CHRONIC REJECTION IN DLA IDENTICAL DOGS AFTER ORTHOTOPIC CARDIAC TRANSPLANTATION

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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. LEIJNSE
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATS VINDEN OP
WOENSDAG 18 APRIL 1979
DES NAMIDDAGS OM 4.15 UUR PRECIES

DOOR

OLAF CORINE KAREL MARIA PENN
geboren te Rantau Prapat (Indonesië)

1979

grafische verzorging:
davids decor alblasserdam

PROMOTOR : PROF. DR. E. BOS

CO-REFERENTEN : PROF. DR. J. NAUTA

PROF. P.G. HUGENHOLTZ

Aan de nagedachtenis van mijn vader
aan mijn moeder
aan Myriam en David-Marc.

Het verschijnen van dit proefschrift en het in het proefschrift beschreven transplantatie-onderzoek werden mede mogelijk gemaakt door steun van de Nederlandse Hartstichting.
Het verschijnen van dit proefschrift werd mede mogelijk gemaakt door het Hippocrates Studiefonds.

Every discovery, however important and apparently epoch making, is but a natural and inevitable outcome of a vast mass of work, involving many failures, by a host of different observers.

Ernest Starling.

CONTENTS

CHAPTER 1

INTRODUCTION

1.1. General considerations

"What you don't know whould make a great book".
Rev. Sydney Smith.

The justification for clinical cardiac transplantation is that it should solve end-stage cardiac disease when no other medical or surgical treatment is available (76).

However, after cardiac transplantation the main barriers to long-term survival and complete rehabilitation include the management of acute rejection episodes, the complications related to the immunosuppressive therapy and the progression of graft arteriosclerosis or chronic rejection (77). Although a progressive increase in patient survival has become apparent (77), many aspects of the process of acute and chronic cardiac allograft rejection have yet to be studied.

Starting in 1969, several investigations in experimental cardiac transplantation have been performed in the laboratory for experimental surgery, Erasmus University, Rotterdam. These studies have focussed primarily on the rejection process after cardiac allotransplantation in dogs.

In 1977 Jongsma reported experimental work on orthotopic versus heterotopic canine cardiac allografts (92). He analyzed these two cardiac transplantation models and concluded that their pattern of acute rejection is different: in heterotopic grafts the rejection process seems to proceed more rapidly than in orthotopic grafts. It was suggested that the impaired coronary circulation of the cardiac allograft in the heterotopic position caused additional ischemic injury to the graft (92). Thus in studies dealing with cardiac allografts the model of orthotopic cardiac transplantation is to be preferred in order to obtain more realistic information.

In 1979 Bos reported on experiments in orthotopic canine cardiac allotransplantation (19). He developed an experimental model for chronic rejection after orthotopic cardiac transplantation. Looking for a method to prolong graft

survival time without the detrimental effects on the recipient by the use of immunosuppressive drugs, Dog Leucocyte Antigen (DLA) matching was successfuly applied to serve this purpose.

Clinically the process of chronic cardiac allograft rejection resembles the process of arteriosclerosis (19, 75). It represents the limiting factor for long-term survival and complete rehabilitation. Therefore, it was decided to study in more detail this standardized model of chronic rejection after orthotopic canine cardiac transplantation in DLA identical beagle littermates, as developed by Bos. In this thesis histopathological and functional changes, as observed during the chronic rejection process, are described.

1.2. Rationale of the study

Chronic rejection or graft arteriosclerosis.

The process of chronic rejection was studied because it represents the limiting factor in long-term survival after orthotopic cardiac transplantation. After necropsy of long-term surviving cardiac allograft recipients, it became obvious that these cardiac allografts are prone to an accelerated atherosclerosis, whereas, at the time of transplantation the coronary vasculature of the cardiac donors was assumed to be free of atheroma (14, 106, 109). The narrowing of the lumina of the coronary arteries could also be demonstrated on angiograms, during follow-up of the patients, as they were routinely performed in some clinics. Death seemed to be the result of myocardial ischemia (77, 149, 150). This progressive coronary arterial narrowing, which occurs in the allografted heart, was designated as chronic rejection or graft arteriosclerosis (75). So far the only therapeutic measure that could be taken to alleviate this imminent danger was the institution of a regimen of antithrombotic agents and the regulation of dietary lipids (77, 82, 98, 129).

Possible explantations for the relationship between the accelerated graft arteriosclerosis after orthotopic cardiac transplantation and the process of rejection are still debated.

In 1973 O'Connell and Mowbray indicated that graft failure could be the result of an endothelial damage of the arteries caused by circulating antibodies in the host. Intimal thickening could be reproduced by them in arteries of rabbits by exposure to immune alloantibodies (134).

Cerilli reported, in 1977, the existence of an antivascular endothelial cell antibody and the absence

of this antibody correlated significantly with graft survival (33, 34). Thus, vascular lesions seem to be the result of a humoral reaction after transplantation, by which the endothelium is directly injured by the circulating antibodies. A unifying hypothesis explaining the connection between the immunological vascular lesions and graft arteriosclerosis was proposed by Kosek (109). He postulated that the primary immunological damage was directed to the endothelium of the arteries and also to the endothelium of the vasa vasorum of the same arteries. This should lead to primary endothelial damage followed by secondary lesions caused by medial hypoxia after obliteration of the vasa vasorum. Fatty changes in the vessel wall are the subsequent result of this direct damage and arterial wall hypoxia (109).

The chronic rejection model.

The model described by Bos provides an opportunity to investigate experimentally the process of chronic rejection after orthotopic cardiac transplantation (19). In this model prolongation of graft survival was achieved by DLA matching in beagle littermates. Immunosuppressive medication was not employed in order to avoid the detrimental effects of these drugs on the recipients. It was concluded that this was an appropriate model to study in detail the course and the characteristic features of chronic rejection (19).

The histopathological changes that were described by Bos consisted of postmortem findings. Based on the supposition that a documentation of sequential histopathologic events could give a better insight into the rejection process and as this could possibly lead to indications for specific treatment in the future, it was decided to study the histopathological course of rejection in this thesis.

The clinical course of chronic rejection in DLA identical beagle littermates after orthotopic cardiac transplantation was also described by Bos (19). He mentioned that no helpful measurements are available to monitor the early onset or the progression of the course of chronic rejection. Voltage changes in the ECG can be satisfactory used to diagnose acute rejection episodes and immediate therapy can be instituted when a voltage drop of 20% is observed (119, 120). However in chronic rejection the ECG fails to show significant changes and can not be used in this way (19). This led us to introduce another technique for sequential documentation on myocardial performance during chronic rejection.

Histopathology during chronic rejection

End-stage cardiac allograft pathology of chronic rejection was described in detail by Kosek (106-109), Bieber (12, 14) and Hollander (88). As mentioned by Bos (19), the predominant histopathological feature of chronic cardiac allograft rejection is found in the larger medium sized vessels of the graft and is defined as chronic obliterative arteritis. However no insight was obtained in the course of the rejection process, because these findings were obtained after postmortem examination.

Therefore the investigations presented in this thesis were carried out in order to study the histopathological changes that develop during the course of chronic rejection. For this purpose a percutaneous myocardial biopsy technique was applied.

Extensive experience with myocardial biopsies was reported by Sakakibara and Konno, who first described their technique in 1962 (103). In 1973, Caves at Stanford University introduced a modified Konno-Sakakibara biotome to obtain right ventricular endomyocardial biopsies. To study the acute rejection process he took biopsies in dogs after orthotopic cardiac transplantation and immunosuppressive therapy. The results of these studies encouraged Caves to take transvenous endomyocardial biopsies in patients following cardiac transplantation (27, 76). As acute rejection is manifested by parenchymal infiltration these myocardial biopsies turned out to be of great value for the diagnosis and treatment of acute rejection episodes.

The endomyocardial biopsy technique presented two problems as we tried it out in our investigations. The technique is not adequate for serial biopsies during long-term survival of the animals because thrombosis of the neck veins easily occurs. Also endomyocardial biopsies are small and consist of endocardium and a small piece of myocardium in which few arteries are seen. Hence another biopsy technique was necessary to analyse the vascular lesions of chronic rejection. The method of taking percutaneous myocardial biopsies with a disposable liver biopsy needle was chosen. These biopsies were taken by introducing the needle through the skin of the chest into the apex of the heart and sampling a piece of left-ventricular myocardium. These weekly biopsies contained enough blood vessels for histopathological examination and allowed us to follow the course of the chronic rejection process.

Myocardial contractility during chronic rejection.

The process of chronic rejection after orthotopic cardiac transplantation, with its slowly developing ischemic changes, might also be reflected in changes in myocar-

dial performance. Studies about myocardial performance during long-term survival comprise cardiac catheterizations after orthotopic cardiac transplantation in man (71, 74, 76). Serial measurements are necessary to detect these changes in myocardial function during the course of rejection and a non-invasive method is preferable for these repeated recordings. A standardized method to determine myocardial contractility using invasive methods, like cardiac catheterization, can not be used readily for serial measurements. Unfortunately conventional echocardiography, while being non-invasive, can not be satisfactorily applied in dogs for anatomical reasons. For our studies on chronic rejection we had to search for another non-invasive method which could reflect changes in contractility. We adapted a method described by Karliner in 1971. By this method the mean velocity of circumferential fiber shortening ($\bar{V}_{cf}$) is determined from the motion of endocardial markers as registered on Roentgencinefilms (96). According to Karliner, the mean velocity of shortening represents a measure of myocardial contractility. As little was known about this method, we also evaluated it to determine if indeed a positive correlation existed between $\bar{V}_{cf}$ and more conventional criteria for myocardial contractility. Thus, if these investigations were positive, once the markers were attached to the heart, a reliable non-invasive method for serial measurements of myocardial contractility would be available.

1.3. Objectives of the study

Based on the considerations described in the previous introduction, the investigations presented in this study focussed upon the following objectives.

First objective.

To describe the histopathological changes that develop during the course of chronic rejection after orthotopic cardiac transplantation in DLA identical beagle littermates, and to determine the time of onset of these changes as demonstrated by means of serial percutaneous myocardial biopsies.

Second objective.

To study myocardial performance during chronic rejection after orthotopic cardiac transplantation in DLA identical beagle littermates by means of a non-invasive technique for recording the motion of endocardial markers.

1.4. Plan of action of this study.

The techniques and the subsequent results of our investigations on chronic rejection after orthotopic canine cardiac transplantation are described in four articles that have appeared in international journals. These articles, reproduced in chapters two to five, are arranged in the order of time of appearance.

The aim of the study was to investigate the histopathology of serial biopsy specimens and myocardial contractility during chronic rejection.

In the first chapter the experiments are introduced and the specific experimental objectives stated.

The second chapter contains the first article: "Serial percutaneous biopsies from canine orthotopic cardiac allografts" (Journal of Surgical Research 18 : 615, 1975). In this chapter the method for taking serial percutaneous myocardial biopsies is described.

The third chapter comprises the second paper entitled: "Orthotopic canine heart transplantation: left ventricular contractility during chronic rejection" (Journal of Thoracic and Cardiovascular Surgery 71 : 526, 1976). In this paper the changes in myocardial contractility during chronic rejection are described. The method we used as a contractility index was to calculate the mean velocity of circumferential fiber shortening ($\bar{V}_{cf}$) from endocardial marker movements on Roentgenfilms (96).

The fourth chapter contains the third article and deals with the "Histopathology of rejection in DLA-identical canine orthotopic cardiac allografts". (Transplantation 22 : 313, 1976). The results of serial percutaneous biopsies taken during chronic rejection are given here. A detailed and systematic histopathological description of the course of chronic rejection is given together with time of onset for the various lesions.

The fifth chapter contains the article: "Myocardial radiopaque markers used to quantify minor axis shortening for follow-up studies in experimental surgery". (European Surgical Research 9 : 364, 1977). The technical details of the method used to measure myocardial contractility during chronic rejection after orthotopic canine cardiac transplantation are given here. One of the shortcomings of this study was that the marker method, though it gave valuable results, presented some technical problems. These problems had to be evaluated later and the results are described in chapter five.

Chapter six is dedicated to the discussion and conclusions. A historical survey of orthotopic cardiac transplantation is given in chapter seven.

CHAPTER 2

Serial Percutaneous Biopsies from Canine Orthotopic Cardiac Allografts[1]

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Submitted for publication November 19, 1974

Chronic rejection is still a major problem in clinical and experimental heart transplantation. Since most of the morphologic descriptions of clinical and experimental material are limited to postmortem findings, little is known about the time of onset and the course of chronic rejection. Biopsies can readily provide useful material for the study of this process.

A transvenous method for obtaining biopsies from canine orthotopic allografts has been described by Caves [2]. After working with a percutaneous technique, we found that it offers certain advantages in canine orthotopic cardiac allografts. The method is described and evaluated in this paper. The results of this technique proved to be of great value for further investigation of the histological aspects of chronic rejection.

MATERIAL AND METHODS

Orthotopic cardiac transplantation was performed in beagles weighing 10–14 kg. The recipients were identical for known DL-A groups of the littermate donors [7]. No immunosuppressive treatment was given. The operation technique was that of Lower, Stofer, and Shumway [1]. Biopsies were taken weekly from 19 beagles after orthotopic transplantation.

The biopsies were taken percutaneously with a Vim Tru Cut disposable liver biopsy needle (6-inch, 15.2-cm cannula; 20-mm specimen notch, Travenol Fig. 1). Dogs were anesthethetized with iv-administered sodium methohexital, intubated, and maintained on pure oxygen.

The left anterolateral chest wall was shaved, cleansed with iodine, and draped with sterile towels leaving only the site for the needle biopsy exposed. The site was easily recognized by visible cardiac pulsations in the fifth intercostal space. The EKG Lead II was recorded on a multichannel recorder and scope (Elema Schonander).

The needle was advanced into the chest cavity toward the epicardial surface of the apex of the left ventricle. Contact with this surface was evidenced by ventricular ectopic beats and pulsatile movements of the needle synchronous with the cardiac cycle. The inner needle was thrust forward into the myocardium as far as the instrument would allow. The cutting cannula was then advanced over the needle and the myocardial specimen was removed. The biopsy specimens were immersed in a formol–saline solution (10%) and stained with hematoxylin–eosin and methyl green pyronin for light microscopy. Chronic rejection was classified morphologically by the criteria of Bieber [5].

RESULTS

One hundred and forty-two biopsy specimens were obtained from 19 dogs. The mean survival time of 17 dogs was 48.8 days (14–124 days). Two dogs are still alive at 315 and 161 days.

[1]This work was supported by Grant 13-29-23 of Fungo (Scientific Medical Research, Netherlands) and Grant 72040 of the Netherlands Heart Foundation.

Adequate material for light microscopy was obtained on 92% of the occasions. Postmortem findings correlated well with those of needle samples taken during the terminal phase. In 76% of the cases, biopsies consisted of a cross section of the left ventricular wall from epicardium to endocardium. Almost every sample contained arteries of different sizes but consisted mostly of small arteries and opened-up collaterals. Segments of major coronary branches were obtained in two cases.

TABLE 1
Number of Biopsies, Survival Days, and Cause of Death of 19 Dogs

Dog	Survival days	Number of biopsies	Cause of death
1	124	12	Chron. rej., rupture of aorta
2	82	9	Chron. rej.
3	32	2	Chron. rej., narcosis
4	315	34	Alive, signs of rej.
5	26	3	Chron. rej., pneumonia
6	17	2	Chron. rej., pneumonia
7	56	7	Chron. rej., hemorrhage
8	29	4	Chron. rej., narc.
9	64	9	Chron. rej.
10	17	2	Chron. rej., narc.
11	35	5	Chron. rej., narc.
12	14	1	Chron. rej., pneumonia
13	14	2	Chron. rej., narc.
14	161	14	Alive, signs of rej.
15	91	13	Chron. rej.
16	28	3	Chron. rej. narc.
17	70	9	Chron. rej., narc.
18	103	10	Chron. rej.
19	28	1	Chron. rej., pneumonia

As a complication of this technique, we lost one dog because of hemorrhage after the percutaneous biopsy. Seven dogs which in previous biopsies had shown extensive damage to the myocardium died at the induction of narcosis (Fig. 2). On 10 occasions, the biopsy material was later found to consist of a blood clot, an organized thrombus, or healthy lung tissue. On postmortem examination the histological features of chronic rejection were present in all grafts. The time of onset as well as changes in these features during the course of chronic rejection could be determined for every dog by means of the biopsies.

Lymphoid infiltration (Fig. 3) does not play an important role in this model and even the spontaneous disappearance of lymphoid perivascular infiltration was seen, although these dogs received no immunosuppressive therapy. The arterial wall is the site of primary damage in chronic rejection. Vascular lesions were a constant finding in this model (Fig. 4).

DISCUSSION

Myocardial biopsies have been a subject for investigation for several years. Leedham [3] reported on biopsies with a Vim Tru Cut disposable needle for studying the course of acute and modified rejection in canine cardiac allografts. However, these allografts were implanted heterotopically. This in itself might alter the morphological features due to changed hemodynamics and functional requirements. Shirey [4] took single percutaneous biopsies from 198 patients with a thin-walled Silverman needle for the diagnosis of myocardiopathy.

Caves [2] described a transvenous method for obtaining myocardial biopsies in canine orthotopic heart allografts. He introduced a modified Konno-Sakakibara bioptome into the external maxillary vein and advanced it into the right ventricle.

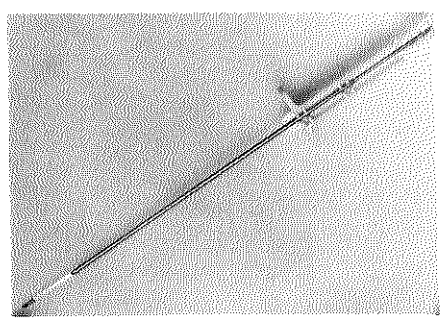

FIG. 1. The Vim Tru Cut disposable liver biopsy needle (Travenol).

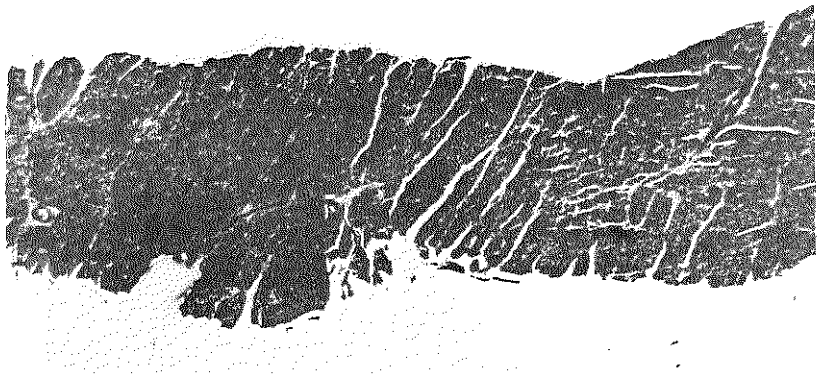

FIG. 2. Example of a percutaneously obtained myocardial biopsy. ×60.

At first, we took myocardial biopsies from orthotopic heart allografts by repeated thoracotomies. We also attempted to take serial transvenous biopsies in this experimental model, but, after about three biopsies, the maxillary vein was thrombosed in every dog. Even leaving a cannula in the vein did not solve the problem of venous thrombosis. Experimentally obtained transvenous biopsies were good but were limited in size and in the incidence of arteries in the samples. This rendered them less useful for the study of vessel changes in chronic rejection. We recently solved the problem of death during the induction of narcosis by taking percutaneous biopsies under local anesthesia. Experimentally, the percutaneous method with a biopsy needle proved to offer certain advantages in the taking of serial biopsies from canine orthotopic cardiac allografts. One is not dependent on vein patency in cases of multiple biopsies in long-term survivors and the biopsy site is not solely confined to the endocardium.

A cross section of the ventricle wall can be

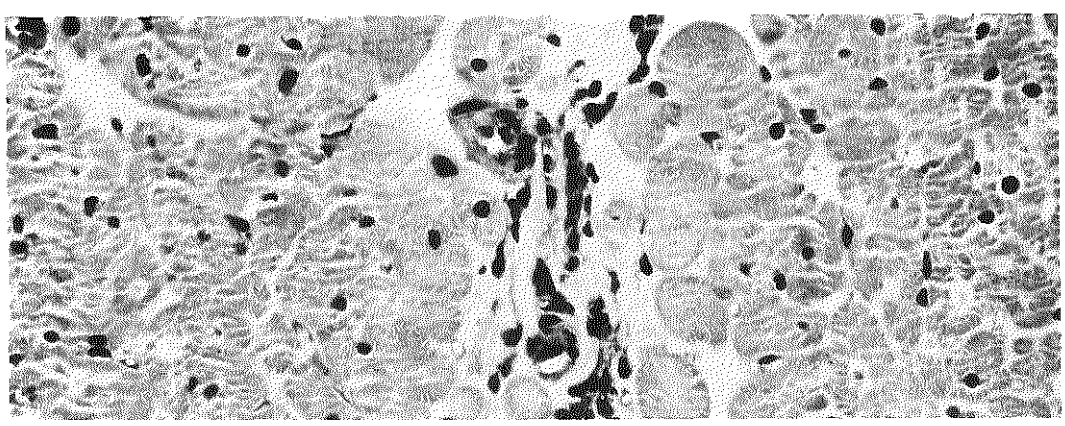

FIG. 3. Dog 4, postoperative week 5. Biopsy shows slight lymphoid perivascular infiltration and myocytolysis. ×380.

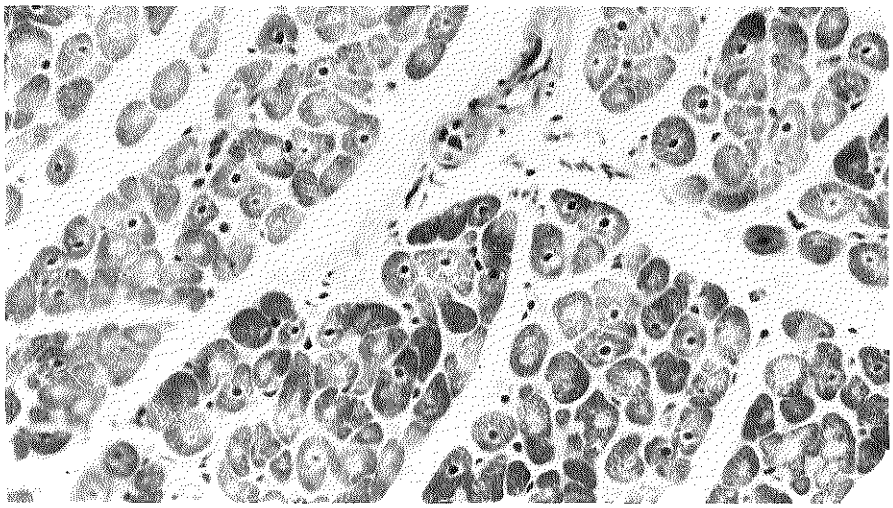

FIG. 4. Dog 4, postoperative week 15. Vascular lesion, intimal proliferation. ×380.

obtained. Arteries are very important in the study of chronic allograft rejection. Few vessels are seen in endocardial biopsies. These arteries are easy to obtain percutaneously. Hemorrhage, cardiac tamponade, damage to coronary arteries, arrythmias, and infection are major objections against percutaneous needle biopsies of the left ventricle, as reported in the literature.

Apart from one case of hemorrhage, none of these complications occurred in our series of 142 punctures. Probably the risk of hemorrhage and tamponade is reduced by the adhesions between the transplanted heart and the inner side of the left thoracic wall and pericardium.

Damage to the coronary arteries appears to be tolerated by the dogs, probably because of adequate collateral circulation. Without detectable consequences for myocardial function, major branches of coronary arteries were obtained twice in the biopsy specimens. Arrythmias, like ectopic beats, were seen only when the tip of the needle touched the epicardium. A similar phenomenon has been described for when the transvenous bioptome touches the endocardium [2]. Infection was never seen.

REFERENCES

1. Lower, R. R., Stofer, R. C., and Shumway, N. E. Homovital transplantation of the heart. *J. Thorac. Cardiovasc. Surg.* **41**;196, 1961.
2. Caves, P. K., Billingham, M. E., Schultz, W. P., Dong, E., and Shumway, N. E. Transvenous biopsy from canine orthotopic heart allografts. *Am. Heart J.* **85**:525, 1973.
3. Leedham, P. W., Baum, M., and Cullum, P. A. Acute and modified rejection of heterotopic canine cardiac allotransplants studied by serial needle biopsy. *Thorax* **26**:534, 1971.
4. Shirey, E. K., Hawk, W. A., Devobroto Mukerji, and Effler, D. B. Percutaneous myocardial biopsy of the left ventricle. *Circulation*, **41**:753, 1970.
5. Bieber, C. P., Stinson, E. B., Shumway, N. E., Payne, R. and Kosek, J. Cardiac transplantation in man, VII. Cardiac allograft pathology. *Circulation* **41**:753, 1970.
6. Bos, E., Meeter, K., Stibbe, J. Vriesendorp, H. M., Westbroek, D. L., de Vries, M. J., Nauta, J., and van Rood, J. J. Histocompatibility in orthotopic heart transplantation in dogs. *Transplant. Proc.* **3**:155, 1971.
7. Vriesendorp, H. M. Rothengatter, C., Bos, E., Westbroek, D. L., and van Rood, J. The production and evaluation of dog allolymphocytotoxins for donor selection in transplantation experiments. *Transplantation* **11**:440, 1971.

