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Changes in the mechanical properties of bioprosthetic valve leaflets made of bovine pericardium, as a result of long-term mechanical conditioning *in vitro* and implantation *in vivo*

P. ZIOUPOS
Department of Biology, University of York, York, UK
J. C. BARBENEL
Bioengineering Unit, University of Strathclyde, Glasgow, UK
J. FISHER
Mechanical Engineering Department, University of Leeds, Leeds, UK
D. J. WHEATLEY
Department of Cardiac Surgery, Glasgow Royal Infirmary, Glasgow, UK

The long-term performance and function of bioprosthetic valves constructed from glutaraldehyde-fixed bovine pericardium depend on the leaflet material retaining its original properties. Prolonged mechanical conditioning or implantation in the human body may alter the mechanical properties, the geometry and the function of the leaflets. Studies of the leaflets of fatigue-cycled valves and explanted valves showed that the leaflets have changed their shape due to permanent deformation. In addition, the leaflets of the explanted valves were thickened. Examination of the mechanical properties of the leaflet tissue showed that the material of both the fatigue-cycled and explanted valves had reduced extensibility compared to the material of uncycled leaflets, which had not been implanted. Such changes may adversely affect the valve function and may also accelerate valve failure in the long term. Analytical description and statistical treatment of the mechanical behaviour of the leaflet tissue showed that it was possible to discriminate between implant and explant tissue and less satisfactorily between uncycled-control and fatigue-cycled tissue.

1. Introduction

The long-term performance of bioprosthetic heart valves is dependent on the flexible leaflets maintaining their properties throughout the lifetime of the valve in the recipient. Mechanical and/or biological modification of these properties *in vivo* can have a detrimental effect in valve function and durability. The examination of the material properties of the leaflet tissue has been an important stage in the development of these valves. However, the effects on the mechanical behaviour of the tissue of prolonged cycling and of implantation in the human body are not yet well established. Extensive cycling of strips of porcine valve material has been shown to make the tissue stiffer and less extensible [1]. The fatigue cycling of strips may, however, not represent the loading experienced by a leaflet in a functioning valve. The preparation of the strips may also interfere with the integrity of the tissue. There is no information available on the effect of long-term mechanical conditioning of bovine pericardial valve tissue. In the present report we describe the mechanical behaviour of leaflet tissue from valves, which have been fatigue-cycled and compare it to appropriately matched uncycled control valves.

The effect of implantation on porcine valve leaflets has never been examined. There are only preliminary data on bovine pericardium which suggest that it may stiffen and thicken after implantation under non-functioning conditions [2]. The changes in hydrodynamic function of explanted valves, which had normal clinical function, has been described in an earlier report [3]. In the present report we describe the mechanical properties of the leaflets of these explanted valves, and of unimplanted control valves of the same type.

The purpose of our investigation was to differentiate the effect of long-term fatigue loading from long-term biological modification on the valve leaflet tissue and also to examine the effect that either/or both of these factors have on the valve's performance in the long term.

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2. Materials

2.1. Uncycled control/fatigue-cycled valves

The fatigue-cycled leaflets came from four pericardial valves (27 mm size) of an improved design [4], which had been cycled in accelerated fatigue-tests (Rowan Ash tester) at 12 Hz for at least 400×10^6 cycles (equivalent to 10 years life) in the laboratory. The valves were subsequently taken apart, the leaflets inspected and only those which had no signs of wear and/or tearing were subjected to tensile tests. The control group of uncycled leaflets came from the same type and size of valves as the fatigued leaflets. They had not been cycled after manufacture and thus the tissue had no history of deformation.

2.2. Implant control/explanted valves

The explanted valves came from a population of 32 pericardial valves explanted in the Glasgow Royal Infirmary over a period of two years [3]. These valves had been implanted for periods between 13 and 84 months. Six of these valves had been considered to function normally when examined clinically with Doppler ultrasound and echocardiography prior to explantation. These six valves were replaced in double-valve reoperations for reasons of precaution along with a failed valve, or because of perivalvular leaks. The selection criterion for the tensile tests was the absence of leaflet tears or calcification. The leaflets finally selected were free from macroscopic calcification and tears, and when viewed with X-rays they also appeared free from calcification in the body of the leaflet. They came from size 29/31 mm Ionescu-Shiley pericardial valves that had been implanted in the mitral position for periods between 20 and 73 months. The control group for these explant leaflets consisted of leaflets originating from Ionescu-Shiley valves (hereafter called implants) of the same size as the explant valves.

