FACTORS IN THE EARLY FAILURE OF CRYOPRESERVED HOMOGRRAFT PULMONARY VALVES IN CHILDREN: PRESERVED IMMUNOGENICITY?

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Methods: Between 1990 and 1995, 48 homograft valves (15 aortic and 33 pulmonary), cryopreserved on-site, were implanted to reconstruct the right ventricular outflow tracts in 44 children (mean age 6.2 ± 6.0 years; range 3 days to 20.2 years). Blinded serial echocardiographic follow-up evaluation was performed for all 45 valves in the 41 survivors. **Results:** Four homograft valves were replaced because of pulmonary insufficiency (3) or stenosis and insufficiency (1). Freedom from reoperation was 90% (70% interval, 84% to 97%) at 50 months. During the follow-up period 15 valves developed progressive pulmonary insufficiency of at least two grades. Three valves developed transvalvular gradients of ≥ 50 mm Hg, and one of these valves was also insufficient. The freedom from echocardiographic failure (two or more grades of pulmonary regurgitation or ≥ 50 mm Hg gradient) was 44% at 50 months (70% confidence interval, 32% to 55%). Young age ($p = 0.03$), low operative weight ($p = 0.04$), small graft size ($p = 0.04$), and homograft retrieval-to-cryopreservation time of less than 24 hours ($p = 0.02$) were significantly associated with failure. The type of donor valve (pulmonic or aortic), donor age, and blood group mismatch were not associated with failure, although blood group mismatch approached significance ($p = 0.05$). **Conclusions:** Homografts function well as conduits between the pulmonary ventricle and pulmonary arteries if long-term valve competency is not crucial. However, many rapidly become insufficient. This has important implications for the choice of a valve if the indication for valve replacement is to protect a ventricle failing due to pulmonary insufficiency. Short periods between homograft retrieval and cryopreservation enhance viability and antigenicity. This may suggest an immunologic basis for the failure. (J Thorac Cardiovasc Surg 1996;112:1170-9)

Homograft valves are widely used in the repair of congenital heart anomalies as conduits between the pulmonary ventricle and pulmonary arteries and as valves to protect a ventricle failing due to pulmonary insufficiency and stenosis. The freedom from

reoperation rates reported for series of cryopreserved valves for right ventricular outflow tract (RVOT) reconstruction in the young have been 55% to 80% at 4 to 6 years.¹⁻⁴ Doubt has been expressed about the durability of homografts in the aortic and pulmonary positions in children.⁵⁻⁷

Adequate homograft function is usually reported in terms of freedom from reoperation, but this is a relatively insensitive measure of valve integrity. Pulmonary insufficiency may be well tolerated if the pulmonary arteries are normal and pulmonary resistance is low. However, many children requiring RVOT reconstruction have abnormal pulmonary vasculature and decreased right ventricular function. Longer follow-up demonstrates that a competent, nonstenotic pulmonary valve is important to preserve ventricular function and optimize exercise capacity.⁸⁻¹⁰

This study was undertaken to examine the inci-

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Table I. Homograft valve patients

Primary diagnosis	No.
Pulmonary atresia or ventricular septal defect	13
Tetralogy of Fallot	8
Complex transposition	8
Truncus arteriosus	5
Double outlet right ventricle	4
Pulmonary stenosis/atresia	3
Aortic stenosis (Ross procedure)	3
Total	44

dence, progression, and determinants of cryopreserved homograft valve failure in the RVOTs of children.

Methods

Patient population. From December 1990 to May 1995 at the IWK Children's Hospital 48 cryopreserved homograft valves (33 pulmonary and 15 aortic) were implanted in 44 patients (22 girls and 22 boys) for reconstruction of the RVOT or pulmonary valve replacement for a variety of congenital lesions (Table I). The mean age at operation was 6.2 ± 6.0 years (range 3 days to 20.2 years), and 15 patients were younger than 1 year. Mean weight was 21.0 ± 18.3 kg (range 2.8 to 72 kg). Forty-one (85%) of 48 patients had undergone at least one previous cardiac operation (Table II).

Homograft procurement and preparation. The 48 homograft valves were harvested en bloc with the heart by a surgeon or a tissue bank technologist under sterile conditions from 39 donors; both valves were used from nine donors (Table III).