CHAPTER 3

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Orthotopic canine heart transplantation: Left ventricular contractility during chronic rejection

Myocardial left ventricular contractility is assessed in 8 orthotopic heart-transplanted dogs during chronic rejection by a noninvasive method. The mean circumferential velocity of shortening ($\overline{V}cf$) is calculated from endocardial marker motion on cinefilms. A model for chronic rejection after cardiac transplantation was obtained by histocompatibility matching in DLA identical Beagle littermates. No immunosuppressive treatment was used. All dogs eventually died of chronic rejection; mean survival time was 112.5 days. LV contractility shows the following pattern: Chronic rejection causes an important contractility decrease ($P < 0.001$) with two significant drops: (1) from the week preoperative to the first week after transplantation ($P < 0.02$) and (2) from week terminal minus one to the terminal week ($P < 0.01$). This study describes the technique and the changes in $\overline{V}cf$ observed over a period of time in a predictable model.

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Until now most studies on allograft rejection following orthotopic cardiac transplantation are directed toward the acute phase of allograft rejection.[1–4] By means of histocompatibility matching a prolonged survival time is obtained experimentally by using littermates with identity between donor and recipient for the DLA complex.[6] Histologically, this type of rejection can be defined as chronic rejection according to the criteria of Bieber and associates.[9] This experimental model for chronic rejection was used to describe the changes in left ventricular contractility during rejection. This is useful not only for describing the phenomenon but also as an index for therapeutic measures during the whole postoperative period. The current problem is that practical diagnostic methods are not yet available for following the course of chronic rejection. Moreover, this problem is complicated by the fact that, with regard to long survival times, an easily repeatable and noninvasive technique is preferable to an invasive one. In acute rejection the electrocardiographic (ECG) voltage proves to be very useful in determining acute rejection episodes but we found it failing in chronic rejection. Hence we tried in this study to apply the method of measuring the mean velocity of circumferential fiber shortening ($\overline{V}cf$). This method was originally described by Karliner and associates[5] and developed with the use of cineangiograms. However, we applied the same calculations on the measurements from myocardial marker movements on roentgen films.[5] Being noninvasive, this procedure is easily repeated once the silver markers are attached to the donor heart. Therefore the aim of this study is to quantify mean circumferential fiber shortening rate (as a contractility index) in dogs during chronic rejection after orthotopic cardiac transplantation with filmed endocardial marker motion.

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This work was supported by Grant 13-29-23 of Fungo (Scientific Medical Research, The Netherlands) and Grant 72040 of the Netherlands Heart Foundation.

Received for publication July 21, 1975.

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Materials and methods

Orthotopic cardiac transplantation was performed in 8 beagles weighing 10 to 14 kilograms. Donor-recipient pairs were identical for the DLA complex.[6, 7] No immunosuppressive treatment was given. The

operative technique is that of Lower, Stofer, and Shumway.[8] Chronic rejection was classified histologically by the criteria of Bieber and associates[9] and assessed by serial percutaneous biopsies[10] and postmortem histological findings.

Fig. 1 shows three pairs of silver clips, each pair connected by a 5 to 7 mm. long rubber thread. These are placed at the apex, the anterior wall, and the posterior wall of the left ventricle. With the help of a harpoon-like device* the sharp-tipped inner clip is rapidly introduced perpendicularly through the wall in such a manner that after insertion the inner clip rests against the endocardial surface and the outer clip against the epicardial surface, as shown in Fig. 2. A small silver ring is sutured at the base of the aorta. The compliant rubber band allows easy separation of the clips during systolic wall thickening. Transmural wall thickness can be measured by the distance between the inner and the outer part of the clip pairs. Transmural silver markers are attached to the donor heart the week before transplantation with general anesthesia and a left thoracotomy in order to obtain control values for each graft. Clip movements are recorded three times a week, with the dog at rest and without anesthesia, on high-speed single-plane cinefilms at 80 frames per second in the lateral projection. The dogs were positioned in such a way that the projected minor axis was maximum. Since $\overline{V}cf$ calculations involve the ratio of two distances, the technique is insensitive to nonparallel beam distortion, or even to minor positioning errors of the dog. Other than centering the image no attempt was made to correct for pin-cushion distortion nor were measurements controlled with respect to respiration.

The time of exposure of cineradiograms together with the ECG is recorded on a Siemens oscillomink, 12 channel, ink-jet, direct-writing recorder. A simplified measure of left ventricular myocardial contractility, i.e., $\overline{V}cf$ calculated from epicardial markers, is used for the evaluation of left ventricular contractility.[5] The mean velocity of circumferential fiber shortening ($\overline{V}cf$) is defined as the extent of shortening of the radius

$$\left(r = \frac{\text{minor axis} + \text{wall thickness}}{2}\right),$$

at the midpoint of the long axis, between end-diastole and end-ejection, divided by the time required for shortening ($t_d - t_s$). This dimension at end-diastole is shown in Fig. 2. Wall thickness is taken into account for purposes of standardization of minor axis because of possible errors introduced by the trabeculae.

$$\overline{V}cf = \frac{r_d - r_s}{(t_d - t_s)\, r_d} \text{ sec}^{-1}$$

End-diastole is defined to occur at the first frame exposed after the onset of the Q-wave and the corresponding outward wall excursion is measured here. End-ejection is defined as the maximum inward wall excursion determined by visual inspection of the film. The mean velocity of shortening of the internal circumference is divided by the end-diastolic internal circumference at the minor equator. The paired Student's t test is applied to the results of $\overline{V}cf$ calculations.

*The original idea for this device was conceived by Dr. R. F. Rushmer, Seattle, Wash.

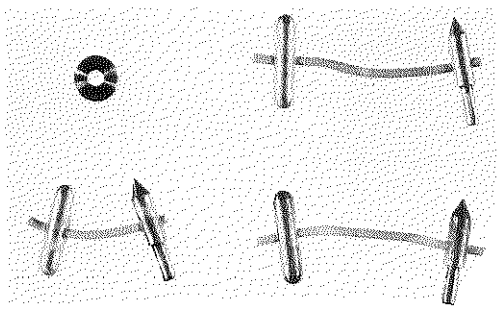

Fig. 1. Three pairs of silver clips and a small silver ring are used to identify wall position and motion.

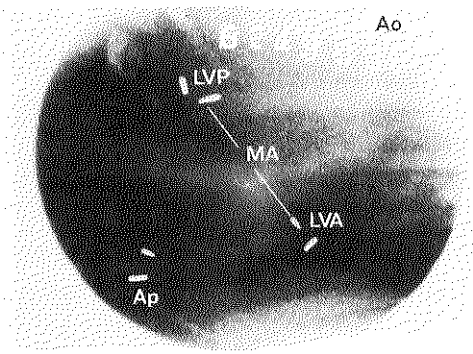

Fig. 2. A cinefilm in the lateral projection. The left ventricular cavity is lined by endocardial markers. *MA* is the minor axis and the velocity of shortening of the axis is measured between the left ventricular posterior wall *(LVP)* and the left ventricular anterior wall *(LVA)*. *AP* means apex and *AO* stands for aorta base.

ECG recordings are taken daily in order to quantify heart rate, detect arrhythmias, and determine R_{II} wave voltage.

Results

Survival. Eight beagles received hearts from DL-A identical littermate donors. The mean survival time is 112.5 days (21 to 494 days). No immunosuppressive therapy was used. All 8 dogs eventually succumbed to chronic rejection and showed ischemic changes of the myocardium due to obliteration primarily of epicardial coronary arteries and of intramyocardial arterioles, as was assessed by serial percutaneous biopsies[10] and postmortem histological findings. Detailed histological descriptions will be published separately.

ECG. Values for ECG voltage are shown in Table I. Only QRS voltage in normally conducted complexes (Lead II) is measured. Their magnitude is expressed in millimeters (10 mm. = 1 mv.). In Table II the mean and standard deviation are shown. The mean control value is 8.86 mm. and except for the unexplained significant drop ($P < 0.02$) in the third postoperative week no other significant change ($P < 0.05$) is found for the entire period, even including the week prior to death.

There appears to be a slight fall in the ECG voltage in the last 2 weeks of survival (terminal and T_{-1} weeks), but this is not significant either.

These results are summarized in Fig. 3, which shows the data, mean, and standard deviation for the control week, the first postoperative week, the week prior to death (T_{-1}) and the terminal week (T). A small and nonsignificant drop in mean values is apparent.

Heart rate. Values for heart rate are mentioned because of possible influence on contractility. These values are shown in Table I and appear relatively constant during the postoperative period. The mean and S.D. together with P values are shown in Table II. The control rate is 136 (S.D. = 25) and the only significant change occurred in the first postoperative week (mean = 149, S.D. = 20, $P < 0.05$, week 0). However, this cannot be regarded as an important index for chronic rejection as it is due in part, at least, to surgical trauma. Body temperature was not recorded.

These results are illustrated in Fig. 4, which shows heart rate during the control week, the first week postoperative, week terminal minus one, and the terminal week. An over-all upward trend is apparent. The increase in the mean rate during the last week is slightly less than the change observed in the first week.

The relative constancy of heart rate during the postoperative period (excluding the last week) is also apparent. A somewhat paradoxical finding is that the change in the first week is significant ($P < 0.05$), while at week T (with respect to the control) it is not, despite an even greater mean difference in heart rate. This is explained by the fact that the pattern of change for each individual data point was more variable than in the first week.

$\overline{V}$cf. The $\overline{V}$cf determinations are shown in Table I and the mean, S.D., and significance levels in Table II. With respect to ECG Lead II and heart rate, the changes which occur in $\overline{V}$cf are much more significant.

The surgical procedure probably causes a significant drop in $\overline{V}$cf ($P < 0.02$) from 2.2 at week 0 to 1.8 at week 1. Furthermore, $\overline{V}$cf remains depressed and no further significant change appears until the last week. At the terminal week (T) and week prior to death (T_{-1}) the $\overline{V}$cf has fallen to 1.38 ($P < 0.01$) and 1.49 ($P < 0.05$), respectively. The final drop in the last week (comparison of week T to T_{-1}) is significant at the $P < 0.01$ level.

These findings are summarized in Fig. 5, which shows $\overline{V}$cf at the control week, week 1, week T_{-1}, and the week of death. The drop in $\overline{V}$cf from week 0 to 1 is significant as well as the change from week T_{-1} to T.

The apparently greater drop between week 1 and T_{-1} is once again not significant (according to the paired T test), because the pattern of change is not sufficiently consistent over this period. In the last week the fall in mean values is less, but the pattern is consistent.

Discussion

Despite identity for the DLA complex between donor and recipient in orthotopic canine cardiac transplantation the allograft is slowly rejected.

Histologically, chronic rejection is characterized by ischemic changes of the myocardium by gradual obliteration of coronary arteries and smaller vessels.[9] These features express the histological difference between chronic and acute rejection. In acute rejection lymphoid infiltration of the myocardium is the most striking phenomenon.[9] Hence endocardial biopsies are reported to provide useful information about the infiltration rate in acute rejection.[12] In chronic rejection, however, lymphoid infiltration is hardly present, with the process being especially directed to the arteries. Biopsies for diagnostic purposes in chronic rejection however should aim at the acquiring of arteries of different sizes.[10]

A striking phenomenon observed in the results is the long period of mean survival time and the divergence in survival times. Observations such as these were never

Table I. *Measured data on ECG_{II}, heart rate, and mean $\overline{V}cf$*

Dog		Week 0	1	2	3	4	5	6	7	8	9	10
1. Dog	R_{II}	7	6	7	3							
BD 1230	HR	160	170	160	160							
	$\overline{V}cf$	1.42	1.29	1.13	0.97							
2. Dog	R_{II}	6	7	5.5	4	7						
BD 2325	HR	130	160	165	170	200						
	$\overline{V}cf$	2.48	2.05	2.40	2.28	2.30						
3. Dog	R_{II}	13	10	8	9	4						
BD 2191	HR	120	150	140	130	180						
	$\overline{V}cf$	1.80	1.45	1.28	1.40	1.21						
4. Dog	R_{II}	7	1	7	3	3	2	4	2	1		
B 644	HR	130	130	140	150	140	150	160	160	180		
	$\overline{V}cf$	2.48	1.35	1.62	1.29	1.43	1.38	1.33	1.26	1.15		
5. Dog	R_{II}	8	10	15	10	12	7	7	9	10.5	9.5	
BD 2165	HR	110	140	120	140	170	150	170	150	170	180	
	$\overline{V}cf$	1.99	1.73	1.68	1.87	1.34	1.84	1.92	1.98	1.54	1.40	
6. Dog	R_{II}		17.5	15	6.5	9	7	7	7	4	4	5
B 1209	HR		140	140	140	160	160	165	180	180	170	160
	$\overline{V}cf$	2.58	2.66	2.33	2.40	2.37	2.27	2.47	2.74	2.12	1.55	1.83
7. Dog	R_{II}	14	9	12	10	11	8.5	11	9.5	7.5	8.5	10
BD 637	HR	120	120	140	120	120	120	140	140	120	140	130
	$\overline{V}cf$	2.47	2.12	1.91	2.03	1.84	1.96	1.59	1.85	1.56	1.76	1.66
8. Dog	R_{II}	7	6	7	4	9	5	4	7	2	7	5
BD 1229	HR	180	180	160	160	130	125	130	140	150	150	160
	$\overline{V}cf$	2.40	1.97	2.34	2.06	2.07	1.76	1.42	2.29	2.16		1.92

Dog		Week 19	20	21	22	23	24	25	26	27	28	29
	R_{II}	5	5	7	6	7	9	5	6	7	6	13
	HR	140	140	120	120	170	120	170	120	180	140	120
	$\overline{V}cf$	1.54	1.78	1.65	1.69	1.70	2.11	1.74	1.82	1.84	1.83	1.73

11	*12*	*13*	*14*	*15*	*16*	*17*	*18*
2	2						
160	150						
1.63	1.43						
13	10	11	15	10	9	11	10
135	150	160	140	140	140	130	150
1.05	1.73	1.37	1.69	1.31	1.33	1.19	
7	5	4	3	9	5	9	6
150	130	140	160	130	130	110	110
1.79	1.60	1.68	1.67	1.61	1.48	1.59	1.68

30	*31*	*67*	*69*	*70*
6	4	12	8	10
170	170	180	150	130
1.57	1.94	1.76	1.52	1.43

Table II. *Mean, S.D., and significance of changes in ECG_{II}, heart rate, and $\overline{V}cf$*

	Week										
ECG_{II}	*C*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	T_{-1}	*T*
Mean	*8.86*	*8.31*	*9.56*	*6.19*	*7.86*	*5.90*	*6.90*	*7.90*	*5.00*	*6.69*	*5.81*
S.D.	*3.24*	*4.73*	*3.84*	*3.09*	*3.39*	*2.51*	*1.95*	*2.97*	*3.95*	*3.61*	*3.76*
Significance of change with respect to week:											
C		NS*	NS	$p < 0.02$	NS					NS	NS
1			NS	NS						NS	NS
T_{-1}											NS

	Week										
Heart rate	*C*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	T_{-1}	*T*
Mean	*136*	*149*	*146*	*146*	*157*	*141*	*153*	*154*	*160*	*154*	*166*
S.D.	*25*	*20*	*15*	*17*	*29*	*18*	*17*	*17*	*26*	*16*	*23*
Significance of change with respect to week:											
C		$p < 0.05$	NS	NS						NS	NS
1			NS	NS						NS	NS
T_{-1}											NS

	Week										
$\overline{V}cf$	*C*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	T_{-1}	*T*
Mean	*2.20*	*1.83*	*1.84*	*1.79*	*1.79*	*1.84*	*1.75*	*2.02*	*1.71*	*1.49*	*1.38*
S.D.	*0.42*	*0.47*	*0.49*	*0.51*	*0.47*	*0.32*	*0.46*	*0.55*	*0.43*	*0.36*	*0.40*
Significance of change with respect to week:											
C		$p < 0.02$	$p < 0.01$	$p < 0.02$						$p < 0.005$	$p < 0.001$
1			NS	NS	NS	NS	NS	NS	NS	NS	$p < 0.025$
T_{-1}											$p < 0.01$

*NS = no significant change up to the $p < 0.05$ level.

reported in experimental skin and kidney transplantations in identical beagle littermates.[12]

An argument in favor of an unexpected short survival time in identical beagle littermates after transplantation is the liability to sudden death caused by fibrillation.

Extremely long survival times are also quite astonishing compared to renal and skin transplantation results. One of the differences in the transplantation procedure of immunological significance is the use of large amounts of mongrel-donor blood for extracorporeal circulation and postoperative management.

In fact, common diagnostic methods used in acute rejection fail to be helpful in chronic rejection. The results are clear enough to show that the ECG fails to be helpful for diagnostic purposes during chronic rejection. This is contradictory to findings in acute rejection where the ECG is one of the leading diagnostic methods.

For $\overline{V}cf$ (mean velocity of circumferential fiber shortening) we found a noninvasive method for serial measurements of myocardial contractility after transplantation. Little is known about this method in dogs so the silver clips were attached to the donor heart 1 week before transplantation in order to obtain $\overline{V}cf$ values in normal dogs.

Measurements are made manually and represent an enormous amount of work which needs mechanization and the whole method needs further perfection to become an easy and quick detection method.

The significant drop in $\overline{V}cf$ the first week after transplantation might be caused by operation trauma and the early onset of immunological damage as was assessed by serial percutaneous biopsies. In fact myocardial contractility never returns to its pretransplantation normal values and strength. The term "operation trauma" meanwhile became a statement for diminished myocardial function in the early postoperative period. Immunological damage, however, which can be expected after 24 hours postoperatively, contributes also to it because serial biopsies show that the amount of lyphocyte infiltration in chronic rejection

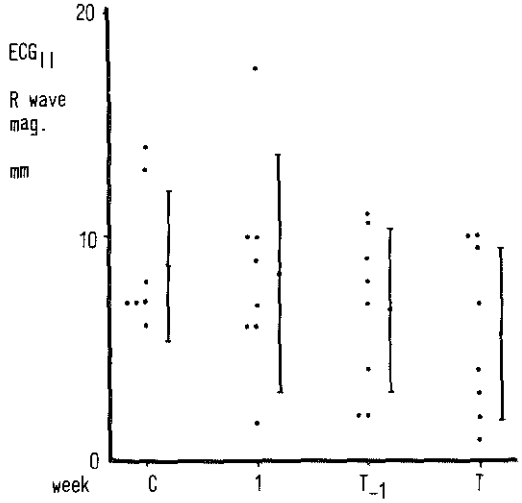

Fig. 3. Data, mean, and S.D. for R II voltage in millimeters (10 mm. = 1 mV.) during the week before transplantation (= the control week = week 0), the first week after transplantation, the week prior to death (T_{-1}), and the terminal week (T). The data are from Table I.

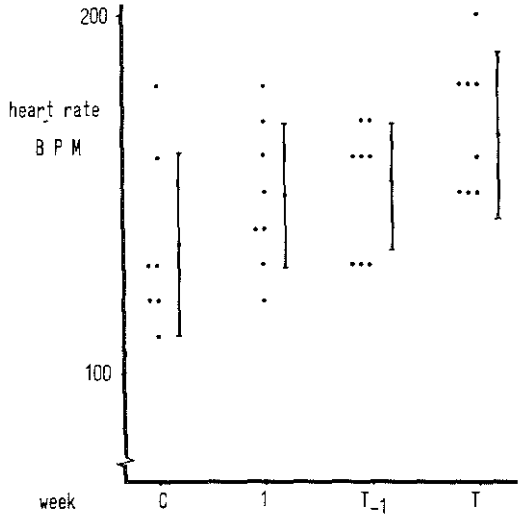

Fig. 4. Heart rate in beats per minute during the week before transplantation (C), the first week after transplantation (1), the week prior to death (T_{-1}), and the terminal week (T). The data are from Table I.

is the greatest in the early postoperative period representing intrinsic myocardial depression.[10] In the following period contractility seems to fall down slowly until it finally turns down definitely shortly before death. Values during the last week are significantly lower than those of control values, representing the difference between healthy muscle and terminal damage.

There are a number of methodological problem areas in this study which should at least be identified. At the moment the reproducibility of $\overline{V}cf$ measurements needs further investigation. The wide variability in $\overline{V}cf$ in Table I may in part be explained by the fact that heart rate was not maintained constant in follow-up measurements nor were measurements made at specific points in the respiratory cycle.

Other potential problems with $\overline{V}cf$ as a contractility index involve sensitivity to preload and afterload.[13] Furthermore, the possibility that there were elevated levels of circulating catecholamines in the first week following transplantation needs to be examined.

Nevertheless, the estimation of the mean rate of circumferential fiber shortening provides a relatively simple and satisfactory method of measuring left ventricular performance and is a more sensitive indicator than ejection fraction.[5]

Quantitative comparison of left ventricular perfor-

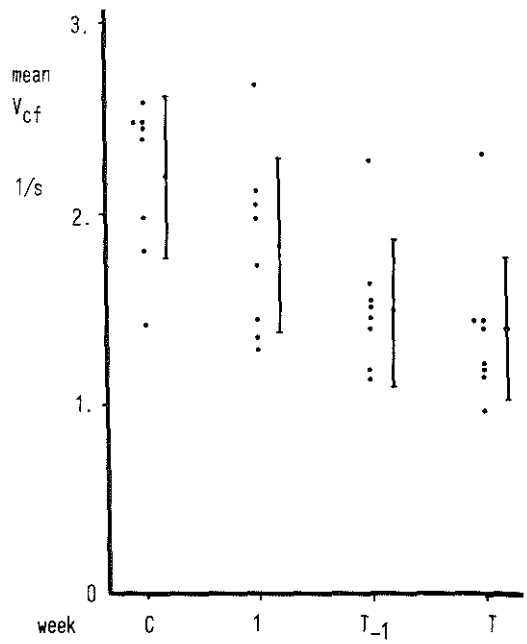

Fig. 5. $\overline{V}cf$ in sec.$^{-1}$ at the week before transplantation (C), the first week after transplantation (1), the week prior to death (T_{-1}), and the terminal week (T). The data are from Table I.

mance among subjects is also possible, since velocity is divided by end-diastolic circumference and expressed per unit of circumferential length, a term analogous to muscle lengths per second in the isolated muscle.

REFERENCES

1 Stinson, E. B., Tecklenberg, P. L., Hollingsworth, J. F., Jones, K. W., Sloane, R., and Rahmoeller, G.: Changes in Left Ventricular Mechanical and Hemodynamic Function During Acute Rejection of Orthotopically Transplanted Hearts in Dogs, J. THORAC. CARDIOVASC. SURG. **68:** 783, 1974.

2 Nelson, R. J., Castagna, J. T., Nedelman, A. L., Feldman, I. M., and Cleveland, R. J.: Hemodynamic Effects of Volume Loading in Dogs Following Cardiac Homotransplantation, Circulation **43, 44** (Suppl. 1): 130, 1971.

3 Chartrand, C., Bryan, B., Kosek, J. E., Dong, E., Angell, W. W., and Shumway, N. E.: Hemodynamics of the Orthotopic Homotransplanted Canine Heart, Ann. Thorac. Surg. **13:** 15, 1972.

4 Stinson, E. B., Griepp, R. B., Bieber, C. P., and Shumway, N. E. Hemodynamic Observations After Orthotopic Transplantation of the Canine Heart, J. THORAC. CARDIOVASC. SURG. **63:** 344, 1972.

5 Karliner, J. S., Gault, J. H., Eckberg, D., Mullins, C. B., and Ross, J.: Mean Velocity of Fiber Shortening, Circulation **44:** 323, 1971.

6 Bos, E., Meeter, K., Stibbe, J., Vriesendorp, H. M., Westbroek, D. L., Vries, M. J. de, Nauta, J., and van Rood, J. J.: Histocompatibility in Orthotopic Heart Transplantation in Dogs, Transplant. Proc. **3:** 155, 1971.

7 Vriesendorp, H. M., Rothengatter, C., Bos, E., Westbroek, D. L., and van Rood, J. J.: The Production and Evaluation of Dog Allolymphocytotoxins for Donor Selection in Transplantation Experiments, Transplantation **11:** 440, 1971.

8 Lower, R. R., Stofer, R. C., and Shumway, N. E.: Homovital Transplantation of the Heart, J. THORAC. CARDIOVASC. SURG. **41:** 196, 1961.

9 Bieber, C. P., Stinson, E. B., Shumway, N. E., Payne, R., and Kosek, J.: Cardiac Transplantation in Man. VII. Cardiac Allograft Pathology, Circulation **41:** 753, 1971.

10 Penn, O. C., McDicken, I., Bos, E., and Nauta, J.: Serial Percutaneous Biopsies From Canine Orthotopic Cardiac Allografts, J. Surg. Res. **18:** 615, 1975.

11 Ross, J., Covell, J. W., and Mahler, F.: Contractile Responses of the Left Ventricle to Acute and Chronic Stress, J. Cardiol. **1:** 325, 1974.

12 Caves, P. K., Billingham, M. E., Schultz, W. P., Dong, E., and Shumway, M. E.: Transvenous Biopsy From Canine Orthotopic Heart Allografts, Am. Heart J. **85:** 525, 1973.

13 Mahler, F., Ross, J., O'Rourke, R. A., and Covell, J. W.: Effects of Changes in Preload, Afterload, and Inotropic State on Ejection Fraction and Isovolume Phase Measurements of Contractility in the Conscious Dog, Am. J. Cardiol. **35:** 626, 1975.

CHAPTER 4

HISTOPATHOLOGY OF REJECTION IN DLA-IDENTICAL CANINE ORTHOTOPIC CARDIAC ALLOGRAFTS[1]

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SUMMARY

The process of chronic rejection is the limiting factor in long term survival after cardiac transplantation.

