was considered to be a more physiological environment and was thought to give better protection than the cervical pouch used in the cervical technique. The average weight of the donor dogs amounted to 10 kg, that of the recipients to 20 kg.

Pre-operative treatment

Food and water were withdrawn 12 hours before the operation,

and the animals were given an intramuscular injection of 1 million units of penicillin and 1 gram of streptomycin. ECG tracings were recorded for both donor and recipient.

Anesthesia

Nembutal (30 mg/kg body weight) was administered intravenously, followed by endotracheal intubation and artificial ventilation.

Preparation of the recipient

Via an inferior median laparotomy, the right iliac vessels were exposed from the aortic bifurcation to Poupart's ligament. Snares of umbilical tape, which could be closed by a tourniquet, were placed around the common iliac artery and vein just proximal and distal to the intended arteriotomy and venotomy. Collateral vessels in these areas were similarly occluded.

Preparation of the donor

After the induction of anesthesia, heparin (4 mg/kg body weight) was administered intravenously. The thorax was opened by a lateral incision in the 4th left intercostal space. The pericardial sac was partially removed and the heart pulled upward. The inferior caval vein and superior caval vein were divided 1 cm beyond the pericardial reflection. The pulmonary veins were divided, preserving a cuff of at least 5 mm on the cardiac side. The aorta and pulmonary artery were then cut approximately 1.5 cm above the aortic and pulmonary annuli.

The graft was washed and cooled in 0.9 per cent saline held at 4°C. The lung veins and caval veins were closed by ligation. Special care was taken not to injure the sino auricular node. Since peri-aortal and peri-pulmonary fatty tissue made it difficult to establish vascular anastomoses, it was carefully removed. The mean cold ischemic time amounted to 15 minutes.

Transplantation procedure

After closure of the tourniquets on the right iliac vein, venotomy and end-to-side anastomosis of the pulmonary artery and the iliac vein were carried out. The tourniquets on the arterial side were then closed and arteriotomy and end-to-side anastomosis of the aorta and the iliac artery were performed. Intermittent flushing with heparin solution was applied during suturing. All anastomoses were made by continuous suture, the venous anastomosis with 5x0 silk, the arterial with 4x0 silk. The venous anastomosis was always made a little proximal to the arterial one. Meanwhile, the graft was cooled uninterruptedly by bathing it in 0.9 per cent saline (4°C). The mean "warm" ischemic time was 45 minutes (range: 30-60 minutes). The size of the anastomoses was estimated from the diameter of the vessels in question, and was on average 1.5 cm.

Resuscitation of the graft

Gradual release of the tourniquets was used to regulate the blood flow to the graft. Air was aspirated from the ventricles. When strong fibrillation occurred, the graft was defibrillated. In some cases contractions started spontaneously, which made defibrillation unnecessary.

Distension of the ventricles had to be avoided by all possible means. When it threatened to occur, the arterial blood supply to the graft was reduced by reclosing one or both arterial tourniquets and by applying delicate massage to the ventricles, sometimes combined with the intraventricular injection of 1 to 2 ml 10 per cent calcium chloride.

After stable heart action had been established and hemostasis was complete, an epicardial electrode was inserted in the atrial wall near the sino auricular node, the wire being passed through the abdominal wall and brought subcutaneously to the back of the neck, where it was exteriorized to prevent damage by biting by the recipient. The wire was used to record epicardial ECGs.

In the last phase of the operation, the abdominal incision was closed.

As early as 1933, Mann and his co-workers discussed the prob-

lems associated with the heterotopic heart transplantation (see p. 95). Although a number of later authors advocate the creation of an atrial septum defect to prevent distension of the left ventricle (Largiader *et al.* 1969; Semb 1971; Shumak *et al.* 1970), according to Mann this does not prevent distension. An atrial septum defect was not made in our experiments, where distension of the left ventricle was prevented by manipulation of the tourniquets and the other measures described above. These methods were almost invariably effective during resuscitation of the graft in our experiments.

Orthotopic transplantation technique

The technique we used for orthotopic transplantation was that of Lower *et al.* (1961). The average weight of the donors and recipients was 20 kg.

The pre-operative management of both dogs was the same as for the heterotopic transplantation. In general, two dogs weighing 20 kg were used as blood donors. Briefly, the procedure was as follows. After heparinization (2 mg/kg body weight), cannulas were inserted into the recipient (venous canulas via the right external jugular vein and the left femoral vein, the arterial canula via the left femoral artery) and connected to an extracorporeal circuit consisting of a Dreissen roller pump with 3/8x5/8 latex tubing and a Temptrol (Q 110) oxygenator. Tubing and blood reservoir were primed with fresh heparinized donor blood (1 ml heparin per 1000 ml blood). The priming was adjusted to the required flow rate (50 ml/kg body weight/minute). The graft was taken from the donor in the same way as for the heterotopic operation, and was then washed, cooled, and prepared for implantation in the recipient.

Meanwhile, the heart-lung bypass was started in the recipient. The animal's temperature was lowered to 30°C, and the bypass was made complete. The aorta and pulmonary artery were cross-clamped, the heart excised, and the graft inserted. The left ventricle was vented and air was removed from the heart cavities. Coronary perfusion was re-established by opening the cross-clamp. Air was aspirated from the left ventricular apex. A Lephophed® drip (1 microgram per minute on average) was started and pulmonary ventilation resumed. When strong fibrillations developed, the graft was

defibrillated. The animal's temperature was brought up to 35°C, and the circulatory volume adjusted. When the graft had taken over the workload of the recipient's circulation and hemostasis seemed adequate, the heart-lung bypass was gradually withdrawn and protamine sulphate was administered (approximately 2 mg/kg body weight). The thorax was drained, the thoracotomy incision closed, and air aspirated. The animal was then turned on its left side. When a stable circulatory condition was obtained, ventilation was stopped. The chest was carefully cleared of blood, the blood loss made up, and the circulatory volume adjusted to central venous and arterial pressures. Extubation was performed when the dog was sufficiently awake. Lastly, the Lephophed infusion was gradually diminished and then terminated.

For the reader interested in the problems encountered in experimental orthotopic transplantation, reference is made to Hardy *et al.* (1966).

Experimental design of Series III

After heparinization of the recipient (2 mg/kg body weight) cannulas were inserted into both the left femoral artery and vein. For transfusion, use was made of fresh heparinized donor blood (1 ml heparin per 1000 ml blood) taken from two unrelated mongrel dogs (volume 1500 ml, the same volume on average as in the orthotopic experiments). The transplantation procedure was similar to that described under *Heterotopic transplantation technique* (page 89). Blood transfusion, given with a roller pump via the left femoral vein, was started at the moment of closure of the tourniquets on the right iliac vein. The blood transfusion rate was 750 ml per hour; the withdrawal rate from the arterial cannula was the same. When 1500 ml was given, the blood balance was adjusted to 0 and protamine sulphate was administered (approximately 2 mg/kg body weight). In general, the operation was then terminated. Decanulation was performed when the dog was awake and in a stable circulatory condition.

Post-operative management

Special attention was paid to post-operative bleeding, which was the main cause of failure (aortic suture line) in the orthotopic series. As soon as thoracic blood drainage stopped, the chest drain was removed. On the first post-operative day the animals were allowed to drink, and on the second day they received food. Routine laboratory tests were performed twice a week, and ECG tracings were taken daily in a standardized manner. The body temperature was controlled, and the uptake of water and excretion of urine were measured.

During the first five days, antibiotics were administered intramuscularly: on day 0: 3 doses of 1 million units penicillin and 0.5 g streptomycin; on days 1 to 6: 2 doses of 1 million units penicillin and 1 dose of 0.5 g streptomycin daily. When the immediated post-operative course was uneventful, the animals were awake and active 3-4 hours after the operation.

A.3. Historical review of cardiac transplantation research

Heterotopic hearts

The cervical transplantation technique

The existence of two heart transplantation models is partially explained by the history of this field of research. The annals of experimental cardiac transplantation commence in the year 1905, when Carrel and Guthrie implanted the heart of a small dog in the neck of a larger one. Fig. A.4 shows the vascular anastomoses they used. Strong fibrillations occurred in the transplanted heart after re-establishment of the circulation, and ventricular contractions started one hour after the operation. "Owing to the fact that the operation was made without aseptic technic, coagulation occurred in the cavities of the heart after about two hours, and the experiment was interrupted" (Carrel 1907).

Man *et al.* (1933) simplified the cervical transplantation in dogs by employing only two vascular anastomoses, as shown in Fig. A.5. These authors gave essential information about pitfalls and errors related to the performance of the heterotopic operation and their management. They discussed the friability of the ascending aorta in the dog and the resulting tendency for bleeding to develop along the aortic suture line; the need for attention to the correct positioning of aortic and pulmonary anastomoses to avoid kinking or twisting of the vascular pedicle; the prevention of air embolism in the coronary arteries by filling these vessels from the venous side; the deleterious effect of distension of the left ventricle and how to avoid it by maintaining a low arterial pressure in the graft's aorta, together with adequate venous drainage by opening up the venous discharge until the heartbeat becomes regular; and the establishment of cardiac tonus to prevent valvular insufficiency. Other measures to stop ventricular distension, such as making an anastomosis between the caval

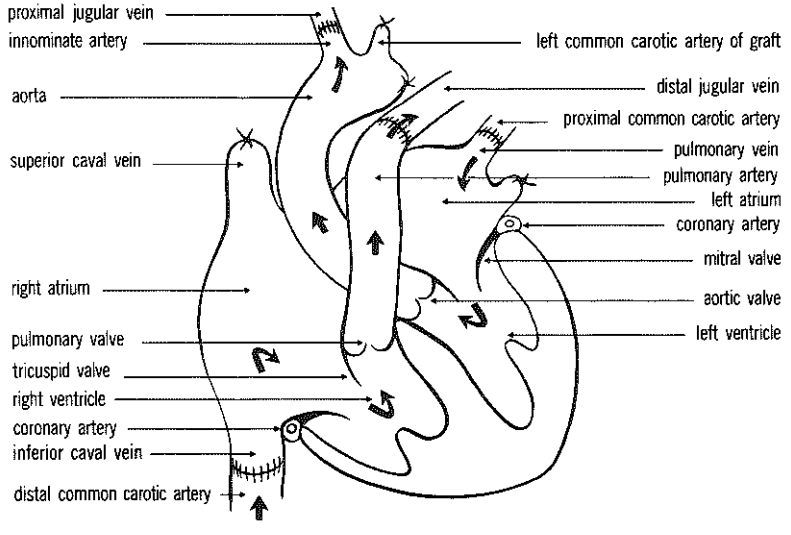


Fig. A.4. The operation used in a dog by Carrel and Guthrie (1906-1907). The arrows indicate the direction of the bloodflow.

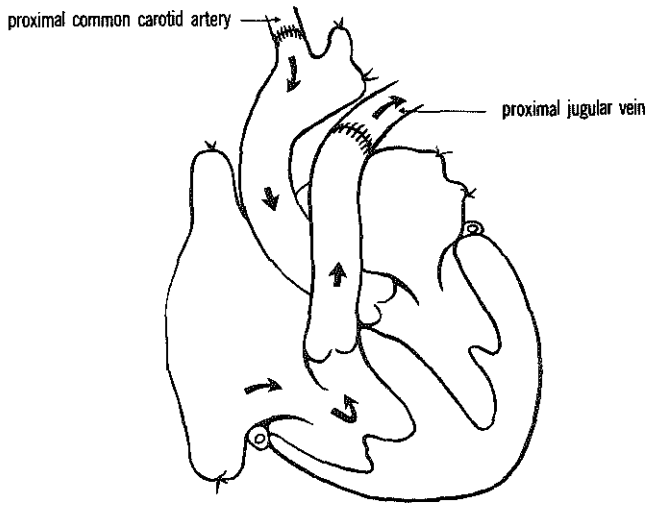


Fig. A.5. The method for heterotopic heart transplantation in dogs used by Mann *et al.* (1933).

vein and pulmonary artery or between the caval vein and jugular vein or the creation of an atrial septal defect, were considered useless. When the coronary circulation was re-established in these experiments, the grafts started to contract at a rate of 100-130 beats per minute. Pharmacological studies to determine the effect of thyroxine on a denervated heart were undertaken.

The mean survival time in these experiments amounted to 4 days (range: 1 to 8 days). The histopathology of the rejected grafts showed infiltration of myocardium by mononuclear and polymorphonuclear cells. Clots were present in the heart cavities. Electrocardiographic manifestations of cardiac allograft rejection were first described by these authors. Unfortunately, the exact number of their experiments is not mentioned. Graft failure was attributed to: ". . . some biological factor which is probably identical to that which prevents survival of other homotransplanted tissues and organs."

The Mann technique and modifications of it have been used by many investigators. Downie *et al.* (1953) described 30 experiments in dogs in which Mann's technique was used and 23 survivals were obtained. The mean graft survival time was 5 days; in two animals the graft survived for 10 days. It was concluded that small donor hearts continue to beat longer than larger ones. The improvement on Mann's results with respect to graft survival time were ascribed to a better vascular operation technique and to the use of antibiotics. In 5 experiments the recipient received blood from the prospective donor before the operation. A "longer survival time" was established.

