A comparison of antibiotic-sterilized, stent-mounted pulmonary and aortic valve allografts in the mitral region of dogs

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Monro, J. L., Gavin, J. B., and Barratt-Boyes, B. G. (1974). *Thorax*, **29**, 323–328. A comparison of antibiotic-sterilized, stent-mounted pulmonary and aortic valve allografts in the mitral region of dogs. The mitral valves of 40 dogs were replaced with antibiotic-sterilized, stent-mounted semilunar valve allografts. Twenty grafts were pulmonary valves and 20 were aortic valves. Six dogs in each group died from causes related to the operation. All remaining dogs with pulmonary valve grafts died of causes related to the allograft itself (vegetative endocarditis (5), peripheral leak (1), cusp rupture (4), cusp shrinkage (4)). In the aortic valve group there were seven deaths from allograft endocarditis and one from a peripheral leak, but six dogs had competent allografts when sacrificed up to 12 months after surgery. It is concluded that the inherent strength and bulk of the aortic valve cusps make this valve a more suitable mitral valve replacement than the more delicate pulmonary valves.

As an alternative to prosthetic valves with their thromboembolic problems, aortic allograft valves have been used with success in large numbers in the aortic region (McDonald *et al.*, 1968; Barratt-Boyes, 1971; Yacoub, Knight, Towers, and Somerville 1973; Layton *et al.*, 1973) and to a lesser extent in the mitral region (Graham, Schroeder, Daily, and Harrison, 1971; Lennox *et al.*, 1971; Barratt-Boyes *et al.*, 1972; Angell, Wuerflein, Chun, and Shumway, 1973). The behaviour of the aortic valve when used as a mitral valve replacement has also been fairly extensively investigated in animals (Howard, Willman, and Hanlon, 1960; Heimbecker *et al.*, 1962; McKenzie *et al.*, 1966; Suzuki and Kay, 1966; Angell, Wuerflein, and Shumway, 1967; Hubka, Siska, and Holec, 1967; Weldon, Ameli, and Morovati, 1967; Braunwald, Fuchs, and Bonchek, 1968; Sugie *et al.*, 1969).

When used in the mitral position the semilunar valve should be at least 24 mm in internal diameter, for with small valves pressure gradients are present in adult patients (Barratt-Boyes *et al.*, 1972). While aortic allografts of greater diameter

than this are unusual, particularly from young donors, larger diameter pulmonary valves are common, and this valve is virtually always free from disease. Accordingly, it was decided to assess the behaviour of the pulmonary valve in the mitral position in dogs. The only previous comparable experimental studies are those of Lower, Stofer, and Shumway (1960), who used first the autologous pulmonary valve and later the untreated pulmonary valve allograft (Lower and Shumway, 1963) to replace the mitral valve. These studies indicated good graft function over a two-year period.

This paper describes a study which compares pulmonary valve allografts with aortic valve allografts used to replace the mitral valve in dogs.

MATERIALS AND METHODS

Aortic and pulmonary valves were dissected under sterile conditions from the hearts of donor dogs and stored in Hanks's balanced salt solution containing, per millilitre, 50 units penicillin, 1 mg streptomycin, 1 mg kanamycin, and 25 units amphotericin B. Before insertion the valve allograft was trimmed and mounted under sterile conditions onto a stent constructed
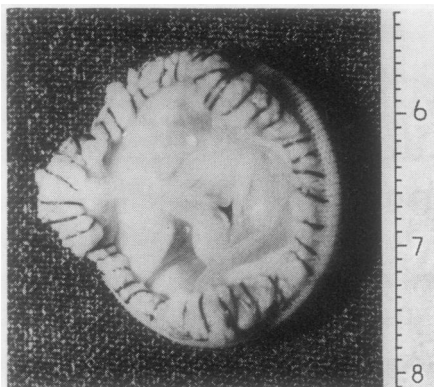
FIG. 1.   *A canine pulmonary valve sutured onto the stent ready for insertion. The stent is constructed of stainless steel wire covered with Dacron cloth.*

of stainless steel wire covered with Dacron cloth (Fig. 1). In each case the valve was tested for competence under pressure (Barratt-Boyes *et al.*, 1972). The valves were kept in the antibiotic solution for eight to 255 days before use and all were proven to be sterile at insertion.

