The role of rejection in aortic valve allograft failure

Ahmad Moustapha, Dalhousie University Faculty of Medicine

Available at: https://works.bepress.com/vivianmcalister/248/
INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
THE ROLE OF REJECTION IN AORTIC VALVE ALLOGRAFT FAILURE

by

Ahmad Moustapha

Submitted in partial fulfillment of the requirements

for the degree of Master of Science

at

Dalhousie University

Halifax, Nova Scotia

May, 1997

© Copyright by Ahmad Moustapha, 1997
The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author’s permission.

L’auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L’auteur conserve la propriété du droit d’auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-24886-0

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Dalhousie University

Department of Pathology

The undersigned hereby certify that they have read and recommended to the Faculty of Graduate Studies for acceptance a thesis entitled "The Role of Rejection in Aortic Valve Allograft Failure" by Ahmad Moustapha in partial fulfillment of the requirements for the degree of Master of Science.

Dated: June 25, 1997

Co-Supervisor: [Signature]

Co-Supervisor: [Signature]

Readers: [Signature]

External: [Signature]
DALHOUSSIE UNIVERSITY

Date: June 24, 1997

AUTHOR: Ahmad Moustapha

TITLE: The Role of Rejection in Aortic Valve Allograft Failure

DEPARTMENT OR SCHOOL: Pathology

DEGREE: MSc  CONVOCATION: October  YEAR: 1997

Permission is herewith granted to Dalhousie University to circulate and to have copies for non-commercial purposes, at its discretion, the above title upon request of individuals or institutions.

[Signature of Author]

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

The author attests that permission has been obtained for the use of any copyrighted material appearing in this thesis (other than brief excerpts requiring only proper acknowledgement in scholarly writing) and that all such use is clearly acknowledged.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Dedicated to my wife Susan Catherine and my daughters
Aisha Noor and Maryam Iman
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table of Contents</td>
<td>v</td>
</tr>
<tr>
<td>Figures and Tables</td>
<td>vi</td>
</tr>
<tr>
<td>Abstract</td>
<td>viii</td>
</tr>
<tr>
<td>Abbreviations and Symbols used</td>
<td>ix</td>
</tr>
<tr>
<td>Presentations resulting from this work</td>
<td>x</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>xi</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>2. Background</td>
<td>3</td>
</tr>
<tr>
<td>2.1. Valve Homografts</td>
<td></td>
</tr>
<tr>
<td>2.2. Cryopreservation</td>
<td></td>
</tr>
<tr>
<td>2.3. Homograft Failure</td>
<td></td>
</tr>
<tr>
<td>2.4. Evidence for Rejection as Cause of Graft Failure</td>
<td></td>
</tr>
<tr>
<td>2.5. Histopathological Features of Acute and Chronic Rejection</td>
<td></td>
</tr>
<tr>
<td>3. Hypothesis and Rationale for Experiments</td>
<td>19</td>
</tr>
<tr>
<td>4. Experiment 1: Development of a Rat Model of Cryopreserved Aortic Valve Allograft Transplantation</td>
<td>20</td>
</tr>
<tr>
<td>4.1. Introduction</td>
<td></td>
</tr>
<tr>
<td>4.2. Materials and Methods</td>
<td></td>
</tr>
<tr>
<td>4.3. Results</td>
<td></td>
</tr>
<tr>
<td>4.4. Discussion</td>
<td></td>
</tr>
<tr>
<td>5. Experiment 2: Aortic Valve Allografts in the Rat: Evidence for Rejection</td>
<td>43</td>
</tr>
<tr>
<td>5.1. Introduction</td>
<td></td>
</tr>
<tr>
<td>5.2. Materials and Methods</td>
<td></td>
</tr>
<tr>
<td>5.3. Results</td>
<td></td>
</tr>
<tr>
<td>5.4. Discussion</td>
<td></td>
</tr>
<tr>
<td>6. Conclusion</td>
<td>70</td>
</tr>
<tr>
<td>7. Bibliography</td>
<td>71</td>
</tr>
</tbody>
</table>

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
FIGURES AND TABLES

Figure 1. Explanted rat aortic valve. 25
Figure 2. Completed heterotopic rat aortic valve transplant. 26
Figure 3. Freshly isolated, not transplanted rat aortic valve. 33
Figure 4. Rat aortic valve leaflet structure. 34
Figure 5a. Fresh syngeneic graft at 56 days (x 20). 35
Figure 5b. Fresh syngeneic graft at 56 days (x 100). 36
Figure 6a. Cryopreserved syngeneic graft at 56 days (x 20). 37
Figure 6b. Cryopreserved syngeneic graft at 56 days (x 100). 38
Table 1. Initial results 39
Table 2. Surgical results and failures 40
Table 3. Experimental groups. 51
Table 4. Preserved leaflets seen at different time points. 51
Table 5. Summary of histological findings. 52
Figure 7. Fresh syngeneic graft at 2 days (x 100). 54
Figure 8. Fresh syngeneic graft at 7 days (x 100).

Figure 9a. Fresh syngeneic graft at 28 days (x 20).

Figure 9b. Fresh syngeneic graft at 28 days (x 100).

Figure 10. Fresh allogeneic graft at 2 days (x 100).

Figure 11. Fresh allogeneic graft at 7 days (x 100).

Figure 12a. Fresh allogeneic graft at 28 days (x 20).

Figure 12b. Fresh allogeneic graft at 28 days (x 100).

Figure 13a. Fresh allogeneic graft at 56 days (x 20).

Figure 13b. Fresh allogeneic graft at 56 days (x 100).

Figure 14a. Cryopreserved allogeneic graft at 56 days (x 20).

Figure 14b. Cryopreserved allogeneic graft at 56 days (x 100).
ABSTRACT

Cardiac valve replacement is the second most common operation performed for heart disease in Canada. Patients receiving a valve replacement, be it mechanical or bioprosthetic, do not regain normal life expectancy. Thirty-five years after the first valve replacements were undertaken a perfect valve substitute has not been developed. Recipients of mechanical valves are at risk of thromboembolism, coagulopathy, endocarditis and cardiac failure. Homografts, cadaveric human cardiac allograft valves, are cryopreserved at -196 °C for storage. When transplanted, recipients need not be anticoagulated and are at much lesser risk of endocarditis. Unfortunately, all homografts ultimately fail and durability seems worse in young patients. In adults, homografts fail after 10 to 15 years. In children up to 45% of these valves have to be replaced after 5 years. The precise cause of homograft failure remains a mystery and the relative roles of different pathological and possibly immunological factors have not been elucidated.

At present the main question is whether homograft failure is due to rejection or mechanical degeneration. The use of cardiac valve transplants between syngeneic and allogeneic strains of rats permits investigations into the role of immune mediated rejection of these grafts. We expanded an already developed microsurgical rat model for aortic interposition allografts to include the aortic valve. Aortic valves were harvested from donor rats and then heterotopically transplanted into the abdominal aorta of a recipient rat using inbred strains. All recipient rats were Lewis and donor rats were either Lewis (syngeneic) or Brown Norway (allogeneic). Grafts were transplanted either fresh or cryopreserved, thus giving four experimental groups: fresh syngeneic, fresh allogeneic, cryopreserved syngeneic, and cryopreserved allogeneic. The valves were then explanted at different time points and histologically examined by two pathologists in a blinded fashion. A total of almost 100 transplants were performed. After 56 days, syngeneic grafts had preserved leaflets while allogeneic grafts were completely destroyed. Cryopreservation did not alter this process. This suggests that rejection plays a role in the failure of allograft valves. Histological features of rejection were observed.

This new model of cryopreserved aortic valve allograft transplantation has been used to demonstrate histological features of acute and chronic rejection in allogeneic rat grafts. Should homografts be indeed immunogenic, then this model could be used to investigate the role of immunosuppression of the recipient or treatment of the graft in allograft survival. This will have important clinical implications for patients with valvular heart disease, particularly children.
### Abbreviations and Symbols Used

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>allogeneic</td>
</tr>
<tr>
<td>BN</td>
<td>brown norway rat</td>
</tr>
<tr>
<td>C</td>
<td>cryopreserved</td>
</tr>
<tr>
<td>CR</td>
<td>chronic rejection</td>
</tr>
<tr>
<td>CTLp</td>
<td>cytotoxic lymphocyte precursor</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>ELAM</td>
<td>endothelial-leukocyte adhesion molecule-1</td>
</tr>
<tr>
<td>F</td>
<td>fresh</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hank's balanced salt solution</td>
</tr>
<tr>
<td>ICAM</td>
<td>intercellular adhesion molecule</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin g</td>
</tr>
<tr>
<td>Lew</td>
<td>lewis rat</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility</td>
</tr>
<tr>
<td>RVOT</td>
<td>right ventricular outflow tract</td>
</tr>
<tr>
<td>S</td>
<td>syngeneic</td>
</tr>
<tr>
<td>SD</td>
<td>Sprague-Dawlew rat</td>
</tr>
<tr>
<td>SMC</td>
<td>smooth muscle cell</td>
</tr>
<tr>
<td>VCAM</td>
<td>vascular cell adhesion molecule</td>
</tr>
</tbody>
</table>
PRESENTATIONS AND PUBLICATIONS RESULTING FROM THIS WORK

Transplant Atlantic Chronic Rejection Symposium
Halifax, Nova Scotia. April 1995

Dalhousie University Department of Surgery Research Day

Canadian Association of University Surgeons

American Association of Thoracic Surgeons 77th Annual Meeting
Washington DC. May 1997

The Terrence Donnelly Cardiac Residents Research Day, University of Toronto
Toronto, Ontario. May 1997

Chapter 5 has been submitted to The Journal of Cardiovascular and Thoracic Surgery as
Moustapha A, Ross DB, Bittira B, Van Velzen D, McAlister VC, Lannon CL, Lee TDG. Aortic
Valve Grafts in the Rat: Evidence for Rejection.

x
ACKNOWLEDGEMENTS

Dr. Vivian McAlister for his encouragement and availability. It is clear that without his ideas, tenacity and enthusiasm this work would never have been started nor completed.