A report on the pattern of failure of the homografted canine heart was published by Wesolowski *et al.* in 1953, who had used the Mann technique in 11 experiments. A mean survival time of 4.5 days was obtained. The ECG of the graft, which was compared with the tracing of lead II of the host heart, showed a voltage decline in the QRS complex of the graft. The authors ascribed this phenomenon to fibrin adhesions and accumulation of fluid around the graft. When the "debris" was removed, the QRS complex of the graft recovered.

Macroscopical examination of the grafts showed fibrin adhesions and petechial bleeding in the epicardium after day 2 or 3. On days 4, 5, and 6, areas of liquefaction and bleeding in the myocardium, dilation of the left ventricle, and thrombi in both atria were observed. Complete degeneration of myocardial tissue was seen on day 6.

Microscopical examination showed extensive mononuclear cell infiltration after day 3, consisting of histiocytes and polymorphonuclear cells, accumulating around small interstitial vessels. The myocardial fibers became swollen and lost their normal structure. Marked edema was present between the myocardial fibers, and congestion of capillaries developed. On day 6, two-thirds of the myocardial tissue was devitalized. Between day 7 and day 10, degeneration and liquefaction became complete.

One graft was covered with a plastic bag to investigate the influence of local and humoral factors. This graft showed a rejection pattern similar to that of the others, supporting the view that rejection is mediated by the circulation.

Sayegh *et al.* (1957) used the Mann technique in 31 dogs in which 21 neonatal and 10 fetal hearts were implanted. The graft survival time amounted to 3 days in 67 per cent of the cases and from 7 to 10 days in the rest. The failures were ascribed to thrombotic occlusion of the anastomoses and distension of the left ventricle. In this series the ECG studies included classification of electrocardiographic manifestations of rejection: ST depression, change in PQ interval, T-wave inversion, broadening of the Q wave, and voltage decline were considered signs of ischemia. Mechanical failure was associated with ST depression, the occurrence of an R wave and a biphasic T wave, broadening of the T wave, lowering of the voltage, and ST elevation. Their concept of reactive failure was vaguely defined and based on one experiment.

Histopathological investigation of the grafts showed fibrin adhesions, occasional thrombi in the pulmonary anastomoses, and total necrosis. Grafts surviving more than 30 hours showed infarction, extensive infiltration by polymorphonuclear cells, subepicardial bleeding, and separation of myocardial fibers due to edema. The supposition that neonatal and fetal heart allografts are less antigenic than grafts from older donor dogs proved to be wrong.

Lee Sae Soon *et al.* (1959) used the Mann method in metabolic studies under normo- and hypothermia, and Barsamian *et al.* (1960) "supercooled" dog hearts and transplanted them according to Mann's technique. Jin (1960) reported about 29 experiments in dogs with the Mann model, the mean survival time amounting to 1 day. He recommended the use of end-to-side anastomoses between graft and recipient. The main causes of failure in his experiments were thromboses on and bleeding from suture lines and distension of the graft. His ECG and histological findings do not include any essentially new information.

Reemtsma *et al.* (1960) used the Mann model for serial studies on myocardial blood flow, oxygen consumption, and carbohydrate metabolism in dogs. In 27 experiments, 3 grafts survived more than 4 days. No definite conclusions could be drawn concerning oxygen and glucose consumption. The lactate measurements suggested an impaired lactate metabolism. The large deviations in the results of the measurements were attributed to the significant occurrence of aortic valve incompetence in the graft.

Bing *et al.* (1961) described metabolic, histological, and histiochemical aspects of homografted hearts in dogs (Mann's technique), but without exact data. The longest reported survival time is 47 days. Active RNA synthesis in the cells of the graft was observed during rejection. Pathological phenomena were classified on the basis of two processes: granulomatous myocarditis comparable with the proliferative phase of rheumatic myocarditis and myocardial necrosis occurring on day 7 or 8. Chiba *et al.* (1962), of the same group, published studies on the metabolism and histology of the transplanted heart. Mann's technique was utilized, and a second-set reaction was induced by an injection of spleen cells from the prospective donor into the recipient 5-7 days prior to transplantation. Sequential transcutaneous needle biopsies were preformed to obtain material from the graft for histo-

logical investigation. Three hours after transplantation a "granulomatous infiltration" was observed, with swelling of the arteriolar intima. Five hours later plasma cells, macrophages, and histiocytes, and 19 hours later Anitschkow and Aschoff cells, were present. An "interstitial myocarditis" with infiltration of lymphocytes and plasma cells was noted after 21 hours. This picture persisted until day 8, when an intensification of the granulomatous myocarditis occurred, together with necrosis of myocardial fibers and an increase of arteriolar intimal swelling. The metabolic studies showed an elevation of the initial pyruvate, lactate, malic dehydrogenase, and aldolase levels. Numerical data are not given in this report.

Reemtsma *et al.* (1962) were the first to use a folic antagonist in cardiac transplantation research to improve the survival time. The Mann technique was employed in 21 experiments in dogs. Amethopterine was administered in an oral dose of 0.1-0.2 mg per kg body weight every other day or three times a week. The mean survival time was 10 days, the maximum 26 days. Animals suffering from side effects of the drug showed malaise, anorexia, and intestinal hemorrhage with diarrhea. Drug intoxication had a deleterious influence on survival time.

Ramos, Bing, Chiba *et al.* (1963) published a paper entitled *The presence of humoral factors in the homograft rejection of transplanted dog hearts*, based on the use of the Mann model in 45 experiments. The recipient was sensitized with spleen cells of the prospective donor prior to transplantation. Radioactive albumin was injected into the graft's sinus valsavae and its distribution in the transplant was determined. An enhanced vascular permeability found one hour after transplantation was considered to demonstrate the importance of humoral factors in "homograft rejection".

Rams *et al.* (1964) performed histochemical and electrolyte studies in 12 experiments in dogs (Mann's technique). The electrolyte values in the graft were normal in the intracellular space; the extracellular space showed edema and an increase of tissue calcium.

In 1964, Reemtsma *et al.* published a second report on immunosuppression, again based on the Mann preparation in dogs. In addition to methotrexate, azathioprine was administered as immunosuppressive agent in 14 experiments. A maximum survival time of 14 days was obtained. In 24 other experiments the heart was placed in the thorax and used as an auxiliary bypass for the recipient's heart. Fourteen of these bypass experiments were considered successful. Post mortem studies of immunosuppressed transplants showed endothelial hyperplasia of arterioles with obvious narrowing of the lumen. The ECG of the recipient's heart (host heart) revealed alterations manifested a week after transplantation. These changes were attributed to an immunological process.

DePasquale *et al.* (1965) elaborated Reemtsma's findings. In their electrocardiographic studies on homologous canine heart transplantation, 10 dogs with a graft implanted according to Mann were immunosuppressed with azathioprine (3-5 mg/kg daily). The survival time ranged from 6 to 21 days. Post mortem examination of the grafts showed a variable degree of necrosis, loss of normal structure of myocardial tissue, mononuclear cell infiltration, and endothelial hyperplasia of the arterioles. No apparent lesions were found in the host heart, but the host heart ECG showed a transient increase of the heart frequency. Four of the animals showed ST deviation and 5 showed T-wave inversion in leads I and II. Negative or biphasic T waves in leads I and III altered the configuration. All animals with a graft survival of more than 9 days showed these ECG abnormalities in the host heart. In 3 animals the graft was removed, and in 2 of them the host heart ECG returned to a rather "normal" configuration within 7 to 12 days. In 2 animals hydrocortisone administration after removal of the transplant restored the pre-operative ECG pattern of the host heart. Six normal dogs and 11 dogs with a renal transplant did not show abnormal ECGs after the administration of azathioprine. The authors suggest that the observed ECG changes in the host heart could be due to circulating heart-specific antibodies, because patients with an immunological myo-

carditis or pericarditis show ECG changes similar to those described in these host hearts. Six days after transplantation, the graft developed ECG signs of rejection in the unipolar leads, including a progressive drop in the voltage of the QRS complex, prolongation of the QRS complex, increase or elevation of the ST segment, and prograssive inversion of the T wave.

Manax *et al.* (1965) performed "cervical heart homotransplantation" in dogs to study the effect of hypothermia, hyperbaria, and chlorpromazine applied for preservation of the donor heart prior to transplantation. The duration of the preservation period was between 24 and 48 hours. The mean survival time amounted to 5 days. The donor hearts showed "slight damage" after preservation, reversible within 2 to 3 days. Numerical data are not given.

Furuse *et al.* (1967) investigated heterotopic allotransplantation of the heart in adult dogs treated with thymectomy and intralymphatic administration of 6-mercaptopurine. The Mann technique was used and an atrial septum defect was made. In the control group (9 animals) the mean survival time was 6.9 days, the maximum 11 days; in the thymectomy group (6 animals) 7.2 days and 9 days, respectively. In 7 animals given 6-mercaptopurine intralymphatically, these times were 6.7 days and 11 days; in 10 animals given thymectomy and intralymphatic 6-mercaptopurine, 9.7 days and 16 days; and in 10 animals given azathioprine, 11.5 days and 25 days, respectively. One dose of 6-mercaptopurine (15 mg/kg) was given 5-7 days prior to transplantation and the same dose once on the transplantation day. Azathioprine was given from the day of transplantation in a daily dose of 5-10 mg/kg. Three animals in the azathioprine group died from drug intoxication.

Rowlands *et al.* (1968) and VanderBeek *et al.* (1968) described rejection of canine cardiac allografts and electrophysiological changes during rejection of canine heart transplantation. The Mann technique was used. In unmodified animals (n = 10) the mean survival time amounted to 7.3 days. Sequential open biopsy specimens were taken from the graft. After 2 days, a mild interstitial edema and perivascular mononuclear cell infiltration were observed in 2 transplants. Some small foci of necrosis were present. These features intensified up to day 6 to 8, after which the small arteries showed distinct endothelial cell proliferation, thickening of vessel walls, and intramural deposits of fibrin; extensive myocardial necrosis was also seen. Only 2 grafts remained "viable" from day 9 to day 14. The survival time of 5 animals treated with ALS, azathioprine, and prednisone is not given. Myocardial tissue from these grafts was "normal" until withdrawal of the immunosuppressive agents, after which the above-mentioned features of rejection soon returned. Electrocardiographic signs of rejection were correlated with the histological findings. Extensive myocardial necrosis developed before ECG sings of rejection appeared (compare the observations of Abbott *et al.* (1964) and MacSween *et al.* (1971) in the rat model).

Hattler *et al.* (1969) performed a histochemical analysis of the rejecting canine cardiac allograft. In 12 experiments (Mann's technique) sequential biopsy specimens were taken for histochemical analysis. Presensitized recipients (n = 5) given a previous graft (3 weeks earlier) rejected the second graft within 36 to 48 hours. An initial increase in the activity of oxidative enzymes was followed by a gradual decrease. Damaged mitochondria were thought to be incompetent to meet the oxygen and energy needs of the myocardial fibers.

Largiader *et al.* (1969) and Bachman *et al.* (1970) measured serum enzymes during cardiac allograft rejection. Dogs provided with a "Mann graft" were used in 30 experiments. The mean survival time in unmodified animals was 8.2 days. Seven grafts developed venous thrombosis and one animal pneumonia. SGPT, SGOT, LDH, CPK, and HBDH levels were determined. Only the CPK values showed an increase correlating with the rejection process. The other enzymes showed rising levels but not significantly related to the course of the rejection process.

Tennenbaum *et al.* (1969) used the lymphocyte transformation test described by Bach and Hirschhorn (1969) for early detection of canine heart allograft rejection. In 10 unmodified dogs (Mann's model) a survival time of 4 to 10 days was obtained. The control value of lymphocyte transformation was on average 3 per cent. Twenty-four hours after transplantation, this value increased to 22 per cent. Three modified animals (azathioprine 2 mg/kg, survival time from 16 to 27 days) showed decreased lymphocyte transformation. A correspondence was found between the histological signs of rejection (in needle biopts) and the lymphocyte transformation percentage. ECG signs of rejection did not parallel these phenomena but lagged behind them.

Seki *et al.* (1970) developed an improved method for heterotopic cardiac allografting, based on a slight modification of Mann's technique. Ten unmodified animals survived from 4 to 11 days (mean 7.8 days). The authors considered graft failure to be related to kinking of the arterial supply, and tried to prevent it by placing the graft in a subcutaneous pouch.

Semb *et al.* (1971) published six papers on heterotopic cardiac transplantation in dogs. They used a modification of the Mann technique, adding an atrial septum defect to prevent distension of the left ventricle. In 12 unmodified animals the mean survival time was 136 hours (5.6 days). Cardiac enzyme levels were measured during rejection in 8 animals; an initial rise of transaminases and LDH was observed during the first 24 to 48 hours after the operation and a final rise of these enzymes at complete rejection of the graft.