The 40 dogs used varied between 15 and 35 kg in weight. Each was anaesthetized with sodium methohexitone and ventilated with halothane, nitrous oxide, and oxygen. Thoracotomy was performed through the right fifth space and cardiopulmonary bypass instituted using a blood and Ringer's lactate prime and a disposable bubble oxygenator. Left atriotomy was performed and the mitral valve excised. The new valve was inserted with about 24 interrupted non-absorbable sutures and the atriotomy was closed.

Postoperatively all dogs were given twice daily penicillin, 1 mega unit, and streptomycin, 0·5 g, for eight days, or longer if indicated. Heparin (5,000 units twice daily) was given intramuscularly for seven days starting the morning after operation. The dogs were kept quiet for the first postoperative month but were then sent to kennels where they were very active. Auscultation was performed at regular intervals and 10 dogs underwent angiography.

Necropsy was performed on all dogs and the heart was removed for examination. Note was made of any blood in the chest, pleural effusions, the state of the lungs, and any abnormal finding. The valve was photographed and placed in phosphate-buffered 5% glutaraldehyde solution at pH 7·4. Seven-micron, paraffin-embedded serial sections of each valve cusp were stained with haematoxylin and eosin, van Gieson's stain collagen, Weigert's stain for elastin, phosphotungstic acid haematoxylin for fibrin, and Gram's stain for bacteria.

RESULTS

Twenty animals received a pulmonary valve allograft and 20 received an aortic valve allograft. In each group there were six deaths at operation or within the first two postoperative days, most commonly from postoperative bleeding.

The outcome in the group receiving pulmonary valves is shown in Table I. Of the 14 operative survivors, five animals developed a vegetative endocarditis, four in the first three weeks and one later. In four of these cases bacteria were

T A B L E I
DOGS RECEIVING PULMONARY VALVES

Cause of Death	No.
Operative death	6
Vegetative endocarditis	5
Large peripheral leak	1
Cusp rupture	4
Cusp shrinkage	4
Total	20

identified in the blood or in histological sections but in one they were not. Early in the series, one dog was exercised on the seventh postoperative day and died next day from a large perivalvular leak. The remaining eight dogs were well initially but all died from valve failure. Four developed a cusp rupture (Fig. 2) with sudden central incompetence and survived an average of 68 days. The other four developed thinning and shrinkage of the cusps (Fig. 3), which caused progressive central regurgitation, and survived an average of 258 days.

In the group receiving aortic valves (Table II) there were also 14 operative survivors, of which seven developed a vegetative endocarditis, two early and five late. In four of these a bacterial
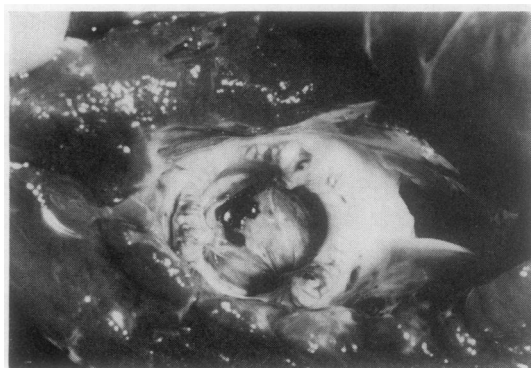

FIG. 2.   *A stent-mounted pulmonary valve seen from the ventricular side from a dog that survived 61 days. One cusp ruptured, causing gross central regurgitation.*
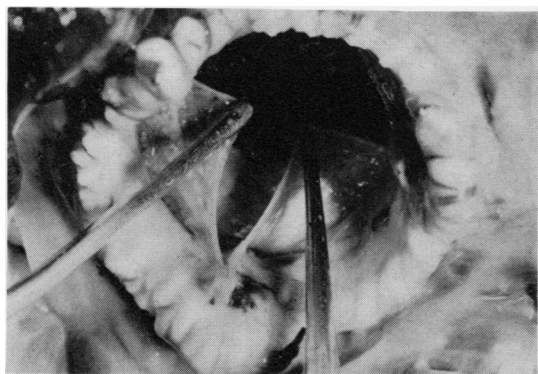
FIG. 3. *The cusps of this pulmonary valve (held open with probes) have become extremely thin and shrunken. The dog survived 135 days and died from severe central regurgitation.*

TABLE II
DOGS RECEIVING AORTIC VALVES

Cause of Death	No.
Operative death	6
Vegetative endocarditis	7
Large peripheral leak	1
Sacrificed when well	6
Total	20

FIG. 4. *A left ventricular angiogram showing the metal stent seated in the mitral ring. There is no mitral incompetence and the aorta is seen filling with contrast.*