3. Methods

The fatigue-cycled and control valves were hydrodynamically tested in a pulsatile flow simulator [5] at a normal cardiac output of 4 l min^{-1} and a rate of 70 beat min^{-1} and their performance assessed on certain parameters of root mean square forward flow (RMS), mean pressure difference, regurgitant volumes during closure and when fully closed, and effective orifice area. These values were determined in the same way as for the explanted valves, the hydrodynamic performance of which has been reported elsewhere [3]. All fatigue-cycled and explanted valves were morphologically examined, photographed and their macroscopic description recorded. They were then stored in a saline solution at 4 °C.

All leaflets were viewed by polarized transmitted light microscopy [6] in order to determine if there was any systematic orientation of the collagen fibres in respect to the two main directions of the leaflet (radial or circumferential). The thickness of all leaflets was measured at the belly and free edge in the saline bath by the use of a Mitutoyo thickness gauge after the pads had been in contact with the tissue for 5 s.

For the tensile tests, 3 mm wide parallel-sided strips were cut circumferentially (parallel to the free edge of the leaflet), two strips from each leaflet (Fig. 1). The leaflets were placed on light card and then the strip specimens were prepared simultaneously using a multiblade cutter. In total 24 strips were prepared from leaflets from the fatigue-cycled valves, 22 strips from the uncycled control valves, and 14 from each of the implant and explant valve groups. The card and tissue strips were mounted vertically in an Instron tensile test machine with a gauge length of 15 mm. The card was removed and the tissue strips cycled at an extension rate of 5 mm min^{-1} between the original gauge length and a peak load of 1 N. All tests were performed in physiological saline bath at 37 °C. A first load point of 1 mN was identified in the first cycle and the fifth, conditioned, cycle. The nominal stress/extension ratio curves were obtained this way.

First, the behaviour of the leaflet tissue in terms of its stiffness and its extensibility at certain prescribed load levels was examined. Subsequently, a simple analytical description of the stress/stretch curves was attempted for all tissue strips and the resulting model parameters were subjected to statistical discrimination analysis.

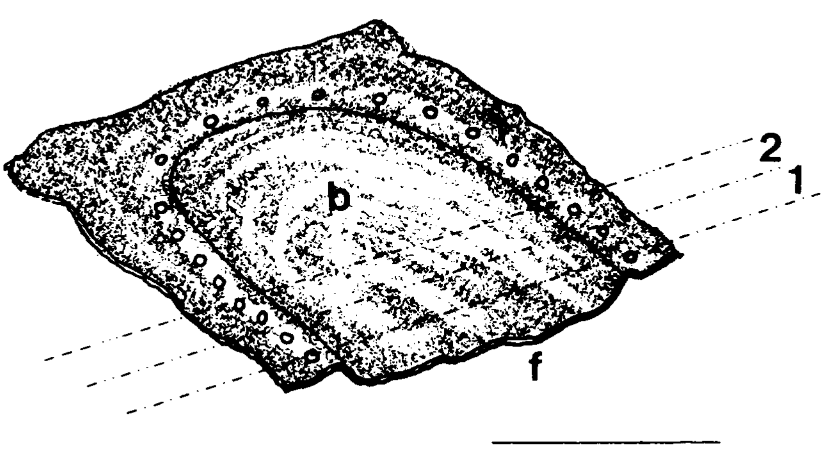

Figure 1 Two strips were prepared from each leaflet; strip 1 was near the leaflet's free edge, f, strip 2 was nearer the leaflet's belly, b.

The loading/unloading behaviour of soft collagenous tissues forms a hysteresis loop indicating a dissipation of energy in the tissue. From the loops we examined, the percentage of the total energy input during deformation that was dissipated in each cycle, and the energy/unit volume dissipated in each cycle. Student's t-test of statistics at a standard level of significance $p = 0.05$ was used for all comparisons throughout this report.

4. Results

Morphologically the fatigue-cycled leaflets appeared to sag and be permanently extended, thus presenting larger dimensions and an enlarged leaflet area to the blood's forward flow. This was accompanied by a small reduction in thickness (Table I) which was not statistically significant.