In 10 unmodified animals and in 6 modified cases, in which azathioprine and prednisone were used, these authors investigated the ECG signs of cardiac rejection. The unmodified grafts showed a progressive voltage decline, nodal rhythm, AV dissociation or heart block, transient Q waves, and ST and T-wave changes. The modified grafts showed "normal" ECGs until the immunosuppressive agents were withdrawn, after which the ECG rejection patterns returned. In 7 unmodified animals angiography of the graft was performed from 1 hour to 144 hours post-operatively. Progressive aortic insufficiency occurred in all cases. Decreased myocardial movements, myocardial enlargement, and narrowing of the coronary arteries were considered important signs of rejection. In one of 2 modified animals (azathioprine and prednisone) the aortic insufficiency was reduced to a minor degree; in the other dog the insufficiency increased and the lumen of the coronary arteries and the myocardial movements remained unaltered.

The pathology of unmodified rejection was studied by the same authors in 18 animals, in 7 of which sequential transcutaneous needle biopsies were performed. The effects of immunosuppression (azathioprine-prednisone) were studied in 6 grafts. The main histological features in the control series consisted of interstitial edema, mononuclear cell infiltration, arteritis, and myocardial necrosis. Immunosuppressive treatment did not completely prevent mononuclear cell infiltration, fibrinoid necrosis of the arterial wall, or focal myocardial necrosis.

Cullum *et al.* (1971) and Leedham *et al.* (1971) described indices of acute rejection after cardiac transplantation and the acute and modified rejection of heterotopic canine cardiac allotransplants studied in serial needle-biopsy specimens. The Mann technique was employed. The 10 control animals survived 5.6 days (range 4-10 days) on average. Animals given azathioprine (3 mg/kg) and hydrocortisone (6 mg/kg) daily survived on average 6.4 days (range: 4-9 days). The authors consider their histological results to correspond with those described by Kosek *et al.* (1968) and Bieber *et al.* (1969).

Dear *et al.* (1973) performed 20 experiments on unrelated random mongrel dogs in which a modification of Mann's technique was used to study the electrocardiographic prediction of unmodified rejection. The mean survival time was 7.5 days. Decrease of the QRS voltage was observed in all 20 cases, bradycardia in 18, arrhythmia in 17, ST deviation in 17, and prolongation of QRS intervals in 15 cases.

Heterotopic hearts

Abdominal transplantation techniques

The rat model

In 1964, Abbott *et al.* described a technique for heart transplantation in the rat (see Fig. A.6). Anastomoses are made between the recipient's abdominal aorta and the donor's ascending aorta end to end, allowing perfusion of the myocardium via the coronary arteries. The coronary venous return drains through the right heart and pulmonary artery, which is anastomosed to the recipient's inferior vena cava. The hemodynamics in the abdominal position are basically similar to those of the Mann model.

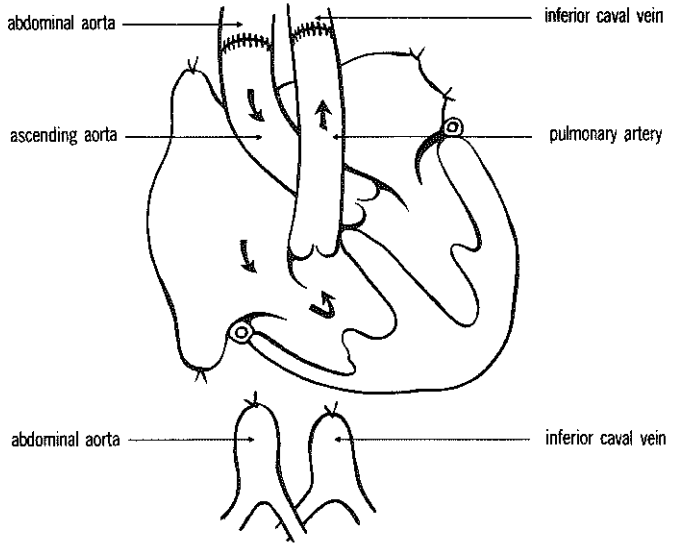


Fig. A.6. Technique developed by Abbott *et al.* (1964) for heterotopic heart transplantation in the rat.

The advent of the rat heart transplantation model brought cardiac transplantation within the reach of laboratories experienced in research with small animals. The advantages offered by the rat model include the cheapness of the animals, their better-defined immunological properties, the simple surgical facilities required, and the gain of time provided by the shorter operation.

Abbott *et al.* performed 9 isografts and 15 "homografts". Isografts (excluding the selectively killed animals) survived more than 130 days (2 animals). The homografts were sacrificed for histological investigation, which showed acute pericarditis, subpericardial necrosis, and occasional thrombi in the left atrium and ventricle. Isografts killed before day 15 did not show visible histological changes. In 1965, Abbott *et al.* elaborated their findings in a paper entitled *The transplanted rat heart, histological and electrocardiographic changes*, which reported the results obtained in 24 "homografts" and 10 isografts. Histological signs

of rejection, which appeared in the graft on day 3, consisted of subendocardial and perivascular infiltration with mononuclear cells. On day 5 this process had become generalized, and interstitial edema and swelling of arteriolar endothelium were present. On day 7 or 8 the graft became necrotic. The isografts did not show distinct pathological features. ECG signs of rejection first appeared on day 7 in the form of flat T waves and defective intraventricular conduction. Discrepancies between the graft pathology and the ECG pattern were obvious.

The rat model with the abdominal technique was subsequently used by many other investigators. Bui-Mong-Hung *et al.* (1966), Tomita (1966), and Ono *et al.* (1969) modified the original technique by changing the end-to-end anastomosis between the graft and recipient to an end-to-side anastomosis (Fig. A.7). This prevented the severe hind-leg ischemia which developed in Abbott's animals. The success rate obtained with this modification amounted to 57 per cent in Abbott's animals. In these studies the animals were selectively killed between the 2nd and 7th days. The graft almost invariably showed thrombi in the left ventricle. The myocardium showed mononuclear cell infiltration, lysis of myocardial fibers, and myocardial necrosis. In outbred albino rats (Tomita 1966) the survival time ranged from 5 to 28 days with a maximum of 167 days. One isograft survived more than 300 days.

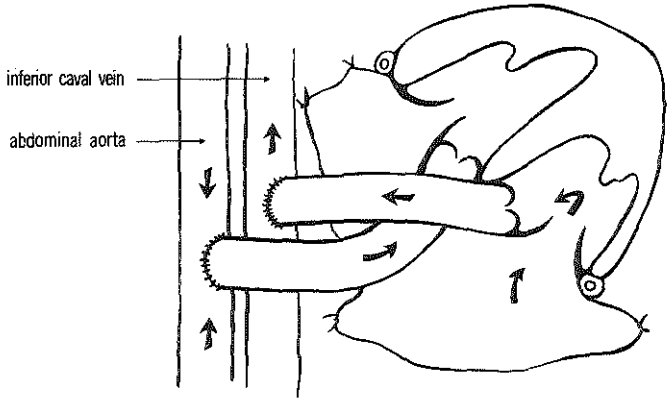


Fig. A.7. The modified design for heterotopic heart transplantation in the rat used by Bui-Mong-Hung *et al.* (1966), Tomita (1966), and Ono *et al.* (1969).

Ono *et al.* (1967) irradiated rat heart allografts. Fifteen allografts given local irradiation (6 doses of 150 R each) had a mean survival time of 14.7 days as against 9.9 days for 15 controls. Isografts (n = 34) survived more than 80 days. After 5 days, the irradiated grafts and controls showed myocardial necrosis. There was less mononuclear infiltration in the irradiated grafts than in the controls. Isografts showed acute epicarditis and patchy infarction. Ono *et al.* (1969, 1972) also investigated other methods of immunosuppression in the rat model by evaluating the immunosuppressive activity of allogenic ALS and thiamphenicol.

Van Bekkum *et al.* (1969) compared the survival time of kidney and heart allografts in several groups of rats treated with the same immunosuppressive agents (azathioprine, prednisone, and ALS). They used the heart transplantation technique shown in Fig. A.7. In the unmodified animals (n = 7) the heart graft survival time ranged from 9 to 11 days, and the

kidney survival time (13 animals) was up to 22 days. Modified heart grafts survived for 30 to 40 days, kidney grafts for 80 to 100 days. Isografts survived more than 365 days. The histological investigation of the unmodified grafts showed mononuclear cell infiltration, arteritis, and infarction necrosis. In the modified grafts, scars resulting from previous infarctions were demonstrated; the isografts showed scattered areas of fibrosis suggesting previous infarctions. It was concluded that rat kidney allografts survive longer than rat heart allografts.

MacSween *et al.* (1971) performed immunological and histological studies on ectopic heart transplants in the rat. Eighteen isografts and 22 allografts were performed. Immunofluorescence investigation of the allograft occasionally showed specific staining (IgG) throughout small-vessel walls 8 days after transplantation. After 10 to 12 days there was positive staining of intima in the large coronary arteries in some cases. Positive immunofluorescence was not observed in either the isografts or in the host hearts. Histologically, thrombus formation was seen in the left atrium and left ventricle 24 hours after operation. After 48 hours mononuclear cell infiltration occurred from the endo- and epicardial sides and in the perivascular areas in combination with interstitial edema. From day 4 to 10 vascular changes were seen, including focal infiltration of the vessel wall of small arteries, endothelial swelling, and medial edema. Extensive disruption of the myocardium, focal areas of interstitial hemorrhage, myocytolysis, and vascular necrosis (fibrin thrombi in small vessels) also developed. The isografts and the host hearts showed no pronounced changes. The discrepancies seen between the ECG and the histological manifestations of rejection resembled those reported by Abbott *et al.*

Laden, Ruszkiewicz and Sinclair (1971, 1972, 1973) investigated the pathogenesis of intimal thickening and the effect on these lesions of various drugs (azathioprine, ALS, dipyridamole, heparin). According to their hypothesis, antibody coats the arterial intima and provokes platelet aggregation, and hyperlipoproteinemia enhances this process.

Sinclair *et al.* (1972) studied the relationship between the histological and immunofluorescence findings in 23 rat cardiac allografts and 4 isografts. Seven grafts with arterial fibrinoid necrosis also showed deposits of gamma and other immunoglobulins in the arterial walls.

Other heterotopic heart transplantation techniques and experience obtained in other animal species

In 1948, Sinitsin described a heterotopic "canine" model with a workload on the left ventricle. This idea was adopted by Marcus *et al.* (1953) and Luisada *et al.* (1954) (Marcus II technique). The vascular anastomosis and the direction of bloodflow are depicted in Fig. A.8.

Marcus and his colleagues also developed a third technique in which the donor heart and both lungs were transplanted to the abdomen of a recipient dog. The graft's aorta was anastomosed into the recipient's aorta, and the graft's right atrium into the recipient's vena cava inferior. The trachea was led out through the recipient's ventral abdominal wall (Marcus III technique). Fifteen experiments according to Mann's technique with a maximum survival time of 45 to 48 hours were reported. Twenty-two experiments performed according to the Marcus II technique gave a maximum survival time of 48 hours. In 8 experiments with the Marcus III technique, the donor organs survived for 9 hours at most. In their later experiments these authors administered cortisone to 9 of their recipients. The survival time was not influenced by this drug. Envelopment of the graft in cellophane or in amnion tissue did not affect the transplant survival time either; in 69 experiments the mean survival time was 6.5 days. The graft's ECG showed alterations on compression, but the phonocardiogram gave normal heart sounds. Angiocardiography was performed in 3 animals. The inotropic

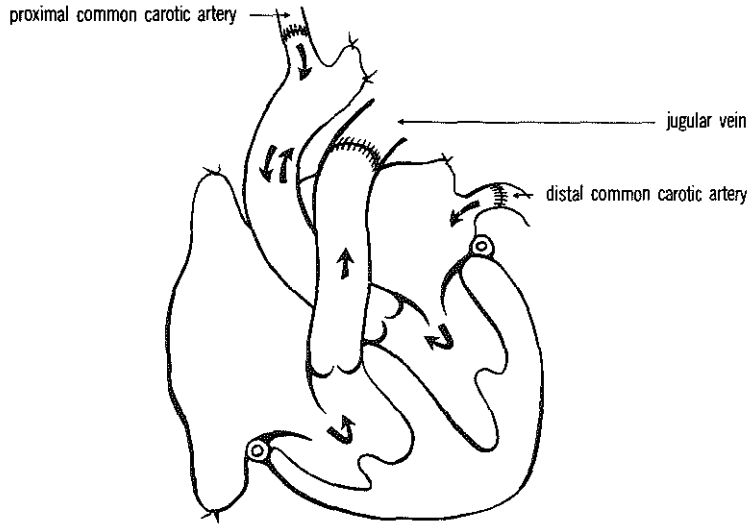


Fig. A.8. The method for heterotopic heart transplantation in dogs used by Sinitsin (1948) and Marcus *et al.* (1953).

effect of adrenalin was apparent in both the graft and the host heart. Digitalis had only a "myogenic" effect on the graft and a myogenic and neurogenic effect on the host heart. Microscopically, the rejected grafts showed an "inflammatory" process and infiltration with mononuclear cells. The authors considered the Sinitsin-Marcus II technique to be more physiological than the Mann technique. The Marcus II model had a lower success rate because of technical failures.