As part of a study of this process in experimental orthotopic heart transplantation, morphological changes during the course of rejection are described in DLA-identical beagle littermates, obtained by serial percutaneous cardiac biopsy. A total of 153 biopsies were performed on 19 dogs. Mean survival time was 88.11 days (14–494 days) without the use of immunosuppressive therapy. Eight dogs, surviving only 4 weeks, showed a histological pattern that resembled delayed acute rejection, with extensive lymphocellular infiltrate, vascular damage, and myocytolysis. In the 11 dogs which survived more than 4 weeks there was a slight and sometimes transient lymphocellular infiltrate. But progressive vascular lesions could be seen from the 2nd week consisting mainly of medial proliferation involving intramural vessels as well as epicardial vessels. Capillary changes were prominent and the rate of capillary damage seems to be an indication of graft survival prognosis.

Despite identity for the DLA complex between donor and recipient the orthotopic cardiac allograft is rejected. Graft survival, however, is prolonged over non-identical littermate transplants (*2*). In non-identical dogs acute rejection usually becomes manifest during the 1st week in the form of capillary and venular injury, accompanied by extensive lymphocyte and monocyte infiltration with degeneration and finally massive necrosis of parenchymal cells (*5*). Chronic rejection occurs during prolonged survival as a widespread arterial lesion with intimal fibrosis, resulting in arterial luminal narrowing with subsequent myocardial ischemia and/or infarction (*1*). These arterial lesions appear to be irreversible and are not readily diagnosed clinically (*5*). Prolonged graft survival by histocompatibility matching allows the development of these histological lesions without the added alterations due to immunosuppressive therapy. In order to follow this histopathological pattern serial percutaneous biopsies were taken after orthotopic cardiac transplantation in DLA-identical beagle littermates. The aim of this study was to complete a systematic description of the course of chronic rejection and to assess time of onset for the various lesions.

[1] This work was supported by Grant 13-29-23 of Fungo (Scientific Medical Research, Netherlands) and Grant 72040 of the Netherland Heart Foundation.

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MATERIAL AND METHODS

Orthotopic cardiac transplantation was performed in 19 beagles, donors and recipients

being littermates of both sexes and identical for the DLA complex. Tissue typing was performed by using a one-stage microcytotoxicity test (*10*), but in these dogs mixed lymphocyte cultures (MLCs) were not done. Mean dog weight was 12 kg and the operative technique was of Lower et al. (*6*). Approximately 1,500 ml of mongrel blood were employed for priming of the heart-lung machine and in postoperative management. No immunosuppressive treatment was given. Daily electrocardiograms (ECGs) were taken and weekly contractility measurements were made, the results of which are published separately (*8*). Following transplantation, percutaneous myocardial biopsies were taken weekly by a previously described technique (*9*). In dogs surviving more than 6 months biopsies were taken monthly. Biopsies were taken from the anterior wall of the left ventricle or its apex during general anaesthesia induced by sodium-methohexital. At necropsy the hearts were removed, opened along the lines of bloodflow, and inspected macroscopically. Biopsy specimens and autopsy samples were fixed in a 4% formol-saline solution and sectioned subserially. Paraffin sections were stained with hematoxylin-eosin, PAS, and methylgreen-pyronin for light microscopy examination.

RESULTS

Mean survival time of the dogs was 88.11 days (14–494 days). Recipient number and sex, sex of donor, number of biopsies, survival time, cause of death, and clinical picture are shown in Table 1. A total number of 153 percutaneous biopsies were obtained. One dog was lost because of hemorrhage as a complication of the biopsy technique. Seven dogs with extensive damage of the myocardium died at the induction of narcosis prior to biopsy. On 10 occasions the biopsy material was found to consist of blood clot, organized thrombus, or healthy lung. Clinical signs of chronic rejection consisted mainly of ascites, hydrothorax, and lethargy.

TABLE 1. Orthotopic cardiac transplantation in DLA-identical beagle littermates

Recipient No. and Sex	Sex of donor	No. of biopsies	Survival time (days)	Type of rejection	Cause of death
1. BD 2149 M	M	1	14	Delayed acute rejection	Pneumonia
2. BD 2199 M	M	2	14	Delayed acute rejection	Narcosis
3. BD 645 M	F	2	17	Delayed acute rejection	Pneumonia
4. B 2176 F	F	2	17	Delayed acute rejection	Heart failure, narcosis
5. BD 1230 M	F	3	26	Delayed acute rejection	Pneumonia
6. BD 2325 M	F	1	28	Delayed acute rejection	Heart failure
7. BD 2191 M	F	3	28	Delayed acute rejection	Heart failure, narcosis
8. BD 295 F	F	4	29	Delayed acute rejection	Narcosis
9. B 636A F	F	2	32	Chronic rejection	Heart failure, narcosis
10. B 2191 F	F	5	35	Chronic rejection	Narcosis
11. B 644 F	M	7	56	Chronic rejection	Hemorrhage
12. BD 2165 M	F	9	64	Chronic rejection	Heart failure
13. BD 2300 M	M	9	70	Chronic rejection	Heart failure
14. B 1209 F	F	9	82	Chronic rejection	Heart failure
15. BD 2216 M	F	12	91	Chronic rejection	Heart failure
16. B 2218 F	F	10	103	Chronic rejection	Heart failure
17. BD 637 M	M	12	124	Chronic rejection	Rupture of aorta
18. BD 2210 M	F	20	350	Chronic rejection	Heart failure
19. BD 1229 M	F	40	494	Chronic rejection	Heart failure

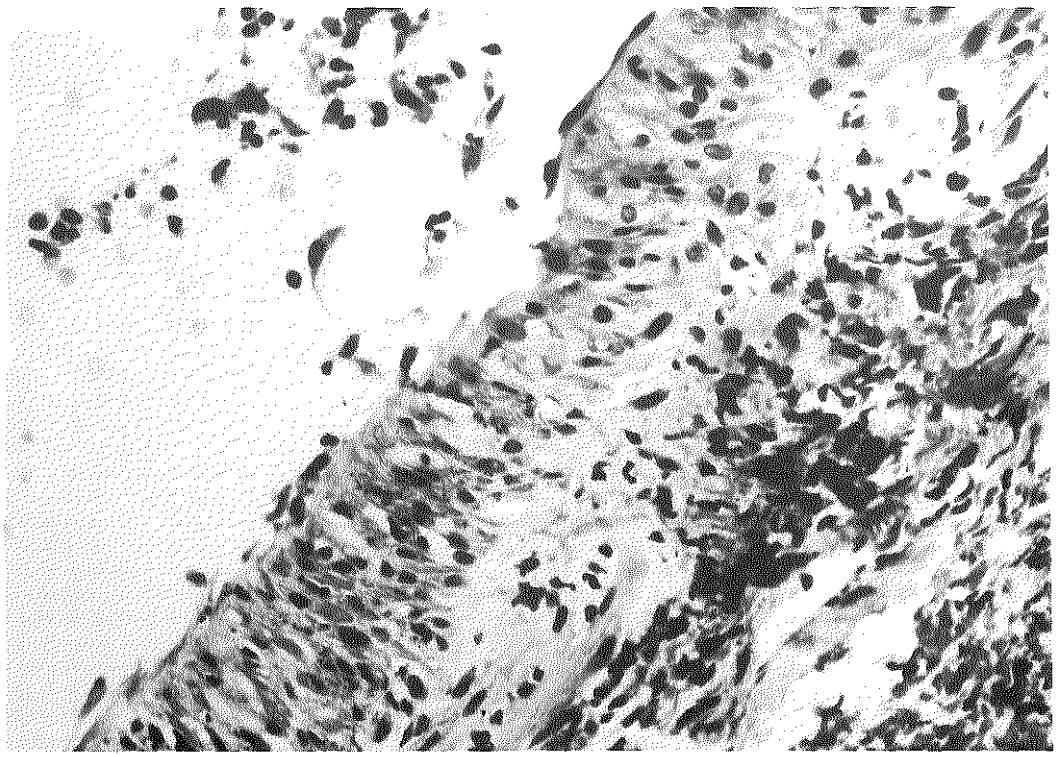

FIGURE 1. Group I, 4th week, lifting of the endothelium and penetration of monocytes in the wall. × 380.

Gross Findings

The allografted hearts from the dogs dying of chronic rejection showed some diffuse ischaemic changes at macroscopic examination. These changes consisted of small fibrotic myocardial scars as a result of vascular occlusion. Very small scars could also be seen at the site of biopsy. In the longest surviving allografts narrowing of coronary arteries was visible.

Weights of the hearts varied from 110 to 165 g (normal, ±85 g).

Histopathological Findings

Based on microscopical features three groups could be discerned.

Group I. The histopathological changes in the group of eight dogs dying in the first 4 weeks were mainly those of delayed acute rejection. In the 1st week all biopsies showed relatively slight muscle damage consisting of swelling of muscle fibers and striations becoming less evident; a mild interstitial oedema was also seen. There were a few infiltrating lymphoid cells in the interstitium and some in the capillaries and venules. Capillary and venular stasis and thrombosis were slight and scattered. The endothelium was swollen in most vessels including the intramural arteries.

In the 2nd week muscle damage was more apparent in the biopsies of grafts surviving 3 or 4 weeks, with a few foci of muscle fiber vacuolisation and a moderate perivascular lymphoid infiltrate. Interstitial haemorrhage, apparently from ruptured engorged capillaries, was now apparent. Second-week biopsies, from grafts of animals dying in the 2nd week, showed histological changes similar to the last biopsies in dogs dying at week 3 or 4. By the 3rd week, in dogs surviving 4 weeks, there was extensive myocy-

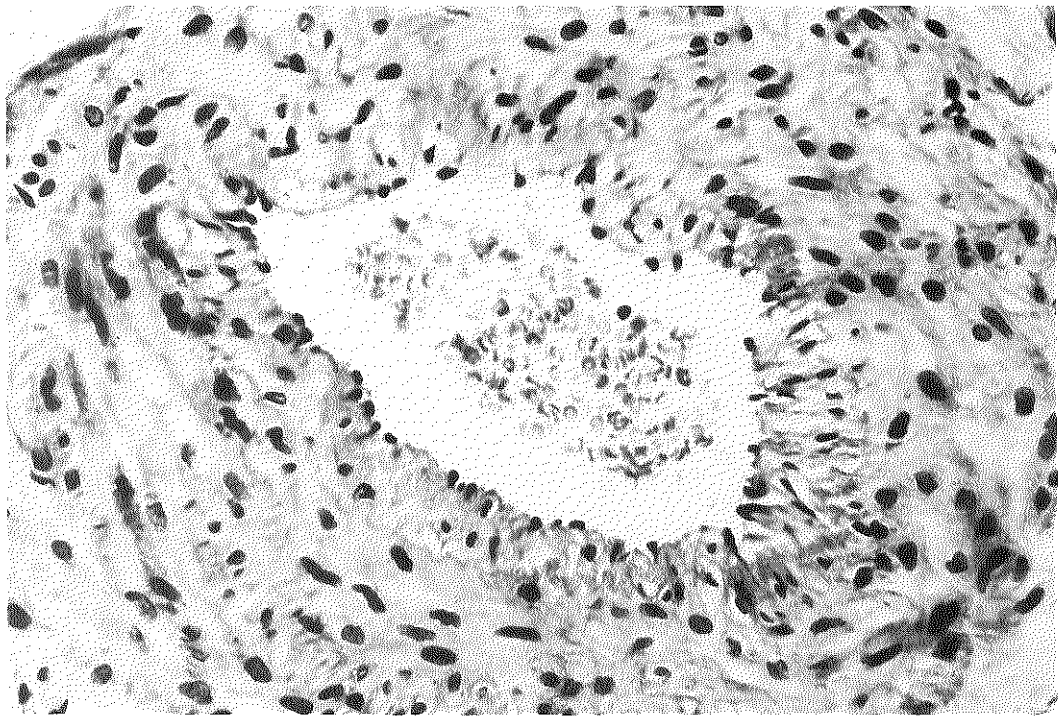

FIGURE 2. Group II. 2nd week. swelling of the endothelium with attachment of lymphoid cells. × 380.

tolysis and oedema, with swelling of muscle fibers, striations becoming less evident, and pyknotic or disappeared nuclei. Lymphoid infiltrate was now more marked and diffuse and consisted of large blastlike lymphoid cells. There was extensive capillary and venular thrombosis and haemorrhage. Dogs dying in this week had more advanced changes, i.e., the damage was equivalent to the last week for the dogs that survived 4 weeks. In the 4th week there was a widespread lymphoid cellular infiltrate and oedema with scattered polymorphonuclear leucocytes. Macrophages were now prominent. There was extensive muscle damage and haemorrhage. The lumina of small vessels were obliterated by the swollen endothelium and monocytes. Larger arteries showed lifting of the endothelium by oedema and penetration of monocytes into the wall (Fig. 1). Capillary channels were now obliterated by microthrombi.

Group II. In the group of eight dogs surviving 9–18 weeks the sequential changes were modified. In the 1st week there was slight swelling of the endothelium of the arteries without lymphoid and polymorph attachment. In the interstitium there was slight oedema and only an occasional lymphoid cell. The myocardial fibres showed little damage.

In the 2nd week a moderate perivascular interstitial lymphoid infiltrate occurred and a few scattered interstitial hemorrhages were present. Small numbers of lymphoid cells were dispersed diffusely in the interstitium. The endothelium of arteries was swollen and lymphoid cells were adherent to this swollen endothelium (Fig. 2). Arteriolar walls were thickened and nuclei were prominent in the walls. Capillaries were dilated and conspicuous with prominent endothelial cells and nuclei (Fig. 3). There was focal muscle damage with vacuolisation.

In the following weeks the lymphoid infiltrate

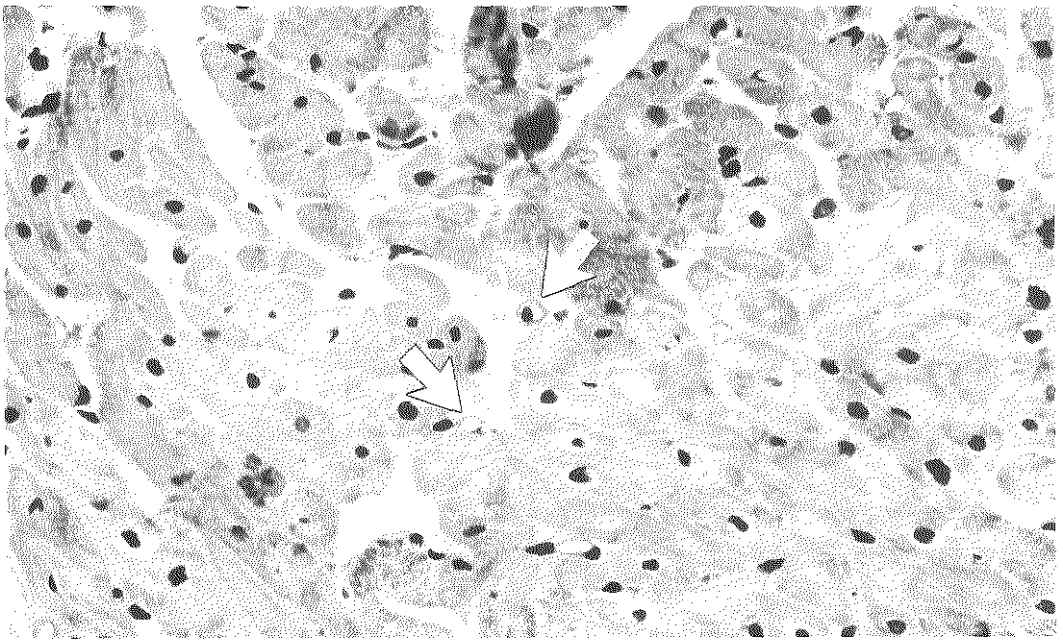

FIGURE 3: Group II, 2nd week, dilated capillaries with enlarged nuclei. (Arrows) × 380.

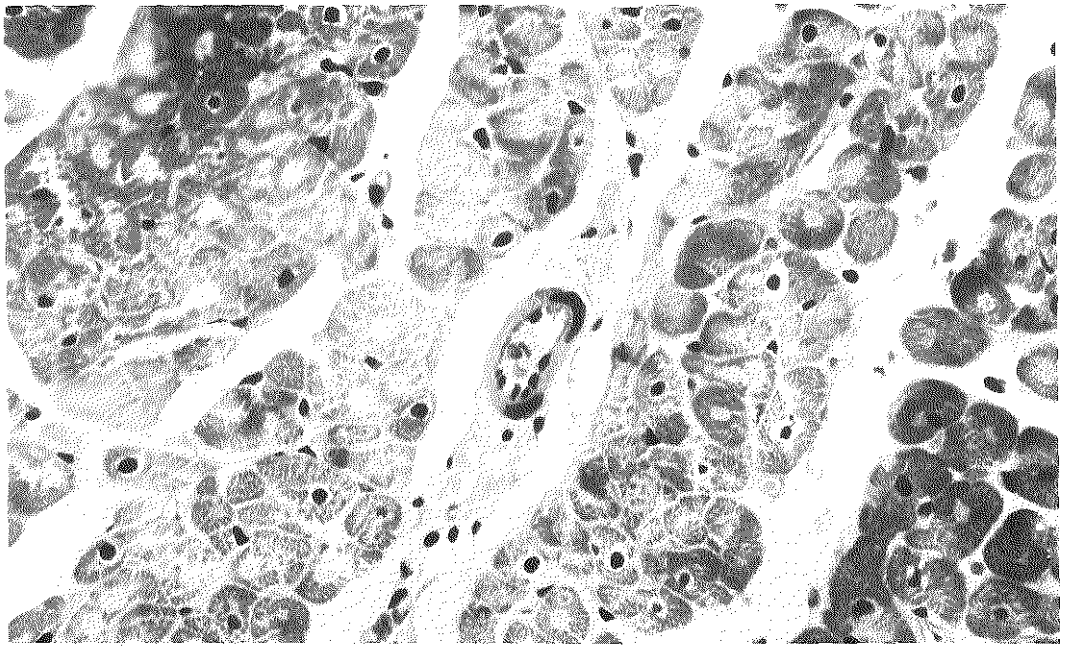

FIGURE 4. Group II, week 15, hyalinisation of arteriolar wall with widely patent lumen. × 380.

became less marked and there was very little interstitial oedema and hemorrhage. The main changes now took place in the arteries and arterioles. In the larger intramural arteries there was medial proliferation and swelling of the endothelium, with great reduction of the lumen. Fibrinoid necrosis, however, was never seen. The walls of the arterioles now showed marked thickening with reduction of the lumen. Occasional vessels of the size of arterioles were seen in which there was no cellular proliferation but instead hyalinisation of the wall and a widely patent lumen (Fig. 4). The swollen endothelium of capillaries and arterioles often blocked the lumen and in these vessels thrombus occurred. The muscle damage appeared to follow the vascular changes, showing mainly atrophy and reduction of fiber diameter. Occasional areas of more severe damage with myocytolysis and fiber necrosis and rarely infarcted areas, associated with vessel occlusion, were biopsied. In most biopsies capillaries were very prominent. Many of them appeared normal, others were dilated, while others with swollen endothelium were obstructed (Fig. 5).

Group III. In the longest surviving three dogs the early histological changes were very slight with only a mild lymphoid infiltrate in a perivascular position. By the 3rd week the intramural arterial and arteriolar walls were showing thickening due to increase in the smooth muscle cells of the media (Fig. 6). In the following weeks this progressed slowly so that the lumen of these vessels was narrowed. A few microthrombi could be seen in some capillaries and monocytes were attached to the endothelium of others. Only small foci of muscle damage could be found. Lymphoid infiltrate was scant and focal (Fig. 7). Arterial medial proliferation progressed in the epicardial and intramural arteries. This change could be found in all biopsies and appeared to be diffuse. The arteriolar walls also became progressively thickened (Fig. 8). Capillaries became much more easily detectable and many were so dilated that their lumen was comparable to that of small arterioles. At the same time vessels with hyalinised walls and dilated lumens also became prominent. A few capillaries had monocytes attached to the endothelium and others were obliterated by thrombus (Fig. 9).

The rate of endothelial swelling and subsequent obliteration in these capillaries appeared slow but all stages of obliteration could be demonstrated. With the progression of the vascular lesions interstitial fibrosis and myocardial fiber atrophy occurred. Small foci of more acute degeneration and infarction could also be found. The cellular infiltrate always remained slight and focal. Coronary arteries at postmortem microscopical examination showed extensive medial proliferation with almost complete obstruction of the lumen (Fig. 10).

DISCUSSION

In this series for the cardiac allografts rejected within 4 weeks, the sequential changes were those of delayed acute rejection, with the dogs dying in the 4th week showing some of the early vascular signs of chronic rejection. In the animals surviving from 4–18 weeks the early acute changes were less marked and subsequently the vascular lesions became dominant features. Small foci of more acute damage continued to occur. One feature of these biopsies was distension and prominence of the capillaries which, however, appeared to be subsequently obliterated by swelling of the endothelium, attachment of monocytes, and thrombosis. Newly opened capillary channels, too, were subsequently obliterated. The arterioles of these biopsies also showed medial proliferation and subsequent obliteration. Other vessels with thickened walls were apparently arterized dilated capillaries with hyalinisation. These vessels seemed to form anastomotic channels. In the longest survivors the early changes were very slight and the more chronic vascular lesions developed gradually. The rate of vascular, and especially endothelial damage apparently occurred slowly enough to allow the opening and dilation of the capillaries to provide an alternative vascular supply for the muscle fibers. Apparently, obliteration of these capillaries occurred very slowly and focally.

In all the biopsies it was shown that there is marked oedema and thickening of the media of

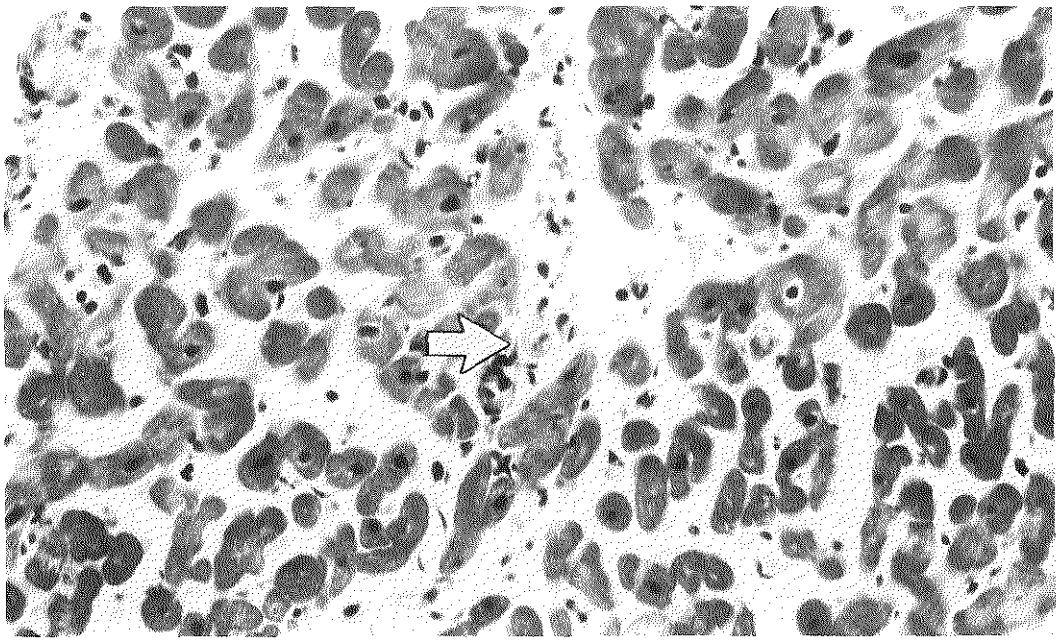

FIGURE 5. Group II, week 17, capillaries, some obstructed (arrow), others with swollen endothelium. × 380.

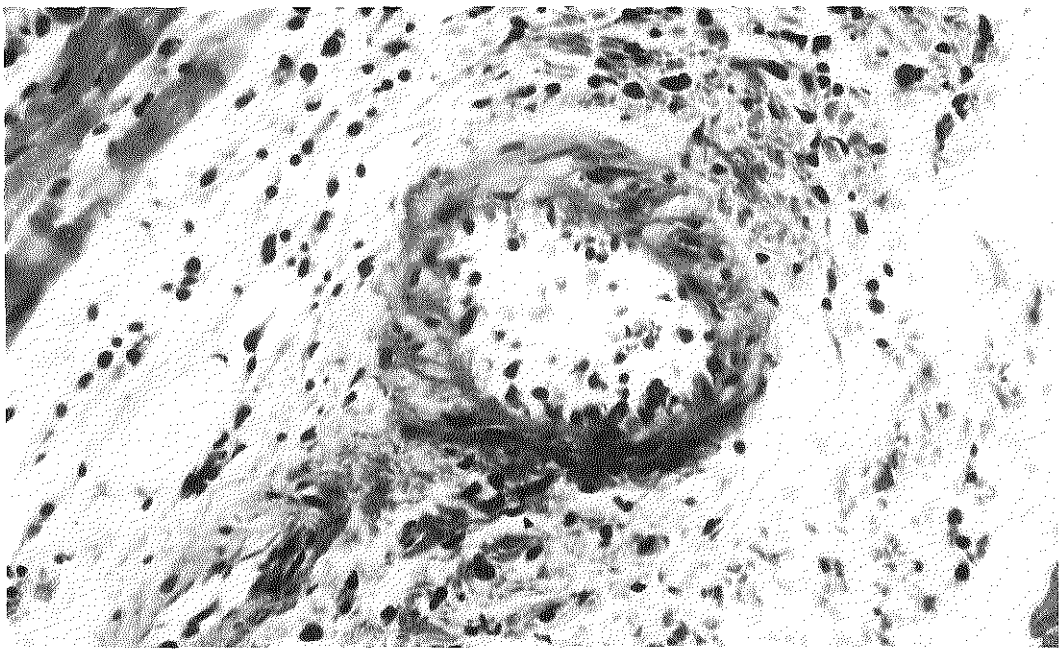

FIGURE 6. Group III. 3rd week, thickening of the arterial wall. × 380.