TABLE III
TIME BEFORE SACRIFICE OF SIX SURVIVING DOGS RECEIVING AORTIC ALLOGRAFTS AND DURATION OF STORAGE OF ALLOGRAFTS IN ANTIBIOTIC SOLUTION BEFORE INSERTION

Dog No.	Survival (days)	Duration of Storage (days)
21	367	8
22	367	102
28	269	205
36	143	45
37	129	22
40	94	8

aetiology was confirmed histologically or by blood cultures. One animal died at 33 days due to a large peripheral leak. The other six animals remained clinically well without mitral regurgitant murmurs and each had an angiogram shortly before sacrifice which confirmed valvular competence (Fig. 4). These six valves were examined at intervals ranging from three to 12 months (Table III). In each case the valves were competent with well aligned cusps (Fig. 5) which were very slightly thinner than normal, but this feature did not vary noticeably between those in position for three months and those in position for one year. In no case had the valve pulled away from the stent and all valves were well accepted into the atrium. Behaviour was not related to the duration of storage before use (see Table III).

Histologically the pulmonary valve showed a host response along the interface between the arterial sleeve of the graft and the host tissue which had proliferated through the stent. This was characterized by the presence of lymphocytes and macrophages and a slow replacement of parts of the arterial wall by host connective tissue. Host tissue also proliferated over the intimal surface of the graft from its margin toward, and sometimes onto, the cusps (Fig. 6). The endothelial cells and
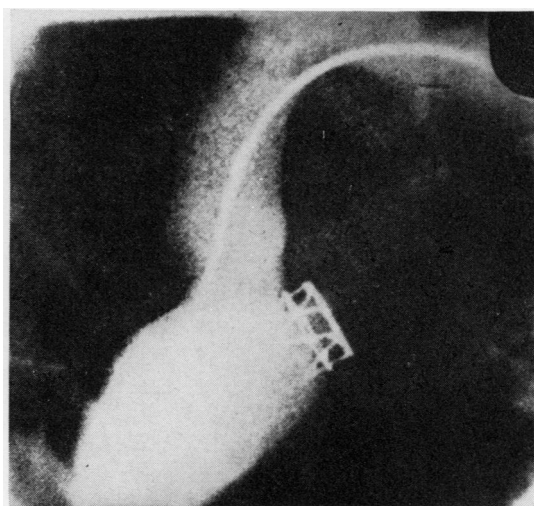

FIG. 5. *A structurally intact aortic valve removed for examination at 129 days and shown by angiogram to be competent.*
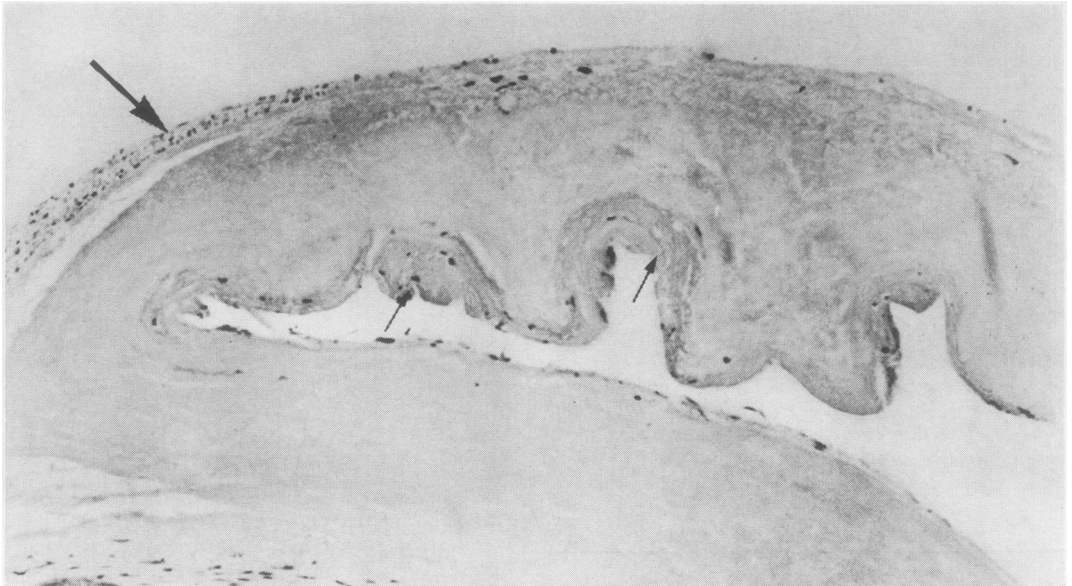
FIG. 6. *Light microscope view of one cusp of the valve seen in Figure 5. The cusp is virtually acellular except for the intimal fibrous sheath tapering onto its base (large arrow) and a few inflammatory cells in the vicinity of deposits of fibrin (small arrows) on its convex surface. The fibrous structure of the cusp is well maintained. (Haematoxylin and eosin ×165.)*