Stansel and Terino (1965) described a single-anastomosis heterotopic cardiac homotransplant used in 16 dogs given 6-mercaptopurine (the doses are not mentioned). The graft's atrial septum was removed, the pulmonary artery ligated, and the tricuspid valve made insufficient, after which the graft's aorta was anastomosed end-to-side into the recipient's abdominal aorta. Complications took the form of irreversible ventricular fibrillation, distension of the left ventricle (if possible prevented by manual compression of the graft), bleeding, and heart block. The mortality rate was 37.5 per cent. The mean survival time was 13.3 days with a maximum of 36 days. Graft failure in the post-operative period was ascribed to infarction, thrombosis, and rejection.

Meisner *et al.* (1968) and Sebening *et al.* (1968) performed 10 heterotopic heart transplantations in the abdomen of dogs. The aorta of the graft was anastomosed end-to-side into the proximal common iliac artery and the pulmonary artery was connected end-to-end with the proximal iliac vein. In these unmodified animals the graft survival time mounted to 8 days. Failures were due to infection and to kinking or twisting of the vascular pedicle. In 10 other experiments performed with Mann's technique, 10 mg/kg azathioprine was administered daily; here, the mean survival time was between 10 and 15 days with a maximum of 36 days.

Childe *et al.* (1969) published a paper on heart transplant rejection in the pig and dog. Mann's technique was used in the dogs, but no mention is made of the technique used in the pigs, the number of animals in this series, or the survival time of the grafts. The survival time of the canine grafts ranged from 3 to 10 days.

Crosby *et al.* (1969) and Reed *et al.* (1969) published three papers concerning heterotopic abdominal canine heart transplantation in allograft combinations used to test various immunosuppressive regimens on this basis. The surgical technique was similar to that of Bui-Mong-Hung *et al.* in the rat (Fig. A.7.). Unmodified recipients (n = 6) had a mean graft survival time of 11 days, for the steroid group (n = 6) this was 9.2 days, for the azathioprine group (n = 6) 19.2 days, for azathioprine + steroids (n = 6) 35.2 days, for low-dose ALS pre-treatment (n = 7) 44.4 days, and for high-dose ALS pre-treatment (n = 6) 38.4 days. The ALS-treated animals received azathioprine and steroids in the post-operative period. The authors consider the post mortem findings in their grafts identical to those made in orthotopic grafts on the basis of a comparison of their findings with Lower *et al.'s* (1965) descriptions of orthotopic canine heart allograft rejection. Their ECGs made during graft rejection showed bradycardia, a voltage drop, broadening of the QRS complex, a shift of the ST segment, and rhythm disturbances.

Folts and Boake (1969) demonstrated changes in coronary blood flow in the homografted canine heart. Twelve dogs received a heart transplanted according to the technique depicted in Fig. A.9. In the donor heart the coronary blood flow was measured periodically during the fortnight before transplantation, use being made of a 2.5 or 3.0 mm electromagnetic flow meter applied to the left circumflex or the left descending anterior coronary artery. These measurements were continued after transplantation. One hour after transplantation the coronary flow was 20-25 per cent lower than the pre-operative value; 50 per cent reduction of the pre-operative flow was reached within 3 to 4 days after transplantation; thereafter, the flow reduction was distinctly progressive until final rejection. At the time of 50 per cent flow reduction, ECG changes appeared (bradycardia, voltage decline, etc.). When the flow had decreased to nil, there was still ECG activity, i.e., bradycardia with AV block. The survival times ranged from 13 hours to 9 days. Extensive infarction necrosis

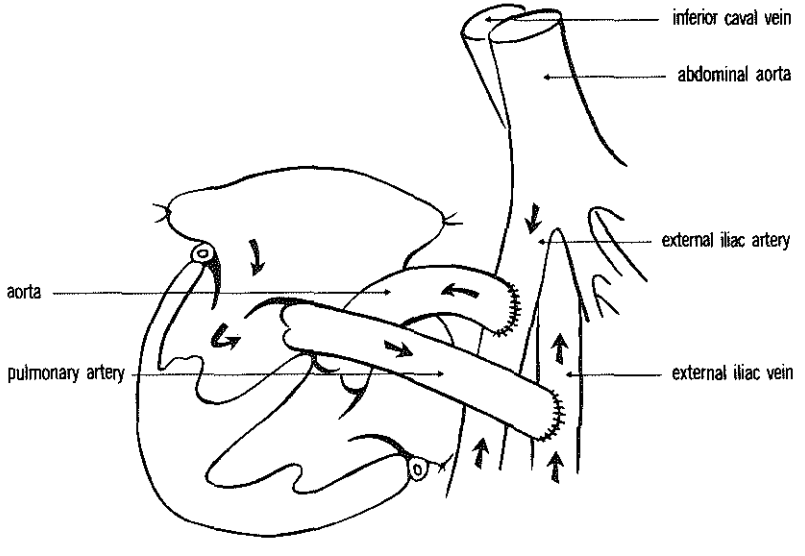


Fig. A.9. Technique for heterotopic heart transplantation in dogs used by Folts and Boake (1969) and Legrain *et al.* (1969).

occurred in all grafts that survived more than 3 days. The authors concluded that during rejection there is a significant reduction of coronary bloodflow caused by denervation of the graft, the absence of a workload on the left ventricle, the disturbed mechanism of the sinus valsalvae, and the blood pressure characteristics of a peripheral artery, which differ from those of the ascending aorta.

Legrain *et al.* (1969) performed simultaneous homotransplantation of heart and kidney from the same donor in bilaterally nephrectomized dogs. The transplantation technique employed was as shown in Fig. A.9. The vascular anastomoses of the renal graft were on the left iliac vessels. In unmodified animals (13) a mean survival time of 9.7 days was attained. Various regimens of immunosuppressive agents were tested. The histological lesions in the heart and kidney were similar as far as the extent and quality of the lesions were concerned. The prolongation of graft survival time achieved in this combination was attributed to the larger amount of antigenic material supplied to the recipient.

Gergely and Coles (1970) obtained prolongation of heterotopic cardiac allografts in dogs by topical radiation. They used the technique shown in Fig. A.7. Sixty-three recipients were irradiated with 2400 rad given in 6 doses over a period of 3 weeks. Nineteen animals died in the early post-operative period due to bleeding, infection, lung embolism, and ileus. In the remaining 44 animals the graft survived for at most 10 weeks. In 10 control animals the maximum graft survival time was 12 days. Twelve dogs received irradiation without operation; the hearts of these animals showed distinct subendocardial, perivascular, and interstitial thickening 75 days after irradiation. In the irradiated grafts the myocardium showed 75 per cent infarction necrosis, and in the control grafts 90 per cent of the myocardial tissue was infarcted.

Heron (1970, 1971, 1972) introduced a model for cervical heart transplantation in the rabbit. Blood-vessel anastomoses were rapdily established by coupling the vessels over an extraluminal teflon prosthesis. These anastomoses are therefore end-to-end and similar to those of Mann (Fig. A.5). This method was later used in the rat.

Histological immunofluorescence, electrocardiographic, and enhancement studies were performed in pregnant and postpartum rabbits and rats. Graft enhancement was demonstrated in mother-child combinations. In isografts in rats, focal infarctions with areas of fibrosis were observed.

Kahn *et al.* (1970-1972) investigated the effects of anticoagulant, immunosuppressive, and platelet-disaggregating agents in pigs and monkeys, using abdominal transplants with the vascular anastomoses and hemodynamics shown in Fig. A.9. The degree of rejection was assessed on the basis of the ^{131}cesium uptake (see Carr *et al.* 1970) of the graft. Heparin delayed cellular infiltration, myocardial necrosis, and arterial intimal proliferation. Twelve monkeys were given azathioprine and prednisone, 5 of them also receiving 7 mg heparin/kg body weight daily and 3 animals 10 mg dipyridamole/kg daily. All of the animals that survived more than 30 days "showed marked vascular changes of chronic rejection regardless of whether they received heparin and dipyridamole as well as azathioprine and prednisone". Shumak *et al.* (1970), Goldman *et al.* (1971), and Mullerworth *et al.* (1972) demonstrated antibodies in cardiac rejection in dogs given an abdominal graft (Fig. A.7). The rejected grafts showed distinct linear sarcolemmal fluorescence. The recipient's heart (host heart) occasionally showed focal myocarditis and sarcolemmal IgG deposits on immunofluorescence investigation. Recipients pre-sensitized by a previous graft, rejected the final transplant within 2 to 12 days. Control animals (n = 17) had a mean survival time of 9.3 days. In recipients given ALS, the graft survived 14.3 days on average.

Marquet, Heystek and van Bekkum (1972) carried out heterotopic heart and kidney allografting in unrelated Indian and Pakistani rhesus monkeys typed for Rh L-A specificities. The heart transplantation technique was similar to that in Fig. A.7 (see van Bekkum *et al.*

1969). The mean survival time of the unmodified heart grafts was 8 days. Fourteen heart recipients treated with ALS and azathioprine survived for 10 to 64 days. Under the same conditions kidney grafts survived for 11 days and from 9 days to 2.5 years, respectively. The heart grafts showed decidedly more infarction necrosis than did the kidney grafts. There were no obvious differences in arteritis between rejected kidney and heart allografts.

Corry *et al.* (1973) were the first to describe heart transplantation in mice. The vascular anastomoses were similar to those shown in Fig. A.7. The objective of these studies was to perform heart transplantation in individuals with precisely defined immunogenetic differences as a basis for the study of organ transplants across single antigen incompatibilities. The results of skin grafts in these animals are clearly defined. Rejection could be established by palpation of the graft. Isografts survived for more than 6 months. Several regimens of antisera against weak and stronger histocompatibilities were tested. Slight differences were found between the results of skin and heart grafts in the same combinations of animals.

Gonzalez-Lavin *et al.* (1974) and O'Connell *et al.* (1974) cleared the canine donor heart of passenger blood cells prior to transplantation by perfusing the heart with a specially designed perfusate. Their surgical technique was identical to that in Fig. A.7. The mean survival time of non-perfused grafts was 7 days, that of perfused grafts 11 days.

Free heart grafts: the third heart transplantation model

Huff *et al.* (1968) developed the "free heart graft" technique. With the use of inbred strains of mice, hearts were "free grafted" to the pinna of adult mouse ears. Only fetal or neo-natal grafts took. The method was subsequently used by Judd and Trentin (1971, 1973) and Svehaj and Schilling (1973). Graft viability could be monitored electrically or visually by viewing pulsatile activity at a magnification of 10.

Milam *et al.* (1971) described the histopathology of such free-heart grafts in control and anti-thymocyte serum-treated recipients. Isografts showed extensive myocardial coagulation necrosis, some viable myocardial fibers remaining in the central portions of the graft. The devitalized tissue was subsequently organized and replaced by fibroblasts and connective tissue. Some small nests of viable contractile myocardium were present 25 months after transplantation. Unmodified allografts showed dense interstitial hemorrhage, diffuse infiltration of polymorphonuclear leucocytes, and edema starting on days 4 to 8. Fewer viable myocardial fibers remained than in isografts. In the following days a very dense infiltrate with polymorphonuclear cells, histiocytes, and mononuclear cells was observed; necrotic allograft tissue was replaced by fibrous connective tissue within 32 days. On day 4, recipients treated with rabbit anti-mouse thymocyte serum (RAMTS) showed graft pictures intermediate between those of unmodified recipients and of isografts. There were lower numbers of polymorphonuclear cells and mononuclear cells, and less edema. The grafts remained contractile until the 48th day. Whem RAMTS was discontinued, the grafts were rejected within 4 weeks. Induction of tolerance could not be obtained.

We consider this method of experimental heart transplantation to represent an *in vivo* heart-tissue culture, and that it should be compared with other methods of avascular grafting rather than with vascular grafts. The limited suitability and completely different pattern of rejection led us to omit this third heart transplantation model from further consideration.

Xenogeneic heart grafting

Dupree *et al.* (1969) transplanted the hearts of *Maccaca speciosa* monkeys to the abdomen of baboons (*Papio doguera*) to study the possibilities of xenogeneic storage of a heart prior to definitive transplantation in an allograft situation, in connection with the question of whether a primate heart could be used as a xenograft in man. The technique was a modification of the one shown in Fig. A.7. In the control animals, 3 grafts survived for respectively 3, 7, and 16 days.

Hagl *et al.* (1971) obtained a mean survival time of 5 days in a fox to dog combination. At autopsy the grafts showed extensive anemic myocardial necrosis or massive hyperemia and granulocytosis.

Van Bekkum *et al.* (personal communication 1970), performed xenografting of hearts from hamster to rat. In unmodified combinations the longest graft survival time amounted to 4 days. At autopsy extensive necrosis was seen in the graft.

Heart grafts as auxiliary cardiac assist devices

The pioneer in this field is Demikhov, who described 24 technical variants applied in 250 operations in dogs to place an auxiliary heart into a recipient's thorax. Forty-three animals died on the operating table, 87 succumbed during the first two days, 97 between the 2nd and 12th day, and 13 between the 12th and 19th day, post-operatively. One dog survived for 32 days (Cooper 1968).