Donors as old as 65 years were accepted. Standard exclusion criteria included historic or serologic evidence of viral infection (e.g., human immunodeficiency virus, hepatitis B or C), malignancy (except nonmetastatic central nervous system tumors), long-term corticosteroid therapy, or evidence of valvular heart disease or cardiac trauma. The valves were incubated in 500 ml of Hanks balanced salt solution containing 40 mg of gentamicin and 500 mg of cefazolin at 4°C for a mean of 31.3 ± 11.5 hours (range 12 to 72 hours). Valves were then inspected, trimmed, and measured, and macroscopically defective valves were discarded. Four small tissue biopsy specimens were obtained from each valve for culture of aerobic bacteria, anaerobic bacteria, fungi, and mycobacteria. The valves were then cryopreserved in Hanks balanced salt solution with 10% dimethylsulfoxide as a cryoprotectant. The valves were cooled in a controlled-rate freezer (Planer KRYO10, Planer Products Ltd., Sunbury-on-Thames, Middlesex, U.K.) at $1^{\circ}\text{C}/\text{minute}$ to a temperature of -80°C and stored in vapor-phase liquid nitrogen below -135°C until used. Valves were transported in a cooler with liquid nitrogen to the operating theater and thawed in a water bath at 39°C .

Operative technique. All valves were implanted by two surgeons. The operations were performed through a median sternotomy with standard cardiopulmonary techniques and mild hypothermia (32°C). The aortic root was

Table II. Previous cardiac surgery

Procedure	No.
Modified Blalock-Taussig shunt	22
Tetralogy of Fallot repair	13
Pulmonary valve replacement	8
Central shunt	6
Aortic valvotomy	6
Rastelli repair	5
Arterial switch	4
RVOT annular patch	4
Pulmonary valvotomy	3
VSD repair	2
Senning	1

RVOT, Right ventricular outflow tract; VSD, ventricular septal defects.

*Three valvotomies were performed in one patient and two in another one.

Table III. Homograft valve characteristics

Source	No.	Mean	Range
Heart transplant recipients	4		
Brain-dead multiorgan donors	11		
Tissue-only donors	24		
Total*	48		
Age of donor		20.3 ± 16.8 yr	3 days to 58 yr
Valve size		17.2 ± 3.7 mm	9 to 25 mm
Ischemic time†		4.4 ± 3.7 hr	1 to 18.5 hr
Retrieval-to-preservation time‡		31.3 ± 11.5 hr	12 to 72 hr

*Both valves were used from nine donors.

†Time from heart cessation to valve incubation at 4°C in Hanks balanced salt solution and antibiotics.

‡Time from onset of valve incubation to onset of cryopreservation.

not routinely crossclamped unless other procedures, such as ventricular septal defect closure or an aortic autograft procedure, were required. In seven patients the pulmonary reconstruction involved implanting the homograft as a bifurcated graft; the others had circumferential distal anastomosis. Proximal extensions of autologous pericardium or homograft material were added in 22 patients. An additional four patients had proximal extensions of Dacron polyester fiber. All proximal extensions were placed as patches and none as tubular grafts. Valve size was determined by the surgeon and was always larger than the minimum acceptable pulmonary annulus diameter as determined by Pacifico and colleagues.¹¹ Pulmonary valves were used preferentially. No attempt was made to match donor and recipient for blood type.

Echocardiographic evaluation. All surviving patients were examined in the immediate postoperative period and reexamined serially with transthoracic echocardiograms every 6 to 12 months. All studies were reviewed in a random and blinded fashion by one pediatric cardiologist. Pulmonary regurgitation (PR) was assessed by looking at

Fig. 1. Actuarial survival of all patients undergoing reconstruction of the right ventricular outflow tract with a cryopreserved homograft.

the regurgitant jet with pulsed color-flow Doppler, particularly its width at the source and how far it entered the corresponding ventricle. The severity was graded semi-quantitatively from 0 to 4+, in a manner similar to that described by Chan and coworkers⁶: 0, absent; 1+, a pinhole jet; 2+, a jet of regurgitation approximately 20% of the valve annulus; 3+, a wide-based jet approximately 40% of the annular width; 4+, a broad jet larger than 40% of the annular width. When possible, observations were made with the short-axis parasternal view. Stenosis was assessed by measuring the peak velocity through the valve with continuous-wave Doppler technique. The highest peak velocity was used in the simplified Bernoulli equation: gradient = $4 \times \text{peak velocity}^2$.