Dr. Tim Lee for his tremendous support, patience and breadth and depth of teaching in all aspects related or unrelated to this work.

Dr. David Ross who provided the original idea for this study. His guidance, ideas, surgical expertise and enthusiasm are greatly appreciated.

Dr. Jim Wright for his outstanding teaching, patience, help with the pathological assessment and for allowing me unrestricted access to his photo-microscope.

Dr. Van-Velzen for his invaluable help with the pathological assessment and description.

Mrs. Mary Wile for her dedication, patience and expertise with which she approached the histological processing. I am confident that without her expertise the slides would not have turned out as well.

I thank Mr. Lewis Page, Mr. André Le Prairie and members of the Regional Tissue Bank, Queen Elizabeth II Health Sciences Centre, Halifax, Canada, for their tremendous help and teaching with the cryopreservation.

Mr. Howard Matheson for allowing me unrestricted access to his computer and office.
1. Introduction

Every year thousands of Canadians require and undergo a cardiac valve replacement. A variety of valve replacements exists including metal, bioprosthetic valves, such as porcine valves, and homografts. Each one of these valve replacements has risks and contraindications. Patients receiving mechanical valves are at risk of thromboembolism, coagulopathy, endocarditis and cardiac failure. Mechanical valves should not be implanted into women who wish to become pregnant. Bioprosthetic valves deteriorate in adults and faster in children. Obstruction occurs secondary to a diffuse intimal peel. Again this valve type is prone to thromboembolic events and infection, and should preferably not be implanted in presence of endocarditis. Calcification of the valve leaflet necessitates replacement after 7-10 years (1, 2). Homografts are human cardiac allograft valves of either cadaveric origin or from explanted hearts transplanted into patients. After retrieval, these homografts are cryopreserved for storage. Homografts have excellent hemodynamics, come in different sizes, and therefore are preferred for the repair of certain congenital cardiac anomalies. When transplanted, recipients need not be anticoagulated and are at much lesser risk of endocarditis. Because of these excellent characteristics homografts have obtained widespread use in children and adults. There is growing evidence that durability of these valves is superior to bioprosthetic valves over long-term follow up. However, all homografts will ultimately fail and durability seems worse in young patients, particularly those less than three years of age. In adults, homografts fail after 10-15 years (1). In children, up to 45% of these valves have to be replaced after 5 years (2).
The precise cause of homograft failure, whether in children or adults, remains a mystery and the relative role of different pathological and possibly immunological factors has not been elucidated. All studies to date investigating cardiac valve allograft failure deal only with noncryopreserved allografts which are not applicable to current clinical practice. At present the main question is whether homograft failure is due to rejection or mechanical degeneration. To address this question we will expand an already developed microsurgical rat model for aortic interposition allografts to include the aortic valve. We will assess the influence of host cellular and immunological effects and cryopreservation on degenerative changes of valves in the rat model. By transplanting fresh and cryopreserved valves between rats of different immunological backgrounds we will be able to identify the relative importance of immunologically mediated transplant rejection as a cause of valve failure. This will have important clinical implications for patients with valvular heart disease, particularly children.
2. Background

2.1. Valve Homografts

Thirty-five years after the first cardiac valve replacements were undertaken, a perfect valve substitute has not been developed. Mechanical valves, but not homografts, require lifelong anticoagulation with warfarin and the patient is therefore at risk of hemorrhage. Bioprosthetic valves deteriorate over 8 to 10 years in adults and faster in children. Mechanical and bioprosthetic valves are prone to thromboembolic events and infection, and should preferably not be implanted in presence of endocarditis. The first fresh aortic valve homografts were inserted heterotopically into the descending aorta by Gordon Murray of Toronto in 1956. These valves were followed for up to 6 years and showed satisfactory function (1, 3). Orthotopic cardiac valve allografts have been used successfully in the replacement of diseased human aortic valves since 1962 and for reconstruction of the right ventricular outflow tract (RVOT) since 1966 (1, 4). It was soon noted that homografts had many advantages over commercially available mechanical and bioprosthetic valves. This included a low transvalvular gradients at rest or with exercise, low rates of thromboembolism and resistance to prosthetic valve endocarditis (5, 6). Thus homograft implantation soon was used extensively for children who are prone to rapidly calcify commercially available bioprosthetic valves and for whom anticoagulation required with mechanical valves is difficult. Homografts allowed for an unrestricted lifestyle with restoration of valve function to normal (2, 3, 7). At present homografts are the valve of
choice for the reconstruction of the right ventricular outflow tract, in the presence of endocarditis and for the replacement of the patients own pulmonary valve as a replacement for the diseased aortic valve. This is the so-called Ross procedure.

In an attempt to develop the perfect valve replacement numerous developments have been made in the areas of procurement, preservation and utilization of homografts (1). For decades, a variety of methods of valve allograft sterilization and preservation existed. This diversity of methods confounded the comparable analysis of longterm valve allograft performance.

Initially, homograft valves were freeze-dried, which involved snap-freezing the valves without any attempt to protect the cellular contents. Accelerated calcification and deterioration occurred in these homografts. Next homografts were sterilized in antibiotic solutions and stored at 4°C in a nutrient medium. These homografts were referred to as “fresh” and had to be implanted by 3-6 weeks. This limited the use of homografts largely to big centers in London, Australia, and New Zealand where large numbers implanted avoided wastage of valves. It became apparent that if they were to become more widely used they would have to be capable of being stored for prolonged periods of time (1). Eventually a technique of controlled-rate freezing after antibiotic sterilization was introduced by Mark O'Brien. This technique is the standard method used for valve storage today although the use of fresh, unfrozen valves is still recommended by some. Most storage techniques have attempted to maximize tissue viability of the stored valves in the assumption that
this would give the best longterm performance of the valve (1, 5, 8). Whether present
techniques result in tissue viability of the graft in situ is not clear and there is evidence to
both support the concept of viability and refute it (5, 9).

A number of studies have demonstrated the superiority of cryopreserved homografts
compared to fresh homografts over longterm follow-up in regards to durability. O'Brien
showed in a study of over 300 fresh and cryopreserved aortic homografts the following
results: freedom from reoperation for valve degeneration at 10 years was 100% for
cryopreserved and 89% for fresh homografts. At 15 years this was 59% for fresh
homografts. There was no statistical difference in survival. Combined, the survival rate
was 83% at 4 years and 60% at 15 years. Both groups had a similar low incidence of
valve-related complications like thromboembolism and endocarditis. The freedom from a
thromboembolic event for both groups combined was 97% at 10 years and 96% at 15
years. The freedom from allograft valve endocarditis for both groups combined was 92%
at 10 years and 89% at 15 years (5). Clarke reported on a group of 47 children who
received aortic root replacement with cryopreserved aortic valve allografts. In the 33
children who were older than 3 years in hospital mortality was 9% with no late deaths.
Primary allograft degeneration was not observed in the 29 children that were followed
over a period of 2.5 months to 4.7 years (mean 2.3 years) (10).

In essence, after almost four decades of developments in homograft procurement,
sterilization, preservation and indications for implantation, the cryopreserved homograft
has established itself as the valve replacement of choice in certain conditions outlined
above. It is difficult to conceive that man-made valves could be more effective and
efficient than the natural tissues. Nowhere is this more striking than when looking at the
heart valves, which have been molded by millions of years of blood flow and pressure
(1).

2.2. Cryopreservation

The modern technique of cryopreservation was introduced by O’Brien in the early
1970’s (11). Cryopreservation, a method of programmed slow freezing with cryoprotection
of the tissue and storage of homografts at below -135°C, allows indefinite storage of these
valves. The main goal of developing this tissue friendly way of valve preservation and
storage was to maintain valve graft viability. O’Brien postulated that viability of
homografts was an important determinant of their durability (5). This is perhaps because it
has been assumed that viability implies the capacity of the homograft to regenerate, to
replace dead or damaged cells, and to repair itself. If regeneration occurs, it may be
expected that structural integrity would be maintained and that longterm function would be
enhanced (12). In 1987 O’Brien reported explanting a cryopreserved homograft at 9 ¼
years because of technical malalignment producing progressive incompetence from the
time of operation. This valve was from a male donor to a female recipient. The valve
contained viable cells and all cultured cells from this explanted valve had a male
chromosomal configuration. This indicated that donor cells and only donor cells had persisted in the leaflet tissue for 9 ¼ years and provided the first evidence of donor cell survival in homograft valves by chromosome analysis.