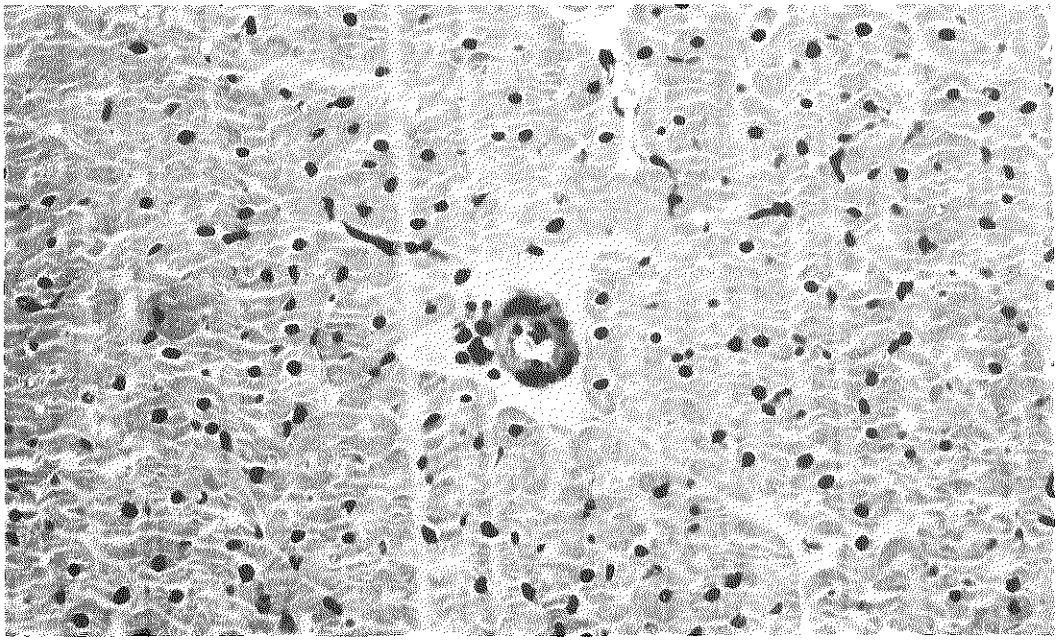

FIGURE 7. Group III, 5th week, scant and focal lymphoid infiltrate. × 380.

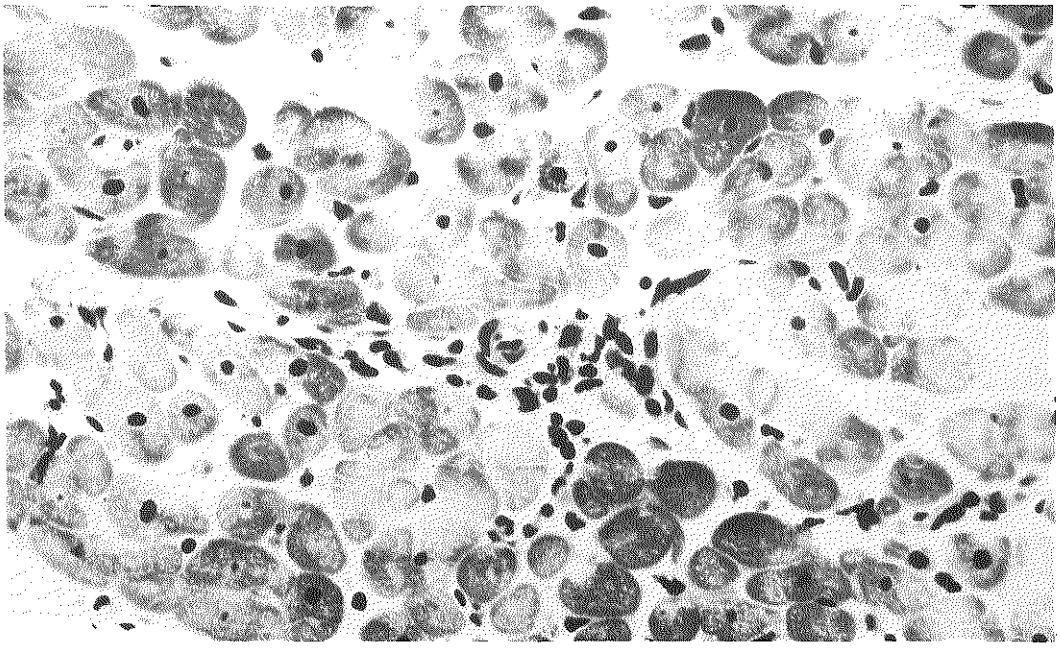

FIGURE 8. Group III, week 20, thickening arteriolar wall. × 380.

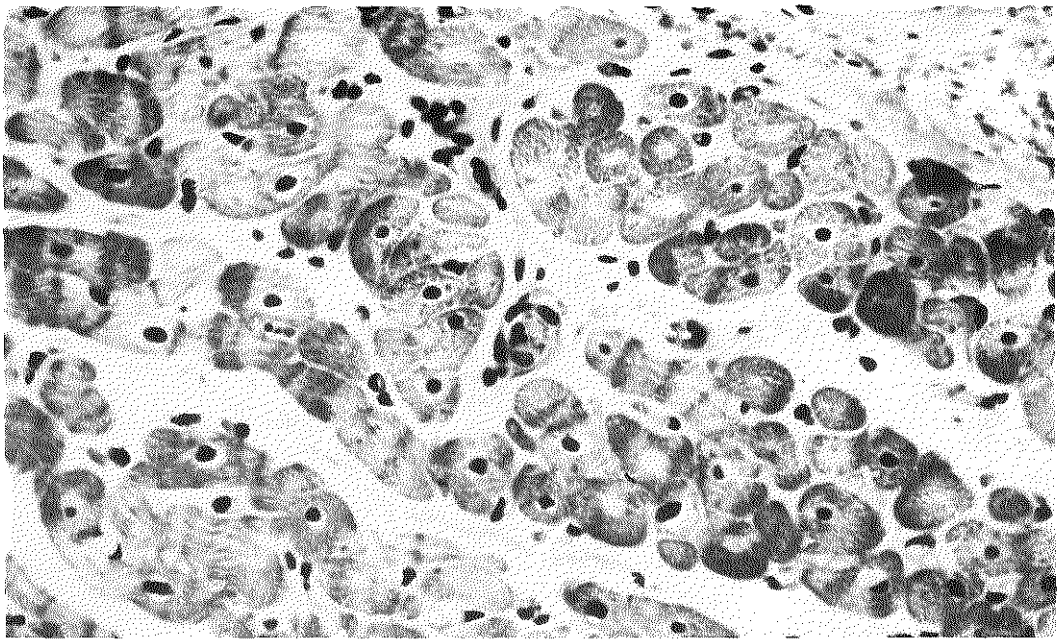

FIGURE 9. Group III, week 65, obliteration of capillary. × 380.

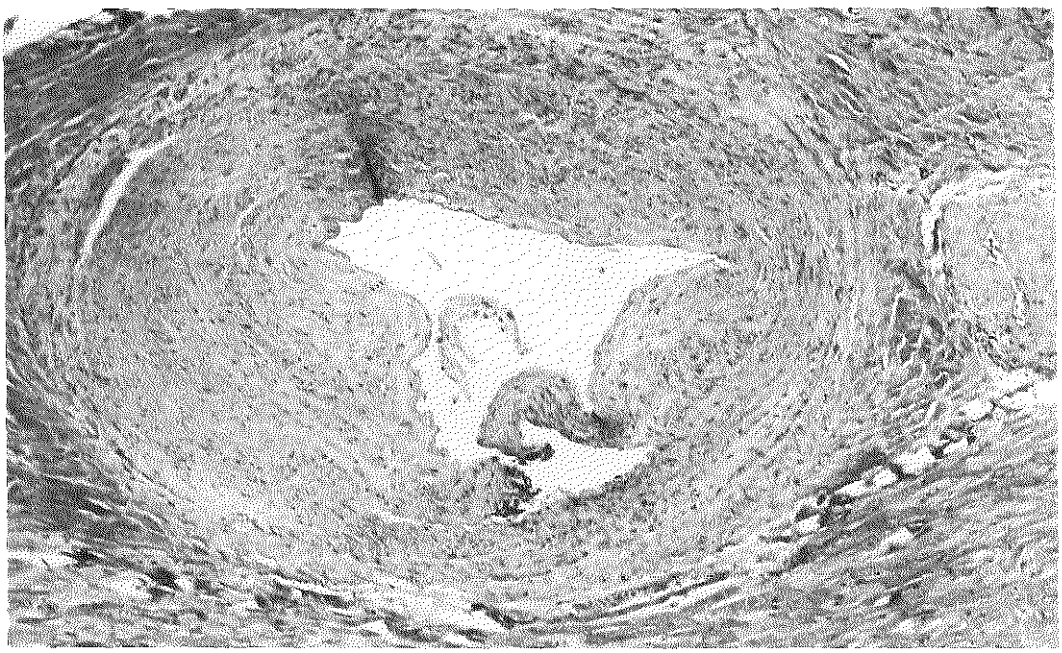

FIGURE 10. Group III, postmortem, coronary artery; severe narrowing of the lumen. × 100.

the vessels of all myocardial layers and that these medial changes in the intramural vessels are as severe as in the epicardial vessels, although the intimal and subintimal lesions are not so marked. If this thickening of the vessels results in rigidity of the arteries and arterioles and inability to dilate, as appears so from the histology, then the influence on myocardial function of the intramural vessel damage may be as significant as that of the epicardial vessel damage. It has been shown that the subendothelium depends for its blood supply on blood flow during diastole, associated with dilation of subendocardial vessels (*3*), and on whether in the ischaemic state the vessels are probably constantly maximally dilated (*7*). The vessels involved are most probably those capable of vasomotion, predominantly arterioles, but the role of the large intramural arteries is unknown. There is evidence, too, that the capillary bed is of importance (*4*). This has two consequences for the transplanted heart, the first being that even with relatively mild main coronary artery obliteration, as occurs in a few long surviving hearts, the subendocardial vessels will still not be able to dilate in diastole with subsequent anoxia of the muscle. Second, if the main coronary arteries are stenosed by intimal proliferation, as occurs in many chronic allografts, the subendocardial vessels will not be able to be in that state of constant dilation seen in the ischemic nontransplanted heart, again with the deleterious effects on the myocardium. This might also explain why the most severe myocardial damage in long surviving cardiac allografts is predominantly subendocardial.

Although the limiting factor in chronic transplants is the degree of damage to the endothelium and subsequent obliterative processes, from our studies the rate of response to the obliterative damage by opening of vascular anastomoses from the capillary bed may be equally important.

Rapid, severe damage will obliterate any collateral vessel that develops whereas a slow obliterative process will allow time for collaterals to develop and the vessels formed will not be immediately obliterated.

A striking phenomenon observed in the results in this series is the long survival times. This was not found in experimental skin and kidney transplantations in identical beagle littermates (*10*). One difference in the transplantation procedure that may be of immunological significance is the use of a large amount of mongrel donor blood for extracorporeal circulation and postoperative management. Possibly, this plays a role in modifying the immune response. Rather disappointing is the conclusion that results described in this article are giving more evidence to the known fact that narrowing of allograft arteries resembles more or less the original disease for which heart transplantation ought to serve as a therapeutic measure.

REFERENCES

1. Bieber CP, Stinson EB, Shumway NE, et al: 1970 Circulation 41: 753
2. Bos E, Meeter K, Stibbe J, et al: 1971 Transplant Proc 3: 155
3. Buckberg GD, Fixler DE, Archie JP, et al: 1972 Circ Res 30: 67
4. Kirk ES, Honig CR: 1964 Am J Physiol 207: 661
5. Kosek JC, Bieber C, Lower RR: 1971 Transplant Proc 3: 512
6. Lower RR, Stofer RC, Shumway NE: 1961 J Thorac Cardiovasc Surg 41: 196
7. Moir TW, Debra DW: 1967 Circ Res 21: 65
8. Penn OC, Brower R, Bos E, et al: 1976 J Thorac Cardiovasc Surg 71: 526
9. Penn OC, McDicken I, Bos E, et al: 1975 J Surg Res 18: 615
10. Vriesendorp HM, Rothengatter C, Bos E, et al: 1971 Transplantation 11: 440

Received 30 December 1975.
Accepted 6 April 1976.

CHAPTER 5

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Myocardial Radiopaque Markers Used to Quantify Minor Axis Shortening for Follow-Up Studies in Experimental Surgery

I. Recording and Analysis Techniques

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II. Comparison with Contractility Indices[1]

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Key Words. Dogs · Konigsberg transducer · Digitizing pen · Contractility · Velocity of shortening · Extent of shortening

Abstract. This work further validates the use of radiopaque epicardial marker motion for quantifying myocardial shortening in long duration follow-up studies. Mean velocity and extent of minor axis shortening were determined in 8 dogs filmed daily for 6–10 days postoperative. As an independent measure of contractility, peak dP/dt was also determined. A useful approximation to the mean ejection slope was developed which uses the end diastolic minor axis 50 msec after the Q wave, and end systolic minor axis 150 msec after the Q wave of the ECG.

Introduction

In order to quantify myocardial shortening after surgery only a few noninvasive direct methods are available for studies in experimental and

[1] This work was supported by grant 74086 from the Netherlands Heart Foundation. Dr. BROWER was supported by the Foundation for Medical Research (FUNGO) which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

clinical cardiology. Such methods have the advantage of being nontraumatic and easily repeated and are therefore of interest especially in long duration chronic preparations. Previous experience in our laboratory [PENN *et al.*, 1976] as well as elsewhere [HARRISON *et al.*, 1963; MITCHELL *et al.*, 1969] has shown that radiopaque myocardial markers can provide direct, accurate, and unambiguous information from man [MCDONALD, 1970, 1972; INGELS *et al.*, 1975], and from a variety of common experimental animals under diverse conditions [RUSHMER, 1954; NOBLE and MILNE, 1969]. Furthermore, mean velocity of minor axis shortening, $\bar{V}_{ma}$, from epicardial and endocardial radiopaque markers had previously been introduced to study functional changes during chronic rejection following orthotopic canine cardiac transplants in dogs [BOS and MEETER, 1971; PENN *et al.*, 1975] and acute myocardial infarction in swine [HEIKKILÄ *et al.*, 1972].

Despite the fact that we had little experience with this method, significant changes in $\bar{V}_{ma}$ were demonstrated [PENN *et al.*, 1976]. However, a great deal of variation in the data was apparent which needed further work to clarify its origin. Encouraged by these preliminary results and convinced of the benefits of a well-tested noninvasive method, the animal experiments described herein were conducted in order to improve the reliability of this method. The intent of these investigations was to: (1) perfect the data recording and analysis techniques; (2) simplify the calculation procedures for routine use; (3) compare $\bar{V}_{ma}$ with well-known variables used to quantify myocardial function, i.e. peak dP/dt and V_{max}-dev, and (4) compare $\bar{V}_{ma}$ values derived from epi- and endocardial markers.

Material and Methods

8 beagles weighing from 10 to 14 kg provided technically adequate data over a period of 1 week to 10 days. The animals were induced and anesthetized with thiopental-natrium. Pancuroniumbromide and fentanyl were employed for muscle relaxation and analgesia, respectively. The dogs were maintained during surgery on 33% O_2 and 67% N_2O. Tidal volume and respiration rate were adjusted to maintain a constant end expired CO_2 of 4–5%. The heart was exposed by a left thoracotomy at the fourth intercostal space. Two types of radiopaque markers were attached to the myocardium: a small silver ring or 0-type marker which was sutured to the epicardium in the first group of 3 dogs; and an H-type marker used in the second group of 5 dogs. This marker consists of a sharp tipped inner clip and a blunt tipped outer clip, connected by a 5- to 7-mm long rubber thread. With the help of a harpoon-like

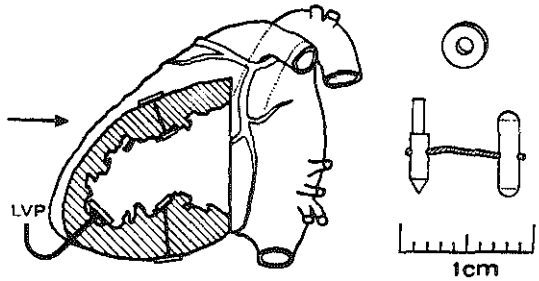

Fig. 1. Two types of markers were used: the H-type consists of two silver markers separated by a rubber band; and the O-type is similar to a 1-mm thick washer. The O-type is used only on the epicardium, while the H-type is used to mark both epi- and endocardium. The sketch of the heart in the left lateral view shows the H-type markers in place. The arrow indicates the direction of the roentgen beam for P-A filming. A Konigsberg pressure transducer is implanted to record LV pressure.

device, described previously [HEIKKILÄ *et al.*, 1972], the sharp end of the H-type marker is introduced through the left ventricular wall. After being released by the device this clip demarcates the endocardium and the blunt tipped clip borders the epicardium.

The markers were attached to the midpoint of the left ventricle wall in a plane perpendicular to posterior-anterior projection (fig. 1).

An elongation of 20% over unstressed length produces in the elastic segment connecting the markers a force of 6 K dyn which is shown below to be small compared to other wall forces developed during systole. We have preferred to implant these markers with the heart exposed so as to be certain of constant marker position during follow-up. However, this is not a fundamental limitation of the method as CARLSSON and MILNE [1967] have shown satisfactory results implanting endocardial markers for other projections using tantalum helices mounted on a specially constructed catheter.

The influence of the silver markers on the surrounding myocardial tissue has been described [PENN *et al.*, 1976]. The radiopaque markers were attached to the myocardium of 5 dogs during periods ranging from 14 to 494 days. In all cases the histological findings were similar. There were no gross signs of tissue degeneration. Macroscopically the markers as well as their rubber bands were embedded in fibrotic material which could be easily peeled off the markers. Microscopically the implants were found to be surrounded by a thin layer of dense fibrous tissue. No signs of any infiltrate were present in these types of foreign material.

Minor axis shortening was followed as CARLETON and CLARK [1968] and ROSS *et al.* [1967] have established that the predominant change in ventricular dimensions involves the transverse diameter.

Following placement of the markers a calibrated Konigsberg pressure transducer (Model P_{20}) was introduced into the left ventricle through the apex and secured by a

purse string suture. Calibration was performed just prior to insertion. In 3 of the dogs this was repeated on autopsy. The pressure sensitivity was found to be constant but some variation in the zero or baseline pressure was apparent. Hence only relative changes within a single pressure recording could be relied on.

Also a bipolar pacemaker electrode was attached to the atrium in order to control the heart rate during recordings. All connecting leads were tunneled subcutaneously to the back, led out between the dorsal aspect of the scapulae and were packed away in a small box strapped onto the dog's back.

Daily postoperative left ventricular pressure recordings, cinefluorography, and ECG recordings were made at rest or during pacing in conscious dogs. Occasionally 10 mg nicomorphine hydrochloride was administered intramuscular in case of excitement during recordings. Positioning of the dogs was mainly frontal for posterior-anterior projection. Care was taken to consistently reposition the dogs on each follow-up day.

Roentgen films of marker position were made at 50 frames a second. All signals: frame and time markers, ECG lead 2, and left ventricular pressure were recorded simultaneously on a UV recorder (paper speed 125 mm/sec).

A 1-cm grid was filmed at the midthoracic level for calibration of the film recording.

Analytic Methods

The myocardial marker motion was recorded on a 35-mm film and projected with a Vanguard projector (Model M 35, case S 20), showing the left ventricle about three times its actual size. From the pressure and ECG tracings the first five consecutive and adequately exposed contractions were chosen for analysis and the corresponding epicardial and endocardial marker distances were measured frame by frame a Vernier caliper reproducible within 0.2 mm of the projected diameter. Two of these five beats were also measured in the diastolic period. No corrections for nonparallel beam distortion of pin-cushion distortion were made (small distance changes were measured and only relative parameters calculated). Contractions directly following extrasystoles or associated with an irregular ECG pattern were ignored.

The measured values were plotted against time to identify the contraction pattern defined as the time course of minor axis shortening. From this, $\bar{V}_{ma}$ and extent of shortening (ExS) were calculated from the diameter change during ejection.

The end diastolic minor axis (M_{ed}) was measured from the exposure made 40–60 msec after the onset of the Q wave of ECG (cineframe interval time = 20 msec).

The minor axis at the end ejection (M_{es}) was measured from the exposure just preceding the moment that dP/dt reached –200 mm Hg/sec; this operational definition of end systole is described elsewhere [MEESTER *et al.*, 1975]. Epicardial and endocardial extent of shortening were calculated as the relative diameter change:

$$ExS = \frac{M_{ed} - M_{es}}{M_{ed}} \times 100\ (\%). \qquad (1)$$

Epicardial and endocardial $\bar{V}_{ma}$ were calculated as the relative diameter change divided by the corresponding time interval ΔT, required for shortening:

$$\bar{V}_{ma} = \frac{M_{ed} - M_{es}}{M_{ed} \times \Delta T} (sec^{-1}). \quad (2)$$

The time of occurrence of the actual maximum (M_{max}) and minimum (M_{min}) diameter was compared with the time of occurrence of the diameters M_{ed} and M_{es} at the moments defined above.

From the pressure recordings peak dP/dt and developed V_{max} (V_{max}-dev) were calculated: V_{max} is the linear extrapolation of dP/dt/P from developed pressure to P = 0 using the least squares criterion. The range of extrapolation from developed pressure extended from 10 mm Hg above ED pressure to 70% of developed peak pressure.

Results

Pattern of Contraction

In both groups of dogs the epicardial minor axis was measured in over 400 beats in order to characterize the pattern of contraction.

By the last third of diastole, *diastasis,* the minor axis had reached its maximum and there was little or no further increase. During *atrial contraction* an a-wave was seen in the LV pressure and the minor axis; in 26% of all beats the a-wave was coincident with the onset of the Q wave of the ECG, in 31% preceded the Q wave by 0–40 msec, and 39% it followed the Q wave by 0–40 msec. There was no apparent a-wave in 4%.

Characterization of the *isovolumic period* revealed seven different patterns as shown in figure 2. In 57% of the measured beats there was shortening over the complete isovolumic period, this was followed by a less rapid shortening during ejection in 9% (type 1), no change in the shortening slope during ejection in 36% (type 2) and a more rapid shortening during ejection in 12% (type 3).

In 43% of all the beats a plateau in shortening was found during opening of the aortic valves: 10% showed a weaker contraction during 20–40 msec (type 4), 8% showed no contraction during 20–40 msec (type 5), in 8% the minor axis increased 1–5 mm (type 6) while in 17% the increase was so strong that the diameter exceeded the maximum value during the a-wave (type 7); however, 14% of these came from 2 dogs filmed in a somewhat more lateral position than the rest of the group.

The most rapid shortening occurred in the first half of *ejection,* and a reduced shortening in the second half.

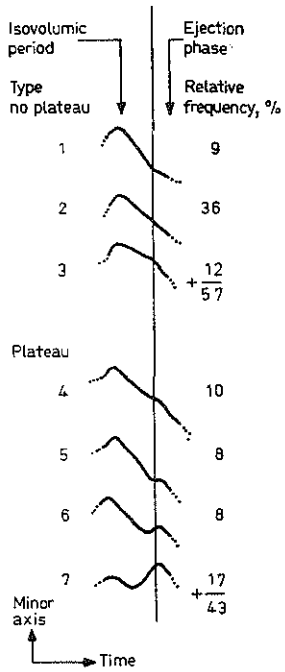

Fig. 2. Seven distinct patterns of minor axis shortening were observed during the isovolumic phase and early ejection phase. These are divided into two major groups: 57% showed no pause or plateau during opening of the aortic valve, while 43% showed either a plateau or a temporal reversal. The reversal in shortening (17%) is generally associated with viewing the minor axis somewhat off the perpendicular and is probably due to heart rotation.

During *isovolumic relaxation* there was normally a 3–6% increase in diameter. In some cases, associated with lower heart rates, a second reduction in diameter was found (0.4–2 mm). Therefore, the LV diameter during this second wave can be up to 2 mm smaller than that at end ejection. Within the first third of diastole, *rapid filling phase,* the minor axis reached 70–90% of its end diastolic value.

Maximum and Minimum Diameter

The moments that the actual maximum and minimum diameters occurred were compared with the criteria described above which are based on the ECG and LV pressure curves. The mean delay time from the onset of the Q wave to the actual maximum diameter was 19 msec (SD =

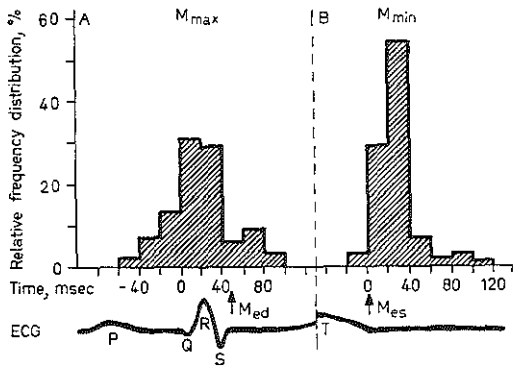

Fig. 3. A The relative frequency distribution of the measured maximum minor axis (M_{max}) within 20 msec windows measured from the Q wave of the ECG is shown. By measuring 50 msec after the Q wave, one is assured in 78% of cases of being in the ejection phase and measuring on the relatively constant slope portion of the ejection curve. *B* The minimum minor axis (M_{min}) is measured with respect to the moment that dP/dt reaches –200 mm Hg/sec since the ECG cannot be relied on to help define end systole.