Statistical analysis. Data are presented as the mean $\pm$ the standard deviation. All statistical tests were conducted with SAS version 6.08 (SAS Institute Inc., Cary, N.C.).¹² Twelve variables were analyzed in a univariate analysis for significant association with echocardiographic failure. Continuous variables were analyzed with Student's *t* test and categorical variables using a χ^2 test. A forward stepwise discriminant function analysis was also conducted to determine which combination of these variables best predicted valve failure. Variables were chosen to enter the model based on a critical F test ($p < 0.05$) from an analysis of covariance in which the variables already added to the model act as covariates and the variable under consideration is the dependant variable.¹² The outcome measure of valve failure was defined as a progression of $\geq 2+$ PR or a transvalvar gradient of ≥ 50 mm Hg. Because of the inherent subjectivity in interpreting echocardiograms, a change of $\geq 2+$ PR or a ≥ 50 mm Hg gradient was thought to be significant and is supported in the literature.⁶

Life tables for freedom from valve failure, reoperation, or death were calculated using the Blossom statistical program (Blossom, BR Cole, National Institutes of

Health, Bethesda, Md.). Confidence intervals (70%) were approximated from the standard error for actuarial survival estimates.

Results

The three early deaths all were patients with complex truncus arteriosus, and there were no late deaths. No patient was lost to follow-up. Actuarial survival was 93% at 50 months (70% confidence interval, 89% to 97%) (Fig. 1).

Four valves (8.9%), have been explanted, resulting in an actuarial freedom from reoperation rate of 90% at 50 months (70% confidence interval, 84% to 97%) (Fig. 2). The first homograft was removed 14 months after implantation in an 11-year-old patient with complex tetralogy of Fallot. The valve had become acutely insufficient because of embolization of a clam shell device that had been used to close a ventricular septal defect 5 months earlier. At reoperation the device was found in the main pulmonary artery. The leaflets of the valve were found to be in good condition, except for a tear. The most recent echocardiogram had shown no regurgitation and no gradient. The new homograft functioned well for 3 months but showed a progressive increase of PR to 4+ at 22 months after implantation.

The second pulmonary valve reoperation was in a 7-month-old child 5 months after implantation. This patient with tetralogy of Fallot and pulmonary atresia developed an RVOT aneurysm, pulmonary regurgitation, and right ventricular failure. At reop-

Fig. 2. Actuarial freedom from reoperation in the 41 survivors.

eration there was no evidence of the valve tissue. The replacement homograft was functioning well, with trivial regurgitation and a moderate gradient of 40 mm Hg seen at the last echocardiogram 2 years later.

A third child, also with tetralogy of Fallot and pulmonary atresia, had a homograft implanted when he was 2 years 9 months old because of continued right ventricular failure after tetralogy repair. This child developed 2+ PR within 5 months of the operation. He remained well clinically, but the regurgitation continued to increase and was 4+ 2 years after the operation. The homograft also became progressively obstructive; the initial gradient was 24 mm Hg, and it had progressed to 90 mm Hg at the time of reoperation. At reoperation 4 years after implantation, the leaflets were still present but furled together. Immediately after the operation the replacement homograft had a 1+ PR grade and a transvalvular gradient of <20 mm Hg. Four months later there is no change on echocardiography, and the child is without symptoms.

The fourth child also suffered from tetralogy of Fallot with pulmonary atresia. The first homograft was inserted in the 11-month-old child because of congestive failure due to high pulmonary artery pressures. The valve developed 2+ PR within 2 months, and 7 months after it was implanted, the homograft was replaced because of worsening congestive failure. The new homograft also rapidly developed 2+ PR. The patient's condition is stable,

but the patient has limited exercise tolerance and remains in right-sided heart failure.