Using in situ hybridization on cryopreserved porcine aortic allografts in the pig model, Hazekamp was able to demonstrate both donor and recipient fibroblasts in the leaflets (9). However no conclusions were drawn with respect to what this observation meant to the durability or functioning of the graft. The main issue to be raised here is whether preserved cellular viability in the graft is advantageous to graft longevity or whether it is disadvantageous since it represents an increased immunological load. Arguments have focussed on the enhanced structural preservation of viable grafts vs. the immunological stimulation they represent. Despite the above-mentioned evidence, there still is much discussion and confusion in the literature concerning the presence of viable cells in the allograft and the role of such cells in the function of the graft. The most ardent proponent of the presence and importance of viability is O'Brien (5, 11). That some donor cells remain alive, for a while after implantation, is probably true, but whether they have any functional role is less clear. O'Brien reported that fibroblasts from cryopreserved allografts were viable as assessed by cell culture, ability to metabolize glucose and on histologic examination at the time of implantation. Three human allografts were explanted due to technical errors at 2, 10, and 20 months following
implantation and all grew fibroblasts in cell culture. However, it was noted that the
degree of cellularity was progressively decreased between the valve explanted at 2
months and the ones explanted later such that the valve explanted at 20 months had very
sparse cellularity and the few remaining fibroblasts had pyknotic nuclei (11).

Cryopreservation has a different effect on the viability of different cell populations
that compose the homograft. As described above, there is good evidence that
cryopreservation preserves some cellular functions and viability of fibroblasts, however
the effects on endothelium and antigenicity are less certain. Lupinetti showed that
cryopreservation resulted in the absence of replicating endothelium in both rat isografts
and allografts as measured by tritiated thymidine uptake 72 hours post transplantation.
As endothelial cells express the major histocompatibility antigens, are more
immunostimulatory than other cellular components of allografts, and are the major site of
leucocyte adhesion molecule expression, it is very crucial to understand their fate during
or after cryopreservation. Yankah reported that in cryopreserved homografts, endothelial
cells had a 70-80% cell survival as assessed by alcian blue dye exclusion. Furthermore
the cryopreserved valves showed normal appearance and the endothelium did express
HLA class I and II antigens measured with indirect immunofluorescence (6, 12). This
antigenicity was not noted in valves refrigerated for 3 days in antibiotic solution.
Cochran found that cryopreserved rat valve leaflets placed in a subcutaneous pouch of a
recipient rat provoked an accelerated second set rejection of a subsequent skin graft. This was not different from the response seen with fresh leaflets and they concluded that cryopreservation preserved the antigenicity of the leaflets (13). This finding was supported by El Khatib who found accelerated second set rejection of skin grafts in a rat model using cryopreserved aortic valve allografts (14). The degree of antigenicity was related to the degree of histoincompatibility of the rats.

In the rejection process endothelial cells express leukocyte adhesion molecules when stimulated by cytokines leading to adherence of leukocytes. Mulligan found that allogeneic but not syngeneic rat aortic grafts caused up regulation of the adhesion molecules endothelial-leukocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1). This upregulation was maximal for fresh valves and was mildly attenuated by prolonged storage at 4°C. It was markedly attenuated by cryopreservation such that by 21 days there was only weak expression of ELAM-1 and VCAM-1 with moderately strong expression of ICAM-1 (15). These findings would indicate a decreased immunogenicity of these valves. Other major factors influencing the viability of cryopreserved heart valves include the duration of warm and cold ischemia, use of sterilisation procedure which optimizes cell viability, selection of appropriate cryoprotectant and cooling times, and rapid thawing and gradual dilution of the cryoprotectant.
2.3. Homograft failure

Despite their advantages, homografts ultimately fail in the aortic position. The freedom from valve related death or reoperation at 10 years is approximately 70% although O'Brien initially reported a freedom from structural deterioration of 99% at 10 years for cryopreserved valves (5). In 1991 O’Brien presented an updated report on over 530 homograft recipients. This showed the actuarial freedom from reoperation for structural degeneration at 14 years to be 95% for cryopreserved homografts and 67% for fresh homografts (8). This has not been reported by other groups. Most homografts function well for 7 to 10 years after which deterioration inevitably appears. Some of the discrepancy in reported results may be due to the nature of defining failure of the valve. Gradual onset of aortic regurgitation can be tolerated for years so that evaluating valve function by freedom from reoperation may underestimate the degree of valve dysfunction. More recent, careful echocardiographic studies show progressive early aortic regurgitation even using the root technique of implantation. Bando presented a group of 326 patients receiving aortic or pulmonary cryopreserved homografts. The 5-year freedom from homograft failure was significantly better for pulmonary homografts, 94% vs. 70% (7). In this study, risk factors for patient mortality and homograft failure were identified by Cox multivariate analysis. These included aortic type of homograft and age 4 years or younger for aortic homograft recipients.
Cryopreserved aortic homografts in children have been reported to deteriorate at an unacceptably high rate. Clarke reported on a group of 47 children who received aortic root replacement with cryopreserved aortic valve allografts. There was a relatively high incidence of early allograft failure in recipients less than three years of age. In this subgroup of 14 children, mortality was 29% (4/14) and 60% (6/10) required reoperation for progressive failure of the homograft with replacement necessary at a mean interval of 1.9 years following the original operation. These explanted valves showed aortic wall calcification, intimal thickening without cellular infiltrates, and severely retracted or absent valve leaflets. An accelerated immunological rejection was postulated as the cause of failure (10).

Other risk factors for homograft failure include low operative weight, small graft size and homograft retrieval-to-cryopreservation time of less than 24 hours. The type of donor valve, donor age, and blood group mismatch were not associated with failure (16).

Amongst a variety of other factors homograft failure has been suggested to have an immunological etiology (1, 2, 10, 17). The idea appears logical as graft matching is not done in current clinical practice and the recipients do not receive immunosuppressive therapy postoperatively. There are a number of reports of immunosuppressive treatment of valve graft recipients despite the fact that current knowledge is insufficient to justify such drastic therapy (1, 10, 18). There is currently a debate with respect to the level of immunogenicity of these valves which relates to the perception of the valve tissue as
antigenic. Such immunogenicity would suggest a positive response from the use of immunosuppressive treatment, but no such effect has been objectively demonstrated. Thus these issues are still unresolved. Replacement valves that fail sclerose, fibrose and calcify, but it is unclear whether this is an expression of rejection or simply degeneration. There is no possible way of studying this in humans. Investigators have looked at a couple of different animal models of aortic valve allografts, including pig and sheep models. These models are expensive, with time consuming surgery and difficult to control as the animals are outbred. The rat model however, is a very good model as it allows to transplant valves between syngeneic and allogeneic rats. This will allow us to differentiate between rejection, which would cause only the allogeneic grafts to fail and structural degeneration which would occur in both allo- and syngeneic valves in a similar fashion.

Shortly after homografts were introduced for aortic valve replacement, they were implanted in the RVOT either to establish continuity from a ventricle to the pulmonary artery in the case of pulmonary stenosis or atresia or to replace an insufficient pulmonary valve when right ventricular dysfunction occurred. These homograft conduits or valves are presently the valve of choice for reconstruction of the RVOT for congenital heart disease. Cryopreserved aortic valves placed as conduits in the RVOT have had an unacceptably low freedom from reoperation rate in some series of 47-70% at five years. Better results have been reported with pulmonary valved conduits with freedom from reoperation rates of
around 90% at five years. Very young children have a much worse freedom from reoperation rate (1, 7) with one series reporting all children less than 1 year of age at the time of conduit implantation requiring reoperation by 5 years compared to only 6% for the older group. Chan showed early pulmonary homograft failure with progression of regurgitation in 35% of patients over a mean followup period of 3 years. As with aortic valve replacements, measuring valve function by freedom from reoperation is a crude and insensitive measure of the integrity of the valve leaflets. Pulmonary insufficiency is well tolerated for a long period in some patients particularly if the arteries are normal and the pulmonary resistance low. Non-valved conduits have been widely used in RVOT reconstruction. There is increasing echocardiographic evidence that there is early and progressive pulmonary insufficiency, such that in Chan’s study there was progression of regurgitation greater than 2 grades in 35% of patients over a mean followup period of 3 years. It was predicted that 50% of patients operated on before 18 months would have severe regurgitation by 15 months postoperatively compared to only 15% operated on after 18 months (19). This has important implications as an increasing indication for pulmonary valve replacement is to protect a failing right ventricle from pulmonary regurgitation, often in the face of distal pulmonary artery abnormalities. Obviously the rapid onset of homograft insufficiency will negate any benefit from such procedures. The problem is magnified by the lack of a suitable alternative for pulmonary valve replacement, particularly in young children who calcify porcine tissue valves quickly.
2.4. Evidence for Rejection as Cause of Graft Failure

It was the initial belief that valve allografts were immunologically privileged, because no microvasculature developed between host and graft, because the cells were rapidly replaced by recipient cells, or because the graft did not contain a significant mass of viable tissue (14). Consequently, human recipients of homografts do not currently receive immunosuppression. The first evidence that aortic valve allografts are immunogeneic was shown by Heslop, who found that aortic valve allografts were weakly antigenic in a rat study and attributed most of the antigenicity to the rim of cardiac muscle included in the graft and not to the leaflet. Subcutaneous implants of aortic valve allografts did not effect reduction in survival time of challenging skin grafts, whereas subcutaneous implants of myocardium did. Both lymphocytotoxic and hemagglutinating antibodies were produced (20).