29 msec). In 78% of all events the maximum was reached within 50 msec of the onset of the Q wave and in 12% the minor axis was found over 50 msec after the onset of the Q wave (fig. 3A). The moment that the minor axis reached its minimum, was not compared with the ECG because of variations in heart rate during the follow-up, but with the LV pressure curve. In 3% of the measurements the minimum diameter occurred before dP/dt reached –200 mm Hg/sec, and 97% occurred later. Mean delay time was 28 msec, SD = 20 msec (fig. 3B).

Derived Variables from the Minor Axis

In the majority of cases a nearly constant slope in the minor axis vs. time plot was observed in the time interval from 50 msec after the Q wave to when dP/dt first exceeds –200 mm Hg/sec. Therefore, $\overline{V}_{ma}$ and the extent of shortening ExS were calculated using the two cineframes just within this time interval. All follow-up marker data are summarized in table I. The mean epicardial $\overline{V}_{ma}$ of all 8 dogs measured daily for 6 days after surgery was 0.72 ± 0.16 sec^{-1} (mean ± SD); however, each dog had a narrower range of $\overline{V}_{ma}$: for all dogs SD = 22% of the mean value, but averaged dog by dog SD = 13% of the mean values (fig. 4). The epicardial ExS was 8.6 ± 1.8%.

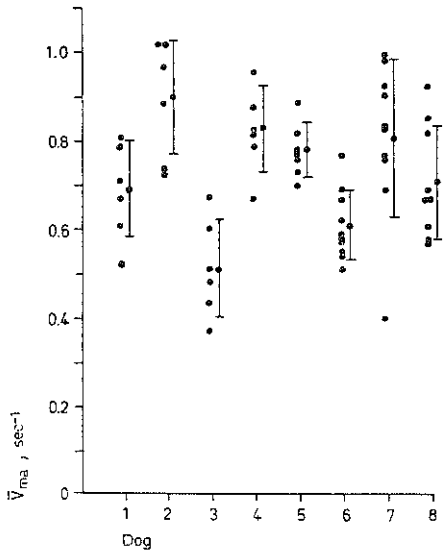

Fig. 4. The mean velocity of minor axis shortening, $\bar{V}_{ma}$, is shown for all 8 dogs on each measurement day (from 6 to 10 postoperative days). Taken as a whole the standard deviation is 22% of the group mean, but each dog has its own and different mean with an individual standard deviation only 13% of the individual mean. Inter-dog variation is due to different marker positioning on the myocardium.

Endocardial $\bar{V}_{ma}$ and ExS were calculated from the group of 5 dogs with the H-type markers: the mean 6-day postsurgical endocardial $\bar{V}_{ma}$ and endocardial ExS were 1.58 ± 0.30 sec^{-1} and 19.3 ± 2.6%, respectively. The epicardial and endocardial $\bar{V}_{ma}$ relation for these 5 dogs over 6 days was:

$$\bar{V}_{ma}\ \text{epicardial} = 0.39 \times \bar{V}_{ma}\ \text{endocardial} + 0.11,$$
$$S_{y,x} = 0.073,\ r = 0.85,\ p \ll 0.001. \qquad (3)$$

Specific frame by frame correlations between epicardial and endocardial diameter were made daily for 6 days for 2 dogs (Dogs 4 and 5). None of these correlations showed a correlation coefficient lower than 0.97.

Left Ventricular Pressure Derived Variables

Left ventricular pressure, peak dP/dt, and V_{max}-dev are summarized in table II. No significant differences were found between the 6 follow-up days. The mean peak dP/dt was 4,322 mm Hg/sec, SD = 924, while the mean V_{max}-dev was 131 sec^{-1}, SD = 20, both from 8 dogs over 6 days.

Table I. Heart rate, mean velocity of minor axis shortening, and extent of shortening (averaged over five consecutive beats) is shown for each dog for the first 6 postoperative follow-up days

	Dog No.	Day 1			Day 2			Day 3		
		HR b/min	$\bar{V}_{ma}$ sec^{-1}	ExS %	HR b/min	$\bar{V}_{ma}$ sec^{-1}	ExS %	HR b/min	$\bar{V}_{ma}$ sec^{-1}	ExS %
Epicardial	1	181	0.82	9.4	107	0.72	10.2	102	0.80	10.7
	2	170	0.75	6.3	120	0.90	8.3	114	0.74	8.1
	3	174	0.38	2.4	168	0.68	6.1	134	0.61	6.7
	4	152	0.68	7.4	152	0.89	8.2	154	0.83	9.7
	5	184	0.90	9.1	177	0.71	7.7	163	0.73	8.6
	6	155	0.78	9.2	150	0.55	6.7	133	0.68	9.5
	7	166	1.00	9.7	164	0.92	10.8	121	0.41	5.9
	8	154	0.70	8.3	129	0.68	8.5	121	0.59	8.3
Endocardial	4	152	1.64	18.0	152	1.92	17.6	154	1.79	20.9
	5	184	2.17	21.9	177	1.92	20.5	163	1.65	19.5
	6	155	1.64	19.2	150	1.21	14.8	133	1.39	19.1
	7	166	2.30	22.2	164	1.77	20.8	121	1.13	16.1
	8	154	1.29	15.2	129	1.20	15.1	121	1.14	15.9

The H-type marker was used in the latter 5 dogs allowing determination of endocardial shortening as shown in the last five lines of the table.

The relation between $\bar{V}_{ma}$ (epicardial) and V_{max}-dev was:

$$\bar{V}_{ma} = 0.0044 \times V_{max}\text{-dev} + 0.14, \quad S_{y.x} = 0.137,\ r = 0.55,\ p < 0.001. \tag{4}$$

The relation between $\bar{V}_{ma}$ (epicardial) and peak dP/dt was:

$$\bar{V}_{ma} = 8.7 \times 10^{-5}\ dP/dt + 0.35, \quad S_{y.x} = 0.142,\ r = 0.50,\ p < 0.001. \tag{5}$$

Discussion

Comparison with other Techniques

At least six techniques have been described for the direct measurement of myocardial dimensions: ventriculography [MITCHELL *et al.*, 1969; HUGENHOLTZ *et al.*, 1969] using contrast injection, ultrasonic echo [BROWER

Table I (continued)

Day 4			Day 5			Day 6			Days 1–6					
HR b/min	$\bar{V}_{ma}$ sec^{-1}	ExS %	HR b/min	$\bar{V}_{ma}$ sec^{-1}	ExS %	HR b/min	$\bar{V}_{ma}$ sec^{-1}	ExS %	HR b/min		$\bar{V}_{ma}$ sec^{-1}		ExS %	
									mean	SD	mean	SD	mean	SD
101	0.62	10.1	134	0.54	6.8	134	0.68	9.3	126	28	0.70	0.11	9.3	1.5
150	1.03	9.8	139	0.98	9.8	135	1.03	9.9	138	20	0.91	0.13	8.7	1.4
130	0.52	6.6	133	0.49	7.1	120	0.44	6.0	143	22	0.52	0.11	5.8	1.7
147	0.84	11.1	137	0.80	10.6	137	0.97	11.4	147	8	0.84	0.10	9.7	1.6
166	0.77	9.3	168	0.78	8.6	177	0.83	9.4	173	8	0.79	0.07	8.8	0.6
121	0.58	8.4	95	0.60	10.3	126	0.56	8.8	130	22	0.63	0.09	8.8	1.2
137	0.85	10.3	128	0.78	11.3	124	0.70	9.0	140	20	0.78	0.21	9.5	1.9
135	0.58	5.9	119	0.68	8.2	121	0.62	8.0	130	13	0.64	0.05	7.9	1.0
147	1.66	22.0	137	1.62	21.3	137	1.78	21.2	147	8	1.74	0.12	20.2	1.9
166	1.77	21.4	168	1.79	19.7	177	1.86	21.1	173	8	1.86	0.18	20.7	1.0
121	1.39	20.2	95	1.28	21.9	126	1.27	19.7	130	22	1.36	0.15	19.2	2.4
137	1.72	20.9	128	1.60	23.3	124	1.60	20.8	140	20	1.69	0.38	20.7	2.5
135	1.35	13.8	119	1.30	15.8	121	1.38	17.5	130	13	1.28	0.09	15.6	1.2

et al., 1975], ultrasonic transit time [SASAYAMA *et al.*, 1976], specially constructed radiopaque markers implanted either by catheterization [CARLSSON and MILNE, 1967; NOBLE and MILNE [1969] or surgery [HEIKKILÄ, 1972; INGELS *et al.*, 1975; PENN *et al.*, 1976], and strain gauge based dimension transducers [RUSHMER, 1954]. It is well known that both ventriculography and ultrasonic echos give inaccurate measurements of end systolic dimensions due to infolding of the trabeculae. Ultrasonic transit time is attractive since it leads to a direct readout of dimension. However, its use in measuring wall thickness is complicated by the need for myocardial surgery to implant the receiver crystal; and if placed on the endocardial surface, it may also be subject to trabeculae infolding. The strain gauge based methods can provide direct readout of dimensions and can be designed to minimize artefacts due to the trabeculae. However, these generally are not well suited for chronic implantation being bulky and in

Table II. The left ventricular developed pressure, peak rate of change of pressure, and V_{max} from developed pressure was determined from the same 8 dogs shown in table I

Dog No.	Day 1			Day 2			Day 3			Day 4		
	LVP mm Hg	dP/dt mm Hg /sec	V_{max} sec^{-1}	LVP mm Hg	dP/dt mm Hg /sec	V_{max} sec^{-1}	LVP mm Hg	dP/dt mm Hg /sec	V_{max} sec^{-1}	LVP mm Hg	dP/dt mm Hg /sec	V_{max} sec^{-1}
1	163	5,827	159	196	6,065	154	176	5,840	172	198	5,613	155
2	147	5,543	139	139	4,887	150	142	4,520	144	156	5,071	178
3	133	3,321	113	121	3,805	138	125	3,627	127	121	2,878	102
4	110	3,692	115	125	4,976	130	150	4,488	135	140	4,329	136
5	168	6,343	149	166	5,387	136	160	3,876	131	174	4,374	140
6	133	4,236	132	140	3,522	115	160	4,451	134	152	4,432	123
7	125	4,511	121	125	3,610	115	131	2,810	105	130	3,649	122
8	126	4,031	109	150	4,430	131	141	3,112	116	161	4,383	95

the long-term having an unstable baseline. This leaves the radiopaque markers, which can provide very accurate and noninvasive follow-up; their major disadvantage being the time-consuming measurement technique requiring frame by frame measurement from cinefilms.

Recording and Analysis Techniques

We have found the consistent positioning of the dogs in relation to the Roentgen apparatus for maximal marker separation in the lateral or AP projections to be crucial for reproducible measurements of ExS and $\bar{V}_{ma}$. It is well known that since the angle Θ between true minor axis and projected minor axis (MA obs. = MA true $\times$ Cos Θ) appears in both numerator and denominator of ExS and $\bar{V}_{ma}$, these calculations are largely self-correcting for repositioning errors. This, however, neglects the fact that the heart rotates during ejection, and depending on the viewing position, this may either over or underestimate the fractional shortening. In order to avoid this problem with systolic rotation it is necessary to either film in the plane perpendicular to rotation of the marker pairs, or use biplane filming techniques. The first possibility is not practicable, since the position of the markers (minor axis) requires filming in a position (direction from base to apex) which is difficult to achieve in animals and gives technically inferior results. The use of biplane roentgen techniques therefore emerges as the only method to give completely reliable

Table II (continued)

Day 5			Day 6			Days 1–6					
LVP mm Hg	dP/dt mm Hg /sec	V_{max} sec^{-1}	LVP mm Hg	dP/dt mm Hg /sec	V_{max} sec^{-1}	LVP mm Hg		dP/dt mm Hg/sec		V_{max}-dev sec^{-1}	
						mean	SD	mean	SD	mean	SD
190	5,426	142	–	–	–	185	15	5,754	243	156	11
140	5,120	156	130	5,360	163	127	7	5,075	360	155	14
137	2,877	125	124	2,388	113	127	7	3,149	532	120	13
132	3,525	132	138	4,036	124	133	14	4,170	536	129	8
175	4,359	153	164	4,544	158	168	6	4,815	897	145	10
155	4,191	127	181	4,452	119	153	17	4,214	358	125	7
145	4,254	140	120	2,984	109	129	9	3,636	672	119	12
148	3,548	85	157	4,420	107	147	13	3,987	548	107	16

measurements. However, this is not a practical solution in many institutions because of the expense and increased time for analysis. On the other hand, it can be shown that the error due to systolic rotation is minimized when the dog is positioned for maximal projected marker separation in the lateral to AP projection. Consistent positioning of the animal in this way thereby achieves two goals: (1) a consistent systematic error, and (2) the minimal systematic error achievable by lateral or AP filming. This is not entirely in agreement with McDONALD [1970] who concluded that cardiac rotation had only small effects. The effects are small only if the correct projection is used.

As the elastic segment exerts a force to hold the markers to the wall, it is pertinent to show that this force does not impede wall thickening during systole. Assuming a mean systolic pressure of 100 mm Hg and the applicability of Laplace's law (radius = 2.5 cm), then wall tension is 332 K dyn/cm. The average tension over the length of the marker for a 30% increase in elastic segment length is 9 K dyn/cm. Thus, the tension contributed by the elastic segment is about 3% of wall tension during systole and should not contribute an important error although a slight deformation of the epicardial surface is apparent.

Also, we did not observe any exaggerated motion of endocardial marker ascribable to rotation or interference with the trabeculae or papillary muscles; the position of the image to maximize marker shortening, effec-

tively minimizes rotation effects. Further, the shape of the marker stabilizes the orientation of the endocardial marker between the trabeculae.

The great variety of patterns of contraction shown in figure 2 confirms and extends results reported by others [McDonald, 1970; Kong *et al.*, 1971; Ingels *et al.*, 1971]. These patterns are important because they have a direct bearing on the analytic methodology appropriate for quantifying marker shortening. For example, Rushmer [1956] was among the first to observe that endocardial minor axis measured from an implanted gauge increases during isovolumic systole. 3 of the 4 types of shortening reported by McDonald [1972] concerning the spectrum of abnormality in LV hypertrophy in patients were seen in our normal dogs. We did not see paradoxical motion in our group however.

The variety in the pattern of contraction complicates the job of defining a simple rule for determining ExS or $\bar{V}_{ma}$ which can be applied uniformly and objectively. Nevertheless, as indicated the results, there was usually a constant shortening slope during ejection which could be estimated by the following rule: measure the minor axis at 50 msec after the onset of the Q wave of the ECG and the minor axis 100 msec later or at the first local minimum. This is similar to the rule proposed by Karliner *et al.* [1971] for use in man.

It was also discovered that respiration could have dramatic effects not only on the LV dimension but also on the contraction pattern. Harrison *et al.* [1963] have previously reported the effect of deep respiration on myocardial dimensions, but they did not report how this can also effect the pattern of contraction. We have found this to be especially apparent during rapid and shallow breathing or panting when the respiration rate would approach or exceed the heart rate. When this condition occurs, a curious 'heterodyne' effect becomes manifest which can be seen in several heart beats as shown in figure 5. The result is an apparent wobble in the beat by beat minor axis with a frequency of $f_r - f_h$ where f_r is respiratory frequency and f_h is heart beat frequency. To average out this wobble it is necessary to average N heart beats, $N = f_h/(f_r - f_h)$ or to carefully select beats for analysis midway through the wobble.

Figure 3 shows the relative frequency distribution in time of the measured maximum (M_{max}) and minimum (M_{min}) minor axis. The timing of M_{ed} was measured with respect to Q wave onset in ECG lead 2. The arrow in figure 3A indicates the 50-msec delay used by Karliner *et al.* [1971] in humans. Our data show that the maximum occurs on average at 20 msec, and that by waiting to 50 msec, one is assured of measuring on

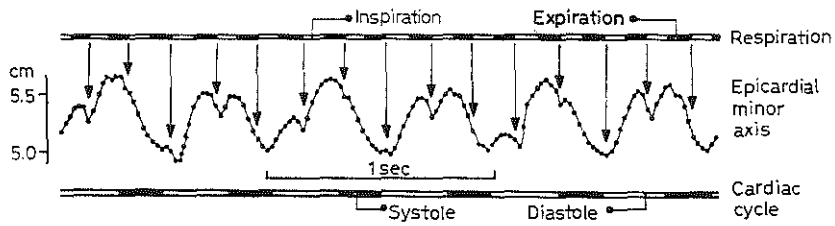

Fig. 5. When there is a shallow and rapid respiration as in panting, respiratory frequency can exceed heart rate. This results in a 'wobble' in the minor axis shortening which is more manifest in diastole than systole. The bar representing the cardiac cycle shows systole as a darkened bar while diastole is open. Similarly for respiration, the darkened bar shows expiration and the open bar inspiration.

the ejection slope in 80% of cases. M_{min} was difficult to pinpoint as the minimum would often be broad and spread out over the latter third of systole as has been seen in echocardiographic studies [BROWER *et al.*, 1975]. This is in agreement with WILDENTHAL and MITCHELL [1969] who also found no discernible pattern in dogs for identifying the moment of end systole based on the dimensional data alone. In figure 3B the arrow represents the point in time where left ventricular dP/dt reaches –200 mm Hg/sec. In fact, the average M_{min} occurred about 30 msec later. As a practical matter in long duration chronic studies, the left ventricular pressure is not usually available. Hence, criterial for identifying M_{es} based on pressure would not be useful in this context. The Q wave of the ECG, being useful as a landmark for establishing M_{ed} is also not useful in this context because of the variation in ejection time between dogs and for different heart rates. Hence, for extent of shortening (ExS) the only practical possibility at this point seems to be to use the first local minimum in minor axis shortening occurring after M_{max}. It is seldom found that M_{min} occurs within 100 msec. Hence, a useful approximation to the mean ejection slope is the following: measure M_{ed} at 50 msec after the Q wave, measure M_{es} 100 msec after M_{ed}. This results in a simple quick and relatively reliable estimate of $\bar{V}_{ma}$.

Finally, the reproducibility of caliper for recording dimensions was better than 0.2 mm of the projected diameter (= 3 × anatomical dimension). A digitizing pen system is available with a resolution of 0.3 mm which would speed up the data acquisition phase. INGELS *et al.* [1975] report satisfactory results with a 0.4 mm resolution. This becomes a crucial consideration only when marker separation is less than 20 mm.

Comparison with Contractility Indices

As a measure of LV contractility peak dP/dt and V_{max} derived from developed pressure (V_{max}-dev) have been studied experimentally [PETERSON *et al.*, 1974] and theoretically [POLLACK, 1970]. The justification for V_{max}-dev and the initial positive experimental findings described by GROSSMAN *et al.* [1972], suggested that V_{max}-dev was a promising approach. However, the problems involved in reliably calculating V_{max}-dev have overwhelmed its other merits.

Nevertheless, it was employed in this study since the 'zero level' pressure was unreliable and V_{max}-dev was the only available measure of contractility independent of preload, albeit this point being very controversial. On the other hand, peak dP/dt while sensitive to preload and afterload is attractive as a practical measurement as most recently suggested by QUINONES *et al.* [1976] who also found that the pre- and afterload dependence was not as severe as previously thought.

The correlation between peak dP/dt and V_{max}-dev was found to be

$$\text{peak dP/dt} = 32.95 \times V_{max}\text{-dev} - 7.10,$$
$$S_{y.x} = 615.5,\ r = 0.72,\ p < 0.001. \qquad (6)$$

The unexplained variation is due in large part to uncertainties in the extrapolation techniques and differential sensitivity of the two quantities to preload and afterload. It is our opinion that the evidence in the literature shows that peak dP/dt is the more reliable and useful measurement.

$\bar{V}_{ma}$, on the other hand, is an ejection phase index and necessarily dependent on afterload as shown by QUINONES *et al.* [1976].

V_{max}-dev, peak dP/dt and $\bar{V}_{ma}$ all have their origins in the same LV contraction and significant correlations are to be expected. But it is overly simplistic to expect that a pressure derived isovolumic index and a geometrically derived ejection index are equivalent to the extent that one can be used as a replacement for the other. Indeed this is shown in the regression between V_{max}-dev and $\bar{V}_{ma}$ and between peak dP/dt and $\bar{V}_{ma}$ as given in equations (4) and (5), respectively.

The low correlation between V_{max}-dev and $\bar{V}_{ma}$ (correlation coefficient $r = 0.55$) was caused in part by the fact that each dog had a different value of $\bar{V}_{max}$ (a different muscle constant) and a somewhat different position of its markers, so that slightly different regions of contraction were measured. This effect is shown in figure 6, where dogs 1 and 7 are plotted separately. Besides the correlation coefficient between peak dP/dt and $\bar{V}_{max}$-dev is 0.72 ($p < 0.001$) and this sets an effective upper limit on the correlations between pressure derived and ejection derived indices.

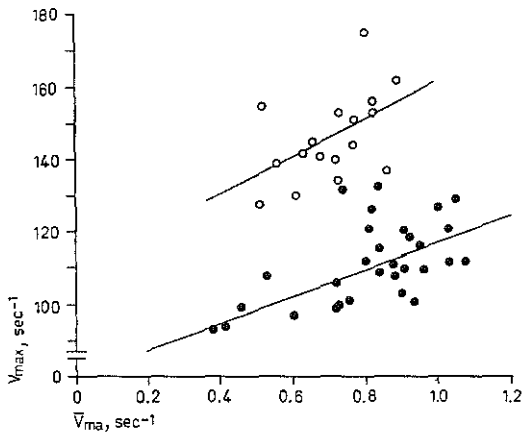

Fig. 6. The correlation between V_{max} and $\bar{V}_{ma}$ is shown for 2 dogs representing measurements taken over 6–10 days and representing 3 measurements per day. There is a significant correlation (with respect to $r = 0$) in each case: dog 1 (O), $r = 0.50$ and dog 7 (●), $r = 0.68$. The different regression lines may be due to different muscle constants. It is unlikely that the explanation lies in $\bar{V}_{ma}$ as in both cases $\bar{V}_{ma}$ is in the normal range.

It should be remarked that these data were all measured at rest and hence, tend to cluster. The correlation coefficients would be greater if the heart was stressed during measurement.

The mean extent of wall thickening was determined during the first week postop.: day 1, 10%; day 2, 10.5%; day 3, 12.5%; day 4, 13.6%; day 5, 14.0%; and day 6, 14.6%. The apparent increase from day 1 becomes significant ($p < 0.05$) by day 3. It is interesting to note that these values are consistently greater than epicardial E_xS and consistently less than endocardial ExS. SASAYAMA *et al.* [1976] reports greater values: a 31% extent of wall thickening 10 days postop., which was chosen to minimize the trauma associated with surgery while we were particularly interested in the early postop. period. Others have also reported wall thickening in the neighborhood of 30%. For example, MITCHELL *et al.* [1969] reported a 25–45% increase in dogs and HEIKKILÄ *et al.* [1972] found a 32% increase in pigs. The explanation for the difference may be: our definition of end diastole and end systole was not based on minimum and maximum wall thickness as the others have used; there may have been foreshortening between the markers even though this caused no problem for minor axis determination; finally we recorded in the early postoperative period

and occasionally used 10 mg of nicomorphine hydrochloride (intramuscular) to calm the dogs during recording sessions.

The correlation between endo- and epicardial $\bar{V}_{ma}$ (see eq. 3) is somewhat less ($r = 0.85$) than might have been expected. There are several reasons for this: variation in wall thickness day to day and dog to dog; inherent measurement errors, and differential excitation of endo- and epicardial layers. Our data show that endocardial $\bar{V}_{ma}$ on the average is double that of epicardial values. One might therefore expect endocardial $\bar{V}_{ma}$ to be a more sensitive measure of LV contraction than epicardial $\bar{V}_{ma}$. Indeed referring to table I, the ratio of average endo- to epicardial $\bar{V}_{ma}$ in 5 of the dogs is 2.07, 2.35, 2.15, 2.17, and 2.00, respectively, but this is accompanied by an increase in the ratio of the standard deviations as well (1.20, 2.57, 1.67, 1.80, and 2.00, respectively). On average there is only a slight improvement in the ratio of mean to SD, so that while endocardial $\bar{V}_{ma}$ is greater than epicardial $\bar{V}_{ma}$, it is not correct to conclude that endocardial $\bar{V}_{ma}$ is much more sensitive or reliable than epicardial $\bar{V}_{ma}$.

The epicardial end diastolic minor axis (M_{ed}) was 50 mm averaged for all dogs over all measurement days. There was no significant ($p < 0.05$) change in M_{ed} from day 1 to day 6. The ratio of pooled standard deviation to mean M_{ed} was 6.1%. Heart rate on day 1 was consistently greater than the average HR over the follow-up week; under normal circumstances it would be expected that M_{ed} at day 1 would be less than subsequent measurements since heart size decreases with HR, everything else held constant. However, on day 1 the dog is still recovering from surgical trauma and anesthesiology and it may be supposed that the expected increase in M_{ed} as a result of this was effectively compensated by the increased HR.

Conclusion

The implantation of radiopaque markers on the myocardium provides a noninvasive methodology available for postsurgical follow-up studies of myocardial shortening. However, several methodological questions needed clarification in order to make this method a practical tool. The work reported herein was conducted to help resolve these questions.