Postoperative echocardiograms were obtained in all 45 valves in the 41 survivors at a mean of 9.8 ± 9.5 days (range 1 to 41 days) after repair. The mean follow-up time was 20.6 ± 15.8 months (range 3 to 52 months). For four patients the echocardiographic windows were inadequate to allow comment on valve function; the echocardiographic follow-up is therefore for 41 (91%) of 45 valves. Three of the patients had initial postoperative echocardiograms showing 2+ or 3+ PR and were excluded from the statistical analysis because they were considered technical failures. Figure 3 shows the extent of pulmonary regurgitation at the last echocardiogram. Only three valves had a gradient of ≥ 50 mm Hg, (Fig. 4) one of these also had 4+ PR. The other two had no PR.

Seventeen (45%) of 38 of the valves failed (15 with $\geq 2+$ PR grades, two with ≥ 50 mm Hg gradients, and one with both). Actuarial freedom from failure was 44% at 26 and 50 months (70% confidence interval, 32% to 55%) (Fig. 5). The mean time to failure was 8.9 ± 6.9 months (range 1 to 26 months).

The univariate analysis showed that young age at operation, low weight, small graft size, and a time for homograft retrieval to cryopreservation of less than 24 hours were significantly associated with the development of $\geq 2+$ PR or a ≥ 50 mm Hg transvalvular gradient. None of the other variables was

Fig. 3. Pulmonary insufficiency (PR) assessed by pulsed color-flow Doppler echocardiography for 38 patients.

Fig. 4. Transvalvular gradient measured by peak velocity (PV) at the valve with continuous-wave Doppler echocardiography in 38 patients. The Bernoulli equation ($\text{gradient} = 4 \times \text{PV}^2$) was used for the calculations.

found to be statistically significantly associated with failure, although blood group mismatch approached significance (Table IV). The multivariate analysis showed that retrieval-to-cryopreservation time of less than 24 hours, blood group mismatch, and small valve size together predicted 84% of the failures. The partial correlation coefficients for each of these

variables were approximately equal (Table V). These three variables are equally important predictors of failure. Weight and age at operation, significant in the univariate analysis, were not significant predictors of failure in the stepwise discriminant function analysis. They were associated with valve size, and when valve size was controlled for in the

Fig. 5. Actuarial freedom from failure (progression of two or more grades of insufficiency or ≥ 50 mm Hg gradient) for the 38 valves analyzed.

multivariate analysis, age and weight became insignificant.

Discussion

All the patients requiring reoperation had tetralogy of Fallot with significant distal pulmonary artery stenosis. In each case when the valve became incompetent and ceased to protect the ventricle, heart failure developed, leading to reoperation. It is these patients in whom the homograft is most needed as a competent valve rather than a mere conduit. Long-standing PR is associated with decreased exercise tolerance and right ventricular dysfunction after repair of tetralogy.^{8,9} Pulmonary insufficiency after RVOT reconstruction is no longer thought to be innocuous.¹³ The low reoperation rate (see Fig. 2) is in keeping with that reported in other series, but it masks the true incidence of valve failure.^{1,14}

Although somewhat subjective, echocardiography provides a more sensitive measure of valve function than freedom from reoperation rates. Echocardiography has been shown to be a reliable method of assessing homograft function.^{6,15}

Several factors have been associated with homograft failure when reoperation was used as the outcome measure: young age,^{1,5-7} small valve size,^{1,7} and the use of aortic valves in the RVOT in some series,^{2,7} but not in others.^{1,6} Our results confirm young age and small graft size but not valve type. However, on the basis of the results reported by others, we preferentially used pulmonary valves

when possible.^{2,7} How the various factors in valve preparation affect valve longevity has not been fully determined.

It has been suggested that homograft valve failure may result from immunologic rejection, which may be heightened in young children,^{5,7,16} and immunosuppression has been suggested by some research.^{5,17,18} Much of the work with cryopreservation has emphasized the maintenance of viability, based on the belief that the durability of homograft valves is related to the viability of fibroblasts, which maintain the valve matrix.^{19,20} Homograft valve viability is determined by the time between donor heart cessation and harvest and by the preservation technique.^{19,21} Cryopreserved valves are thought to retain viability, but they also retain their immunogenicity.^{18,22-24} Viability and immunogenicity decline as the duration of wet storage at 4° C increases.²⁴⁻²⁶ The length of time the valve is sterilized before freezing (i.e., retrieval-to-preservation time) is thought to be an important determinant of immunogenicity. There may be a trade-off between retained structural integrity and retained immunogenicity, both of which are preserved by cryopreservation.