The explant leaflets presented extensive fibrous overgrowth. Their outflow surface, adjacent to the frame, was covered with a thin layer of fibrous tissue which extended 1–2 mm on to the flexing portion of the valve leaflet. This produced a restricted asymmetrical orifice for forward flow. The unloaded explant leaflets also appeared extended and were thickened (Table I). The use of polarized light microscopy confirmed that the orientation of the preferential direction of the collagen bundles, which normally exists in the pericardium [6], was absolutely random in respect to the two main directions of the leaflet (radial/circumferential) in all four groups of leaflet tissue. The existence of a preferential orientation in the collagen fibres results in the presence of an anisotropic material, which possesses a predominant direction in its mechanical properties. Inspection of the leaflets originating from the same valve and from different valves indicated that the material had been treated as if it were isotropic during the construction of all the valves of the present study. In the hydrodynamic performance tests no major changes were seen in the function of the fatigued valves after cycling in the laboratory for 400×10^6 cycles compared to the characteristics of the uncycled valves. However, the geometry of the leaflets had been altered by permanent sagging. In contrast, as shown elsewhere [3], all the hydrodynamic parameters that characterize the function of valves, were significantly different for the explanted valves compared to controls of the same type, reflecting a deteriorating function after implantation. There were significant changes in the mechanical behaviour between the groups under comparison. Table II shows the average extension ratio values at a peak load of 1 N for strips from fatigue-cycled and control valve leaflets. The statistics showed that: (i) at manufacture the extensibility of strip 1 (nearer the leaflet's free edge) did not differ from strip 2 (nearer the leaflet belly), and (ii) that fatigue cycling induced a significant difference only between the properties of strip 2 between uncycled and fatigue-cycled valve leaflet tissue. This indicated that the manufacturing procedure produced strips 1 and 2 with similar properties, but strip 1, which was included in the leaflets' coaption area was protected from the effects of fatigue cycling, while only strips 2 were adequately stretched during cycling. Consequently, it was decided that only the mechanical behaviour of strips 2 should be examined for all four groups of tissue in the present report. These explant tissue strips were significantly less extensible than the implant tissue strips (Table II).

The nominal stress/extension ratio curves had an exponential shape and this suggested that plots of the incremental modulus of elasticity versus the stress level might be linear. The nominal stress/extension ratio curves were digitized and as a result there was some scatter in the modulus/stress plots. However, they appeared in general to be straight and linear regression lines had correlation coefficients in the range 0.945–0.989 for the uncycled and between 0.948 and 0.989 for the fatigue-cycled tissue. The slope of the modulus/stress curves, also called Fung's modulus [7], reflects the convexity of the curve and it can be

TABLE I Thickness values prior to tensile tests. Leaflets originate from four valves for each of the fatigue-cycled and uncycled control groups, and from three valves from each of the implant and explant groups. One leaflet was lost in the control group and two leaflets from each of implant–explant groups during the preparation

	Thickness (mm)			
	Control	Fatigue-cycled	Implants	Explants
	0.50	0.45	0.64	0.75
	0.47	0.48	0.64	0.72
	0.37	0.45	–	–
	0.45	0.38	0.40	0.48
	0.49	0.42	0.40	0.55
	0.48	0.42	–	–
	0.44	0.54	0.45	0.70
	0.42	0.45	0.45	0.80
	–	0.44	0.45	0.80
	0.44	0.41		
	0.45	0.46		
	0.44	0.38		
	$n = 11$	$n = 12$	$n = 7$	$n = 7$
Mean	0.45	0.44	0.49	0.69
S.D.	0.036	0.044	0.105	0.124

t-tests at a level of significance $p = 0.05$ gave: (i) control/fatigue-cycled, not significantly different, (ii) control/Ionescu–Shiley implants, not significantly different, (iii) implants/explants, significantly thicker explants.