Myocardial markers and a Konigsberg pressure transducer were applied to the hearts of 8 healthy dogs. To determine the best way to use marker motion to quantify changes in the vigor of the left ventricular con-

traction, serial measurements were made daily from 6 to 10 days post-operative. $\bar{V}_{ma}$ (mean velocity minor axis shortening) and ExS (extent of shortening) were determined from epi- and endocardial markers. As an independent measure of contractility peak dP/dt and V_{max} from developed pressure were also calculated. The implantation of pressure transducers, which would not usually be done in long duration chronic studies, allowed us in this case to compare $\bar{V}_{ma}$ with peak dP/dt and V_{max}-dev.

It is clear that the reproducibility of the method for determining $\bar{V}_{ma}$ is improved over previous work. Special attention must be given to the positioning of the dogs with respect to the roentgen apparatus during filming. Position changes are directly seen in the absolute values of the minor axis and this information must be used to position the dog for maximum minor axis so as to minimize the effects of heart rotation during ejection. Normalization of minor axis change to end diastolic dimensions removes the necessity to perform a grid calibration, but does not compensate for systematic errors due to heart rotation during systole. The significant correlation between $\bar{V}_{ma}$ and peak dP/dt or V_{max}-dev showed that $\bar{V}_{ma}$ provides information on myocardial contractility, but the inherent errors in calculating V_{max}-dev, and the differential sensitivity to preload, afterload and heart rate preclude substituting one for the other. $\bar{V}_{ma}$ measured from epicardial markers was consistently less than from endocardial markers, but was no less sensitive nor accurate.

We suggest that as a practical rule for the efficient measurement of $\bar{V}_{ma}$ in subsequent work, that the calculation be based on frames measured 50 and 150 msec after onset of the Q wave of the ECG. In most cases this provides two measurement points approximating the slope of the minor axis shortening.

References

Bos, E. and Meeter, K.: Histocompatibility in orthotopic heart transplantation in dogs. Trans. Proc. *3:* 155–156 (1971).

Brower, R. W.; Dorp, W. G. van; Vogel, J. A., and Roeland, J. R.: An improved method for the quantitative analysis of M-mode echocardiograms. Eur. J. Cardiol. *3:* 171–179 (1975).

Carleton, R. A. and Clark, J. G.: Measurement of left ventricular diameter in the dog by cardiac catheterisation. Circulation Res. *22:* 545–558 (1968).

Carlsson, E. and Milne, E. N. C.: Permanent implantation of endocardial tantalum screws: a new technique for functional studies of the heart in the experimental animal. J. Ass. can. Radio. *19:* 304–309 (1967).

GROSSMAN, W.; HAYNES, F.; PARAKOS, J. A.; SALTZ, S.; DALEN, J. E., and DEXTER, L.: Alterations in preload and myocardial mechanics in the dog and in man. Circulation Res. *31:* 83–94 (1972).

HARRISON, D. C.; GOLDBLATTT, A.; BRAUNWALD, E.; GLICK, G., and MASON, D. T.: Studies on cardiac dimensions in intact, unanesthetized man. Circulation Res. *13:* 448–467 (1963).

HEIKKILÄ, J.; TABAKIN, B. S., and HUGENHOLTZ, P. G.: Quantification of function in normal and infarcted regions of the left ventricle. Card. Res. *6:* 516–531 (1972).

HUGENHOLTZ, P. G.; KAPLAN, E., and HALL, E.: Determination of left ventricular wall thickness by angio cardiography. Am. Heart J. *78:* 513–522 (1969).

INGELS, N. B.; DAUGHTERS, G. T.; DAVIES, S. R., and MCDONALD, I. B.: Stereo photogrammetric studies on the dynamic geometry of the canine left ventricular epicardium. J. Biomech. *4:* 541–550 (1971).

INGELS, N. B.; DAUGHTERS, G. T.; STINSON, E. B., and ALDERMAN, E. L.: Measurement of midwall myocardial dynamics in intact man by radiography of surgically implanted markers. Circulation *52:* 859–867 (1975).

KARLINER, J. S.; GAULT, J. H.; ECKBER, D.; MULLINS, C. B., and ROSS, J., jr.: Mean velocity of fiber shortening. Circulation *44:* 323–333 (1971).

KONG, Y.; MORRIS, J. J., and MCINTOSH, H. D.: Assessment of regional myocardial performance from biplane coronary cineangiograms. Am. J. Cardiol. *27:* 529–537 (1971).

MCDONALD, I. G.: The shape and movements of the human left ventricle during systole. Am. J. Cardiol. *26:* 221–230 (1970).

MCDONALD, I. G.: Contraction of the hypertrophied left ventricle in man studied by cineradiography of epicardial markers. Am. J. Cardiol. *30:* 587–594 (1972).

MEESTER, G. T.; BERNARD, N.; ZEELENBERG, C.; BROWER, R. W., and HUGENHOLTZ, P. G.: A system for the on-line analysis of cardiac catheterization data. Catheter. cardiovasc. Diag. *1:* 113–131 (1975).

MITCHELL, J. H.; WILDENTHAL, K., and MULLINS, C. B.: Geometrical studies of the left ventricle utilizing biplane cinefluorography. Fed. Proc. *28:* 1334–1343 (1969).

NOBLE, M. I. M. and MILNE, E. N. C.: Left ventricular filling and diastolic pressure volume relations in the conscious dog. Circulation Res. *24:* 269–283 (1969).

PENN, O. C.; BROWER, R. W.; LEICHER, F.; MEESTER, G. T., and BOS, E.: Orthotopic canine heart transplantation: left ventricular contractility during chronic rejection. J. thorac. cardiovasc. Surg. *71:* 526–532 (1976).

PENN, O. C.; MCDICKEN, I.; BOS, E., and NAUTA, J.: Serial percutaneous biopsies from canine orthotopic cardiac allografts. J. surg. Res. *18:* 615–618 (1975).

PETERSON, K. L.; SKLOVEN, D.; LUDBROOK, P.; UTHER, J. B., and ROSS, J. jr.: Comparison of isovolumic and ejection phase indices of myocardial performance in man. Circulation *49:* 1088–1101 (1974).

POLLACK, G.: Maximum velocity as an index of contractility in cardiac muscle: a critical evalution. Circulation Res. *26:* 111–127 (1970).

QUINONES, M.; GAASCH, W. H., and ALEXANDER, J. K.: Influence of acute changes in preload, afterload, contractile state, and heart rate on ejection and isovolumic indices of myocardial contractility in man. Circulation *53:* 293–302 (1976).

Ross, J., jr.; Sonnenblick, E. H.; Covell, J. W.; Kaiser, G. A., and Spiro, D.: The architecture of the heart in systole and diastole. Circulation Res. *21:* 409–421 (1967).

Rushmer, R. F.: Continuous measurements of left ventricular dimensions of intact, unanesthetized dogs. Circulation Res. *2:* 14–21 (1954).

Rushmer, R. F.: Initial phase of ventricular systole: asynchronous contraction. Am. J. Physiol. *184:* 188–194 (1956).

Sasayama, S.; Franklin, D.; Ross, J.; Kemper, W. S., and McKoun, D.: Dynamic changes in left ventricular wall thickness and their use in analyzing cardiac function in the conscious dog. Am. J. Cardiol. *38:* 870–879 (1976).

Wildenthal, K. and Mitchell, J. H.: Dimensional analysis of the left ventricle in unanesthetized dogs. J. appl. Physiol. *27:* 115–119 (1969).

CHAPTER 6

DISCUSSION AND CONCLUSIONS

The model, developed by Bos (19), for chronic rejection after orthotopic canine cardiac transplantation in DLA identical beagle littermates was further investigated in this thesis. Bos postulated that this standardized model could be used to study the course and the characteristic features of chronic rejection in more detail. The sequential changes as they occur in the cardiac graft, during this type of rejection, still had to be documented and no conventional parameters, such as daily ECG recording, proved useful for monitoring the early onset of these observed changes.

The first aim of this study was to describe the histopathological changes that develop during the course of chronic rejection after orthotopic cardiac transplantation in DLA identical beagle littermates by means of serial percutaneous myocardial biopsies and to determine the time of onset of these changes.

Secondly, left ventricular contractility during chronic rejection was studied by means of a non-invasive technique by recording the motion of endocardial markers.

The techniques and the subsequent results of our investigations are described in four articles presented in the previous chapters.

Chapter two deals with the percutaneous technique that was used to obtain serial biopsies from the canine cardiac allografts.

Chapter three presents the results of the measurements of left ventricular contractility during chronic rejection.

Chapter four presents the results of the histopathological examinations of the myocardial biopsy specimens obtained during the process of chronic rejection.

Chapter five further evaluates the method to calculate myocardial contractility from the motion of radiopaque markers. The reason for a more detailed study on this method is explained in the last part of this discussion (page 75).

Serial percutaneous biopsies from canine orthotopic cardiac allografts.

The method for taking serial percutaneous myocardial biopsies from the left ventricle is described in the second chapter.

The biopsies were taken, under general anaesthesia, with the aid of a disposable liver biopsy needle, which was inserted through the skin of the thorax into the apex of the heart. One hundred and forty two biopsies were obtained from nineteen beagles after orthotopic cardiac transplantation. Only one dog was lost because of intrathoracic bleeding after a biopsy was taken. Biopsies of the left ventricular wall, obtained by this method, were found to contain adequate material for light microscopic examination.

Potential complications of the percutaneous technique for cardiac biopsies include hemorrhage, resulting in cardiac tamponade, damage to the coronary arteries, arrhythmias and infection. Apart from one case of hemorrhage, none of these complications occurred in our series. Probably the risk of hemorrhage is reduced by the formation of adhesions between the transplanted heart and the inner side of the left thoracic wall and the previously opened pericardium.

It has been suggested that the percutaneous biopsy technique could very likely produce cardiac tamponade or bleeding when the formation of adhesions was inhibited by the administration of immunosuppressive drugs. We did perform percutaneous biopsies in two mongrel dogs on immunosuppressive therapy after orthotopic cardiac transplantation, but no complications were seen. This indicates that the percutaneous biopsy technique does not necessarily create cardiac tamponade after orthotopic heart transplantation in combination with immunosuppressive therapy.

This technique for serial percutaneous biopsies, which was not previously described, proved to be a convenient method for obtaining cardiac biopsies in which canine orthotopic cardiac allograft histopathology could be studied.

The results of percutaneous biopsies taken from heterotopic canine cardiac allografts were reported by Leedham in 1971. With a liver biopsy needle serial biopsies were taken from cardiac allografts transplanted to the neck in dogs (110a).

To obtain biopsies from orthotopic canine cardiac allografts, Caves reported the transvenous approach of a myocardial bioptome in 1973 (29). He employed a modified Konno-Sakakibara bioptome (103) to deter-

mine the histopathological changes which occur following cardiac transplantation. The transvenous approach proved to be very successfull for the early diagnosis of acute rejection crises and clinical application soon followed (27, 28, 32). No morbidity or mortality related to the transvenous endomyocardial biopsy method has been reported and it certainly deserves a first consideration when myocardial biopsies are considered in clinical cardiac transplantation.

Unfortunately the transvenous method proved unworkable in our long-surviving beagles, because the biopsies had to be taken very frequently and over a long period of time. Having already sacrificed the left external jugular vein at the time of transplantation, the right external jugular vein thrombosed early after only a few transvenous biopsies. As a consequence the percutaneous method was introduced.

At first the percutaneous myocardial biopsies, described above, were taken after general anaesthesia was induced in the dogs; later on we succeeded in taking serial percutaneous myocardial biopsies after orthotopic cardiac transplantation using local anaesthesia. This provided a safer procedure because general anaesthesia was tolerated very badly by the dogs suffering from the end-stage of the chronic rejection process.

The greatest advantage of the percutaneous biopsy technique is that most of the samples consist of a cross section of the left ventricular wall, from epi- to endocardium. In contrast to the endomyocardial biopsies, the histopathological changes can be investigated in all myocardial layers.

In *conclusion,* the percutaneous myocardial biopsy technique proved to be very valuable since it is an accurate method for diagnosing the histopathological changes of chronic rejection in the living transplanted dog. It could also provide tissue for other studies like electronmicroscopy, immunofluorescence and histochemistry, of the canine cardiac allograft.

Another application could be its use for documenting the histopathological changes in the model of chronic rejection, modified by immunosuppressive therapy, anticoagulants or a balanced diet.

Histopathology of rejection in DLA-identical canine orthotopic cardiac allografts.

The results of the histopathological examinations of the myocardial biopsy specimens obtained during the process of chronic rejection are described in the fourth chapter. In the animals surviving 4-18 weeks the vascular lesions became dominant

features after the second week post-transplantation. Small foci of parenchymal infiltration were observed during the first weeks after transplantation but they disappeared later. The vascular changes consisted of endothelial swelling, marked edema and thickening of the media of the vessels, leading to obliteration of the lumen. Ischemic changes, like muscle degeneration and infarction, could be discerned later during the course of chronic rejection; these could be secondary to the chronic obliterative arteritis. These vascular changes were observed in all myocardial layers. Medial changes in the intramural vessels were as severe as in the epicardial vessels, although intimal and subintimal lesions were not so marked in the epicardial vessels. As the course of chronic rejection was progressing, the process of chronic obliterative arteritis was found to lead to a generalized myocardial ischemia. At postmortem examination the coronary arteries showed extensive medial proliferation with almost complete obstruction of the lumen.

Myocardial biopsies were examined by light microscopy with conventional techniques. Hattler (87a) stated that histochemical methods show changes in dog cardiac allografts before these can be appreciated by light microscopy. It has to be assumed that the time of onset of the immunologic injury must take place earlier than the above mentioned second week. However, light microscopy failed to reveal the arterial changes, characteristic of chronic rejection, before that time.

Bos stated that (19) the predominant histopathological feature of chronic cardiac allograft rejection is found in the larger medium sized vessels of the graft. From our findings it seems as if all myocardial arteries of the allograft are affected. Gross changes were observed in the coronary arteries and their main branches, but there was evidence too that the capillaries were damaged. One feature observed in the serial biopsies was distension and prominence of the capillaries which appeared to be subsequently obliterated by swelling of the endothelium, attachment of monocytes and thrombosis. The arterioles of these biopsies also showed medial proliferation and subsequent obliteration. Other vessels with thickened walls were apparently arterized dilated capillaries with hyalinisation. Although the limiting factor in chronic rejection is the degree of damage to the endothelium and subsequent processes, it seems from our studies that the rate of response to the obliterative damage by opening of vascular anastomoses from the capillary bed may be equally important. Rapid severe damage will obliterate any collateral vessel that develops whereas a slow obliterative process will allow time for collaterals to develop and the newly formed vessels will not become immediately obliterated.

The clinical picture in some of the longest surviving dogs after orthotopic cardiac transplantation resembles the syndrome

of tricuspid insufficiency, showing severe ascites and venous congestion. Bos (19) described extensive valvular lesions as another result of the humoral or antibody-mediated immune response of the host during chronic rejection. It is obvious that no information could be gathered about the valvular injuries during the course of chronic rejection by means of the biopsy technique.

The chronic obliterative arteritis is supposed to be the result of a humoral or antibody-mediated immune response. In 1973 O' Connell and Mowbray indicated that these vascular lesions started with arterial intimal thickening produced by alloantibodies (134). In 1977 Cerilli isolated an antivascular endothelial cell antibody that could be responsible for these lesions (33, 34). According to Kosek the chronic obliterative arteritis, which is characteristic for chronic rejection, will eventually lead to graft arteriosclerosis (109). The arterial injury can lead to intimal accumulation of modified smooth muscle cells which are prone to fatty changes and are the basic cells of atheromas. At the same time medial hypoxia is caused by damage to the endothelial cells of the vasa vasorum. As mentioned by Hollander (88) no influence has been observed of immunosuppressive treatment on the occurrence of this obliterative arteritis.

Clinically only the administration of warfarin , dipyridamole and the restriction of dietary lipids are instituted as the therapeutic regimen to reduce the incidence of graft arteriosclerosis in the transplanted heart as described by Griepp (77).

Myocardial contractility during chronic rejection

Orthotopic canine heart transplantation:
left ventricular contractility during chronic rejection.

The results of the measurements of left ventricular contractility during chronic rejection are described in the third chapter. The method that was used for the contractility measurements was to calculate the mean velocity of shortening in the circumferential fibers ($\overline{V}_{cf}$) from the motion of radiopaque endocardial markers. Being representative of myocardial contractility, the calculated values for $\overline{V}_{cf}$ should reflect changes in left ventricular pumping performance during chronic rejection. For reasons explained later (page 76), the term $\overline{V}_{cf}$ was replaced by $\overline{V}_{ma}$ (Mean velocity of minor axis shortening) in chapter five, without affecting the value of the findings of chapter three.

To determine left ventricular contractility during chronic

rejection endocardial markers were attached to the donor heart a week before transplantation. Donors and recipients were beagle littermates and identical for the DLA histocompatibility complex. Before and after orthotopic cardiac transplantation the marker movements were recorded on Roentgen cinefilms in eight recipients with a mean survival time of 112.5 days.

As a result of the analysis of marker motion during the process of chronic rejection two significant drops in left ventricular contractility could be determined. The first drop occurred between the preoperative measurements and those of the first week after transplantation ($P < 0.02$). This drop may have been caused by the operative trauma and the onset of the rejection process, although the characteristic histopathological changes of chronic rejection were not demonstrated before the second week after transplantation.

The second drop in myocardial contractility occurred in the week before death ($P < 0.01$). This drop probably represents the exhaustion of reserve mechanisms after longstanding immunological injury. In the last week the percutaneous biopsies reveal the lesions of an end-stage myocardial ischemia. At postmortem examination the coronary arteries of the cardiac allografts showed extensive medial proliferation and nearly complete obliteration of the lumen.

Between the first week after transplantation and the week before death there is a decrease in left ventricular contractility, but this drop proved to be not statistically significant. In this period of time myocardial contractility either is only slightly impaired or the technique failed to reveal more subtle changes. Values calculated the week before death are significantly lower compared to those of the week before transplantation, representing the difference in performance of injured and healthy myocardial muscle.

During the process of chronic rejection in these dogs the values for the ECG voltage in limb lead II and for heart rate were also collected. No significant changes could be found in these values.

It is *concluded* that a certain pattern of depression of left ventricular contractility could be discerned during the course of chronic rejection. A progressive decrease in myocardial function seems to be apparent in the period of time from transplantation till death. Shortly after transplantation and in the week preceding death a statistically significant depression of left ventricular contractility could be established.

When the results of the histopathological investigations, as described in chapter four, are taken into account, it seems as if the decrease in myocardial contractility is based upon a progressive myocardial ischemia caused by the chronic obliterative arteritis that is characteristic

for chronic rejection. However, this could not be established for the decrease in myocardial contractility in the first week after transplantation. Explanations for this particular phenomenon are myocardial depression by the operation trauma or the onset of immunological damage which could not be detected by light microscopy.

The method of determining left ventricular contractility, by calculating the mean velocity of shortening of the circumferential fibers ($\bar{V}_{cf}$), proved to be a useful method for studying myocardial performance during chronic rejection. However, some problems related to this method and to the way we applied it in our study were identified.

We have not been able to prove that the method could be used for early diagnosis of the chronic rejection process. The possibility exists however that a refined technique would show more subtle changes in cardiac performance during the slow development of chronic rejection.

Also a great deal of variation in the data was apparent and a certain discomfort existed about the fact that no significant changes in left ventricular contractility had been identified between the first and the last week after transplantation. As we had little experience with this method further work was needed to clarify the origin of this imperfection.

Encouraged by the results and convinced of the benefits of a well-tested non-invasive method it was decided that other experiments ought to be conducted in order to improve the reliability of this method. These experiments are described in chapter five.

The search for a non-invasive method to determine myocardial function was necessitated by the need to study left ventricular performance during chronic rejection. Because frequent measurements of left ventricular performance were desirable and over a long period of time, a non-invasive method was indicated. As little was known about this subject at that time, the solution to this problem was found in a combination of two techniques: the insertion of endocardial markers and the calculation of the mean velocity of circumferential fiber shortening.

The mean velocity of circumferential fiber shortening ($\bar{V}_{cf}$) was introduced by Karliner in 1971 (96) as a simplified measure of left ventricular contractility. The $\bar{V}_{cf}$ was derived from angiograms by determining the systolic excursion of the left ventricular minor axis. It was concluded that the estimation of the mean rate of circumferential fiber shortening provided a reliable method of measuring left ventricular contractility.

As we wanted to avoid diagnostic catheterizations and angiograms in our study it was decided to mark the internal minor axis of the left ventricle by the insertion of endocardial radiopaque markers. From the marker movements, as recorded on

Roentgen cinefilms, the systolic excursion of the left ventricular minor axis could be determined and the velocity of shortening could be calculated.

The insertion of the endocardial markers was carried out with the help of a harpoon-like device. The original idea for this device was conceived by Dr. R.F. Rushmer, Seattle, Washington. It was described by Heikkilä in his study about the quantification of function in normal and infarcted regions of the porcine left ventricle (87b).

Myocardial markers have never been very popular. Certainly when echocardiography appeared on the diagnostic scene, there seemed even less need for such a laborious technique. However, the echocardiogram has not yet proved itself workable in the dog, although sector scanning techniques may provide an answer in the future, as recently reported by Meltzer (127). At the moment echotechniques do not provide reliable information on general myocardial function or regional contraction patterns.

To determine the regional pattern of contraction of the myocardium after coronary bypass surgery, epicardial markers were introduced in the Rotterdam University Hospital by Brower (21). From Brower's work it is clear that the attachment of radiopaque markers can provide useful information especially when multiple serial measurements are required in the postoperative period.

Only two studies have been reporting the use of radiopaque markers for follow-up measurements after orthotopic cardiac transplantation.

Matilla and Ingels (126) used intramyocardial markers in 1972 to determine general myocardial function after orthotopic canine cardiac transplantation. After inserting tantalum coils into the left ventricular wall at the time of transplantation, the effects of atrial pacing were studied. At that time it was found that only cardiac output was significantly changed by alterations in heart rate.

Later in 1977 Ingels implanted tantalum coils in eleven patients at the time of orthotopic cardiac transplantation. It was found that, after atrial pacing, augmentation of heart rate produced a reduction in end-diastolic and end-systolic volume, while cardiac output was little changed with increased rate. He concluded that end-diastolic volume reduction was brought about by abbreviated filling (91).

The effect of denervation on myocardial performance has not been taken into account in our experiments. In their studies Ingels and Mattila (91, 126) investigated the fact that cardiac transplantation results in the loss of direct, autonomically mediated mechanisms for regulation of cardiac output because of denervation. Dong (59) showed a persistent increase in blood volume following cardiac transplantation, possibly due to a loss of the Bainbridge reflex in the denervated heart. The transplanted heart was supposed to have a fixed stroke volume, thus

cardiac output could only be increased by the augmented venous return combined with an increase in heart rate. This could not be proved in the study of Ingels and Mattila about donor left ventricular response to atrial pacing. A more normal stroke volume response to increased heart rate was found. It was stated that the transplanted, denervated heart possesses an apparently normal intrinsic mechanism for regulation of stroke volume (91, 126).

Myocardial radiopaque markers used to quantify minor axis shortening for follow-up studies in experimental surgery.

The method of determining left ventricular contractility from endocardial marker movements is further validated in chapter five.

Several reasons can be enumerated for these extended investigations:

1. Despite the fact that we had little experience with this method, significant changes in myocardial contractility were demonstrated during the process of chronic rejection. However a great deal of variation in the data was apparent which needed further work to clarify its origin.
2. The measurement of marker motion was carried out manually from frame by frame cinefilm projections, which makes the method a very laborious technique.
3. Little was known about the mean velocity of shortening being representative for myocardial function. Therefore, we wanted to establish for ourselves the correlation between the mean velocity of shortening and well-known variables used to quantify myocardial function.
4. Our calculations of myocardial contractility were derived from endocardial marker motion. Endocardial markers are more difficult to insert than epicardial markers. If epicardial markers provide us with the same information this could lead to a simplification of the method.

Therefore the animal experiments described in chapter five were conducted in order to:

1. Perfect the data recording and analysis techniques.
2. Simplify the measurements and calculation procedures for routine use.
3. Compare the mean velocity of shortening with well-known variables used to quantify myocardial function, i.e. peak dP/dt and developed $V_{max.}$
4. Compare the values for the mean velocity of shortening derived from epi-and endocardial markers.

Epi- and endocardial markers were attached to the hearts of eight

beagles. After placement of the markers, a Koningsberg pressure transducer was introduced into the left ventricle. Also a bipolar pacemaker electrode was attached to the atrium in order to control heart rate during the recordings. Daily postoperative left ventricular pressure recordings, cinefluorography and ECG recordings were made. This was performed up to 10 days after surgery at rest or during pacing in conscious dogs.

It has to be mentioned that the term $\overline{V}_{cf}$ (mean velocity of circumferential fiber shortening) was not correctly used in chapter three. Instead of the circumferential fiber shortening, the shortening of the minor axis had been calculated. This is of no consequence for the findings in chapter three but as the term $\overline{V}_{cf}$ was misleading it was replaced by $\overline{V}_{ma}$ (mean velocity of minor axis shortening) in chapter five.

From these experiments it is *concluded* that the reproducibility of the method for determining the mean velocity of minor axis shortening could be improved over previous work. Special attention had to be given to the positioning of the dogs with respect to the Roentgen apparatus during filming. Position changes are directly seen in the absolute values of the minor axis.

A digitizing pen system was introduced to speed up the data acquisition phase.

A significant correlation between the mean velocity of minor axis shortening and peak dP/dt or developed V_{max} (correlation coefficient R = 0,55) showed that $\overline{V}_{ma}$ provides information on myocardial contractility, but has a different sensitivity to loading and heart rate changes.

The mean velocity of minor axis shortening measured from epicardial markers was consistently less than from endocardial markers, but was no less sensitive nor accurate.