Viable endothelial cells are thought to provide the strongest antigenicity.^{24,27,28} Endothelial cells are the most susceptible to ischemic damage and, when stored in antibiotic solution, are nonviable after 24 hours to 14 days, depending on the type of donor and the length of ischemic time before processing.^{7,25,27} Lang and associates demonstrated

Table IV. Results of statistical analysis

Variables	p Value*	Test used
Continuous		
Young age at operation	0.03	Student <i>t</i> test
Low weight at operation	0.04	Student <i>t</i> test
Small graft size	0.04	Student <i>t</i> test
Donor age	0.32	Student <i>t</i> test
Valve ischemic time†	0.76	Student <i>t</i> test
Categorical		
Retrieval to preservation time <24 or >24 hr‡	0.02	χ^2
Graft type (pulmonic or aortic)	0.58	χ^2
Graft or host size mismatch§	0.58	χ^2
Blood group mismatch	0.05	χ^2
Previous cardiac surgery	0.88	χ^2
Donor type (organ or tissue)	0.44	χ^2
Use of Dacron	0.68	χ^2

*Rounded off to two decimal places.

†Time from heart cessation to valve incubation at 4° C in antibiotic and Hank's balanced salt solution.

‡Time from onset of valve incubation to onset of cryopreservation.

§Valve size >95th percentile for the patient's weight as described by Pacifico, Kirklin, and Blackstone.¹¹

that, after 12 to 24 hours of storage at 4° C before cryopreservation, endothelial cells of the homografts retained the capacity to proliferate, but those stored for 3 weeks at 4° C did not.²⁹ Although homograft immunogenicity has been demonstrated to be retained despite the attenuation of endothelial viability,²⁴ it is apparent that the precise nature of homograft valve viability and immunogenicity is not understood.

Five valves in our series had retrieval-to-preservation times of less than 24 hours. Four of the 17 valves that failed had retrieval-to-preservation times of less than 24 hours, and none of the competent valves had a time less than 24 hours. The fifth valve had an initial postoperative echocardiogram showing a 2+ insufficiency and was thus excluded from the analysis, although at the most recent echocardiogram it was judged to have 3+ insufficiency. Valves with short retrieval-to-preservation times (<24 hours) were significantly more likely to fail ($p = 0.02$).

Blood group incompatibility approached significance in the current series ($p = 0.05$) and was a significant predictor of failure in the multivariate analysis (see Table V). The significance of blood group incompatibility has long been postulated and its importance suggested by some,³⁰ although other studies have failed to demonstrate this.^{31,32} However, ABO matching has often been carried out to

Table V. Results of stepwise discriminant function analysis

Variable*	Partial r^2	F statistic	p Value
Retrieval to preservation time <24 hours	0.145	6.121	0.018
Graft size	0.143	5.857	0.020
Blood group mismatch	0.157	6.361	0.017

*These three variables together predicted 84% of the failures.

some extent,^{5, 7, 27, 33} and it is recommended by several investigators.^{24, 27} Homograft ischemic time was not shown to be a significant factor in valve failure in our series ($p = 0.76$), although the ischemic times were all quite short and relatively consistent (Table IV).

Results from earlier work with stored antibiotic-sterilized valves (i.e., fresh valves) appear to be at least as good and perhaps better than those achieved with the cryopreserved valves, particularly considering the longer follow-up.³³⁻³⁶ This may be the result of decreased antigenicity, which has been demonstrated in homografts stored for several weeks.^{24, 27} However, the great variability in patient selection, age, surgical technique, perioperative mortality, procurement and preservation techniques, and follow-up make a valid comparison impossible.³⁷ Our experience and that of others with cryopreserved homograft degeneration in young children has emphasized the need to further investigate the cause and mechanism of this early failure.^{5, 6, 16}

Factors other than rejection and growth must be considered in assessing the mechanisms of homograft valve failure.¹⁴ Geometric distortion has been recognized as a factor in aortic homograft replacement and is largely responsible for the switch from a subcoronary insertion to root replacement. This may be a factor in the pulmonary position as well. However, all the valves in our study were implanted as intact cylinders. Christie and Barratt-Boyes demonstrated that the loss of valve leaflet extensibility over time leads to increasing valve incompetence.³⁸ This could explain long-term failure, and the effect may result from decreased viability, immunologic rejection, or a combination of the two in conjunction with subtle geometric distortion. Although immunologic factors do not fully explain valve failure, they cannot be ignored as a factor in early homograft valve failure, particularly in young children.