fibroblasts of the graft disappeared, leaving the graft tissue virtually acellular (Fig. 6) apart from the scattered focal infiltrates of fibrin, inflammatory cells, and macrophages. These macrophages appeared to ingest both the intracuspal fibrin and the fibrous matrix of the cusp. They were most numerous in the vicinity of cusp rupture. The collagen and elastin fibres of the graft valves were well preserved except for some disruption in the vicinity of the cellular infiltrates. The aortic valves had inherently more robust cusps but in all other respects were histologically similar to the pulmonary valves. These histological findings are described and discussed in greater detail elsewhere (Gavin and Monro, 1974).

DISCUSSION

This study has clearly shown that the antibiotic-treated dog pulmonary valve allograft, although initially competent, invariably failed when placed in the mitral position. This is in marked contrast to the aortic valve allografts which, apart from those which developed vegetations, functioned well up to one year after insertion.

Allograft failure was due to incompetence, but the mechanism varied between endocarditis, cusp rupture, and cusp shrinkage. The high incidence of endocarditis observed in this study is similar to that reported by other workers in this field (Berghuis *et al.*, 1964; McKenzie *et al.*, 1966; Weldon *et al.*, 1967; Buch, Kosek, Angell, and Shumway, 1971) and is presumably due to the bacteraemias which frequently occur in dogs (Nelson and Noyes, 1954; Das and Rush, 1965). Fortunately, endocarditis is a rare complication in man (Clarkson and Barratt-Boyes, 1970).

Cusp rupture has been the main cause of late allograft incompetence in man and on histological study the mechanism seems to be similar in dogs to that demonstrated in humans (Gavin, Herdson, and Barratt-Boyes, 1972). Cusp shrinkage, on the other hand, had not been recognized as a cause of late incompetence in man either with pulmonary or aortic allografts and its exact cause in the dog is uncertain. In transferring these experimental results to man considerable caution is therefore required. It does, however, seem likely from follow-up of our patients receiving antibiotic-treated pulmonary valve allografts for mitral valve disease that late cusp rupture is relatively common (Barratt-Boyes *et al.*, 1972) and that, as suggested by the present experimental study, this valve is therefore unsatisfactory in the high-pressure mitral position.

The method of allograft valve preparation is now recognized as vitally important. In the present experimental study the same antibiotic solution as that in use clinically since 1968 (Barratt-Boyes, 1971) has been employed. With this method, tissue culture (Girinath, Gavin, Strickett, and Barratt-Boyes, 1974) and electron microscopic studies (Gavin, Monro, Wall, and Chalcroft, 1973c) indicate that the fibroblasts are structurally damaged and following even short periods of contact with the antibiotics they do not survive implantation in the host for longer than a few weeks (Gavin, Herdson, Monro, and Barratt-Boyes, 1973b). Persistent normal function of the leaflet is thus dependent primarily on the integrity of the fibrous tissue. On a longer term basis the growth of a host intimal fibrous sheath onto and into the leaflet will give further support. As in this study, which extended to 12 months only, the intimal fibrous sheaths had rarely grown as far as the cusps, we conclude that it was the inherent strength and bulk of the aortic cusp which enabled them to withstand both the haemodynamic load and the focal depredations of macrophages better than the more delicate pulmonary cusps.

Other workers have suggested that permanent survival and proliferation of the transplanted fibroblasts in the leaflet is necessary for satisfactory long-term function and that this can be achieved only by avoiding antibiotics completely (untreated or 'fresh' grafts) or using lower antibiotic concentrations for shorter periods of time (Angell *et al.*, 1967, 1973). Our detailed histological studies of antibiotic-treated, untreated, and chemically treated grafts in humans convince us that the good long-term function of antibiotic and untreated allografts is related to the integrity of the ground substance of the leaflet and ultimate leaflet replacement by proliferating host fibroblasts (Gavin *et al.*, 1972, 1973a, 1973b).

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