Reemtsma (1964) worked on the same problem and was able to maintain functioning of the auxiliary graft for at most 72 hours (see page 98)

Orthotopic hearts

Orthotopic heart transplantation proved to be impossible without the help of supporting techniques. In dogs, Demikhov (1951) used end-to-side anastomoses between corresponding vessels of the recipient's heart and the graft. After these anastomoses had been completed, the blood supply to the recipient's heart was closed and the organ removed. In 1955, two survivals were obtained among 22 animals, one for 11.5 hours and the other for 15.5 hours (Cooper 1968). Neptune *et al.* (1953) carried out complete heart plus lung transplantations under hypothermia (21-25°C) on three occasions, the longest survival amounting to 6 hours. Goldberg *et al.* (1958) and Webb *et al.* (1957-1959) performed canine orthotopic heart transplantations under extra-corporeal circulation. Goldberg preserved a left atrial rim to avoid time-consuming pulmonary vein anastomoses, and Webb used couples to make caval anastomoses. The survival times obtained by these authors ranged from 21 minutes to 2 hours and from 30 minutes to 7.5 hours, respectively. Blanco *et al.* (1958) performed complete homotransplantation of canine heart and lung under extra-corporeal circulation. The mean survival time was from 30 minutes to 4.5 hours.

In 1959, Cass and Brock left a rim of both of the recipient's atria intact, thus eliminating the need for time-consuming and tedious pulmonary vein anastomoses (Cooper 1968). This technique was adopted by Lower and Shumway (1960) and Lower, Stofer and Shumway (1961) with more success in a series of 8 heart allotransplantations in dogs, 5 of the recipients surviving for 6 to 21 days. In the post-operative period the animals showed rather normal exercise tolerance and ate and drank normally. With the onset of rejection, the animals became lethargic and dyspnoic. The course was lethal within about 24 hours after the onset of symptoms. The ECG taken 5 hours prior to death seemed normal, except for

T-wave inversions, primarily in leads II, III, and AVF.

The post mortem examination of the heart revealed it to be ecchymotic and edematous with a fibrinoid pericarditis and generalized dilatation. Microscopically, the sections showed severe myocarditis, massive round-cell infiltration, patchy necrosis, interstitial hemorrhage, and edema. The regional lymph nodes were large, but microscopical examination showed a non-specific increase in the numbers of plasma cells and histiocytes.

Since these studies, the technique of Lower and Shumway has been employed in almost all experimental orthotopic heart transplantations, and since 1967 it has also been used in human cardiac transplantation. Barnard (1968) and Cooley *et al.* (1968) modified the method slightly to preserve the integrity of the sino auricular node. Instead of opening the back of the right atrium from the inferior caval orifice to the superior vena cava orifice, the stump of the superior vena cava was ligated and the opening was made from the inferior vena cava orifice, i.e., more laterally. This modification eliminated the risk of trauma in the area where the superior vena cava joins the right atrium, the site of the sino auricular node. Caves and Dong (1973) added some details to the experimental technique currently in use in dogs. The aorta suture line was reinforced with mattress sutures, and the ischemic time of the donor heart was shortened by excising it after stable perfusion had been established in the recipient and its heart had been removed. Immediately after completion of the aortic suture line the aortic cross-clamp was removed, thus re-establishing coronary perfusion of the graft. These measures reduced the ischemic time of the cooled donor heart to approximately 26 minutes. The pulmonary artery suture line was doubled. An epicardial atrial pacemaker was implanted until a stable heart rhythm was obtained. The final result of surgery according to the original technique of Lower and Shumway is shown in Fig. A.10.

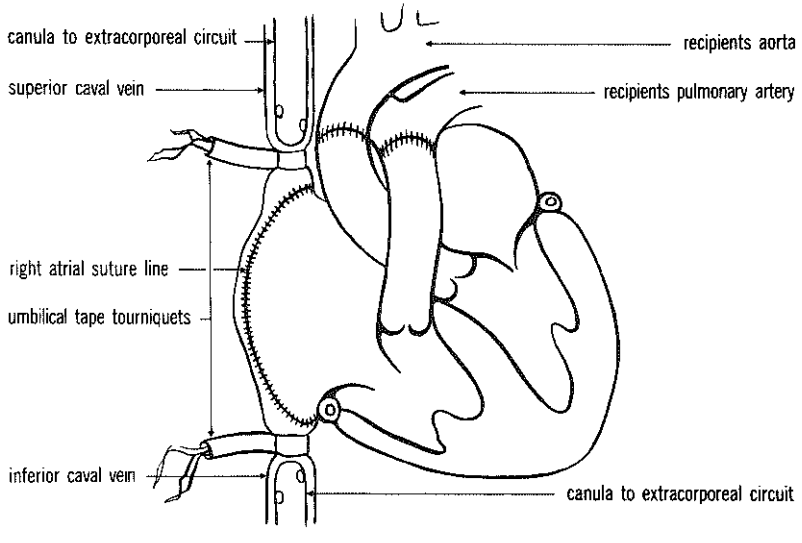


Fig. A.10. The orthotopic heart transplantation technique used in dogs and developed by Lower, Stofer and Shumway (1961).

The Stanford school

Lower and Shumway founded a famous school of orthotopic cardiac transplantation, one which has contributed greatly to our knowledge of this subject. The following survey summarizes their work and the work of their associates and of the surgeons they trained.

Hurley *et al.* (1962) published a paper entitled *Isotopic replacement of the totally excised canine heart* based on some 20 experiments in which a survival rate of 75 to 80 per cent was obtained. Their paper *An approach to extra corporeal surgery of the heart* (1962) concerned autotransplantations in 9 dogs in which survival times varying from 6 hours to 9 months (in 4 cases more than 5 months) were obtained. Failures were due to hemorrhage from the aortic suture line, rhythm disturbances, lung complications, and infections. Physiological studies revealed altered but physiologically sound hemodynamics.

In 1965, Hurley *et al.* described Stokes-Adams attacks in orthotopically transplanted canine hearts, and recommended the use of an epicardial pacemaker in the post-operative period. In 1968, Hurley and Kosek described an atypical rejection of the canine heart in a dog which received the heart of a littermate and survived for 5 months. The rejection patterns observed in the graft were different from those of the unrelated control and immunosuppressed dogs, and were explained on the basis of histocompatibility.

Lower *et al.* (1965) reported on long-term survival of cardiac homografts and suppression of rejection crises in the cardiac homograft. Various immunosuppressive drugs were used, i.e., hydrocortisone, methylprednisolone, 6-mercaptopurine, and azathioprine. The control dogs (n = 20) survived from 4 to 21 days (mean survival time 7 days). Twelve animals given continuous immunosuppression survived for 6 to 35 days (mean 17 days). These animals showed drug toxicity, infections, and gastrointestinal bleeding as serious complications. Seven animals were immunosuppressed for 8 to 18 days; in this group rejection occurred within 5 to 12 days after the drugs were withdrawn. Of 6 animals given immunosuppressive treatment only on indication, 5 survived from 1 to 3 months. The prevalence of complications was distinctly lower in this last group, the animals ate better, and wound healing progressed faster. The paramount parameter for the determination of rejection was considered to be a drop in the voltage in lead II of the ECG. The microscopical features of rejection were described and the irreversible character of myocardial fiber and arteriolar wall damage was assessed.

In 1966, Lower *et al.* discussed the electrocardiograms of dogs with heart homografts. Rhythm and voltage alterations were found in the ECGs after transplantation. In the immediate post-operative period these rhythm disturbances were attributed to anoxia and trauma in the graft during surgery. After day 4, these disturbances might be suggestive for rejection. In two papers, *Rejection of the cardiac transplant* by Lower *et al.* (1969) and *Rejection of the transplanted heart* by Lower (1969), serum enzyme determination levels were reported. According to these authors, elevation of LDH levels may confirm impending rejection. The histopathology of acute rejection was associated with mononuclear cell infiltration of the graft and interstitial hemorrhage and edema. Chronic rejection was characterized by coronary artery narrowing due to intimal proliferation presumably resulting from immunological damage to the endothelium.

Shumway and his co-workers wrote many editorials on experimental orthotopic cardiac transplantation (see e.g. Shumway *et al.* 1964, 1967, 1969). Chartrand *et al.* (1968, 1969, 1972) and Stinson *et al.* (1972) investigated the hemodynamics of orthotopic homotransplanted hearts, and concluded that atrial pacing is imperative in the early post-operative period. In the resulting unanesthetized unmodified dog the cardiac output, stroke volume, mean systolic ejection rate, peak velocity, and acceleration were reduced immediately after the operation. Twenty to 48 hours post-operatively, all values were within normal limits,

and 48 hours before the decease of the animals the contractile force of the graft decreased. Twelve hours before death, the animal showed low cardiac output, low stroke volume, reduced systolic and increased diastolic pressures, and high venous pressure. In no instance did electro-cardiographic signs precede hemodynamic impairment.

Stinson *et al.* (1972) measured coronary bloodflows in orthotopically transplanted canine hearts during rejection. They applied 2-3 mm electromagnetic flow probes around the circumflex branch of the left coronary artery. In 4 unmodified animals and 2 recipients treated with prednisolone and azathioprine and measured twice daily from 6 hours postoperatively until death, the mean flow was 30 to 40 ml per minute (24-48 hours after the operation), and 24 hours before death there was a sharp decline to 12 ml per hour. In the meantime the coronary resistance increased, compared with the early post-operative values. Immunosuppressive treatment prolonged the interval in which normal values persisted, but rejection was nevertheless preceded by the same decline of the coronary flow. Rejection was assessed by post mortem investigation of the grafts.

Kosek, Hurley and Lower (1968) and Kosek *et al.* (1969) gave the first fully detailed reports on the histopathology of orthotopic canine cardiac homografts, based on the data obtained in 105 allografts and 36 autografts and describing the unmodified and the modified rejection patterns of grafts influenced by immunosuppression. The evolution of the process was followed in material obtained by open biopsies. In a paper on arteries in canine cardiac homografts, the ultrastructural findings during acute rejection were assessed (Kosek *et al.* 1969). In a paper entitled *Heart graft arteriosclerosis*, Kosek, Bieber and Lower (1971) gave their views on the origin of graft arteriosclerosis, which they attributed to arterial hypoxia due to compression of vasa vasorum by mononuclear cell infiltration.

Bieber *et al.* (1969) found a correlation between pathological damage to the conducting system of the cardiac canine graft and rhythm disturbances detected by electrocardiography. Rejection was graded from 0 to 4. When stage 4 was reached, arrhythmia was always present. The controls (5 autografts) did not show this pattern.

Caves introduced the transvenous endomyocardial biopsy by which rejection could be easily assessed, followed, and, if necessary, treated more specifically (Billingham *et al.* (1973)). Sewell *et al.* (1969) discussed epicardial ECG monitoring in cardiac homograft rejection. The use of epicardial wires for ECG recording is recommended to differentiate between pleural and/or pericardial effusions and rejection, voltage decline in lead II being considered to be the best electrocardiographical parameter for rejection. A voltage decline of 35 per cent is highly suggestive for rejection.

Graham *et al.* (1969, 1970) and Weymouth *et al.* (1970) investigated the reversibility of dog cardiac allografting by means of immunosuppression and local graft irradiation. The effect of the suppressive measures was judged on the basis of open biopsies and ECG changes. In approximately 60 per cent of the cases (n = 54) reversal of rejection was obtained, partial reversal in about 15 per cent, and none in about 25 per cent. Irradiation alone was given in 10 animals (300 R daily for 5 days) when the ECG voltage declined to 50 per cent of the original value. Reduction of rejection was obtained in 6 cases. In 2 animals no difference was observed between pre- and post-irradiation biopsy specimens, and in 2 animals the abnormalities increased after irradiation.

The significance of histocompatibility was shown by Bos *et al.* (1970, 1971, 1972) in orthotopic heart transplantation in dogs. Prospective donor selection with leucocyte antisera was applied to littermate beagles on the assumption that iso-antisera could segregate the parental chromosomes carrying the major histocompatibility locus DL-A (Vriesendorp *et al.* 1971). The survival time obtained in identical series of beagle littermates (n = 8) ranged from 21 to 247 days (mean 96 days); in non-identical combinations (n = 8) survival ranged 9 to 45 days (mean 20 days).

Boyd *et al.* (1970, 1971) used standard histocompatibility techniques to determine major and weak histocompatibility barriers in various series of dogs prior to orthotopic heart transplantation. In DL-A-compatible closely bred beagle-to-beagle combinations (15 animals) the mean survival time was 37 days; in DL-A incompatibles (n = 13) the mean survival time was 8.6 days. In unrelated random mongrel-to-mongrel combinations (28 animals) the mean survival time was 10 days. In DL-A-compatible beagle littermate combinations (6 animals) the mean survival time was 53.2 days; in incompatibles (n = 7) this was 7.3 days. In unrelated DL-A-compatible beagle-to-beagle combinations (9 animals) the mean survival time was 26.3 days and in incompatible animals (n =6) it was 6.3 days. These investigations demonstrated that the methods of histocompatibility testing used could prolong survival time in compatible combination of dogs, but could not prevent rejection.

Cleveland *et al.* (1970) and Nelson *et al.* (1969, 1970) evaluated the canine cardiac allograft function after transplantation by measuring the left ventricular ejection time (9 animals). Normal values were restored in the first week after the operation. Prolonged left ventricular ejection times occurred during arrhythmia and rejection. The importance of synchronic atrial contractions for the hemodynamic function of the transplanted graft and its ultimate longer survival, was demonstrated.