Conclusions

The cryopreserved homograft is effective as a conduit between the pulmonary ventricle and the pulmonary arteries. However, the early development of significant incompetence limits its usefulness for protecting the ventricle from high pulmonary artery pressures when a competent valve is essential. Dysfunction of the valves is common and progressive over the first 6 to 12 months (see Fig. 5). This early failure is more in keeping with rejection than the inevitable tissue degeneration seen in late failure.³⁸ Small graft size was a significant factor in valve failure, but a valve larger than the 95th percentile for weight was not.¹¹ The practice of oversizing valves to allow for growth appears to be justified.³⁸ We found no difference in aortic versus pulmonary valves ($p = 0.58$), although pulmonary valves were used whenever possible; 33 pulmonary and 15 aortic valves were used.

Our finding of short retrieval-to-preservation time and blood group incompatibility as significantly associated with homograft valve failure lends some clinical support to the suggested immunologic nature of early homograft failure in children. Although some surgeons have used a variety of immunosuppressive regimens on small numbers of patients, the current state of knowledge does not seem to support this as a routine practice.^{16, 32, 39}

Continued work is required to assess the significance of the immune response to cryopreserved homograft valves. Some studies have shown an immune response to valves in the laboratory.²²⁻²⁴ The nature of this response needs to be characterized and methods to modify it should be assessed, initially in the laboratory and then clinically. Because of the small number of patients operated on at any single institution and the great variability in such a heterogeneous group of patients, multicentered randomized studies will be necessary to assess the efficacy of any clinical intervention. Interventions aimed at modifying the immunogenicity of the donor valve and the recipients response appear to be indicated.

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Discussion

Dr. Richard A. Hopkins (*Washington, D.C.*). I thank the Association for the opportunity to comment on this paper and the authors for providing me a copy of the manuscript before this meeting. I think this is an extremely important paper. I agree with the authors' premise for doing the study, and I agree with their conclusions. I think it is a very important biologic and clinical experiment, which sheds tremendous light on the biology of cryopreserved homografts.

I and my colleagues have had experience with about 200 homograft implants; the last 100 have been monitored, as your implants were, with every-6-month echo studies. Our results parallel your findings in terms of survival. However, we have only had two patients who required replacement for homograft failure in the right-sided position and only three others who developed increasing right ventricular pressures or high-grade insufficiency which ultimately required replacement. We had little difficulty with progressive or late regurgitation in the right ventricular outflow tract position. We had a freedom from structural failure rate of about 91%, as opposed to your 44%. Our replacement valves were assessed at 5.5 years, or 2000 days, and your valves were evaluated at about 1500 days. I think it could be illuminating to understand the differences in these two series, because the comparison could give us a clue about what to avoid in the future.

Your results confirm that young age and small graft size are risk factors, and we agree with this but not with whether the aortic or pulmonary valve is used. It is interesting that your failures seem to be in other than the truncus series, which means that the neonates do not seem to be a problem in your series. We agree with that finding, but it is at variance with other studies, and I would like you to comment on the neonatal issue.

Your finding that short retrieval-to-preservation times correlated with failure is the central and most important finding of your study. It supports our own contention that cryopreservation should be good but not too good. Your cold ischemic time after harvest but before the start of incubation in Hanks solution may be the key to this study. What fluid did you place these homografts in for the 4° C storage time? In other institutions where the processing is done off-site, storage is most often done in lactated Ringer's solution, which is our preference, because it effectively kills the endothelium.

We think the geometric factors are important ones and that the reconstruction must be performed to reduce hydraulic turbulence. We agree with your conclusion that oversizing is good as long as it does not lead to distortion. However, the proximal reconstruction is very important. Did you use polytetrafluoroethylene (PTFE)* hoods to smooth the fluid flow entry architecture into the conduit to optimize hydraulics and avoid turbulence just below the valve leaflets? Did you avoid distal Dacron graft extensions?