$\overline{V}_{ma}$ values in these healthy dogs were found to be in the range of $\overline{V}_{cf}$ values described in chapter three for the preoperative measurements. There was no significant change in values for the mean velocity of minor axis shortening obtained from these healthy dogs in the postoperative period. It would be expected that the mean velocity of minor axis shortening was less on the first day postoperative as a result of surgical trauma to the heart. However, this could not be reproduced. A possible explanation for this feature is that the insertion of the markers themselves, hardly imposes damage to the heart.

The studies described in this thesis were executed in the hope that a little more clarity could be brought to the chronic rejection process.

Based on the

results described above it can be stated that the objectives, which this thesis focussed on, were fulfilled.

The value of the findings, described in this study, for *further experiments* are at least fourfold:

1. The technique for serial percutaneous biopsies could provide tissue for other studies relating to canine orthotopic cardiac allograft transplantation.
2. The description of the chronic obliterative arteritis, as presented in this thesis could lead to a determination of the specific agents responsible for it.
3. The assessment of left ventricular contractility during chronic rejection may provide some insight on the influence of the immunological injury on myocardial performance.
4. The attachement of radiopaque markers to the myocardium provides a non-invasive method to gather information on general myocardial function or regional contraction patterns for follow-up studies.

CHAPTER 7

HISTORICAL SURVEY OF ORTHOTOPIC CARDIAC TRANSPLANTATION

"Never in history of man so much has been said to so many about so little for the benefit of so few".

Dr. John C. Callaghan's paraphrase on
Sir Winston Churchill's famous words.

It was in the year 300 B.C. that the surgeon Pien CH'iao was visited by two travelers, Kung Wu of Lu and Chi Ying of Chao, who requested that he should treat them. Pien CH'iao reportedly gave the two men a potion that rendered them unconscious for three days, during which time he interchanged both their hearts and stomachs. The two men awoke fully recovered and went their separate ways home.

Carrel and Guthrie brought cardiac transplantation out of these mythological atmospheres in 1905. Introducing previously unknown suturing techniques they transplanted successfully the heart of one dog into the neck of a larger one. After re-establishing the circulation, the transplanted heart manifested strong contractions until intracavitary coagulation occurred some 21 hours after transplantation; sepsis was implicated as the major factor limiting survival (23, 24), but by means of these experiments it was demonstrated that a denervated heart could continue beating. Before the development of modern methods for cardiopulmonary bypass it was not possible till 1959 to perform orthotopic cardiac transplantation. The history and the development of various models of heterotopically transplanted hearts have been described in detail by Jongsma (92) and Hairston (78). The various studies of the heart in an ectopic position have added considerably to the knowledge of the transplanted heart and its susceptibility to the process of rejection (78, 124, 167), its vulnerability to hypoxia (115, 165) and the protective effect of hypothermia (16, 166). When a successful complete cardiac replacement was first performed by Lower and Shumway in 1959 several conditions had been worked out. Especially modern techniques for cardiopulmonary bypass, for myocardial protection during the period of obligatory anoxia and for the conversion of numerous venous anastomoses to a long atrial suture line, made it possible to succeed the first time in cardiac

replacement. In dogs only the fragile supravalvular aorta, having the consistency of blotting paper, still presents a major technical problem (117, 118). Hemodilution did not prove to be helpful in the experimental laboratory. Blood prime provided the best method for extracorporeal circulation and general hypothermia was not needed (145-147). Survival of 6 to 21 days was achieved by this technique. Yet electrocardiographic studies revealed a progressive decrease in the voltage of the QRS complex and postmortem pathologic changes in the myocardium were characteristic for the homograft rejection mechanism (113, 116, 120, 122).

Studies were then performed by Willman and Hurley on autotransplanted hearts to investigate the characteristics of the totally denervated heart (90, 121). No deleterious effects were noted from denervation itself. A hemodynamic pattern was found, which proved to be compatible with life. The physiologic impairment which accrues from the transplantation procedure appeared small and transient. Evidence for re-innervation across the tissue barrier had also been sought. In fact sympathetic and parasympathetic reinnervation proved nearly complete in the autograft series 14-18 months after excision and reimplantation of the heart (55, 61, 90, 102, 104, 105, 170-174).

With respect to control of homograft immunity, imuran (azathioprine) and steroids constituted the best therapy in restraining the forces of rejection and voltage changes in the QRS complex of the electrocardiogram, reflecting graft invasion, were used as guide line in the use of chemotherapy (119, 120).

Kantrowitz, Kondo and Hurley also studied the effect of minimization of antigenicity and found that the immune mechanism was somehow suspended after cardiac replacement in littermates and puppies. However these donor-recipient combinations were not spared homograft rejection and some program of treatment for homograft rejection remains a "sine qua non" for long-time survival (90, 95, 99, 101).

From 1959 till 1967 orthotopic cardiac transplantation was continuously investigated in the laboratory (35-37, 59, 131). The only clinical experiment during this period was performed by Hardy who replaced a diseased human heart by that of a chimpanzee in 1964. The outcome of this xeno-transplant was unsuccessful (83-87).

In March 1967 senator Mondale from Minnesota, now vice-president of the United States of America, together with senator Ribicoff, proposed the appointment of a commission to advise the Congress on the validity of a program on heart transplantation. One thing that excited the Mondale-Ribicoff Committee was the matter of the furor over cardiac transplantation, quite unlike that which occurred with transplantation efforts either

in the kidney or with the liver. It was Owen H. Wangensteen from Minneapolis, the surgeon, who volunteered to appear before this Committee to remind the members that whereas the kidney and the liver received little attention in the Scriptures, it takes more than four pages to enumerate all the citations to the heart.

In December 1967, Barnard performed the first human to human transplantation in Cape Town, South Africa (2-5, 11, 114, 130, 160). This operation, as might be expected, met with reactions ranging from acclaim to condemnation. But for many centers, that were confronted with patients suffering from incurable or end-stage cardiac disease, the starting shot was fired which resulted in 135 transplant operations in the ensuing 18 months. This included one other xenograft operation by Cooley, who used a sheep heart, which also rejected acutely (10, 42-45, 79, 80).

To investigate the possibilities of xenografts for human cardiac transplantations, Neville implanted calf, sheep and goathearts into dogs. They all reacted similarly: a short period after the release of the aortic clamp, bluish patchy areas became visible in the myocardium, fibrillation ensued and cardiac action could not be reinstituted. The same phenomenon was described by Cooley in the sheep heart which he used for the one transplantation in man. Histology showed obstruction of the capillaries by agglutinated erythrocytes. However when the experiments were reversed and the dog heart was orthotopically placed in the goat, the donor heart reacted well and appeared not different than when it was placed in another dog. As far as could be ascertained the reason for failure of the first group of xenografts was due in part to the size of the red blood cells. Goat and sheepcells are much smaller than dogcells and as a result the capillaries are concomitantly decreased in size in these animals. On the other hand the smaller cells of goat and sheep can easily traverse the larger myocardial capillaries of the dog (47). Other mechanisms however cannot be excluded.

In that time also three orthotopic cardiac transplantations were performed in infants. The longest surviving infant underwent the only combined heart and lung transplantation and lived for 14 hours before dying of pulmonary complications. The indications for cardiac replacement in those children were terminal cardiac failure due to atrioventricularis communis, endocardial fibroelastosis and pulmonary atresia (46, 94).

In the period from 1968 till 1969 the overall mortality in the first two days after transplantation was about 20 percent. For the surviving patients postoperative care had to be organized. This postoperative care was concentrated along three lines: maintenance of a satisfactory cardiac output, suppression of the immunologic reaction to the transplanted heart and the prevention or treatment of infection (2, 97, 157).

Fighting rejection created a lot of new complications which made Callaghan from Edmonton cry out in despair: "Basic biologists must come to our aid. It is apparent that a deep biological problem exists. Remembering the reasons for antigen formation of the cell membrane explains simply why the antigen provides a password to prevent bacteria and virus, like communists, from taking over. This is rejection. To alter it explains the infections" (46). Today the problem remains unsolved.

After some time it became apparent that after long time survival cardiac allograft recipients showed at autopsy atheroma of the coronary arteries with marked luminal narrowing whereas the donor had been free of atheroma at the time of transplantation. Also coronary graft sclerosis was confirmed later by coronary angiography in surviving individuals and the process that was leading to this rapidly developing coronary artery obliteration was called chronic rejection. It was regarded a sad irony that the same disease was reproduced in the coronary arteries of the transplanted heart which had determined the dysfunction of the recipients heart (56, 63, 74, 109, 149, 161).

After 1969 most of the clinical cardiac transplantation programs came to an end. Partly because of the numerous problems that had become evident and partly because the experience with direct coronary artery bypass surgery had allowed many surgeons to expand considerably operative indications for patients with severe coronary artery disease. The efforts were continued only by a few centers that had been in cardiac transplantation from the cradle: Stanford, U.S.A., Cape Town, S.A. and Richmond, U.S.A. (30, 39, 40, 48, 49, 57, 60, 71, 81, 123, 128, 133, 143, 152, 155, 156, 158).

Since the original observations of Lower in 1966, the voltage drop in the sum of the standard leads on the ECG had remained the most important indication of acute rejection episodes. The measurement of a 20 % reduction in R-wave voltage was regarded as an indication of acute rejection (120, 144). A more sensitive diagnostic technique for acute rejection was introduced by Caves in 1973 by means of the transvenous right-ventricular biopsy method (15, 28, 29, 32).

The mainstay of immunosuppressive medication has always been azathioprine (imuran) and prednison (2, 97). Actinomycin C was also used and anti-lymphocyte-globulin (ALG) was administered later to reduce the amounts of azathioprine and steroids (58, 132). Also in 1973 two modifications in the immunosuppressive therapy were introduced. Cyclophosphamide was incorporated in the on-going therapy schemes (40) and ALG was replaced by antithymocyte globulin (ATG) (6, 13, 62, 73, 138, 154).

In 1974 Barnard again led the way by performing

an auxiliary heart transplantation. The indication for this type of transplantation was to bypass the recipients diseased left ventricle but to leave the recipients hypertrophic right ventricle in place to cope with the longstanding elevated pulmonary artery pressure (7, 8). Another xenotransplant, an auxiliary baboon heart, followed in 1978 in Cape Town, however this ended without success. The method for left heart bypass by auxiliary heart transplantation was originally designed for patients with an increased pulmonary vascular resistance but it has replaced the method of orthotopic cardiac transplantation in Cape Town ever since.

The Stanford Group achieved a progressive increase in patient survival after orthotopic cardiac transplantation, as experience in dealing with the complex postoperative care of the cardiac transplantation recipient accumulated and improved. At Stanford alone 150 patients have thus far received cardiac allografts. At the moment survival of the entire group is 52% at one year and 27% at 5 years. The one year survival rate for patients operated on in successive years from 1968 rose from 22% to finally 70% in 1978 (91a).

The major factors responsible for increased survival time after clinical cardiac transplantation appear to be improved management of acute rejection episodes by utilizing endomyocardial biopsies for timely diagnosis and rabbit anti-human thymocyte globulin for treatment. Improvement in long-term survival was also effected by control for graft sclerosis. Cardiac retransplantation appears to be a reasonable option both in patients with unremitting acute rejection injury and in patients who develop obliterative graft arteriosclerosis and subsequent graft failure. Progressive improvement in survival in recipients of cardiac transplants argues strongly for increased application of this technique as a therapy for end-stage myocardial insufficiency (64, 65, 66, 76, 77, 82, 89, 93, 129, 108, 137).

SUMMARY

The course of chronic rejection after orthotopic cardiac transplantation in DLA identical beagle littermates is described in this thesis. This rejection process was investigated in two ways.

First, the histopathological changes, that develop during the course of chronic rejection after orthotopic cardiac transplantation, were studied by means of serial percutaneous myocardial biopsies. The time of onset of these histopathological lesions during this process were investigated as well.

Second, the early diagnosis and follow-up of the process of chronic rejection after orthotopic canine cardiac transplantation were studied by registering myocardial wall motion. This was achieved by attaching silver markers to the donor heart and recording these marker motions by serial cineradiography.

Four articles were published describing the material, methods, and results of these investigations. These papers are reprinted in this thesis in chapter two to five.

In the second chapter, the method for taking the percutaneous myocardial biopsies from the left ventricle was described. Thanks to this technique, adequate serial biopsy specimens were obtained to study the histopathological changes after orthotopic cardiac transplantation in DLA identical beagle littermates.

The results of the histopathological investigation of these biopsies were described in the fourth chapter. The histopathological lesions which develop during the course of chronic rejection seem to be a progressive narrowing of the lumen of arteries and arterioles in the myocardium. It was found that this luminal narrowing was based on endothelial swelling and medial proliferation.

These vascular changes could be observed to begin in the second week after transplantation. During the course of chronic rejection these lesions became dominant features and secondary ischemic changes could be discerned. It appears as if

this process of chronic rejection leads to a progressive and generalized myocardial ischemia.

Myocardial function during chronic rejection after orthotopic canine cardiac transplantation was described in chapter three. From endocardial marker motion values for myocardial contractility have been calculated, before and after transplantation.

During the rejection process two significant drops in myocardial contractility could be determined. The first drop occurred between the preoperative measurements and those of the first week after transplantation ($P < 0.02$). The second drop occurred in the last week ($P < 0.01$). In the intervening period myocardial contractility seems to be depressed, but not significantly.

In the fifth chapter the non-invasive method to determine myocardial function by measuring marker motion was further developed, so that its utility could be improved for clinical and experimental use.

SAMENVATTING

In dit proefschrift wordt het verloop beschreven van het proces van chronische afstoting zoals dat zich voordoet na orthotope harttransplantatie bij DLA identieke beagles. Dit afstotingsproces werd op twee manieren vervolgd. Ten eerste werd getracht door middel van percutane myocard biopsien de histopathologische veranderingen in de hartspier, en het tijdstip van ontstaan ervan, vast te stellen.

Ten tweede werd een poging ondernomen door het onderzoeken van de bewegingen van endocardmarkers te komen tot een vroegtijdige registratie en tot het vervolgen van de functie van de hartspier.

Een viertal artikelen werden over methoden van onderzoek en de resultaten ervan gepubliceerd. Deze publicaties zijn in dit proefschrift bijeengebracht in de hoofdstukken twee t/m vijf.

Het tweede hoofdstuk beschrijft de percutane techniek voor het nemen van biopsien uit de linker ventrikel van de DLA identieke beagles, na orthotope harttransplantatie. Dankzij deze techniek werden biopsien verkregen die geschikt waren voor licht-microscopisch onderzoek, zodat hierin de histopathologische kenmerken van het chronische afstotingsproces konden worden bestudeerd.

De resultaten van het histopathologische onderzoek staan vermeld in het vierde hoofdstuk. Het blijkt dat dit proces zich voornamelijk afspeelt in arterien en arteriolen van het transplantaat en dat het zich uit in een zwelling van het endotheel, proliferatie van de media en dientengevolge vernauwing van het lumen.

Vanaf de tweede week na transplantatie beginnen deze afwijkingen steeds duidelijker waarneembaar te worden. Wanneer de vaatafwijkingen in ernst toenemen ontstaan ook fibrose in het interstitium, atrofie van de spiervezels en gebieden van myodegeneratie met infarcthaardjes. Het lijkt alsof het verlies aan functie van het myocard tengevolge van chronische afstoting gebaseerd is op een gegeneraliseerde ischemie van het myocard.

De functie van het myocard tijdens chronische afstoting na orthotope harttransplantatie bij deze honden is beschreven in hoofdstuk drie. De contractiliteit

van het myocard werd berekend uit de bewegingen van de endocardmarkers. In het verloop van het afstotingsproces worden twee significante dalingen waargenomen in de berekende waarden voor de contractiliteit van het hart. De eerste ($P < 0.02$) valt direct na transplantatie en de tweede ($P < 0.01$) in de week voor overlijden. In de tussenliggende periode lijkt de contractiliteit van het hart eveneens afgenomen, doch deze daling is niet statistisch significant.

In het vijfde hoofdstuk wordt de niet-invasieve methode, die in hoofdstuk drie werd gebruikt, om de contractiliteit van het myocard te bepalen, verder uitgewerkt. Verschillende manieren waardoor deze techniek geperfectioneerd kan worden voor verdere klinische en experimentele toepassing, zijn hierin aangegeven.

REFERENCES

1. W.W. ANGELL, N.E. SHUMWAY, Resuscitative storage of the cadaver heart transplant. Surgical Forum 17: 224, 1966.
2. C.N. BARNARD. The operation. A human cardiac transplant: an interim report of a successful operation performed at Groote Schuur Hospital, Cape Town. S.A. Medical Journal 41:1271, 1967.
3. C.N. BARNARD. What we have learned about heart transplants. Journal Thorac.Cardiovasc.Surg. 56:457, 1968.
4. C.N. BARNARD. Human heart transplantation. The diagnosis of rejection. Am.Journal of Cardiology 22:811, 1968.
5. C.N. BARNARD. Human cardiac transplantation. An evaluation of the first two operations performed at the Groote Schuur Hospital, Cape Town. Am. Journal of Cardiology 22:584, 1968.
6. C.N. BARNARD. The present status of heart transplantation. S.A. Medical Journal 49:213, 1975.
7. C.N. BARNARD, J.G. LOSMAN. Left ventricular bypass. S.A.Medical Journal 49:303, 1975.
8. C.N. BARNARD, et al. The advantage of heterotopic cardiac transplantation over orthotopic cardiac transplantation in the management of severe acute rejection. Journal Thorac.Cardiovasc.Surgery 74:918, 1977.
9. E.M. BARSAMIAN, O.E. OWEN et al. The transplantation of dehydrated and supercooled hearts. Surgical Forum 10:100, 1959.
10. W.A. BAUMGARTNER, N.E. SHUMWAY, E.B. STINSON et al. Current expectations in cardiac transplantation. Journal Thorac.Cardiovasc.Research 4:525, 1978.
11. W. BECK, C.N. BARNARD, V. SHRIRE. Heart Rate after cardiac transplantation. Circulation 40:437, 1969.
12. C.P. BIEBER, E.B. STINSON, N.E. SHUMWAY. Pathology of the conduction system in cardiac rejection. Circulation 39:567, 1969.
13. C.P. BIEBER, R.B. GRIEPP, E.B. STINSON et al. Radio-immune assay of heterologous serum γ-globulin in patients receiving rabbit antihuman thymocyte globulin. Transplantation 20:393, 1975.
14. C.P. BIEBER, N.E. SHUMWAY et al. Cardiac transplantation in man. VII cardiac allograft pathology. Circulation 41:753, 1970.
15. M.E. BILLINGHAM, N.E. SHUMWAY et al. The diagnosis of canine orthotopic cardiac allograft rejection by venous endomyocardial biopsy. Transplantation proceedings V:741, 1973.
16. R.J. BING, S. GUBJARNASON et al. Transplantation of the heart. Circulation 25:273, 1962.
17. E. BOS, J.J. VAN ROOD et al. Orthotopic cardiac allotransplantation in mongrel dogs and beagles. European Surgical Research 2:93, 1970.
18. E. BOS, H.M. VRIESENDORP, J. NAUTA et al. Histocompatibility in orthotopic heart transplantation in dogs. Transplantation proceedings, III:155, 1971.
19. E. BOS. Models in orthotopic canine cardiac allotransplantation, Thesis, Rotterdam, 1979.
20. A.D. BOYD, R.R. LOWER, F.C. SPENCER et al. Role of DLA system of canine histocompatibility in cardiac transplantation. Transplantation proceedings III:152, 1971.
21. R.W. BROWER, H.J. TEN KATEN, G.T. MEESTER. A direct method for determining regional shortening after bypass surgery from radiopaque markers in man. Am. J. Cardiol. 41:1222, 1978.

22. J.P. CACHERA, C. DUBOST et al. Transplantation orthotopic du coeur. Premiers résultats expérimentaux. Mémoires de l'académie de Chirurgie 92:376, 1966.

23. A. CARREL, C.C. GUTHRIE. The transplantation of veins and organs. American Medicine 10:1101, 1905.

24. A. CARREL. The surgery of blood vessels, etc. Johns Hopkins Hospital Bulletin 18:18, 1907.

25. M.H. CASS, R. BROCK. Heart excision and replacement. Guy's hospital reports 108:285, 1959.

26. P.K. CAVES, E. DONG. Modified technique for orthotopic cardiac transplantation in dogs. Journal of Surgical Research 15:100, 1973.

27. P.K. CAVES, N.E. SHUMWAY et al. Diagnosis of human cardiac allograft rejection by serial cardiac biopsy. Journal Thorac. Cardiovasc.Surg. 66:461, 1973.

28. P.K. CAVES, N.E. SHUMWAY et al. Percutaneous transvenous endomyocardial biopsy. JAMA 225:288, 1973.

29. P.K. CAVES, N.E. SHUMWAY et al. Transvenous biopsy from canine orthotopic heart allografts. Am. Heart Journal 85:525, 1973.

30. P.K. CAVES, N.E. SHUMWAY et al. Results of 54 cardiac transplants. Surgery 74:307, 1973.

31. P.K. CAVES, N.E. SHUMWAY et al. Immunosuppression with cyclophosphamide in dogs following cardiac transplantation. Transplantation proceedings, V:517, 1973.

32. P.K. CAVES, N.E. SHUMWAY et al. Serial transvenous biopsy of the transplanted human heart. Improved management of acute rejection episodes. Lancet, 4 May 1974:7862.

33. J. CERILLI, J.E. HOLLIDAY, D.P. FESPERMAN, M.R. FOLGER. Antivascular endothelial cell antibody – its role in transplantation. Surgery 81:132, 1977.

34. J. CERILLI, J.E. HOLLIDAY, D.P. FESPERMAN. Role of antivascular endothelial antibody in predicting renal allograft rejection. Transplantation proceedings, 9:771, 1977.

35. C. CHARTRAND, E. DONG, N.E. SHUMWAY. Hemodynamics of the orthotopic homotransplanted heart. Surgical forum 19:238, 1968.

36. C. CHARTRAND, N.E. SHUMWAY et al. Atrial pacing in the postoperative management of cardiac homotransplantation. The annals of thoracic surgery 8:152, 1969.

37. C. CHARTRAND, N.E. SHUMWAY et al. Hemodynamics of the orthotopic homotransplanted canine heart. The Annals of thoracic surgery 13:15, 1972.

38. J.W. CHILDS, A.P. MONACO, R.R. LOWER et al. Acquired tolerance for cardiac allografts. Surgical Forum 23:204, 1972.

39. D.A. CLARK, N.E. SHUMWAY et al. Cardiac transplantation in man. VI Prognosis of patients selected for cardiac transplantation. Annals of Internal Medicine 75:15, 1971.

40. D.A. CLARK, N.E. SHUMWAY, D.C. HARRISON et al. Cardiac transplantation in man; review of first three years' experience. Am.Journal of Medicine 54:563, 1973.

41. R.J. CLEVELAND, R.R. LOWER. Transplantation of canine cadaver hearts after short-term preservation. Transplantation 5:904, 1967.

42. D.A. COOLEY, R.D. LEACHMAN et al. Human heart transplantation. Experience with twelve cases. Am. Journal of Cardiology 22:804, 1968.

43. D.A. COOLEY, et al. Transplantation of the human heart. JAMA 205:479, 1968.

44. D.A. COOLEY, R.D. BLOODWELL, G.L. HALLMAN. Cardiac transplantation for advanced acquired heart disease. J. Cardiovascular Surgery 9:403, 1968.

45. D.A. COOLEY, J.D. MILAM, et al. Human cardiac transplantation. Circulation, 39 & 40 (Suppl. 1): 1-3, 1969.

46. D.A. COOLEY, R.D. LEACHMAN et al. Organ transplantation for advanced cardiopulmonary disease. Annals of thoracic Surgery 8:30, 1969.

47. D.A. COOLEY, R.D. LEACHMAN et al. Cardiac transplantation as palliation of advanced heart disease. Archives of Surgery 98:619, 1969.

48. D.A. COOLEY, R.D. LEACHMAN et al. Clinical experience in transplantation of the human heart: report of ten cases. Transplantation proceedings 1:703, 1969.

49. D.A. COOLEY, J.D. MILAM et al. Cardiac transplantation: general considerations and results. Annals of Surgery 6:892, 1969.

50. D.K.C. COOPER. The donor heart. The present position with regard to resuscitation storage and assessment of viability. Journal of Surgical Research 21:363, 1971.

51. T. COOPER, V.L. WILLMAN, C.R. HANLON et al. Transplantation of the heart. Archives of Surgery 89: 915, 1964.

52. J.G. COPELAND, E.B. STINSON et al. Successful orthotopic transplantation of canine hearts after in vitro preservation for 24 to 28 hours. Surgical Forum 23:202, 1972.

53. J.G. COPELAND, E.B. STINSON. In vitro preservation of canine hearts for 24 to 28 hours followed by successful orthotopic transplantation. Annals of Surgery 178:687, 1973.

54. R. CORTESINI, S. BEVILACQUA et al. Perspectives on heart substitution: temporary implantation of artificial heart followed by heart allograft. Transplantation proceedings 9:305, 1977.

55. W.M. DAGGETT, V.L. WILLMAN, C.R. HANLON et al. Work capacity and efficiency of the autotransplanted heart. Circulation (Suppl. 1) XXXV & XXXVI : I-96, 1967.

56. M.E. DEBAKEY. Human cardiac transplantation. J. Thorac.Cardiovasc.Surgery 55:447, 1968.

57. M.E. DEBAKEY et al. Human Cardiac transplantation. Clinical Experience. Journal Thorac. Cardiovasc. Surgery 58:303, 1969.

58. W.J. DONAWICK, G.F. FREGIN et al. Cardiac and skin heterograft rejection: suppression with antilymphocyte serum. Transplantation proceedings, III:551, 1971.