TABLE II Extension ratio values at peak load of 1 N

	Position of strip	Mean	S.D.
Control			
$n = 11$	1	1.221	0.061
$n = 11$	2	1.229	0.061
Fatigue-cycled			
$n = 12$	1	1.214	0.004
$n = 12$	2	1.119	0.028
Implants			
$n = 7$	2	1.254	0.027
Explants			
$n = 7$	2	1.202	0.031

TABLE III Fung's moduli over the whole stress range, at the stress/stretch origin and incremental modulus at 1 N peak load presented as means with the standard error in parentheses

	Control	Fatigue-cycled	Implants	Explants
Overall Fung's modulus	27.5 (1.57)	31.4 (1.63)	26.7 (2.20)	35.7 (3.16)
Fung's modulus at the origin	27.1 (3.54)	36.7 (2.49)		
Incremental modulus at 1 N (MPa)	20.3 (1.61)	25.5 (2.03)	20.0 (2.77)	18.2 (1.04)

seen along with the incremental moduli at a 1 N load level in Table III.

Although strips from fatigued leaflets were less extensible, the Fung's moduli, which were produced for the whole stress/stretch curve were not different to the uncycled control, but they were so in the low stress region below 0.2 MPa. Separate regression lines in the low stress region gave Fung's moduli $\alpha_{\text{uncycled}} = 27.1$ significantly lower than $\alpha_{\text{fatigued}} = 36.7$. The incremental moduli at peak load (1 N) were also not different. Both these findings indicated that the curve had stiffened mostly in the near-origin low-stress region. Comparison between implant and explant tissue gave a Fung's modulus significantly higher for the explants indicating an overall stiffening effect over the whole stress range. However, the incremental moduli for implants/explants were again not different at peak load of 1 N mainly because of the increased thickness of the explants, which produced lower nominal stress values.

The satisfactory linear relationship between moduli and stress was translated into a stress/stretch relationship by the use of the exponential formula

$$s = \beta \{\exp[\alpha(\lambda - \lambda_0)] - 1\} \quad (1)$$

where λ is the extension ratio (stretch), λ_0 being the extension ratio at the first detectable load (1 mN) on the strip, α is the Fung's modulus and β is a scaling constant in MPa units. Such a relationship has been suggested in the past for collagenous tissues [8] and similar forms have been used for pericardia of various species [9–13] and valve tissue in particular [14]. The fit for all four groups of tissue was performed in two steps: (i) α was assigned the average value of the slopes of the modulus/stress lines within each group, (ii) linear regressions of the quantity $\{\exp[\alpha(\lambda - \lambda_0)] - 1\}$ versus stress were performed and the slopes yielded an average value for β. The fit produced with this method on the averaged behaviour within a group, together with the individual stress/stretch traces can be seen for all four groups in Figs 2 and 3.

The loading/unloading hysteresis loops showed that the percentage of total input energy, that was dissipated in the tissue was higher in the first cycle than in the following cycles for all four groups of tissue. It has been suggested [15] that the amount of energy dissipated in the first loop is a function of the degree of preconditioning of the material, more preconditioning leading to a decrease in the energy dissipated in the first loop. Indeed, in the present tests the energy/unit volume in the first loop of the uncycled control tissue was found to be higher (12.2 kJ m^{-3}), but not significantly different from that in the first loop of the fatigue-cycled tissue (9.1 kJ m^{-3}), which following fatigue cycling had been left to rest for 3 days before the tensile tests. This indicated that the material has a fading memory regarding the extensive long-term fatigue cycling. On the other hand, it was found that the percentage of energy dissipated in the conditioned loop of the fatigue-cycled tissue strips (27.1%) was significantly higher than in the conditioned loop of the uncycled material (17.9%). In this respect the overall stiffening of the stress/stretch curve had an adverse effect on the performance of the tissue.