Were you able to examine your explanted valve leaflets for the presence of inflammatory cells, macrophages, fibrous sheathing, and most importantly, to look for preservation of donor matrix or endothelial cells? Have you been able to compare those with your homografts after the preservation techniques and before implantation as a comparison of the cell biology at the time of implantation?

I think this is a superb and critically important study. I applaud the authors for accomplishing it and the Association for selecting it for presentation. I greatly appreciate the opportunity to have discussed its contents.

Dr. Baskett. Thank you very much, Dr. Hopkins. Regarding the neonatal issue, we did not look specifically at the diagnosis; we looked at age as a continuous variable. Because it is quite a heterogeneous group and there are only 44 patients, we did not elect to look at the specific diagnosis because we would not have the statistical power to say anything about that subject.

With regard to the ischemic times, I welcome your comment that part of the problem may be that our valves are preserved too well with these very short ischemic times. When the valves are explanted, they are placed in Hanks balanced salt solution and then taken to the tissue bank, where they are stored at 4° C in Hanks balanced salt solution with the antibiotics that I listed.

We did not use PTFE in the reconstruction of the outflow tract. In most cases we used proximal extensions of autologous pericardium. In four of the older patients Dacron was used. Two of the valves with Dacron failed, and two of them did not, which was not a significant finding.

With regard to the explanted valves, the four valves that were removed were taken out 6 months to 2 years later. By the time they were removed, they were completely acellular. A pathologist reviewed the H&E stained slides of these valves and said there was nothing left to look at. Unfortunately, with a retrospective study you are not able to look at the explants.

However, we have established a rat model in our transplantation laboratory to look at what immunologic factors may be involved in the failure of the homografts. Perhaps if I could just show another slide?

(Slide) This is a section taken from an explanted valve that was implanted in the abdominal aorta of a rat. This is a syngenic graft at 8 weeks after implantation; and as you can see, there is some intimal thickening, but the valve leaflet itself looks quite normal.

(Slide) This slide is taken from a partially outbred rat and is partially allogeneic. At 4 weeks after implantation the valve leaflets are quite edematous and filled with a mononuclear type infiltrate.

These are some of the preliminary things that we are working on to qualify the immune response.

Dr. Patrick G. Hogan (Brisbane, Queensland, Australia). We are also interested in laboratory aspects of the immune response in a rat model and in human studies. The big problem is establishing the link between the data we have available for humans and whether the valve is actually damaged. Have you seen, given that you have got retrospective echocardiographic data, any suggestion of nodularity or thickening of the valve? If not, do you think that is something that could be looked for in the future?

Dr. Baskett. Thank you, Dr. Hogan. In the early echocardiograms that we reviewed (we had an initial postoperative one for each patient, usually within a week of his or her operation), the valves, except those we excluded, looked very good. As we monitored them, we could see an increase of insufficiency or transvalvular gradient, but we could not see any leaflet abnormalities at all. It is a very good point. Thank you.

Dr. Flaviano M. Lupinetti (Seattle, Wash.). I would like to clarify some of our previous work that showed that various neglectful methods of preservation could reduce but not abolish immunogenicity. It seems that the valve can run but it cannot hide. I notice that in your analysis three of the four factors related with failure—young age, small size, and small graft—were perhaps really the same factor, and perhaps an analysis of covariance would have shown that only one of those was truly related to valve failure.

I wonder if there was also a difference in the duration of ischemia in the smaller valve such that an analysis of covariance or a multivariate analysis may have eliminated the ischemic time as a factor leading to valve degeneration?

Dr. Baskett. Thank you, Dr. Lupinetti. We did perform a multivariate analysis, and with your permission, I will show it. We performed a forward stepwise discriminate function analysis for the variables I listed. As Dr. Lupinetti mentioned, the age, graft size, and weight of the patient are related. When you run these factors in a multivariate analysis, graft size comes out as the most significant of the three; when you control for it, the other two drop out. The three variables—retrieval-to-preservation time less than 24 hours, small graft size, and blood type mismatch—were all significant in the multivariate analysis. Ischemic time was not.

*Gore-Tex hoods, registered trademark of W. L. Gore & Associates, Inc., Newark, Del.