Cochran found that cryopreserved rat valve leaflets placed in a subcutaneous pouch of a recipient rat of provoked an accelerated second set rejection of a subsequent skin graft. This was not different from the response seen with fresh leaflets and they concluded that fresh grafts were immunogenic and that cryopreservation preserved the antigenicity of the leaflets (13). This finding was supported by El Khatib who found accelerated second set rejection of skin grafts in a rat model using valved aortic grafts (14). The degree of antigenicity was related to the degree of histoincompatibility of the rats.
Despite laboratory evidence that aortic allografts are antigenic and elicit an immune response current clinical practice does not attempt to minimize or control such a response. Some clinicians have attempted to match valves for ABO blood group compatibility but there is no evidence that this is beneficial for long-term valve survival (2, 17, 21).

Yacoub found that major histocompatibility (MHC) Class I antigen was expressed on the endothelial surface of freshly explanted human valves and that this expression gradually diminished such that by 48 hours after procurement there was no identifiable Class I expression found. This was the same for “fresh” valves stored in nutrient media and for antibiotic sterilized valves. Class II antigen, thought to be important for antigen presentation was not present on the endothelial surface but was found in the stroma of the valve on dendritic cells. Again, this expression diminished over the next 48 hours in both groups. Viable endothelial cells remained, thus the reduction in MHC I and II expression was not due to a lack of endothelial cells (22). It is not known if expression of MHC I is induced during rejection of these valves, such induction of MHC I expression does occur in the myocardium of hearts undergoing acute rejection.

Numerous animal studies have suggested evidence for immunologic rejection of aortic allografts. Yankah found that rats receiving fresh viable aortic grafts from syngeneic donors had preserved cellular elements 150 days after implantation while incompatible allogeneic valves had no identifiable cellular elements by this time and there was
disorganization of collagen fibers with breaks in their structures. Rats receiving allogeneic valves had accelerated rejection of subsequent valve donor strain skin grafts indicating an enhanced second set reaction and this was proportional to the degree of histoincompatibility between strains. Direct immunofluorescence revealed anti-rat Ig on the endothelium of allogeneic donor allografts at day 20, but this was diminished by day 50 in accordance with the destruction of endothelial cells observed at that time (23).

Yacoubs's findings are supported by a rat study performed by Lupinetti. Fresh aortic valves and those stored 1 day expressed Class I antigen, but grafts stored for longer periods at 4°C had no detectable antigen present. Grafts stored up to 21 days in this medium had diminished, but still present, immunogenicity as measured by accelerated skin graft rejection. This appeared to correlate with the decline in percentage of viable endothelial cells from 95% at day 0 to 64% at day 21. If rejection does indeed cause the early failure observed in children then this reduced immunogenicity observed with prolonged storage at 4°C may account for the apparent improvement in results found by Ross with this storage technique (24).

Zhao et al found that there were donor-specific immune responses to fresh aortic grafts in allogeneic (Lewis to DA) but not syngeneic (DA to DA) rat strains (25). Mixed lymphocyte culture showed a significant increase in stimulation index in the allogeneic group suggesting induction of CD4⁺ helper cells while limiting dilution analysis of
splenocytes showed a significant increase in the frequency of cytotoxic lymphocyte precursor (CTLp) cells. Flow cytometry showed stimulation of donor-specific anti-T cell antibody.

It has been shown that recipients of homovital, non-cryopreserved homografts produce donor HLA-specific antibodies of the immunoglobulin G (IgG) class, and that recipients of cryopreserved homografts form IgG- and T-cell mediated reactions to donor HLA antigens (26, 27). However it is unknown whether this alloreactivity ultimately results in the observed histopathological changes that lead to valve allograft failure.

2.5. Histopathological Features of Acute and Chronic Rejection

Acute rejection usually occurs days to weeks after transplantation and is initiated by T-cell-dependant immunity. It is characterized by a lymphocytic infiltrate accompanied by plasma cells, eosinophils, and a few mast cells or neutrophils. A variety of strong immunosuppressive drugs have decreased the risk of early graft failure due to acute rejection. The vast majority of solid organ transplants functions well at the end of their first year of existance, but only less than half of renal transplants from cadaveric donors survive six years. The mechanism underlying this slow but steady graft deterioration is now well recognized and termed chronic rejection. It is the most common cause of allograft failure at the present time. The pathologic manifestations of chronic rejection vary with the type of
organ transplanted. Many descriptive pathological studies in clinical and experimental transplantation have identified findings which are consistently present in chronically rejecting organs. These are persistent perivascular inflammation, concentric and generalized intimal thickening, called allograft arteriosclerosis, and complete loss of smooth muscle cells (SMC) in the media, called medial necrosis. Amongst these the hallmark histopathological manifestation of chronic rejection is a progressive thickening of the arterial tree within the affected organ due to neointimal proliferation. This process was therefore termed transplant arteriosclerosis. The underlying mechanisms of transplant arteriosclerosis are not completely understood (21, 28-30).
3. **Hypothesis and Rationale for Experiments**

From the evidence above it is reasonable to believe that valve failure may be an immune mediated process. To study this process more closely, with the goal in mind to develop treatments to ameliorate it, I developed the following hypotheses.

1. Aortic valve allografts are immunogenic.

2. Rejection, or a similar process plays a role in aortic valve allograft failure.

3. Cryopreservation alters the immunogenicity of aortic valve allografts.

I wished to answer these questions through controlled experimentation using a rat model for aortic valve allografts. I would assess the importance of antigenic differences between the donor and recipient by transplanting aortic valves between syngeneic and allogeneic rat combinations. The role of chronic rejection in valve failure would be monitored by histological examination of the explanted aortic allografts looking for the characteristic features of acute or chronic rejection. To establish the contribution of cryopreservation to immunogenicity aortic valve allografts would be transplanted fresh or after cryopreservation. However in order to test the hypotheses I first would have to perfect aortic valve cryopreservation and transplantation in the rat. Then I could assess the function with MHC disparate, fresh and cryopreserved valves.
4. **Experiment 1: Development of a Rat Model of Cryopreserved Aortic Valve Allograft Transplantation**

4.1. **Introduction**

Different animal models exist that examine the aortic valve allograft in large animals, like pig and sheep (9, 31). These are models of orthotopic or heterotopic transplantation which are technically difficult and much more expensive than small animal models, resulting in only few transplanted animals per study. In addition these models do not allow a controlled comparison of syngeneic versus allogeneic transplantation as inbred strains do not exist in these animals. These problems can be overcome by using a small animal model such as the rat. Inbred strains of rats have been used widely for renal and cardiac allograft studies. While a rat aortic valve allograft model has been described by Yankah (23), a model of transplantation of cryopreserved rat aortic valves, using techniques similar to clinical homograft preservation, is not available. In addition histopathological changes after valve transplantation are poorly described. Once mastered, this model would allow the study of aortic valve allograft transplantation under controlled immunogenetic conditions, as well as the assessment of a larger number of experimental subjects. Thus I expanded the model of rat aortic valve transplantation to include cryopreserved allografts.

The aim of this experiment was to develop a rat aortic valve allograft model using fresh and cryopreserved aortic valves. This included developing a method of valve retrieval
and coronary artery ligation, cryopreservation of these valves and the actual transplantation. After the rats survived a specified time period, we had to be able to explant a patent graft and be able to identify preserved valve leaflets on histological examination. With exception of a brief description of the surgical transplantation procedure alone (23, 32) none of the other steps mentioned above have been described elsewhere.

4.2. Materials and Methods

Animals:

Initial transplants were done on semi-outbred Sprague Dawley (SD) rats weighing 225-400g (Harlan Sprague Dawley, Indianapolis). Subsequently, Lewis (Lew) and Brown Norway (BN) rats (Harlan Sprague Dawley, Indianapolis) were used at 225 to 300g. All animals were housed and fed ad libitum at the Dalhousie University Animal Care Center in accordance with the guidelines of the Canadian Council of Animal Care (33).
Preoperative Care and Anaesthesia:

For both the donor and recipient rats food and water was not restricted preoperatively. An intraperitoneal injection of 65 mg/kg of sodium pentobarbital was used to anaesthetize the rats, for induction and maintenance. Anaesthesia was produced in 3 to 5 min, lasting 45 to 90 min, with the animals remaining quiet for several hours after the operation. None of the animals received antibiotics or heparin at any time.