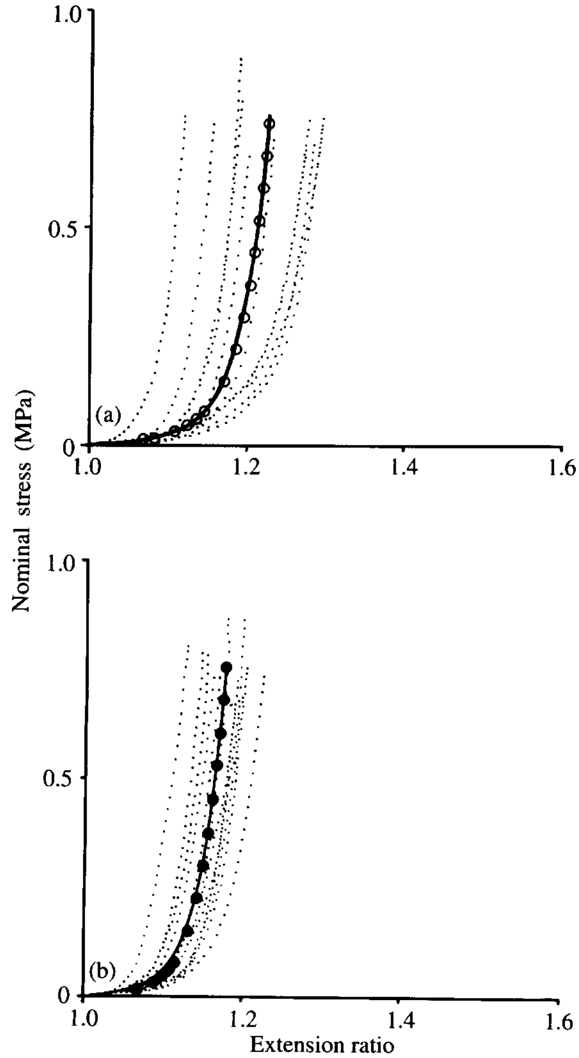

Figure 2 Stress/extension ratio curves for all (a) control and (b) fatigue-cycled strips (···), their average behaviour ((○) control; (●) fatigue cycled), and its description (—) by use of Equation 1. Average parameter values: control, $\alpha = 27.5$, $\beta = 1.38 \times 10^{-3}$ (MPa), $\lambda_0 = 1.0014$; fatigue cycled, $\alpha = 31.4$, $\beta = 2.79 \times 10^{-3}$ (MPa), $\lambda_0 = 1.0003$.

5. Discussion

5.1. Valve and leaflet function

Pericardial heart valves were introduced into clinical use in 1973, and showed improved hydrodynamic and

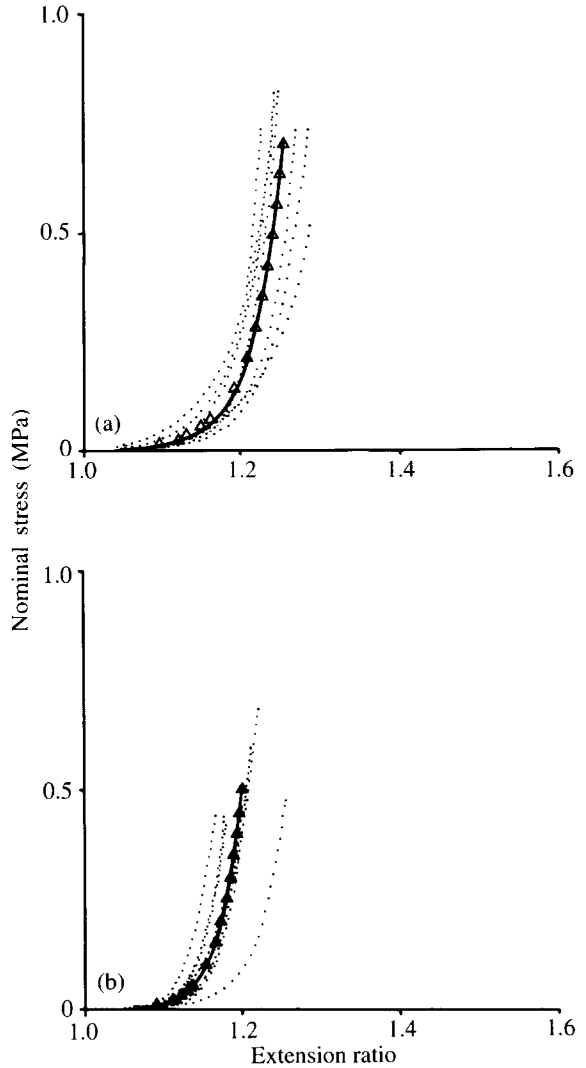

Figure 3 Stress/extension ratio curves for all (a) implant and (b) explant strips (···), their average behaviour ((△) implant; (▲) explant), and its description (——) by use of Equation 1. Average parameter values: implants, $\alpha = 26.7$, $\beta = 0.80 \times 10^{-3}$ (MPa), $\lambda_0 = 1.0004$; explants, $\alpha = 35.8$, $\beta = 0.43 \times 10^{-3}$ (MPa), $\lambda_0 = 1.0036$.