Kontos *et al.* (1970) and Norvell and Lower (1973) studied the degeneration and regeneration of the nerves of the heart after orthotopic transplantation; these authors used anatomical methods and measured electrical and pharmalogical responses in autografts and allografts from 2 weeks to 5 years after transplantation. Autonomic re-innvervation was demonstrated 6 months after autotransplantation in all animals. In allografts, autonomic reinnervation was achieved despite immunosuppressive treatment, but compared with the results in the autotransplants, parasymphatic re-innervation was delayed.

Shanahan *et al.* (1972) and Dagget *et al.* (1973) described the functional metabolic and structural conditions in 5 chronic canine cardiac autografts observed from 13 months to 5 years after transplantation. No ultrastructural differences were found between the autografts and normal hearts. Quantitative reaction to vagal stimulation was demonstrable. In 2 dogs only 1 vagal trunk functioned. The qualitative effect of vagal response was hard to assess. The left ventricular function of 4 of the 5 dogs was depressed, the left ventricular end diastolic pressure was increased, and the contractile forces were decreased. Sympathetic reinnervation was shown to be present in 3 dogs. Myocardial catecholamine levels were still on the denervated level 5 years after transplantation. Myocardial mitochondrial metabolism was undisturbed.

Childs *et al.* (1972) tried to obtain tolerance of an orthotopic graft in random mongrel recipients. After depriving them of their lymphoid elements by thymectomy and ALS treatment, the dogs were injected with bone marrow cells from their donors. In 3 cases the survival time ranged from 24 to 133 days; in the other 3 animals a mean survival time of 6 days was obtained.

Caves *et al.* (1973) tried to diagnose rejection on the basis of measurements of reactive lymphocyte blastogenesis. This method proved to be effective in 37 orthotopic heart transplantation experiments. An increase in reactive lymphocyte blastogenesis coincided with the occurrence of mononuclear cell infiltrates in the graft as assessed in serial biopts.

Penn *et al.* (1975) introduced the method of serial percutaneous biopsies to observe histological changes during chronic rejection after unmodified orthotopic cardiac transplantation in DL-A identical beagles (Vriesendorp *et al.* 1971; Bos *et al.* 1971). The transvenous method of Billingham *et al.* (1973) had the disadvantage that the external jugular vein became blocked by thrombosis after three biopsies. In Penn *et al.'s* studies nineteen dogs were subjected to 153 biopsies. One animal died due to hemorrhage after the puncture. The mean survival time in these dogs was 88.11 days. Progressive vascular lesions were seen from the 2nd week and consisted of medial proliferation involving intramural vessels as well

as epicardial vessels. In dogs (n = 8) surviving for 4 weeks, the biopsy specimens also showed lymphocellular infiltrate and myocytolysis. In the specimens from dogs surviving longer a slight and sometimes transient lymphocellular infiltrate was observed (Penn *et al.* 1976). The same authors (1976) also assessed left ventricular contractility during chronic rejection in 8 of these dogs by a non-invasive method. The mean circumferential velocity of shortening was calculated from endocardial marker motion on cinefilms. These dogs all died from the effects of chronic rejection. It was concluded that chronic rejection causes an important decrease in contractility.

The St. Louis school

Another school whose work, mainly on cardiac autotransplantation, has become well known is that of Cooper, Willman, and Hanlon. In 1962, Cooper *et al.* and Willman *et al.* reported on the effect of myocardial catecholamine and histamine after autotransplantation, the mechanism of cardiac failure after excision and reimplantation of the canine heart, and on heart autotransplantation in general. Their excision and reimplantation technique was different from that of Lower *et al.* (1961). Stepwise, they divided the vena cava superior and inferior and immediately resutured them between umbilical tape snares tied apart over a catheter maintaining the caval continuity. Then the caval catheters were joined to the venous line of an extra-corporeal circuit, and extra-corporeal circulation was started. The aorta and pulmonary artery were clamped and the left atrium divided and resutured, after which division and suturing of the aorta and pulmonary artery were performed. Forty dogs were used in these experiments, 27 of which died within 2 days. In a series of 50 experiments the main cause of failure was bleeding from the aortic suture line; a second cause was failure of the heart to re-establish the circulation after perfusion. Thrombosis in caval anastomoses and disrupture of the wound also occurred. The myocardial tissue showed catecholamine depletion after excision and resuturing. In Willman *et al.'s* initial work (1961, 1962) the post-operative course of dogs surviving more than 2 days was unfavorable: 6 animals died within 4 weeks showing clinical evidence of congestive heart failure confirmed in 4 cases by the post mortem findings. All of the animals had rhythm disturbances. The normal sinus arrhythmia was lost and the heart rate failed to respond to activity. In sham operations in which the animals were subjected to perfusion, hypotermia, and cardiac arrest, 3 out of 4 survived without signs of cardiac decompensation. These findings were rather contradictory in relation to the results obtained by the Stanford group in the field of autotransplantation. The differences have never been fully explained.

Willman *et al.* (1963, 1964) and Cooper *et al.* (1964) demonstrated the return of neural responses in the canine heart. A year after autotransplantation, vagal stimulation decreased heart rate and stellate ganglia stimulation caused cardiac acceleration. The response of the graft to various drugs was identical to that in normal dogs. These phenomena were interpreted as demonstrating that connection had been re-established with the extracardiac nervous system.

Cooper *et al.* (1964) compared the pathology of 5 orthotopic canine autografts with that of 2 allografts. The autografts showed a reduction of perimysial plexus and interstitial fibers; the myocardial structures were rather normal. The allograft showed rejection. Measurements of glucose and hexokinase in 5 dogs given a cardiac autograft (Willman *et al.* 1964) showed a rise in cardiac glycogen content and an increase in hexokinase activity in the transplanted heart.

In 1966, Willman *et al.* demonstrated increased coronary bloodflow two months after reimplantation of 10 dog hearts as compared with the pre-operative values. This phenomenon was attributed to the total extrinsic denervation of the excised heart. In 1967, Willman *et al.*

described the effect of blood volume expansion brought about with blood and Ringer's lactate solution in 9 dogs whose heart had been re-installed and in 9 normal dogs. The post-operative observation period was from 3 weeks to 3 months. The transplanted animals showed an expended blood volume and an increased left atrial pressure; the cardiac output was not depressed. After blood transfusion the diuretic response was lower in the experimental than in the control group, whereas the response to volume loading with Ringer's lactate solution was similar in both groups.

Willman and Hanlon (1969) were the first to attempt cardiac transplantation in primates. In 18 baboons re-implantation of the animal's heart was carried out, and in 2 animals an allotransplantation was performed. The allografted animals received azathioprine. One of them survived for 11 days.

Barnhorst, Olson and Willman (1969) investigated the metabolism of the autotransplanted canine heart by measuring the concentration of derivates related to the myocardial energy production. These measurements were done in left ventricular myocardial specimens obtained by open biopsy. The authors could not find any impairments in the energy metabolism of the hearts that had been excised and re-implanted two years before.

Work done in other centers

Hardy *et al.* (1964) designed a retrograde coronary artery perfusion technique to preserve and transplant hearts, and applied this method in 20 infant calves. In 1966, the same group reported the results of heart transplantation obtained in 142 experiments in dogs, 39 of which survived from 7 hours to 7 days. The pitfalls and sources of errors encountered in performing orthotopic cardiac transplantation in dogs are described in detail.

Kondo *et al.* (1965) performed heart transplantation in puppies with the Lower-Shumway operation technique and applied profound hypothermia. The anoxic arrest lasted on average for 45 ± 5 minutes. Of 40 puppies, 24 survived more than 24 hours, 13 more than a week, and 1 more than 112 days. In 1973, Kondo and Hardy *et al.* reported on puppies operated on under profound hypothermia. Fifteen recipients were pre-sensitized with skin grafts and spleen cells from their prospective heart donors. In 11 control experiments the survival time was between 7 and 17 days (mean 11.7 days). In 8 recipients given skin grafts and spleen cells, acute rejection occurred within 75 hours. Puppies sensitized only by two spleen-cell injections survived for 5, 5, 6, 6, and 12 days.

Benzing *et al.* (1969) used couples to establish rapid aortic and pulmonary anastomosis in orthotopic heart transplantation in dogs. The junction between the vena cava superior and the right atrial wall was preserved according to Barnard and Cooley (see page 109). Later, these authors (1972) described a new method to detect cardiac allograft rejection by means of the so-called C3 inhibition test.

Whiffen *et al.* (1967) performed "normothermic orthotopic heart transplantation". The donor heart was perfused according to Robiscek *et al.* (1963), and excised according to Willman *et al.* (1962). The vascular anastomoses were established rapidly by using graphite benzalkonium-heparin-coated couples. Of their 20 animals, 1 survived for 11 days. The causes of failure are given in detail.

In France, orthotopic heart transplantations were initially carried out with the use of total heart-lung bypass and profound hypothermia (Cachera *et al.* 1966), but later profound hypothermia was used exclusively. The objective was to evaluate the physiological, morphological, and bio-immunological properties of allogeneic heart grafts in young dogs (Cachera *et al.* 1968, 1970). These experiments finally resulted in 3 long-term survivors (731, 558, and 827 days). These animals received heterologous anti-dog lymphocyte globulin and azathioprine for 260, 330, and 596 days, respectively. After withdrawal of the im-

munosuppressive agents, the recipients showed a clinical state of tolerance for their grafts. Pharmacodynamic responses could be demonstrated in 2 animals after the administration of atropine, acetylcholine, or norepinephrine. The excercise performance of these 2 dogs was better than those of the single non-responding dog.

Lacassagne (1969) and Fernandez (1972) described the electrocardiographic findings in auto- and allograft recipients in the French experiments. In autografts they observed atrioventricular blocks, a drop of the voltage of the QRS complexes in 10-50 per cent of all animals, and repolarization distrubances. In 12 dogs given a cardiac allograft and post-operatvie immunosuppressive treatment (ALS and azathioprine) a voltage drop in the QRS complex of the graft's ECG during 20 rejection crises was only related to rejection in 4 cases. These authors consider the use of an epicardial ECG electrode to be essential to establish the real significance of a voltage decline. Rhythm disturbances, heart blocks, and repolarization changes were also judged to be important signs of rejection.

Léandri *et al.* (1967, 1969, 1970, 1974) described the histological features of rejected orthotopic canine cardiac grafts. Autografts showed scattered areas of scar tissue and fibrosis.

The British work in this field includes the original contributions of Cass and Brock (see page 108). More recently, orthotopic heart transplantation was carried out in pigs by Cullum *et al.* (1970) and Calne (1974). The porcine heart seems to be susceptible to prolonged cold ischemia.

In Germany and Austria, attempts to perform experimental orthotopic cardiac transplantation have been rather unsuccessful (Meisner *et al.* 1968; Schober *et al.* 1969). Nasseri *et al.* (1968) reported on 28 orthotopic experiments in dogs, mainly performed under profound hypothermia, in which 3 animals survived more than 24 hours. Kraft-Kinz *et al.* (1969) obtained survival times of up to 2.5 hours in 8 dogs.

In The Netherlands, many experimental orthotopic heart transplantations in dogs were done by Bos *et al.* (1970, 1971, 1972) (see page 111); and the Department of Thoracic Surgery of the Leiden University Hospital, in close collaboration with The Radiobiological Institute TNO, Rijswijk, performed orthotopic cardiac allografts in rhesus monkeys under profound hypothermia. The pathological findings were reported by Hollander and Zurcher (1972) and Hollander (1973). In these studies unmodified animals (n = 5) survived from 6 to 27 days (mean 13.2 days). The classical description of unmodified cardiac allograft rejection is given. Arteritis was only observed in the longest survivors. Modified animals (n = 11) (azathioprine and ALS) survived from 11 to 128 days (mean 45 days). The rejection pattern consisted of mononuclear cell infiltration, and all animals showed severe arteritis after 14 days. Modified animals given a combination of azathioprine, prednisone, and ALS aurvived from 10 to 104 days (mean 45 days). The histological picture was the same as that described for animals treated with azathioprine. The pathology of the cardiac allografts was compared with that of renal allografts in rhesus monkeys; no obvious differences were noticed. It was concluded that the kidney allograft can serve as a model for the study of arteritis in allograft rejection in general.

Marquet *et al.* (1971) compared the results of orthotopic and heterotopic heart allografts in rhesus monkeys. On average, the unmodified heterotopic animals survived 7 days and the orthotopic 13.2 days. The modified heterotopic animals survived for a mean duration of 48 days, the orthotopic 45 days. The pathology of the heterotopic grafts showed an additional feature described as "subendocardial infarction necrosis", defined as a lesion probably due to the unfavourable hemodynamic situation in the heterotopic heart.

Xenogeneic orthotopic heart grafting

Very little information is available on the subject of xenogeneic orthotopic heart transplantation. Neville *et al.* (1969) described the microcirculation of the transplanted heart after orthotopic xenografting of calf, sheep, goat and pig hearts in canine recipients. The failures in these experiments were attributed to the size of the donor erythrocytes, which were larger than those of the recipients. Early erythrocyte aggregation occurred, as demonstrated microscopically. In another group of experiments canine hearts were transplanted into calf, sheep, and goat recipients, which survived for longer periods than those of the first series (exact data are not given). These grafts did not show erythrocyte aggregation at post mortem examination.