59. E. DONG, N.E. SHUMWAY et al. Effect of cardiac transplantation on the homeostatis of blood volume (P). Circulation 35 & 36 (Suppl. II): II-98, 1967.

60. E. DONG, N.E. SHUMWAY et al. Review of four years experience with clinical heart transplantation at Stanford University Medical Center. Transplantation proceedings IV:787, 1972.

61. E. DONG, R.R. LOWER, N.E. SHUMWAY et al. Performance of the heart two years after autotransplantation. Surgery 56:270, 1964.

62. E. DONG, R.B. GRIEPP, E.B. STINSON, N.E. SHUMWAY. Clinical transplantation of the heart. Annals of Surgery 176:503, 1972.

63. C. DUBOST. Scientific and ethical problems in organ transplantation. Annals of thoracic Surgery 8:95, 1969.

64. J.G. DUGUID. Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. J. Pathol.Bact. 58: 207, 1946.

65. P.R. EMMONS, M.J.G. HARRISON, A.J. HONOUR, et al. Effect of a pyrimidopyrimidine derivate on thrombus formation in the rabbit. Nature 208:255, 1965.

66. P.R. EMMONS, M.J.G. HARRISON, A.J. HONOUR et al. Effect of dipyridamole on human platelet behaviour. Lancet 2:603, 1965.

67. J.A. FEEMSTER, R.C. LILLEHEI. Hypothermic-hyperbaric pulsatile perfusion for preservation of the canine heart. Transplantation proceedings 1:138, 1969.

68. M.H. GAIL. Does cardiac transplantation prolong life? Annals of internal medicine 76:815, 1972.

69. M. GOLBERG, E.F. BERMAN, L.C. AKMAN. Homologous transplantation of the canine heart. Journal of the Intern.coll.Surgeons, 30:575, 1958.

70. B.S. GOLDMAN, J.H. CROOKSTON et al. Antibodies in the serum and on the heart of dogs with cardiac allografts. Transplantation proceedings III:515, 1971.

71. A.F. GRAHAM, D.C. HARRISON et al. Does cardiac transplantation significantly prolong life and improve its quality? Circulation (Suppl. III), Vols, XLVII & XLVIII:116, 1973.

72. R.B. GRIEPP, N.E. SHUMWAY et al. Determinants of operative risk in human heart transplantation. Am. Journal of Surgery 122:192, 1971.

73. R.B. GRIEPP, N.E. SHUMWAY et al. Use of antithymocyte globulin in human heart transplantation. Circulation, (Suppl. I) to vols. XLV and XLVI:147, 1972.

74. R.B. GRIEPP, N.E. SHUMWAY et al. Coronary arteriography following cardiac transplantation. JAMA, 221:147, 1972.

75. R.B. GRIEPP, N.E. SHUMWAY et al. Management of long-term survivors of heart transplantation. Transplantation proceedings, VII (Suppl. 1):595, 1975.

76. R.B. GRIEPP, N.E. SHUMWAY et al. Increasing patient survival following heart transplantation. Transplantation proceedings 9:197, 1977.

77. R.B. GRIEPP, E.B. STINSON, C.P. BIEBER, B.A. REITZ, J.G. COPELAND, P.E. OYER, N.E. SHUMWAY. Control of graft arteriosclerosis in human heart transplant recipients. Surgery 81:262, 1977.

78. P. HAIRSTON. Heart transplantation: past, present and future. Journal Thorac.Cardiovasc.Surg. 50:1, 1965.

79. J.D. HALLER, M.M. CERRUTI. Heart transplantation in man: compilation of cases. January 1, 1964 to October 23, 1968. Am.Journal of Cardiology 22:840, 1968.

80. J.D. HALLER, M.M. CERRUTI. Heart transplantation in man: compilation of cases II. January 23, 1964 to June 22, 1969. American Journal of Cardiology 24:554, 1969.

81. G.L. HALLMAN, D.A. COOLEY et al. Factors influencing survival after human heart transplantation. Annals of Surgery 170:593, 1969.

82. N.J. HARDIN, C.R. MINICK, G.E. MURPHY. Experimental induction of atheroarteriosclerosis by the synergy of allergic injury to arteries and lipid rich diet. III The role of earlier acquired fibromuscular thickening in the pathogenesis of later developing atherosclerosis. Am. Journal pathol. 73:301, 1973.

83. J.D. HARDY et al. Heart transplantation in man. JAMA 188:114, 1964.

84. J.D. HARDY, W.R. WEBB et al. Heart transplantation in infant calves: evaluation of coronary perfusion to preserve organ during transfer. Annals New York Academy of Sciences 120:786, 1964.

85. J.D. HARDY, R.D. WILLIAMS et al. Heart transplantation in dogs. Procedures, physiologic problems, and results in 142 experiments. Surgery 60:361, 1966.

86. J.D. HARDY, C.M. CHAVEZ. The first heart transplant in man: developmental animal investigations with analysis of the 1964 case in the light of current clinical experience. Am.Journal of Cardiology 22:772, 1968.

87. J.D. HARDY, C.M. CHAVEZ. The first heart transplantation in man: historical re-examination of the 1964 case in the light of current clinical experience. Transplantation proceedings 1:717, 1969.

87a B.G. HATTLER, P.A. EBERT, R. COSSON. A histochemical analysis of rejecting dog cardiac allografts. Surgical Forum XX:139, 1969.

87b J. HEIKKILÄ, B.S. TABAKIN, P.G. HUGENHOLTZ. Quantification of function in normal and infarcted regions of the left ventricle. Card. Res. 6:516, 1972.

88. C.F. HOLLANDER, C. ZURCHER. Pathology of cardiac and renal allograft rejection in the rhesus monkey. Medical Primatology II:107, 1972.

89. S.A. HUNT, N.E. SHUMWAY et al. Does cardiac transplantation prolong life and improve its quality? An updated report. Cardiovascular Surgery 54:III-56, 1976.

90. E.J. HURLEY, N.E. SHUMWAY et al. Isotopic replacement of the totally excised canine heart. Journal of surgical research 2:90, 1962.

91. N.B. INGELS, E.B. STINSON et al. Effects of heart rate augmentation on left ventricular volumes and cardiac output of the transplanted human heart. Circulation (supp. 2) 56:II-32, 1977.

91a. S.W. JAMIESON, E.B. STINSON, N.E. SHUMWAY. Cardiac transplantation in 150 patients at Stanford University. Brit. Med. J. 1:93, 1979.

92. C.K. JONGSMA. The problem of the heart transplantation model, experimental orthotopic and heterotopic canine heart allografts. Thesis, Rotterdam, 1977.

93. D.R. KAHN, H. SLOAN et al. Effect of anticoagulants on the transplanted heart. Journal Thorac.Cardiovasc.Surg. 60:617, 1970.

94. A. KANTROWITZ, H.E. CARSTENSEN et al. Transplantation of the heart in an infant and an adult. Am. Journal of Cardiology 22:782, 1968.

95. A. KANTROWITZ, J.L. SHERMAN et al. Clinical and experimental observations in heart transplantations. Transplantation proceedings 1:727, 1969.

96. J.S. KARLINER, J. ROSS et al. Mean velocity of fiber shortening. A simplified measure of left ventricular myocardial contractility. Circulation 45:323, 1971.

97. A.S. KEATS, A. GOLDSTEIN. Observations during anesthesia for cardiac homotransplantation in ten patients. Anesthesiology 30:192, 1969.

98. P. KINCAID-SMITH. Modification of the vascular lesions of rejection in cadaveric renal allografts by dipyridamole and anticoagulants. Lancet 2:920, 1969.

99. Y. KONDO, A. KANTROWITZ et al. Immediate and delayed orthotopic homotransplantation of the heart. Journal Thorac.Cardiovasc.Surg. 50:781, 1965.

100. Y. KONDO, F. GRÄDEL, A. KANTROWITZ. Homotransplantation of the heart in puppies under profound hypothermia: long survival without immunosuppressive treatment. Annals of Surgery 162:837, 1965.

101. Y. KONDO, F. GRÄDEL, A. KANTROWITZ. Heart homotransplantation in puppies: long survival without immuno-suppressive therapy. Circulation (Suppl. 1) 31 & 32:1-181, 1965.

102. Y. KONDO, J.L. MATHENY, J.D. HARDY. Autonomic reinnervation of cardiac transplants: further observations in dogs and rhesus monkeys. Annals of Surgery 176:42, 1972.

103. S. KONNO, S. SAKAKIBARA. Endomyocardial biopsy. Bull. Heart Inst. pp 1-24, 1966.

104. H.A. KONTOS, R.R. LOWER et al. Early reinnervation of the heart after cardiac autotransplantation. J. Clin.Invest. 47:56, 1968.

105. H.A. KONTOS, R.R. LOWER, et al. Responses to electrical and reflex autonomic stimulation in dogs with cardiac transplantation before and after reinnervation. J. Thorac.Cardiovasc.Surg. 59:382, 1970.

106. J.C. KOSEK, E.J. HURLEY, R.R. LOWER. Histopathology of orthotopic canine cardiac homografts. Laboratory investigation 19:97, 1968.

107. J.C. KOSEK, R.R. LOWER et al. Histopathology of orthotopic canine cardiac homografts and its clinical correlation. Transplantation proceedings 1:311, 1969

108. J.C. KOSEK, R.R. LOWER et al. Arteries in canine cardiac homografts. Ultrastructure during acute rejection. Laboratory Investigation 21:328, 1969.

109. J.C. KOSEK, C. BIEBER, R.R. LOWER. Heart graft arteriosclerosis. Transplantation proceedings III:512, 1971.

110. R.D. LEACHMAN, D.A. COOLEY et al. Transplantation of human heart. Medical Communications, Texas Medical Center.

110a. P.W. LEEDHAM, P.A. CULLUM et al.. Acute and modified rejection of heterotopic canine cardiac allotransplants studied by serial needle biopsy. Thorax, 26:534, 1971.

111. S.S. LEE, W.R. WEBB. Cardiac metabolism as influenced bij ischemia, refrigeration and enzyme precursors. Am. surgeon, 25:776, 1959.

112. R.H. LEVEY, S.C. BANSAL, G. MATHÉ, A. MONACO, F.T. RAPAPORT, R.E. WILSON. Panel discussion on future techniques for avoidance of rejection. Transplantation proceedings IV:549, 1972.

113. D.B. LONGMORE, W. WELCH et al. Transplantation of the heart and both lungs. II Experimental cardiopulmonary transplantation. Thorax 24:391, 1969.

114. J.H. LOUW. Ex unitate vires. S.A. Medical Journal 41:1257, 1967.

115. R.R. LOWER, N.E. SHUMWAY. Studies on orthotopic homotransplantation of the canine heart. Surgical Forum 11:18, 1960.

116. R.R. LOWER, N.E. SHUMWAY, et al. Complete homograft replacement of the heart and both lungs. Surgery 50:842, 1961.

117. R.R. LOWER, R.C. STOFER, N.E. SHUMWAY. Homovital transplantation of the heart. Journal Thorac. Cardiovasc. Surg. 41:196, 1961.

118. R.R. LOWER, N.E. SHUMWAY et al. Successful homotransplantation of the canine heart after anoxic preservation for seven hours. American Journal of Surgery 104:302, 1962.

119. R.R. LOWER, E. DONG, N.E. SHUMWAY. Suppression of rejection crises in the cardiac homograft. Annals of Thoracic Surgery 1:645, 1965.

120. R.R. LOWER, E. DONG, F.S. GLAZENER. Electrocardiograms of dogs with heart homografts. Circulation XXXIII:455, 1966.

121. R.R. LOWER. Cardiac transplantation without complete cardiac denervation. American Heart Journal 72:841, 1966.

122. R.R. LOWER, J.C. KOSEK et al. Experiences in heart transplantation: technic, physiology and rejection. Am. Journal Cardiology 22:766, 1968.

123. R.R. LOWER, V.E. KEMP et al. Current state of clinical heart transplantation. Transplantation proceedings III:333, 1971.

124. F.C. MANN, W.M. YATER et al. Transplantation of the intact mammalian heart. Archieves of Surgery 26: 219, 1933.

125. D.R. MARTIN, W.M. YATER et al. Primary cause of unsuccessful liver and heart preservation. Cold sensitivity of the ATP-ase system. Annals of Surgery 175:111, 1972.

126. S. MATTILA, E. DONG et al. The effects of atrial pacing on the synergy and hemodynamics of the orthotopically transplanted canine heart. Circulation XLVIII: 386, 1973.

127. R.S. MELTZER, R.L. POPP. Two dimensional echocardiographic quantification of infarct size alteration by pharmacologic agents. Submitted for publication.

128. B.J. MESSNER, D.A. COOLEY et al. Survival-times after cardiac allografts. The Lancet 1:954, 1969.

129. C.R. MINICK, G.E. MURPHY. Experimental induction of atheroarteriosclerosis by the synergy of allergic injury to arteries and lipid rich diet. II Effect of repeatedly injected foreign protein in rabbits fed a lipid-rich, cholesterol poor diet. Am. J. Pathol. 73:265, 1973.

130. M. NELLEN. Experience in Cape Town. Br. Journal of Hospital Medicine 2:1284, 1969.

131. R.J. NELSON, R.J. CLEVELAND. Hemodynamic effects of volume loading in dogs following cardiac homotransplantation. Circulation XLIII & XLIV (Suppl. 1):130, 1971.

132. J.J. NORA, D.A. COOLEY et al. Lactate dehydrogenase isozymes in human cardiac transplantation. Science 164:1079, 1969.

133. J.J. NORA, D.A. COOLEY et al. Rejection of the transplanted human heart: indexes of recognition and problems in prevention. The new England Journal of Medicine 280:1079, 1969.

134. T.X. O'CONNELL, J.F. MOWBRAY. Arterial intimal thickening produced by allo-antibody and xeno-antibody. Transplantation 15:262, 1973.

135. E. PROCTOR, G. MATTHEWS, J. ARCHIBALD. Acute Orthotopic transplantation of hearts stored for 72 hours. Thorax 26:99, 1971.

136. E. PROCTOR, G. MATTHEWS. A simple method for short-term (6 - hours) transport of the dog heart prior to orthotopic transplantation. Thorax 25:477, 1970.

137. A.K. RIDER, C.P. BIEBER, D.C. HARRISON. Atherosclerosis in long-term survivors of human cardiac allograft transplantation. Circulation 46 (Suppl. II) :76, 1972 (Abstract).

138. A.K. RIDER, N.E. SHUMWAY et al. The status of cardiac transplantation, 1975. Circulation 52:531, 1975.

139. D. ROSS. Supply and demand. British Journal of Hospital Medicine 2:1288, 1969.

140. R.C. ROSSEN, K.K. MITTAL et al. Immunofluorescent and serologic studies of the humoral antibody response to cardiac allotransplantation in man. Transplantation proceedings III:445, 1971.

141. N.W. SALOMON, N.E. SHUMWAY et al. Alterations in total and regional myocardial blood flow during acute rejection of orthotopic canine allografts. Journal Thorac. Cardiovasc. Surg. 4:542, 1978.

142. A.M. SAUNDERS, C. BIEBER. Pathologic findings in a case of cardiac transplantation. JAMA 206:815, 1968.

143. P.K. SEN. Human heart homotransplantation. Am. Journal of Cardiology 22:826, 1968.

144. B.H. SEWELL, R.R. LOWER et al. Epicardial ECG in monitoring cardiac homograft rejection. Circulation 39 (Suppl. I) :1, 1969.

145. N.E. SHUMWAY, R.R. LOWER. Special problems in transplantation of the heart. Annals New York Academy of Sciences 120:773, 1964.

146. N.E. SHUMWAY, R.R. LOWER, R.C. STOFER. Transplantation of the heart. Advances in Surgery 2:265, 1966.

147. N.E. SHUMWAY, W.W. ANGELL, R.D. WUERFLEIN. The heart:progress in transplantation of the heart. Transplantation 5:900, 1967.

148. N.E. SHUMWAY, E. DONG, E.B. STINSON. Surgical Aspects of cardiac transplantation in man. Bulletin of the New York Academy of Medicine 45:387, 1969.

149. J.F. SILVERMAN, L. WEXLER et al. Coronary arteriography in long-term human cardiac transplantation Circulation 50:838, 1974.

150. J.F. SILVERMAN, R.B. GRIEPP, L. WEXLER. Radiographic changes in cardiac contour following cardiac transplantation. Diagnostic Radiology, 111:303, 1974.

151. T.L. SPRAY, D.C. WATSON, W.C. ROBERTS. Morphology of canine hearts after 23 hours' preservation and orthotopic transplantation. Journal Thorac. Cardiovasc. Surg. 73:888, 1977.

152. E.B. STINSON, N.E. SHUMWAY et al. Initial clinical experience with heart transplantation. American Journal of Cardiology 22:791, 1968.

153. E.B. STINSON, N.E. SHUMWAY et al. Cardiac transplantation in man. III Surgical aspects. The American Journal of Surgery 118:182, 1969.

154. E.B. STINSON, N.E. SHUMWAY et al. Cardiac transplantation in man. II Immunosuppressive therapy. Journal Thorac. Cardiovasc. Surg. 58:326, 1969.

155. E.B. STINSON, N.E. SHUMWAY et al. Cardiac transplantation in man. VIII Survival and function. Journal Thorac. Cardiovasc. Surg. 60:303, 1970.

156. E.B. STINSON, N.E. SHUMWAY et al. Experience with cardiac transplantation in fourteen patients. Laval Medical 41:125, 1970.

157. E.B. STINSON, N.E. SHUMWAY, J.S. REMINGTON et al. Infectious complications after cardiac transplantation in man. Annals of Internal Medicine 74:22, 1971.

158. E.B. STINSON, N.E. SHUMWAY et al. Results of human heart transplantation at Stanford University. Transplantation proceedings III:337, 1971.

159. E.B. STINSON, G. RAHMOELLER et al. Changes in left ventricular mechanical and hemodynamic function during acute rejection of orthotopically transplanted hearts in dogs. Journal Thorac. Cardiovasc. Surg. 68:783, 1974.

160. J.G. THOMSON. Heart transplantation in man-necropsy findings. British Medical Journal 2:511, 1968.

161. J.G. THOMSON. Production of severe atheroma in a transplanted human heart. The Lancet 2:1088, 1969.

162. D. TRUNKEY, F.O. BELZER et al. Preservation of canine hearts. Cryobiology 6:515, 1970.

163. H.M. VRIESENDORP. Major histocompatibility complex of the dog. Thesis, Rotterdam, 1973.

164. D.C. WATSON. Consistent survival after prolonged donor heart preservation. Transplant proceedings 9:297, 1977.

165. W.R. WEBB, W.L. SUGG, R.R. ECKER. Heart preservation and transplantation:experimental and clinical studies. Am. Journal of Cardiology 22:820, 1968.

166. W.R. WEBB, W.A. NEELY et al. Practical methods of homologous cardiac transplantation. Journal Thorac. Surgery 37:361, 1959.

167. S.A. WESOLOWSKI, J.F. FENNESSEY. Pattern of failure of the homografted canine heart. Circulation 8:750, 1953.

168. G.M. WILLIAMS, B. DEPLANQUE, R. LOWER, D. HUME. Antibodies and human transplant rejection. Annals of Surgery 170:603, 1969.

169. G.M. WILLIAMS, R.R. LOWER et al. Participation of antibodies in acute cardiac-allograft rejection in man. The New England Journal of Medicine 281:1145, 1969.

170. V.L. WILLMAN, C.R. HANLON et al. Autotransplantation of the canine heart. Surg. Gynec & Obst. 115:299, 1962.

171. V.L. WILLMAN, C.R. HANLON et al. Mechanism of cardiac failure after excision and reimplantation of the canine heart. Surgical Forum 13:93, 1962.

172. V.L. WILLMAN, C.R. HANLON et al. Neural responses following autotransplantation of the canine heart. Circulation 27:713, 1963.

173. V.L. WILLMAN, T. COOPER, C.R. HANLON. Return of neural responses after autotransplantation of the heart. Am. Journal of Physiology 207 (1): 187, 1964.

174. V.L. WILLMAN, C.R. HANLON et al. Response of the autotransplanted heart to blood volume expansion Annals of Surgery 166:513, 1967.

ACKNOWLEDGEMENTS

Prof.dr. E. Bos, cardiac surgeon, has conducted the experiments described in this thesis with his characteristic meticulousness, paying attention to the smallest detail. Without him working alongside, neither the experimental work nor the documentation would have been completed.
Prof.dr. J. Nauta, cardiac surgeon, offered his friendship and was always prepared to offer his valid criticism and help as well.
Prof. P.G. Hugenholtz, cardiologist, never lost interest in the myocardial marker method and greatly helped to improve the quality of this manuscript.
Much is owed to Dr. R.W. Brower who directed the interpretation of the results of the $\bar{V}_{cf}$ calculations and led the way to the perfection of this technique for experimental use. He also did his utmost to correct the English usage in this thesis, together with his wife Joan.
Dr. H.M. Vriesendorp, M.D., Primate Center TNO, Rijswijk, always has been to me the shining example of a researchers intrepidity. He was also so kind to advise on immunology.
Prof. dr. C.F. Hollander, M.D., Experimental Gerontology TNO, Rijswijk, has been of great help by giving his opinion about pathology.
The other co-authors of the articles, I.Mc.Dicken, M.D., G.T. Meester, M.D., F. Leicher, M.D., and H.J. ten Katen, Ing. have all contributed by sharing the burden of preparing medical reports.
The team of theatre nurses, anaesthesiologists and extracorporeal circulation technicians of the surgical laboratory, which includes: Mrs. J. Dortland-de Kam, Mrs. M. J. Kesten-Lagerman, Mrs. A.M. Westerveld-Vogelaar, Mrs. M.A. van Seters-van Essen, Mr. E. Ridderhof, Mr. E. Colly, Mr. J. Poortman and Mr. A. Kok; all gave their best efforts to accomplish many successful orthotopic cardiac transplantations.
The team of biotechnicians and analysts of the surgical laboratory, which included: Mr. J. Kasbergen, Mr. R. Meyer, Mr. R. Spruyt, Mr. P. Kreeft, Mr. R. Naessens, Mr. J. van Dongen, Mr. R. van Bremen and Mr. W.P. van Schalkwijk; have taken care of the beagles and supplied necessary auxiliary information.
Secretarial help was provided, without complaint, by Mrs. M.G. Hussaarts-Martin,

Mrs. F.A.M. Wever-van Os, Mrs. L. Polak-van Noort and Mrs. W.M. v.d. Enden-de Geus.
Mr. E.J.W. Davids greatly contributed to the publication of this thesis with his indefatigable cooperation.
Gratitude is also expressed to my collegues in general surgery and cardiothoracic surgery who made it possible that time could be spent in the laboratory.
The Audiovisual Service of the Erasmus University supplied the graphs and tables.
Financial support for this thesis was supplied by the Dutch Heart Foundation and the Hippocrates Studiefonds.

Much is indebted for the vast amount of moral support by my beloved wife Myriam and to little David-Marc, who, by his natural charm, tried to keep me from working.

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Het middelbaar onderwijs werd gestart op het Willibrordus college "Katwijk", de Breul, Zeist, terwijl in 1963 het eindexamen gymnasium B werd behaald aan het St. Odulphus lyceum te Tilburg. In datzelfde jaar werd een aanvang gemaakt met de medicijnenstudie aan de Rijksuniversiteit te Leiden. Vanaf 1967 tot 1969 was hij werkzaam als studentenassistent aan de afdeling thoraxchirurgie van Prof.dr. A.G. Brom in het Academisch Ziekenhuis te Leiden. In 1969 was hij mede werkzaam bij de fabricage van heterografts (varkenskleppen) voor klinisch gebruik, onder leiding van Dr. W. Geldof. In 1971 werd samengewerkt met Dr. H. Wijnen aan follow-up studies na Vineberg operaties in Leiden's experimentele laboratorium voor chirurgie (hoofd H. Stol, dierenarts). Na het behalen van het artsexamen in januari 1972 werd de militaire dienst aangevangen. Gedurende deze tijd was hij verbonden aan de inspectie van de geneeskundige dienst te Den Haag (hoofd kolonel-arts R.G. Nypels), als reserve eerste luitenant arts, doch was te werk gesteld bij de afdeling thermodynamica (hoofd Dr. A. Keijzer, arts) van het Instituut Preventieve Geneeskunde (TNO) te Leiden. In april 1972 voegde hij zich bij het team van Prof.dr. E. Bos voor het onderzoek op het gebied van de experimentele harttransplantaties. Na het beëindigen van de militaire dienst werd in november 1972 begonnen met een stage bij het catheterisatie laboratorium (hoofd G.T. Meester, cardioloog) van het Thoraxcentrum van het Academisch Ziekenhuis, Dijkzigt, te Rotterdam. In april 1973 werd een aanvang genomen met de opleiding tot algemeen chirurg bij de afdeling heelkunde (hoofd wijlen Prof.dr. H. Muller, chirurg) van het Academisch Ziekenhuis, Dijkzigt, te Rotterdam. Het transplantatie onderzoek werd tijdens deze opleidingsperiode gecontinueerd. In 1976 werden zes maanden doorgebracht bij Prof.dr. C.N. Barnard in Kaapstad, Zuid-Afrika. Dit om experimenteel en klinisch de gevolgen van zijn nieuwe operatie (dubbele-harttransplantatie) te kunnen bestuderen. De algemeen chirurgische opleiding werd afgebroken in december 1976 om over te gaan naar de opleiding tot cardiopulmonaal chirurg aan de afdeling thoraxchirurgie (hoofden Prof.dr. J. Nauta en Prof.dr. E. Bos) van het Academisch Ziekenhuis, Dijkzigt, te Rotterdam.