short-term haemodynamic function compared to porcine bioprostheses. In recent years the incidence of early failure in pericardial valves in the absence of calcification has caused concern to many surgeons and in some instances caused valves to be withdrawn from further clinical use. Recent improvements in pericardial valve designs [4, 16] have been introduced in an attempt to reduce the incidence of early valve failure, and it is expected that calcification will be the predominant factor affecting the long-term durability of these valves, as it is for all soft-tissue valves. If the patients are to benefit from the superior hydrodynamic function of the pericardial valves, then their haemodynamic function has to be maintained throughout the lifetime of the valve or at least until macroscopic calcification has a marked effect on valve function.

Examination of the hydrodynamic performance of fatigue-cycled valves revealed that these valves showed little change in their function after 400×10^6 cycles in the laboratory durability tests in a sterile solution of glutaraldehyde. However, implantation markedly affected the hydrodynamic performance of the valves, which showed increased pressure difference, restricted and asymmetric leaflet opening. Both the fatigue-cycled and the explanted material showed similar reduction in extensibility. However, in the laboratory the reduced extensibility of cycled valves did not have a marked effect on valve function, indicating that the altered hydrodynamic function of the explanted valves could be due to the fibrous tissue overgrowth and leaflet thickening. Leaflet thickening *in vivo* was probably caused by absorption of proteins and constituents of the blood into the collagen matrix. The pressure needed to buckle a spherical shell is proportional to the thickness cubed, and thus leaflet thickening presumably increases leaflet bending stresses and valve opening pressures. Fibrous tissue growth around the edge of the frame similarly affected function by preventing full leaflet opening and reversal of the leaflet curvature. Considerable efforts are made during pericardial valve design and manufacture [4] to ensure synchronous leaflet opening and a symmetrical orifice for forward flow, and the behaviour of the valve in this respect depends critically on the properties of the leaflet material.

The examination of the mechanical properties of the leaflet material showed that modification can occur in relatively short periods of time, especially *in vivo*. The most consistent measure to express the alteration occurring *in vitro* and *in vivo* was the extensibility of the tissue at a specified load level. Most of the change occurred in the low stress region (< 0.2 MPa) as indicated by the increased incremental modulus of elasticity in this region. When the fatigue-cycled leaflets were observed in the light microscope, a small reduction in the crimping of the collagen fibres was evident and this was in agreement with similar qualitative observations on porcine leaflet tissue [1]. Thus the degree of permanent elongation of the leaflets was probably due to alterations in the fibre geometry in the most compliant region of low stress for the tissue. This meant that the same measure, the reduction in extensibility, is also expressing the amount of permanent extension (sagging) of the leaflets.

5.2. Discriminant function analysis

The use of the simple exponential formula (Equation 1) allowed a first quantitative description of the modification of the material properties. However, as is usually the case in tissue mechanics, analytical descriptions are often under examination as well as the material properties themselves [13]. Parameters α, β were treated as variables of a bivariate function and the distinction between groups was examined by discriminant analysis. A successful discrimination between two populations should be capable of separating the domain of (α, β) pairs in two territories. Discriminant analysis is always calculated from the observed data and does not require knowledge of the underlying probability mechanisms. It is recommended, though, especially with the use of a linear discriminant function, that suitable transformations of the original data may be performed in order to improve the performance of the function. This was accomplished by using the natural logarithms of α and β and thus rendering linear the initially non-linear envelopes of the bivariate function (Fig. 4).

A SPSS-X statistical package was used to produce linear discriminant functions. The linear discriminating boundary tacitly assumes that the covariance matrix is the same for both populations and by looking at Fig. 4 one can see that this is probably true with only the envelope of the uncycled-control tissue assuming a different shape from the rest.

However, the control valve leaflets of this study had not been subjected to quality control, which would have rejected leaflets with properties greatly different from the average. The performance of the discriminant function was 65% correct classification for the control/fatigue-cycled and 75% for the implant/explant groups.

Wilk's lambda (Λ, equivalent to F-ratio in univariate statistics) for control/fatigue-cycled had a level of significance of $p = 0.13$ rejecting the hypothesis that the two groups were different at $p = 0.05$. On the contrary, implant/explants had a level of significance $p = 0.03$ and thus the two groups could be positively discriminated.