Surgical Technique:

Donor operation. Both, the donor and the recipient operations were carried out on a rectangular piece of plastic. Rat aortic valves with a short portion of thoracic aorta were transplanted heterotopically into the abdominal aorta of recipient rats. For the retrieval of these aortic valves, the rats were anaesthetized and then shaved from the abdominal and thoracic region. The four paws were then taped to the plastic table and a midline skin incision made. The skin was reflected, and the fascia was cut with a similar incision along the midline. The abdominal aorta was located after the intestines were reflected to the right and transected below the renal arteries to bleed the animal. This significantly decreased the bleeding during the cardiac dissection. Next the diaphragm and rib cage were opened, and a triangular flap of tissue from the upper thorax was removed to expose the heart and its surrounding vessels. The right and left atria were removed, as well as the pulmonary trunk and its attachments to the aortic outflow tract. This exposed the left and right coronary
arteries which were tied or sutureligated with 10-0 nylon suture. Identification and dissection of the coronary arteries was best done by leaving the heart in situ attached to the ascending aorta for as long as possible to facilitate traction and exposure. The aorta was intermittently flushed with saline via the left ventricle to prevent its desiccation. The ventricular myocardium was then removed. The aortic valve was located, and one cusp was sutured to the aortic wall using a separate 10-0 nylon suture. Any excess ventricular muscle was removed from the valve. The aorta was then transected, leaving an ascending aorta conduit of 5-8 mm and preserved in cold isotonic saline solution prior to transplantation (Figure 1). Both the donor and recipient operations were performed using a Weck OM-1206 ceiling mounted operating microscope under 7x to 10x magnifications with standard microsurgical instruments.

**Recipient operation.** For the recipient operation, a midline laparotomy was performed to expose the abdominal aorta. The bowel was eviscerated to the right using moist cotton swabs and wrapped in moist gauze. The aorta was then mobilised from the inferior vena cava from the level of the left renal artery almost down to the aortic bifurcation and divided between two vessel clamps. The previously obtained aortic valve allograft was then interposed between the stumps of the recipient aorta by proximal and distal anastomosis using a continous 10-0 nylon suture under the microscope. A stay suture was first placed at
6 o'clock. The next suture was placed right adjacent and then run counterclockwise until it could be tied to the stay suture. The aortic stumps were flushed intermittently with cold normal saline. No systemic heparinization nor antibiotics were used. The distal clamp was released first, to allow washout of air and debris, followed by the proximal clamp. At this stage usually minor oozing occurred from the anastomotic needle holes and this was easily controlled with compression for a minute using a dry gauze (Figure 2). Once hemostasis was secured the abdominal contents were then returned into the peritoneal cavity and the wound closed in layers. Throughout the procedure, the peritoneum and contents were kept moist with normal saline.

Postoperative care:

The recipient rats were laid on their sides in their cages on a warming table where they were kept until awake. Then they were returned to the Animal Care Centre. Postoperatively the animals received water and food ad libitum. For the following five days, the animals were examined daily for graft patency. This was done by assessing movement and colour of the back legs. Ischemia of the legs generally indicated graft thrombosis. Rats with paraplegia or plegia of one hindpaw were sacrificed.
Figure 1. Explanted fresh aortic valve from a Lewis rat
Figure 2. In-situ view of completed heterotopic rat aortic valve transplant. Arrow shows graft in place.
Cryopreservation:

Valves were used immediately after harvesting (fresh) or following two to eight weeks of cryopreservation. Cryopreservation was performed at the Regional Tissue Bank, Queen Elizabeth II Health Sciences Centre, in exactly the same way homografts are cryopreserved (34). The explanted aortic valve allografts were incubated in a 500 ml bottle of Hank's balanced salt solution (HBSS) containing 40 mg of gentamicin and 500 mg of cefazolin and stored at 4°C for 24 hours. The valves were then removed from this solution and cryopreserved in HBSS with 10% dimethylsulfoxide (DMSO) as a cryoprotectant. Each valve was placed in a separate 4" x 6" sterile polyethylene pouch (Kenpak Convertors Incorporated, City of Commerce, California, and Kapak Corporation, St. Louis Park, Minnesota) and 70 ml of HBSS with 10% DMSO were added and the bags heatsealed. These bags were each placed in another outer aluminum foil bag and again heatsealed. The packaged aortic valves were then each placed in a separate casette and into an ice bath pending initiation of the cryopreservation process. Next these cassettes were placed into the pre-cooled freezer chamber of a programmeable controlled rate freezer (Planer KRYO 10, Diamed Laboratory Supplies, Mississauga, Ontario, Canada) and cooled at a rate of -1°C/min to -5°C/min to a temperature of -80°C, then labelled and stored at a temperature below -135°C (Revco Ultralow Freezer, Rheem Manufacturing Company, Asheville, North
Carolina). Cooling is monitored by four temperature probes, which are placed throughout the cooling chamber and between the cassettes and recorded as a cooling curve. For transplantation the packaged cryopreserved valve was thawed in a water bath at 37°C for ten minutes, removed from the package and implanted.

Histology:

The grafts were explanted with a short segment of adjacent recipient aorta and immediately fixed in 10% buffered formalin for at least 24 hours. Using a syringe and a #25 plastic canule 10% buffered formalin was gently injected into both ends of the explanted aortic graft to remove any air and ensure proper fixation. The leaflet area was marked with india ink on the outside of the graft to allow better recognition. The grafts were then embedded in paraffin and completely sectioned through using 4 μm slides until we identified the leaflets. Then alternate slides were stained. Unstained slides and blocks were preserved for future reference and use. Hematoxylin-Eosin and van Giesson-Elastica stains were obtained from each graft. All histological processing was performed by the same person to allow consistency.
4.3. Results

*Technical:* Initially the main difficulty was related to the explantation of the aortic valve and identification of the coronary arteries. Literature on the anatomy of laboratory rats did not address these problems. I had to work out my own approach for the identification and dissection of the coronary arteries in rats. This was best done by leaving the heart in situ attached to the ascending aorta for as long as possible to facilitate traction and exposure. Each coronary artery was then ligated with a 10-0 suture. Experience and improved surgical technique helped to overcome these problems. The next difficulty was the size discrepancy between the recipient abdominal aorta and the larger proximal end of the graft, which is the left ventricular myocardium and anterior leaflet of the mitral valve. Initially we used interrupted sutures but this resulted in longer clamp time and more bleeding. The problem was finally resolved by spatulating both the proximal and distal end of the recipient aorta, by trimming the myocardium as much as possible and by running the suture.

*Cryopreservation:* Major difficulties were not encountered with respect to cryopreservation and histological preparation of the grafts. It became evident that horizontal crosssections of the valves were more appropriate for our purposes than longitudinal sections. No difference was seen in complications using cryopreserved as
compared to fresh grafts. Mortality of rats receiving a cryopreserved graft was 2/20 (10%) as compared to 3/71 (4%) in rats receiving a fresh graft. Thrombosis and aneurysm formation were observed in 3/15 (15%) rats receiving a cryopreserved graft compared to 9/71 (13%) in rats receiving a fresh graft. Because of the relative small numbers a statistical evaluation was not performed. Cooling curves were similar to homograft cooling curves.

**Operative outcomes:** Success was defined as a transplanted animal that lived through the expected time period and at planned sacrifice had a patent graft. Graft occlusion usually resulted in a paraplegic, limping or dead animal (Table 1). However, 7 animals that did not show any of these signs and at explantation, the graft was found to be thrombosed. Reason for failure in the first 91 rat aortic valve transplants is shown in Table 2. In the first 8 animals we had a combined mortality and morbidity of >70% mainly because of technical problems related to bleeding and thrombosis. In the next 40 animals we had 2 postoperative deaths, 3 anastomotic aneurysms, and 5 thrombosed graft. This gave a combined mortality and morbidity of 25%. Of the last 43 transplants, 41(95%) were successful. Causes of failure in this group were thrombosis (11). Both the aneurysmatic and obliterated grafts were considered surgical failures and were not included in the histopathological examination.
Pathology:

*Normal valve.* In the freshly isolated, not transplanted valves normal histology of the rat aortic wall at the leaflet level demonstrates a single layer intima, the media with spindle cells and elastic fibers, and adventitia with its fibroconnective tissue. Figure 3 shows a crosssection of the aortic valve at the level where three valve leaflets are identified. At higher magnification (Figure 4) one can see the leaflet structure consisting of a fibrocollagenous center with a single cell layer endothelium covering each surface. We consistently identified the commissure at the base of the leaflets. This helped us to identify where the leaflets should have been when they were destroyed.

*Cryopreserved, nontransplanted valve.* After 22 months of cryopreservation the nontransplanted valve shows normal histology with exception of some mild edema of the leaflet. The leaflet has retained its endothelium.

Fresh syngeneic valve. At 56 days in the fresh syngeneic grafts (Figure 5a and b) the adventitia and media look normal. There are focal areas of medial cell loss of varying degrees and diffuse intimal thickening. The leaflets are thin and appear normal. Both, the leaflet and the neointimal proliferation are covered with endothelium.
*Sprague Dawley transplants.* At 27 days post transplant in this semi-outbred strain combination there was a massive infiltration of the leaflets with lymphocytes and macrophages, such that only few leaflet stromal cells were recognizable. Beginning retrovalvular thrombus formation was present. Endothelium on the leaflets was partly preserved and not present on the aortic graft. A mild and only focal neointimal proliferation was present and consisted mainly of inflammatory cells. The media was normal. The adventitia showed a moderate and diffuse lymphocytic infiltrate. At 41 days focal neointimal proliferation had progressed and still consisted mainly of inflammatory cells. Areas of complete medial necrosis were now present. The lymphocytic infiltrate in the adventitia persisted.