SUMMARY

Chapter I

I.1.

There are two heart transplantation models, a heterotopic and an orthotopic model. Both are used for research on the same subjects, especially in dogs, but their specific value for heart transplantation studies has never been defined. This curious fact led to the present investigations in dogs, in which differences between the two models were analysed and related to the position of the graft and its hemodynamic function.

In the heterotopic grafts a connection is established between the aorta of the graft and a systemic artery of the recipient. The pulmonary artery is connected to a systemic vein. The lung veins and the superior and inferior caval veins of the graft are ligated. The blood flows from the recipient's artery into the graft's aorta, and when the aortic valves function normally the blood can only enter the coronary arteries. It returns through the coronary veins and is ejected by the right ventricle. The right ventricle performs 10 per cent of the normal workload. The left ventricle has no workload at all, unless the aortic valves are incompetent.

The orthotopic graft occupies the same position and performs all of the functions of the heart it replaces.

I.2.

We reviewed the literature on heart transplantation research in dogs to identify practical and theoretical differences between the two models. The heterotopic model proved to have many practical advantages (costs, experimental animals, technical equipment, supporting techniques, etc.).

Analysis of the position of the graft and the hemodynamic functions showed that the heterotopic graft has disadvantages: positive pressure during inspiration, tendency of the vascular pedicle to twist, thrombotic occlusion of the venous connections, progressive aortic incompetence, absence of the normal force-velocity-length relationships and pressure-volume-flow relationships, and the tendency to more severe arteritis and myocardial necrosis. The orthotopic model did not show these features at all or only to a limited degree.

I.3.

These discrepancies showed that a detailed experimental study on these heart transplantation models, preferably performed in dogs, was required to obtain answers to the following questions:

1) Are the differences indicated by the literature real?
2) What are the determinants of these differences, and what do these differences actually consist of?
3) What is the value of each of the models for heart transplantation research?

Chapter II

II.1.

Unrelated random dogs were chosen for the experiments. Twenty-four of the animals were not modified by the administration of immunosuppressive agents (*Series I*: 10 heterotopic, 14 orthotopic). Twenty-five were treated with azathioprine and prednisone (*Series II*: 12 heterotopic, 13 orthotopic).

The operations were performed according to standard techniques, and the pre-, per-, and post-operative conditions were identical in the heterotopic and orthotopic experiments, except for the extracorporeal circulation used in the orthotopic experiments. The influence of this factor was investigated by the performance of 7 heterotopic experiments in which the per-operative conditions and the amount of blood administered were the same as those in the unmodified orthotopic experiments (*Series III*).

II.2.

Identical measurements made in heterotopic and orthotopic grafts were quantitated and compared. These measurements included graft survival time, ECG recordings (voltage decline and decrease of the heart rate, which are electrocardiographical signs of rejection), and histopathological analysis of the rejected graft. Macroscopical and microscopical examinations were preformed in all cases. The autopsy methods are explained and the microscopical signs of rejection described. The main features of microscopical rejection were selected and quantitated according to a scale constructed for these purposes.

Chapter III

III.1.

The *graft survival time* of unmodified grafts did not differ significantly ($p > 0.10$) between the two types of graft. Orthotopic modified grafts survived significantly longer than heterotopic modified grafts ($p < 0.002$). The results of the grafts in Series III did not differ from those of the heterotopic grafts in Series I ($p > 0.10$). The influence of the chosen immunosuppressive treatment on graft survival time was not insignificant for heterotopic grafts ($p > 0.05$), but was significant for orthotopic grafts ($p < 0.01$).

In the *ECG picture*, the voltage decline was significantly more pronounced in the unmodified heterotopic graft than in the orthotopic graft ($p < 0.05$). A significant difference was not found for

modified grafts ($p > 0.50$).

The decrease in the heart rate was not significantly different between the models in unmodified ($p > 1.0$), but was significantly different in modified grafts in favor of the orthotopic model ($p < 0.05$): bradycardia appeared "earlier" in heterotopic than in orthotopic grafts.

Bradycardia of the graft could be produced in all heterotopic experiments by turning the animal on its back. This phenomenon did not occur in the orthotopic experiments.

The results of the heterotopic grafts of Series III with regard to voltage decline and heart rate were the same as those in Series I (heterotopic grafts).

III.2.

Post-mortem examination of unmodified heterotopic recipients did not reveal any abnormalities. Orthotopic unmodified recipients invariably showed signs of congestion of organ systems due to failure of the graft.

The grafts were examined for mononuclear cell infiltration (MNI), rupture of small interstitial vessels (RIV), myocytolysis (M), arteritis (Art), and infarction necrosis (IN). Semi-quantitative analysis of these features did not show differences between orthotopic and heterotopic grafts as far as MNI and M were concerned. RIV was more striking in orthotopic and Art and IN more striking in heterotopic grafts ($p < 0.01$). Significantly more thrombi were present in heterotopic grafts, especially in the venous outlet ($p < 0.001$).

The unmodified heterotopic graft seemed to be destroyed mainly by Art and IN, the unmodified orthotopic graft by RIV, M, and MNI. Features of rejection were more pronounced in the heterotopic grafts at the moment of cardiac arrest.

The results of analysis of the histological signs of rejection in the heterotopic grafts of Series III were the same as those of Series I (heterotopic grafts) ($p > 0.1$).

Post-mortem examination of modified recipients showed the occurrence of drug toxicity, which amounted to between 50 and 60 per cent. In heterotopic experiments the death of the recipient was always related to drug toxicity. In the orthotopic experiments death

was due to graft rejection, usually in combination with drug intoxication. Microscopically, it was possible to differentiate between rejection and drug intoxication.

Microscopical examination of the grafts and semi-quantitative analysis of the data showed a reduction of MNI, RIV, and M in modified material in both models. Under modification, Art and IN showed a change from "acute" to more "chronic" features and there was no longer a significant difference between these lesions, but the lesions appeared earlier in the heterotopic grafts when the results were considered in relation to graft survival time. There was no influence of immunosuppressive treatment on the incidence and localization of thrombi.

The influence of immunosuppression was significant in heterotopic grafts for MNI, M, Art, and IN (p for all four: < 0.01), and non-significant for RIV and T ($p > 0.05$ for both). In orthotopic grafts this influence was significant for MNI, RIV, and M ($p < 0.05$, 0.01, and 0.01, respectively) and non-significant for Art, IN, and T ($p > 0.05$ for all three).

Chapter IV

IV.1

In this section the findings of our experiments are discussed in relation to the data we collected from the literature concerning investigations in dogs. Our results confirm the differences between the orthotopic and heterotopic heart transplantation models indicated by the various publications and corroborate our view that these differences are related to differences in position of the graft but much more to differences in hemodynamics.

IV.2.

We arrived at the hypothesis that the myocardium of the heterotopic graft in this experimental set up is "susceptible" to ischemia, owing to its impaired coronary circulation, and that ischemic myocardial tissue is subject to a different rejection process.

The impaired coronary circulation of the heterotopic graft allows prolongation of the interaction between the graft tissue and the recipient's immunological offensive mechanisms, which results in more ischemia. The rejection process is more rapid in heterotopic grafts than in orthotopic grafts. In unmodified recipients this fact is not reflected by the graft survival time, but the patterns are clearly distinguishable in the ECG results and the histological findings. In modified animals the difference is significant for graft survival time as well.

Differences between our findings and those of others made in other modified species are attributed to differences in immunosuppressive therapy (differences in the agents and regimens used) and differences related to the species of animal, the clearance of passenger cells, and the supportive techniques applied.

The influence of extracorporeal circulation and blood transfusion in our unmodified heterotopic grafts was not sufficiently strong to explain the differences we found between the two models.

IV.3.

The divergent physiological behavior, the histological rejection pattern, and the weak response to immunosuppressive therapy, mean that the heterotopic model in the described experimental design is not always recommendable for fundamental research in the field of heart transplantation. This model may, however, have some value for the investigation of rejection arteritis and the suppression of this phenomenon.

The orthotopic model remains a satisfactory model for prospective human transplantation. The shortcomings of this model are related only to the rejection process, which prevents it from being completely successful.

IV.4.

Approximately 277 human heart transplantations have been performed all over the world, the longest survival being 6.8 years (Bergan, August 1975). The 5-year survival rate obtained by the

Stanford team is 20 per cent (Schroeder *et al.* 1976).

The main cause of failure is the development of occlusive lesions in the coronary arteries, but many complications are also attributed to immunosuppressive treatment needed by the recipient throughout life. "Graft arteriosclerosis" has been ascribed to the action of circulating immune complexes. A specific treatment for this process has not been found yet.

When the problem of rejection is finally solved, transplantation as a treatment for human heart diseases in a terminal stage will deserve renewed interest.

The title page of this volume represents our evaluation of the two experimental heart transplantation models. An ace of hearts is superior to a two of hearts, but only when hearts are trump. In heart transplantation surgery, rejection – symbolized here by the red background – is still trump.

Appendix

A.1a.

Eight heart catheterization studies were performed in heterotopic grafts, four in Series I and four in Series II. On all occasions pathological pressures were demonstrated to be present in the ventricles, and the contractility of the myocardial tissue was clearly diminished compared to the controls. Immunosuppressive treatment slightly reduced the deterioration of pressure and contractility.

Angiography of the grafts showed very low myocardial excursions in all grafts and valvular insufficiencies in approximately 50 per cent of the cases.

Liability to bradycardia was also found to occur in all grafts when the recipient was turned onto its back.

A.1b.

An immunofluorescence investigation was undertaken in un-

modified dogs to test the hypothesis that "immune myocarditis" occurs in the recipient's "normal heart" (host heart) in the heterotopic experiments. The material for this investigation was chosen from Series I and Series IV, the latter series having been especially designed for this purpose. ECG studies of the host heart after transplantation showed changes suggestive of immune myocarditis. Control ECG studies in dogs demonstrated that the ECG alterations of the host heart in the post-operative period are consistent with the criteria for the normal canine ECG. The above misconception concerning the host heart ECG arose because initially the criteria for the human ECG were applied to the canine ECG. Moreover, immunofluorescence studies on the host heart at the time of rejection (9 host hearts) and 1 week thereafter (8 host hearts) did not reveal any immunofluorescence.

This investigation also revealed a positive correllation between the histological and the immunofluorescence findings in the graft, which showed that there is an immunological basis for the rejection process in the heterotopic graft.

A.2.

The surgical procedures employed in the experiments and the pre- and post-operative management are described here.

A.3.

Detailed information about heart transplantation research from the beginning up to 1976 is given in this section.

SAMENVATTING

Hoofdstuk I

I.1.

Er zijn twee harttransplantatie modellen, een heterotoop en een orthotoop. Beiden worden vooral bij honden gebruikt voor veelal gelijksoortige onderzoekingen, maar de specifieke waarde van beide modellen met betrekking tot harttransplantatie onderzoek werd nooit vastgesteld. Dit merkwaardige feit bracht ons ertoe de beide modellen in het species canis nader te analyseren. De verschillen tussen beide modellen betreffen de positie waarin het transplantaat geplaatst is en de hemodynamische functie der transplantaten.

Het heterotope transplantaat is verbonden met zijn gastheer door middel van een vaatanastomose tussen de aorta van het transplantaat en een systeem arterie van de ontvanger. De arteria pulmonalis is verbonden met een systeem vene. De longvenen en de vena cava superior en inferior zijn geligeerd. Het bloed stroomt vanuit de arterie van de recipient in de aorta van het transplantaat. Wanneer de aorta kleppen sufficient zijn, kan het bloed slechts de coronair arteriën doorstromen. Het bloed keert terug naar de rechter ventrikel via coronair venen en sinus coronarius en wordt dan weggepompt. De rechter ventrikel verricht slechts 10 procent van de normale arbeid, de linker ventrikel verricht in het geheel geen arbeid, tenzij de aortakleppen insufficient zijn.

Het orthotope transplantaat heeft dezelfde positie en de volledige functie van het hart, dat werd verwijderd.

I.2.

We analyseerden praktische en theoretische verschillen tussen orthotoop en heterotoop harttransplantatie model, verkregen uit publicaties over harttransplantatie onderzoek verricht bij honden. Het heterotoop model bleek vele praktische voordelen te bezitten (kosten, proefdieren, technische uitrusting, hulptechnieken enz.).

Analyse van de positie van het transplantaat en zijn hemodynamische funktie toonde echter nadelen voor het heterotope transplantaat: positieve druk gedurende de inspiratie, neiging tot afknikken van de vaatsteel, thrombose van de anastomosen (voornamelijk de veneuze verbinding), progressieve aorta insufficientie, afwezig zijn van de normale "force-velocity-length" relatie en afwezig zijn van de "pressure-volume-flow" relatie en neiging tot uitgesproken arteritis en infarct necrose als uiting van het histologisch afstotingsproces van het transplantaat. Het orthotope model toonde deze verschijnselen niet, of in geringe mate.

I.3.

Deze diskrepanties tussen beide modellen brachten ons ertoe, een diepgaander onderzoek van beide modellen te verrichten in het species canis, daarbij vroegen we ons het volgende af:

1) Kunnen we de verschillen, die met behulp van de literatuur werden aangetoond, bevestigen?
2) Door welke oorzaken ontstaan deze verschillen? Wat is de aard van deze verschillen?
3) Wat is de individuele waarde van beide modellen met betrekking tot harttransplantatie onderzoek?