In the manufacture of modern bioprosthetic valves, computer-aided design of the leaflet geometry and finite element methods (FEM) are employed ever more often. A prerequisite for FEM is the production of the constitutive equations for the material. In this respect, Fig. 4 presents in the simplest possible way the description of the mechanical behaviour of the leaflet material before and after extensive cycling and also before and after implantation. This should considerably assist in designing valves of improved function and durability, because the magnitude and distribution of stresses in the leaflet have a key role in the long-term survival of the leaflet.

Modern pericardial valves allow a considerable degree of intervention either in the choice of the leaflet's shape [4] or in the preparation of the material. Studies have confirmed that fixation under stress or stretch produces a stiffer material [17–19]. In the present uncycled control valve leaflets, it was observed that a correlation of $r = -0.903$ existed between the incremental modulus at peak load 1 N and the extension ratio values at this level. These leaflets had been constrained to take the shape of a mould and thus they were under a variable degree of prestretch during fixation. It was also surprisingly clear that the effect of extensive cycling depended on the position of the strip on the leaflet. Studies on the distribution of stresses in valve leaflets, especially by the use of FEM [20, 21], point out the in-homogeneity of the stress field. We show here for the first time, that this field not only exists but also has a marked effect on the local properties of the leaflet. It may be possible in the future to construct bioprosthetic leaflets with heterogeneous properties over the leaflet area by successfully controlling local restrictions at fixation or even exploiting inherent material anisotropy [6].

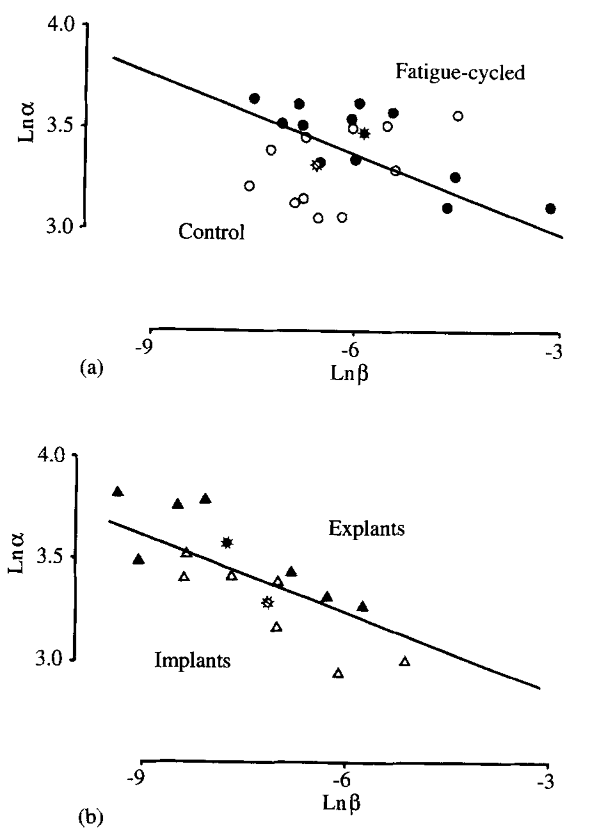

Figure 4 Linear discrimination between groups on the natural logarithms of parameters α, β; (*) average behaviour. The discriminant functions were: (a) control/fatigue-cycled, $4.936 \ln \alpha + 0.623 \ln \beta - 12.814 = 0$; (b) implants/explants, $7.714 \ln \alpha + 0.942 \ln \beta - 19.302 = 0$.

6. Conclusions

The long-term performance of pericardial bioprosthetic valves and their material was examined after fatigue cycling and implantation. It was found that both valve and material will continue to operate for longer (> 10 years) time periods with no obvious changes in the hydrodynamic performance of the valve, provided that the material escapes the classic modes of primary tissue failure such as the development of holes and tears caused by poor valve design. However, the quality of the material itself deteriorates, producing leaflets with altered geometry, which are permanently sagged, with increased stiffness and increased dissipation of energy per cycle. After implantation these same mechanical changes will occur plus a biological modification, which includes fibrous tissue overgrowth and leaflet thickening, which adversely affect the hydrodynamic performance of the valve. Application of discriminant analysis on the parameters expressing the stress/extension ratio behaviour of the tissue before and after the modification, showed that the course of modification may be predictable and this is valuable for valve design in the future.

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