*Cryopreserved syngeneic valve.* Cryopreserved syngeneic grafts at 56 days (Figures 6a and b) showed an unremarkable adventitia. The media showed extensive areas of medial necrosis and only focal areas of normal media were present. There was moderate and diffuse neointimal proliferation. Preserved leaflets were present. Both the leaflet and the neointimal proliferation were covered with endothelium. No retrovalvular thrombus was seen. In the aortic conduit neointimal proliferation was rather excentric.
Figure 3. Horizontal crosssection of a freshly isolated, not transplanted rat aortic valve showing normal histology with three valve leaflets (arrows).
Figure 4. Higher magnification showing leaflet structure consisting of a fibrocollagenous center with a single cell layer endothelium covering each surface (arrow).
Figure 5a. Horizontal crosssection of a fresh syngeneic graft at 56 days with preserved leaflets (arrow) (magnification x 20).
Figure 5b. Fresh syngeneic graft at 56 days showing normal morphology of leaflet, media and adventitia, but progressing intimal thickening (arrow) (magnification x 100).
Figure 6a. Cryopreserved syngeneic graft at 56 days showing preserved leaflet (arrow) and diffuse concentric intimal thickening (magnification x 20)
Figure 6b. Cryopreserved syngeneic graft at 56 days showing loss of cellularity in the media (arrow) (magnification x 100).
Table 1. Surgical results of first aortic valve allograft transplants

<table>
<thead>
<tr>
<th>Transplant No.</th>
<th>Animal combination</th>
<th>Survival</th>
<th>Cause of Death</th>
<th>Histology obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SD-SD</td>
<td>3 days</td>
<td>Sacrificed *</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>SD-SD</td>
<td>No</td>
<td>Bleeding</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>SD-SD</td>
<td>49 days</td>
<td>Sacrificed</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>SD-SD</td>
<td>41 days</td>
<td>Sacrificed</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>SD-SD</td>
<td>40 days</td>
<td>Sacrificed</td>
<td>No b</td>
</tr>
<tr>
<td>6</td>
<td>SD-SD</td>
<td>6 days</td>
<td>Bleeding c</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>SD-SD</td>
<td>27 days</td>
<td>Sacrificed</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>BN-Lew</td>
<td>13 days</td>
<td>Unknown d</td>
<td>No</td>
</tr>
</tbody>
</table>

SD: Sprague-Dawley; *: plegia left hindpaw; b: graft found obliterated at time of sacrifice; c: confirmed at necropsy; d: no necropsy done.
Table 2. Causes of failure in the first 91 aortic valve allograft transplants

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Number</th>
<th>Successful</th>
<th>Death</th>
<th>Thrombosis</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-8</td>
<td>3 (38%)</td>
<td>3\textsuperscript{a,b}</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>9-48</td>
<td>30 (75%)</td>
<td>2\textsuperscript{c}</td>
<td>5</td>
<td>3\textsuperscript{d}</td>
<td></td>
</tr>
<tr>
<td>49-91</td>
<td>41 (95%)</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} cause of death unknown (1)

\textsuperscript{b} bleeding (2)

\textsuperscript{c} causes of deaths unknown

\textsuperscript{d} anastomotic aneurysms
4.4. Discussion

Using the rat a satisfactory model of cryopreserved aortic allograft transplantation is possible. Although rat aortic valves are tiny in comparison to homograft valves, similar freezing curves are seen during cryopreservation. The smaller rat valves are also maintained well over time. No technical difficulty encountered during development of the model could be attributed to cryopreservation and all technical problems appeared to resolve with surgical practice. Careful histology is required to see the leaflets. Horizontal sections are preferable to longitudinal sections. Each section should be 4 μm thick. The leaflets can be identified on the unstained sections allowing selection for staining.

After developing a rat aortic valve transplant model, we are now able to use this model for investigation of a possible immunological cause of aortic valve allograft failure. After almost 100 transplants our current success rate is >90%. This small animal model will allow aortic valve allograft transplantation of a larger number of animals and between inbred strains to examine and compare fresh versus cryopreserved and syngeneic versus allogeneic grafts. Results of this study will help eliciting whether these valve allografts are immunogenic and whether cryopreservation alters the immunogenicity of aortic valve allografts. Should indeed these grafts be immunogenic, then this model could be used to
investigate the role of immunosuppression of the recipient or treatment of the graft in allograft survival.
5. Experiment 2: Aortic Valve Allografts in the Rat: Evidence for Rejection

5.1. Introduction

A number of studies have demonstrated the superiority of cryopreserved aortic valve allografts compared to other bioprosthetic valves over longterm follow-up in regards to durability and low incidence of valve-related complications (5, 8, 11, 35). Despite their advantages, however, aortic valve allografts do ultimately fail in the aortic position and the effective life of the average aortic homograft in an adult is about 15 years (1). In children valve failure occurs faster for reasons that remain unknown (4, 10, 17). The etiology of homograft failure in children and adults is most likely multifactorial, and includes mechanical, immunological and other factors. In particular the role of immune mediated rejection in the failure of allograft heart valves in man is uncertain. While there is clear evidence of a donor-specific systemic immune response to allograft aortic valve implants in humans and rats, the effects of such a response are unclear (25-27). Short retrieval to cryopreservation times which would be expected to maximize viability and therefore antigenicity have been associated with increased failure of allograft valves in children (16). Serial sampling of human allografts for systematic histopathological examination is not feasible and grafts are rarely removed shortly after implantation when such damage may be occurring. The use of heart valve transplants between syngeneic and allogeneic strains of rats permits investigations into the role of immune mediated
rejection of these grafts. Evidence suggesting an immunological basis has been presented by numerous authors (16, 20, 23, 24, 36). This study describes the pathological changes in rats, over time, following transplantation of syngeneic versus allogeneic and fresh versus cryopreserved valves and to identify changes indicative of rejection. This could either be in form of an acute or chronic rejection process. Acute rejection is microscopically characterized by a lymphocytic infiltrate accompanied by plasma cells, eosinophils and a few neutrophils. Chronic rejection however has been described in human and animal solid organ transplants and in the rat aortic allograft model and includes a persistent perivascular inflammation, concentric and generalized intimal thickening, termed graft arteriosclerosis, and complete loss of smooth muscle cell nuclei in the media, termed medial necrosis (28, 29). An indepth examination of the pathological changes and mechanisms underlying valve failure may help define the relative role, time sequence and magnitude of the main components of an immunological reaction. This would have important implications for the choice and timing of therapeutic interventions aimed at modifying this response.
5.2. Materials and Methods

Animals:

Lewis and Brown Norway rats (Harlan Sprague Dawley, Indianapolis) weighing 225 to 300 g were used. Different strain combinations were used with all recipient rats being Lewis and donor rats being either Lewis (syngeneic) or Brown Norway (allogeneic). Grafts were transplanted either fresh or cryopreserved, thus giving four experimental groups: fresh syngeneic, fresh allogeneic, cryopreserved syngeneic, and cryopreserved allogeneic. All animals were housed and fed ad libitum at the Dalhousie University Animal Care Center in accordance with the guidelines of the Canadian Council of Animal Care (33).

Surgical technique:

Aortic valve transplants were performed as described in the previous chapter. Briefly, an intraperitoneal injection of 65 mg/kg of sodium pentobarbitol was used to anaesthetize the rats. None of the animals received antibiotics or heparin at any time. Donor operation: The donor aortic valve was removed including a cuff of ventricular muscle and the ascending aorta. The coronary arteries were tied with a 10-0 nylon suture (Ethilon, Ethicon Inc.). One cusp of the valve was sutured to the aortic wall in order to render the valve incompetent and prevent thrombosis. Any excess myocardial muscle
was removed from the valve. The donor graft was then placed in cold isotonic saline solution prior to transplantation for the fresh experiments or cryopreserved as described. Both the donor and recipient operations were performed using a Weck OM-1206 operating microscope under 7x to 10x magnifications. **Recipient operation:** A midline laparotomy was performed to expose the abdominal aorta. The aorta was then mobilised from the inferior vena cava from the level of the left renal artery almost down to the aortic bifurcation and divided between two vessel clamps. The previously obtained aortic valve allograft was then interposed between the stumps of the recipient aorta using a continuous 10-0 nylon suture.