Hoofdstuk II

II.1.

De proefdieren van het onderzoek waren niet aan elkaar verwante willekeurig gekozen honden, geselecteerd op afmeting en gewicht. Vierentwintig werden niet met immunosuppressiva behandeld

(*Serie I*: 10 heterotoop, 14 orthotoop). Vijfentwintig honden werden behandeld met azathioprine en prednison (*Serie II*: 12 heterotoop, 13 orthotoop). De operaties werden volgens standaard technieken verricht. De pre-, per-, en postoperatieve condities der proefdieren waren identiek met uitzondering van het gebruik van de hartlongmachine in de orthotope experimenten. De invloed van deze factor werd onderzocht door middel van een contrôle serie: *Serie III* (7 niet met immunosuppressiva behandelde heterotoop getransplanteerde proefdieren, die dezelfde behandeling kregen qua bloedtoediening als in de orthotope experimenten).

II.2.

Identieke metingen werden verricht aan beide modellen. De metingen werden gekwantificeerd en hun waarden vergeleken. De metingen waren: overlevingstijd van het transplantaat, ECG metingen (voltage daling en daling van de hart frequentie werden gekozen als electrocardiografische verschijnselen van afstoting) en analyse van de histologie van het afgestoten transplantaat. Macroscopisch en microscopisch onderzoek van het gehele materiaal werd verricht. Sectie methoden werden beschreven evenals de microscopische verschijnselen van het afstotingsproces, waarvan er 5 werden uitgekozen voor kwantitatieve analyse volgens een voor dit doel ontworpen schaal.

Hoofdstuk III

III.1.

De overlevingstijden van de onbehandelde transplantaten toonden geen significant verschil ($p > 0.10$). Behandelde orthotope transplantaten overleefden significant langer dan identiek behandelde heterotope transplantaten ($p < 0.002$). Er was geen verschil tussen overlevingstijden van heterotope transplantaten uit Serie III en Serie I ($p > 0.10$). De toegepaste immunosuppressieve behandeling had geen significante invloed op de overleving van heterotope transplantaten ($p > 0.05$), wel op die van orthotope transplantaten ($p < 0.01$).

Wat het *ECG onderzoek* betreft, voltage daling was significant meer aanwezig in onbehandelde heterotope dan orthotope transplantaten ($p < 0.05$). Verschillen in voltage daling waren niet significant aanwezig in behandelde transplantaten ($p > 0.5$). Daling van hartfrequentie was niet significant verschillend in onbehandelde ($p > 1.0$), maar significant verschillend in behandelde transplantaten. Bradycardie trad in een vroeger stadium op in de heterotope transplantaten, dan in de orthotope transplantaten ($p < 0.05$).

Bradycardie van het transplantaat kon in alle heterotope experimenten worden opgewekt door de ontvanger op zijn rug te leggen. Dit verschijnsel was afwezig in de orthotope experimenten.

De resultaten van Serie III met betrekking tot voltagedaling en hartfrequentie waren identiek aan die van Serie I (heterotope transplantaten).

III.2.

Sectie van de onbehandelde proefdieren leverde geen orgaan afwijkingen op in de heterotope ontvanger, in de orthotope ontvanger bestond stuwing van orgaan systemen als uiting van "failure" van het transplantaat.

De transplantaten werden onderzocht op: infiltratie van mononucleaire cellen (MNI), ruptuur van kleine interstitiële vaatjes (RIV), myocytolyse (M), arteritis (Art) en infarct necrose (IN), Semi-quantitatieve analyse van deze verschijnselen in onbehandelde transplantaten gaf geen duidelijke verschillen wat betreft MNI en M. RIV was duidelijker in orthotope en Art en IN duidelijker in heterotope transplantaten ($p < 0.01$) Thrombi waren significant meer aanwezig in heterotope dan in orthotope transplantaten, vooral op de veneuze anastomose ($p < 0.001$). Het onbehandelde heterotope transplantaat leek voornamelijk te worden afgestoten door Art en IN, het onbehandelde orthotope transplantaat door RIV, M, en MNI. De verschijnselen van afstoting waren meer uitgesproken in het heterotope transplantaat op het moment van stilstand van het transplantaat.

De resultaten van Serie III waren gelijk aan die van Serie I (heterotope transplantaten) voor alle histologische criteria ($p > 0.1$).

Sectie van de behandelde proefdieren toonde het bestaan van "drug intoxicatie" aan in 50 tot 60 procent van de behandelde

dieren. Wanneer de heterotope recipient stierf, voordat het transplantaat stilstond was er altijd sprake van "drug intoxicatie". In de orthotope experimenten werd de dood van de ontvanger meestal veroorzaakt door een combinatie van "drug intoxicatie" en afstoting. Met behulp van het microscopisch onderzoek konden rejectie en drug intoxicatie van elkaar gedifferentieerd worden. Microscopisch onderzoek van de behandelde transplantaten en semi-quantitatieve analyse van de gegevens toonde reductie van MNI, RIV, en M aan in beide modellen in ongeveer gelijke mate. Art en IN veranderden van "acute" naar meer "chronische" vormen en er bestond geen significant verschil meer tussen deze lesies in beide modellen. De lesies traden echter wel vroeger op in het heterotope transplantaat, wanneer de overlevingstijd ervan werd "medebetrokken" in de beoordeling. De toegepaste immunosuppressieve behandeling had geen significante invloed op het aanwezig zijn van thrombi.

In de heterotope transplantaten was het effect van immunosuppressieve behandeling op MNI, M, Art en IN significant (p voor alle vier < 0.01), op RIV en T niet significant (p voor beiden > 0.05). In de orthotope transplantaten was dit significant voor MNI, RIV en M (p respectievelijk < 0.05, 0.01 en 0.01) en niet significant voor Art, IN en T ($p > 0.05$ voor alle drie).

Hoofdstuk IV

IV.1.

In deze paragraaf worden onze experimentele bevindingen besproken met betrekking tot de gegevens die we uit de literatuur over onderzoekingen bij honden verzamelden. Onze resultaten bevestigen de verschillen die werden opgemerkt en zij versterken onze opvatting dat deze verschillen veroorzaakt worden door verschillen in positie van het transplantaat maar bovenal door verschillen in hemodynamiek.

IV.2.

Onze stelling is, dat het myocardium van het heterotope trans-

plantaat in de proefopstelling zoals beschreven, "vatbaar" is voor het ontstaan van ischemie ten gevolge van een gebrekkige coronair circulatie en dat in ischemisch hartspier weefsel een ander afstotingsproces ontstaat. De gestoorde coronair circulatie van het heterotope transplantaat begunstigt langduriger contact tussen het immunologische afweermechanisme van de gastheer en het weefsel van het transplantaat, hetgeen weer tot meer ischemie aanleiding geeft. Heterotope transplantaten worden derhalve sneller afgestoten, hetgeen in onbehandelde dieren niet tot uiting komt in overlevingstijd van het transplantaat, maar wel in voltage daling en histologisch afstotingspatroon. In behandelde dieren wordt het verschil in overlevingstijd van het transplantaat wel duidelijk.

Verschillen tussen onze bevindingen en die, waarbij een ander behandeld "proefdier species" werd gebruikt, werden toegeschreven aan verschillen in immunosuppressieve behandeling (verschillende medicamenten en verschillende schema's), verschillen met betrekking tot het species van het gebruikte proefdier, het al of niet verwijderen van bloedcellen van de donor, nog aanwezig in het transplantaat tijdens de operatie en verschillen in hulp technieken en in hoeveelheden toegediend bloed. In onze experimentele opzet bleek het gebruik van hartlong-machine en het toedienen van bloed geen significante verschillen op te leveren tussen onze onbehandelde heterotope transplantaten.

IV.3.

Het afwijkend fysiologisch karakter, het histologisch afstotingspatroon en het geringe effect van immunosuppressie op het heterotope harttransplantatie model, maken het in de beschreven proefopstelling minder geschikt voor fundamenteel onderzoek. Het model heeft mogelijk wel enige waarde voor het onderzoek van afstotings arteritis en het behandelen ervan.

Het orthotope model blijft het voorkeurs model voor harttransplantatie onderzoek vooral met het oog op toepassing bij de mens. De beperkende factor voor dit model is het afstotingsproces, dat volledig succes tot nu toe onmogelijk maakt.

VI.4.

Tot augustus 1975 zijn ongeveer 277 harttransplantaties bij de mens uitgevoerd. De langste overleving bedraagt 6,8 jaar. De vijfjaars overleving bedraagt in het patiënten materiaal van het Stanford team thans 20 procent (Schroeder *et al.* 1976). Chronische afstoting van het transplantaat, veroorzaakt door "arteriosclerotische" veranderingen van de coronair arteriën, is de voornaamste oorzaak van mislukking. Ook worden veel complicaties beschreven van de langdurige immunosuppressieve behandeling. "Graft arteriosclerosis" ontstaat waarschijnlijk voornamelijk ten gevolge van circulerende immuun complexen. Een specifieke behandeling van dit proces is nog niet gevonden. Wanneer het probleem van de chronisch afstoting is opgelost, zal deze methode ter behandeling van patienten met hartziekten in een eind stadium, grotere belangstelling verdienen dan thans het geval is.

De titel pagina van dit boek symboliseert onze opvatting over de twee harttransplantatie modellen. Wanneer harten troef is, is hartenaas superieur aan harten twee; in de harttransplantatie chirurgie is afstoting, hier gesymboliseerd door de rode achtergrond, nog immer troef.

Appendix

A.1a.

Acht hartcatheterisaties werden in heterotope transplantaten verricht. Viermaal werd een transplantaat uit Serie I en viermaal een transplantaat uit Serie II onderzocht. Bij alle onderzoekingen werden pathologische drukken gevonden in de ventrikels, de contractiliteit van het hartspier weefsel was sterk verminderd in vergelijking met de contrôle waarden. Immunosuppressieve behandeling beïnvloedde de achteruitgang van de functie slechts in geringe mate.

Angiografie toonde minimale myocard excursies in alle transplantaten en klep insufficientie in ongeveer 50 procent van de gevallen. Neiging van het transplantaat tot bradycardie werd vastgesteld bij alle proefdieren, wanneer ze op de rug werden gelegd.

A.1b.

Immunofluorescentie onderzoek werd verricht in onbehandelde proefdieren om de hypothese betreffende een "immunomyocarditis" in het normale hart van de heterotope recipient (host heart) te testen. Het materiaal werd gekozen uit Serie I en uit Serie IV, een speciaal voor dit doel gecreeërde serie. ECG studies van het "host heart" na transplantatie toonden namelijk veranderingen die suggestief waren voor het bestaan van een "immunomyocarditis". Uit contrôle ECG studies in honden bleek echter dat "ECG veranderingen" in het host heart ook aanwezig waren in het contrôle materiaal. Deze misvatting ontstond omdat in eerste instantie de criteria van het menselijk ECG werden toegepast op die van de hond. Noch bij immunofluorescentie onderzoek van het host heart tijdens stilstand van het transplantaat (9 host hearts), noch bij immunofluorescentie onderzoek 1 week na stilstand van het transplantaat (8 host hearts), werden afwijkingen gevonden suggestief voor een immunomyocarditis.

Het immunofluorescentie onderzoek toonde tevens de positieve correllatie aan tussen de bevindingen van histopathologie en immunofluorescentie van het transplantaat, waarmee een immunologische basis voor het heterotope rejectie proces aannemelijk werd gemaakt.

A.2.

In deze paragraaf werden de operatie technieken, die voor de experimenten gebruikt werden, beschreven. Tevens wordt de pre-, en de postoperatieve behandeling toegelicht.

A.3.

Dit historisch overzicht bevat nagenoeg alle gegevens over experimenteel harttransplantatie onderzoek vanaf de begin periode tot 1976.

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CURRICULUM VITAE

C.K. Jongsma werd 10 oktober 1936 te Oosterbeek geboren.

Hij behaalde in 1957 het Gymnasium B diploma (Johannes Calvijn Lyceum te Rotterdam).

De medische studie verrichtte hij aan de Vrije Universiteit te Amsterdam en aan de Stichting Klinisch Hoger Onderwijs te Rotterdam (artsexamen 25 mei 1966).

De opleiding tot algemeen chirurg ontving hij van Prof. Dr. P.J. Kooreman en Dr. G.A.A. Olthuis in het Zuiderziekenhuis te Rotterdam. In het jaar 1970 werkte hij als assistent of de afdeling Thorax Chirurgie van het Academisch Ziekenhuis Dijkzigt te Rotterdam onder leiding van Prof. Dr. J. Nauta. In die periode werd de basis voor het ontstaan van dit proefschrift gelegd. Inschrijving in het specialisten register algemene heelkunde volgde op 1 augustus 1972.

Vanaf 1 augustus 1972 tot 1 april 1975 was hij wederom aan de afdeling Thorax Chirurgie van het Academisch Ziekenhuis Dijkzigt verbonden in de functie van wetenschappelijk hoofdmedewerker.

Sinds 1 april 1975 is hij als chirurg verbonden aan het Havenziekenhuis te Rotterdam.