**Cryopreservation.** Valves were used immediately after harvesting (fresh) or following two to eight weeks of cryopreservation. Cryopreservation was performed using standard protocols for homograft preservation at the Regional Tissue Bank, Queen Elizabeth II Health Sciences Centre. The explanted aortic valve allografts were incubated in Hank’s Balanced Salt Solution (HBSS) containing gentamicin (80mg/l) and cefazolin (1g/l) and stored at 4°C for 24 hours. The valves were then removed from this solution, cryopreserved in HBSS with 10% dimethylsulfoxide (DMSO) and packaged separately in pouches (Kenpak Convertors Incorporated, City of Commerce, California, and Kapak Corporation, St. Louis Park, Minnesota). The packaged aortic valves were placed in the pre-cooled freezer chamber of a programmeable controlled rate freezer (Planer KRYO 10, Diamed Laboratory Supplies, Mississauga, Ontario, Canada) and cooled at a controlled...
rate of -1°C/min to -5°C/min to a temperature of -80°C, then labelled and stored at below -135°C (Revco Ultralow Freezer, Rheem Manufacturing Company, Asheville, North Carolina). For transplantation the packaged cryopreserved valve was thawed in a water bath at 37°C for ten minutes, removed from the package and implanted.

Experimental groups:

The animals were sacrificed for explantation and histological examination of the grafts at different time points following transplantation according to Table 3. Fresh grafts were explanted at 2, 7, 28 and 56 days. Cryopreserved valve grafts were explanted at 56 days. This timing was chosen based on our previous experience in mouse and rat aortic allografts in which we have shown that chronic rejection is evident by 28 days following transplantation and well established by 56 days (37). Surgical failures include death of the animal before planned time of sacrifice, obliteration of graft at time of explantation and aneurysms at the anastomotic site of the graft. These were excluded from analysis.

Histology:

The grafts were explanted with a short segment of adjacent recipient aorta and immediately fixed in 10% buffered formalin for at least 24 hours. Using a syringe and a #25 plastic cannula 10% buffered formalin was gently injected into both ends of the explanted aortic graft to remove any air and ensure proper fixation. The leaflet area was
marked with India ink on the outside of the graft. The grafts were then embedded in paraffin and completely sectioned through with 4 μm thickness sections and stained with Hematoxylin-Eosin. We used longitudinal sections for the 2 and 7 day grafts and horizontal sections for the 28 and 56 days.

Analysis:

Histopathological examination was performed by two pathologists blinded to the original protocol and study design. All layers of the aortic valve transplants were systematically examined.

5.3. Results

Surgical results: Out of 56 transplanted animals 54 animals survived the planned time period to sacrifice. At sacrifice 45 grafts were patent and acceptable for histopathological assessment. 6 grafts were obliterated and 3 developed aneurysms. Animals were lost in each group to that the minimum number in the 28 and 56 days group was 5 (Table 4). No differences were seen in complications using cryopreserved grafts as compared to fresh grafts.

Histological Results: Tables 4 and 5 summarize the histopathological findings of the rat aortic valve allograft at the examined time points. Fresh syngeneic grafts. At 2 days in the fresh syngeneic aortic valve grafts (Figure 7) the myocardial cuff showed
evidence of beginning myocellular necrosis. There was a mild infiltration of the myocardial cuff and adventitia with polymorphonuclear neutrophils (PMN’s). The media looked normal. No intima was present. The leaflet is preserved but lost its endothelium. At 7 days (Figure 8) there was an extensive necrosis of the myocardial cuff. Concomitantly a massive infiltration of PMN’s and macrophages throughout the myocardial cuff, adventitia and media was present. The intima was lost completely and PMN’s were adherent to the lumen. At 28 days in the fresh syngeneic grafts (Figures 9a and 9b) sections at the valvular level and at the more distal aortic level show the adventitia, media, and leaflet to be normal. Focal intimal thickening was present. The endothelium was generally present on intima and leaflet. No retrovalvular thrombus was seen. At 56 days the fresh syngeneic grafts (Figures 5a and 5b) continued to show no changes in the adventitia. There were focal areas of medial cell loss of varying degrees, but the elastin layers were continuous. There was diffuse, but concentric intimal thickening and more prominent than at 4 weeks. The leaflets were thin, appeared viable and without significant changes. Both the leaflet and the neointimal proliferation were still covered with endothelium.

*Fresh allogeneic grafts.* At 2 and 7 days in the fresh allogeneic aortic valve grafts the histopathology does not differ significantly from the syngeneic graft (Figures 10 and 11). At 28 days in the fresh allograft sections at the valvular level (Figures 12a and 12b) showed the adventitia to be densely infiltrated with mainly lymphocytes and
macrophages. Areas of prominent vascularity in the adventitia were identified. Medial necrosis was variable but in many places full thickness. There was limited presence of inflammatory cells within the wall itself. The arrangement of spindle cells and elastic fibers appeared normal in the unaffected media. The changes in the media were far less prominent in the aortic conduit than in the aortic root. The aortic lumen showed retrovalvular thrombus development, with in many cases early organisation of the thrombus. There was lumen narrowing. Focal areas of mild to moderate intimal thickening were present consisting mainly of noninflammatory cells and covering the leaflets on the luminal side. As yet there were limited changes to the leaflet itself other than edema, considerable, often complete loss of vitality of valvular stromal cells and loss of the lining endothelium. At 56 days in the fresh allogeneic transplants (Figures 13a and 13b) the dense lymphocytic cell infiltrate in the adventitia remained. There was almost complete and diffuse medial necrosis. Free valve leaflets were not present. The aortic lumen showed complete retrovalvular thrombosis. The thrombus was now organized with some hemosiderin deposits. Leaflet remains or leaflet ghosts were identified within the thrombus. Significant neointimal proliferation with lumen narrowing was seen between the leaflet ghosts and the lumen. Distally in the aortic conduit the intima was still moderately to markedly thickened consisting of mainly spindle cells and a few inflammatory cells.
Table 3. Number of rats in each experimental group at each time point

<table>
<thead>
<tr>
<th>Group</th>
<th>Days Post-Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Fresh syngeneic</td>
<td>1</td>
</tr>
<tr>
<td>Fresh allogeneic</td>
<td>3</td>
</tr>
<tr>
<td>Cryopreserved syngeneic</td>
<td>--</td>
</tr>
<tr>
<td>Cryopreserved allogeneic</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 4. Preserved leaflets seen at different time points

<table>
<thead>
<tr>
<th>Group</th>
<th>28 days</th>
<th>56 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh syngeneic</td>
<td>7/7</td>
<td>3/5</td>
</tr>
<tr>
<td>Fresh allogeneic</td>
<td>8/8</td>
<td>0/6*</td>
</tr>
<tr>
<td>Cryopreserved syngeneic</td>
<td>2/5</td>
<td></td>
</tr>
<tr>
<td>Cryopreserved allogeneic</td>
<td>0/6**</td>
<td></td>
</tr>
</tbody>
</table>

Data shown are grafts with preserved leaflets/total successful grafts

* Leaflet ghosts were seen in 5/6 grafts
** Leaflet ghosts were seen in 6/6 grafts
Table 5. Schematic representation of the histological appearance of the aortic valve allograft at different time points

<table>
<thead>
<tr>
<th>Grafts</th>
<th>Intimal thickening</th>
<th>Medial necrosis</th>
<th>Perivascular inflammation</th>
<th>Preserved Leaflets</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS 2 days</td>
<td>0</td>
<td>0</td>
<td>0→+</td>
<td>Yes</td>
</tr>
<tr>
<td>FA 2 days</td>
<td>0</td>
<td>0</td>
<td>0→+</td>
<td>Yes</td>
</tr>
<tr>
<td>FS 7 days</td>
<td>0</td>
<td>+→++</td>
<td>++</td>
<td>Yes</td>
</tr>
<tr>
<td>FA 7 days</td>
<td>0</td>
<td>+→++</td>
<td>++</td>
<td>Yes</td>
</tr>
<tr>
<td>FS 28 days</td>
<td>0→+, focal</td>
<td>0</td>
<td>0</td>
<td>Yes</td>
</tr>
<tr>
<td>FA 28 days</td>
<td>+→++</td>
<td>+→++</td>
<td>+++</td>
<td>Yes</td>
</tr>
<tr>
<td>FS 56 days</td>
<td>+→++</td>
<td>0→++, focal</td>
<td>0</td>
<td>Yes</td>
</tr>
<tr>
<td>FA 56 days</td>
<td>++→+++</td>
<td>+++</td>
<td>++</td>
<td>no, leaflet ghosts</td>
</tr>
<tr>
<td>CS 56 days</td>
<td>++</td>
<td>+++</td>
<td>0</td>
<td>Yes</td>
</tr>
<tr>
<td>CA 56 days</td>
<td>++→+++</td>
<td>+++</td>
<td>+</td>
<td>no, leaflet ghosts</td>
</tr>
</tbody>
</table>

F = fresh; C = cryopreserved; S = syngeneic; A = allogeneic; 0 = none; + = mild; ++ = moderate; +++ = severe
**Cryopreserved syngeneic grafts** at 56 days (Figures 6a and 6b) showed an unremarkable adventitia. The media showed more extensive areas of medial necrosis than in the fresh syngeneic grafts at 56 days and only focal areas of normal media were present. There was moderate and diffuse neointimal proliferation. Preserved leaflets were present. Both the leaflet and the neointimal proliferation were covered with endothelium. No retrovalvular thrombus was seen. In the aortic conduit neointimal proliferation was rather excentric.

**Cryopreserved allogeneic grafts** at 56 days (Figures 14a and 14b) showed a moderate to severe mononuclear cell infiltrate in the adventitia consistent of mainly lymphocytes and macrophages. There was complete and diffuse medial necrosis. Free valve leaflets were not present but leaflet ghosts were identified in all grafts. The aortic lumen showed complete retrovalvular thrombosis. Similar to the 56 day fresh allogeneic grafts the thrombus was organized with hemosiderin deposits. Between the leaflet ghosts and the lumen there was significant neointimal proliferation with lumen narrowing. In the aortic conduit there still was neointimal proliferation but less prominent and excentric. Endothelium was seen occasionally.
Figure 7. Longitudinal section through the leaflet and myocardial cuff of a fresh syngeneic rat aortic valve graft at 2 days. Beginning myocellular necrosis and mild infiltration with polymorphonuclear neutrophils (arrow) is noted (magnification x 100).
Figure 8. Fresh syngeneic graft at 7 days showing massive infiltration of polymorphonuclear neutrophils in media (arrow) and adventitia (magnification x 100).
Figure 9a. Horizontal crossection of a fresh syngeneic graft at 28 days showing preserved normal leaflets (arrow) (magnification x 20).
Figure 9b. Fresh syngeneic graft at 28 days showing normal morphology of preserved leaflet, normal media and adventitia with mild, focal intimal thickening (arrow) (magnification x 100).
Figure 10. Longitudinal section through the leaflet and myocardial cuff of a fresh allogeneic rat aortic valve graft at 2 days. Histopathology is similar to syngeneic counterpart. Again beginning myocellular necrosis and mild infiltration with polymorphonuclear neutrophils (arrow) is noted (magnification x 100).
Figure 11. Fresh allogeneic graft at 7 days showing preserved leaflet. Inflammatory cell infiltration of media and adventitia (arrow) is seen (magnification x 100).
Figure 12a. Fresh allogeneic graft at 28 days showing preserved, but dysplastic leaflet (arrow) (magnification x 20).
Figure 12b. Section through the dysplastic leaflet and sinus of valsalva of a fresh allogeneic graft at 28 days showing retrovalvular thrombus development (arrow) (magnification x 100).
Figure 13a. In the fresh allogeneic graft at 56 days no preserved leaflets are seen. Marked concentric intimal thickening is present (arrow) with lumen narrowing (magnification x 20).
Figure 13b. Fresh allogeneic graft at 56 days showing leaflet ghost (small arrow) with neointimal proliferation towards the lumen (open arrow) and complete medial necrosis (large arrow). Lymphocytic infiltration of the adventitia persists (magnification x 100).
Figure 14a. In the cryopreserved allogeneic graft at 56 days no preserved leaflets are seen. Like in the fresh allogeneic grafts marked concentric intimal thickening is present (arrow) with lumen narrowing. Diffuse lymphocytic infiltration of the adventitia persists (open arrow) (magnification x 20).
Figure 14b. Cryopreserved allogeneic graft at 56 days showing leaflet ghost (small arrow) with marked neointimal proliferation towards the lumen (open arrow) and complete medial necrosis (large arrow). Hemosiderin is seen in the organized retrovalvular thrombus (magnification x 100).
5.4. Discussion

Homograft valves are commonly used in cardiac surgery, often in children and young adults. Unfortunately they will fail and require replacement, particularly in children (4, 10, 16, 17). The precise cause of this failure is unknown but one main area of research has been into the possible immunogenicity of these homografts. It has been shown that recipients of homovital, non-cryopreserved homografts produce donor HLA-specific antibodies of the immunoglobulin G (IgG) class, and that recipients of cryopreserved homografts form IgG- and T-cell mediated reactions to donor HLA antigens (26, 27). However it is unknown whether this alloreactivity ultimately results in the observed histopathological changes that lead to valve allograft failure. This study demonstrates that aortic valve allografts in the rat model fail through retrovalvular thrombosis and neointimal proliferation. In addition widespread medial necrosis and a lymphocytic adventitial infiltration are present in the allografts but not in the syngrafts. These findings are characteristic of acute and chronic rejection and have been described in the rat aortic allograft model (28, 29), which does not include the aortic valve. Retrovalvular thrombosis in the allograft could therefore represent one additional expression of alloreactivity. Potential effector mechanisms include secretion of cytokines, growth and other factors by lymphocytes, endothelial cells and smooth muscle cells, resulting in the upregulation of cell adhesion molecules and leading to thrombosis (28-30). Already in the early time points there is a progressive, massive infiltration of the
adventitia with PMN's in both the syn- and allogeneic grafts. This non-specific inflammatory reaction is at least partly caused by the concomitantly transplanted myocardial cuff which undergoes ischemic necrosis. We postulate that this PMN invasion then spills over to the media resulting in medial cell loss again in both the syn- and allogeneic grafts, which could be the source of release of cytokines, growth and other factors. This results in intimal thickening in the syngeneic transplants which was observed beginning at 28 days and progressing at 56 days. At each time point allogeneic grafts demonstrate more extensive neointimal proliferation than their syngeneic controls, as intimal thickening in the allogeneic transplants is a result of both, non-specific inflammatory response to the necrotic muscle cuff and ongoing allostimulus of the graft itself. The importance of the transplanted myocardial cuff has been referred to earlier. Heslop et al. found that aortic valved conduits were weakly antigenic in a rat study and attributed most of the antigenicity to the rim of cardiac muscle included in the graft (20). Both lymphocytotoxic and hemagglutinating antibodies were produced. Interestingly, they found no acceleration of second-set rejection of donor strain skin. By 28 days the fresh syngeneic graft has regenerated its media and one would expect that eventually intimal thickening in the syngeneic grafts should regress once the media is restored (37), however we did not look at this beyond 56 days. In the allogeneic grafts the allostimulus persists and therefore the media does not regenerate but continues to mount an immune-mediated response resulting in further medial necrosis and thus further intimal thickening. Syngeneic grafts are expected to not generate an immune response and survive.
indefinitely (28, 29, 37, 38). The fact that we observed intimal thickening in our fresh and cryopreserved syngeneic grafts at 56 days leads us to believe that the ultimate histopathological morphology of this group of grafts has not yet been reached. Intimal thickening in syngeneic grafts has been briefly mentioned in the literature without an attempt for explanation (38).

Beyond the first 7 days which were characterized by a non-specific inflammatory response and throughout all further time periods a chronic lymphocytic infiltrate persists in the adventitia of the allogeneic grafts. This has been described by others (20, 28, 29) and could be another source of ongoing production of cytokines and other growth factors responsible for smooth muscle cell destruction, migration or transformation, resulting in medial necrosis and neointimal proliferation.

Leaflet viability may be important for long term allograft valve function. In this study the leaflets of fresh syngeneic grafts retained normal morphology and cellularity for up to 56 days. Fresh allogeneic leaflets were dysplastic at 28 days, and demonstrated an obliterative thrombosis in the sinus of valsalva at 56 days. Cryopreservation did not alter the leaflet morphology of syngeneic grafts nor did it prevent retrovalvular leaflet thrombosis in allogeneic grafts. However cryopreserved syngeneic grafts showed more extensive medial necrosis than their fresh counterpart which could be partly due to some cytotoxic effect of cryopreservation.

A criticism of this study is that it is a non working valve model which may explain
the thrombosis of the valves that was seen in the rats and is not seen in humans. Nevertheless the preservation of valve leaflets exclusively in the syngeneic rats is strong evidence of immune mediated damage occurring only in the allogeneic grafts. We postulate that this thrombosis is a marker for such damage. In human allografts this injury may lead to the loss of viability which will ultimately cause the failure of the valve.

This study demonstrates that aortic valve allografts in the rat model undergo histopathological changes that are characteristic of both acute and chronic rejection. This rejection process ultimately leads to allograft valve failure via a progressive obliterative retrovalvular thrombosis which is destructive to the valve leaflets. Further studies are needed to determine whether similar events might be occurring in homografts, and which mediators are involved. Based on these and other results, investigations into therapies to modify this response appear justified. This could be achieved by either tissue typing and matching donor and recipient or by subjecting the recipient to immune suppression for an unknown time period.
6. Conclusion

This study investigates the mechanisms of failure of aortic valve allografts in a well-established rat model. The study addresses the role of donor/recipient antigenic differences in valve survival, the role of rejection in valve allograft failure and the role of cryopreservation in this phenomenon. Solving these questions will be critical to improve functional longevity of cardiac valve allografts particularly in children who suffer most of the adverse effect of valve allograft failure.

The disappointing results with aortic homografts in children and young adults has led to the use of the patient's own pulmonary valve as a replacement for the diseased aortic valve with the use of a homograft for the explanted pulmonary valve. This is the so-called Ross procedure. If the long-term results of this autograft are confirmed to be superior to homografts then this will become the operation of choice. As there is very little long-term follow-up on young children with autografts it is not known if the homografts will perform well in the pulmonary position in this group. Despite the possible advantages of the Ross procedure, it still leaves the patient with a homograft valve in the pulmonary position which will ultimately deteriorate. However, homografts will still be the valve of choice for aortic valve endocarditis and there will be an increasing number of homografts implanted to reconstruct the RVOT in autograft patients.
7. **Bibliography**


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


34. Manual of Regional Tissue Bank, Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia.


