

Mitral valve replacement in dogs using pig aortic valve heterografts

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Mitral valve replacement using pig aortic valve heterografts has been performed in 27 dogs, siting the grafts in the 'atrial position'. Buffered acid formaldehyde sterilization offered the advantages that it is simple and, by de-naturing the proteins of the graft, may minimize 'rejection' phenomena. It may offer some self-sterilizing property to the graft within the host post-operatively. The question whether heterograft valves will ultimately calcify remains unanswered. The aortic valve has been shown to function satisfactorily in place of the mitral valve for up to four months, producing normal haemodynamic studies. The 'atrial position' of the graft has the advantage that the left ventricular cavity becomes totally available for its pumping activities. The fate of these animals depended upon developing mitral incompetence around the grafts and thrombus, leading to mitral stenosis, rather than to rejection phenomena. The operation described requires accuracy in orientating the graft. Mounting heterografts (with almost the whole of the aortic wall cut away) in a Dacron-covered metal frame pre-operatively provided a valve in a range of sizes which can be inserted in the same manner as any prosthesis in current use. Methods of sterilization and storage and the ultimate fate of heterografts *in vivo* require further study. Failure such as calcification will probably not develop suddenly. Such a valve could be replaced if necessary. The continued investigation and clinical use of heterografts appears to be justified when viewed against the uncertain outcome of the other methods of valve replacement.

The perfection of cardiopulmonary bypass techniques in recent years has enabled the surgeon to expose, excise, and replace any of the four heart valves. The problem facing cardiac surgeons at the present time is which type of replacement valve will serve their patients most effectively in their future lives. There are three alternative valves available.

The manufactured prosthesis casts the shadow of thromboembolism across the patient's future life. Anticoagulation is generally prescribed for these patients, but this carries its own problems in regulating adequate dosage and brings its own complications, which may be of serious consequence. The recently described problem of 'ball variance' may prove to be of considerable importance in view of the large numbers of such valves which have now been inserted in patients the world over. Recent improvements in the prostheses, covering metallic parts with cloth to encourage cellular infiltration and the replacement of the sialastic ball with a metallic ball in some valves, may minimize these objections.

Secondly, there is the homograft valve, whose value has been effectively demonstrated by D. N. Ross of London (1962; 1966) and Barratt-Boyes of Auckland (1964; 1965). The ultimate fate of these grafts, with the possibility of eventual calcification, is as yet unknown, as indeed is the longevity of the prosthetic devices.

Because of the difficulty in obtaining suitable supplies of homografts in some countries a third alternative, the heterograft valve, is currently under study in some centres.

It is not necessary to tabulate the advantages and disadvantages of the prosthetic and 'tissue' valve at length. The main appeal of the latter over the former is that a more physiological flow pattern occurs across the grafted tissue valve, and anticoagulation is not required post-operatively.

The technique of inserting homografts and heterografts in the subcoronary position in the aortic root is established (Duran and Gunning, 1962). It has been clear for some time that the incidence of thromboembolism is higher when prostheses are inserted in the mitral position as compared with the aortic. The need for a valve graft to replace the damaged mitral valve appealed

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to us as a necessary field for study. Others have attempted to replace the mitral valve in animals and in humans with mitral homo- and heterografts (O'Brien and Gerbode, 1964; Berghuis, Rastelli, Van Vliet, Titus, Swan, and Ellis, 1964; Hubka, Šiška, Brozman, and Holek, 1966). At the present time this technique is not acceptable for human valve replacement, since stenosis or incompetence has resulted in a high proportion of cases. The chief obstacle which remains is the manner in which the grafted chordae tendineae may be firmly attached to the recipient's papillary muscles without resultant disruption or fibrosis and subsequent chordal shortening.

The following considerations prompted the present study. First, the mitral valve does not lend itself well to grafting (vide supra). Secondly, the mitral annulus in disease varies considerably in size, and a range of valve grafts may be required from the replacement valve bank. Thirdly, animal valves, if accepted by the human body, are more available than human valves. Fourthly, attempts to graft the aortic or pulmonary valve into the mitral annulus, positioning the graft within the body of the left ventricle, have not been satisfactory. The graft occupies too much space and disruption of the pseudo-chordae from the wall of the left ventricle or roots of papillary muscles have caused failures (McKenzie *et al.*, 1966; O'Brien and Gerbode, 1964; Hubka *et al.*, 1966). More promising results have been obtained by placing grafts of aortic and pulmonary valves from the mitral annulus upwards into the left atrium in the so-called atrial position (Lower, Stofor, and Shumway, 1961; Suzuki and Kay, 1966). This appears to have certain advantages. The whole of the left ventricular cavity is left empty and is available to deliver an adequate stroke volume to the systemic circulation. This may be of critical importance in some cases of mitral stenosis, where the cavity of the left ventricle is particularly small. In some cases of pure mitral incompetence, or in mixed mitral valve lesions, the left atrium may reach cavernous size. In these there is an abundance of space between the inferior pulmonary veins and the mitral annulus which might with advantage house the valve graft. The work of Suzuki and Kay (1966) was not published at the time the present study was begun, but their encouraging results gave us added confidence that this procedure was worthy of further investigation.

OPERATIVE TECHNIQUE

Twenty-seven dogs (weighing 17.2 to 38.6 kg.) under-

went mitral valve replacement, with the use of pig aortic valve heterografts. The first 14 dogs acted as a pilot study. These heterografts were sterilized and stored in β -propiolactone and some muscle was left on the graft below the base of the cusps. This constituted a hazard, as will be described. The dogs were anaesthetized with intravenous nembutal and the blood donors with methohexitone sodium (Brevital). An endotracheal tube was passed and positive pressure ventilation was used throughout the operation delivering room air. One hundred per cent oxygen was administered once bypass had been discontinued. The left femoral artery was exposed for cannulation for the return of oxygenated blood from the heart-lung machine. The right femoral vein and artery were cannulated for pressure monitoring. A standard left thoracotomy was made along the upper border of the fifth rib. The pericardium was opened in front of and behind the phrenic nerve and a tape was passed around the nerve to retract it. The right atrial appendage was accessible and a single venous return catheter, which drained by gravity to the heart-lung machine, was inserted. The Gerbode-Osborn-Bramson disc oxygenator or the membrane lung was used with a prime of blood and up to one-third dilution, using Hartman's solution, correcting acidosis with sodium bicarbonate before perfusion started. Once bypass had begun the pulmonary artery was cross-clamped and positive pressure respiration was cut down as much as possible to minimize the return of pulmonary venous blood into the left atrium. The left atrial appendage was opened between stay sutures after the heart had been electrically fibrillated. It was found unnecessary to use a left ventricular vent. The mitral valve was excised by dividing the chordae tendineae from their papillary muscles and then incising the mitral valve cusps circumferentially, leaving a reasonable rim of cusp base attached to the mitral annulus. This increases the strength of the soft mitral annulus for holding sutures. The annulus was then measured with graded obturators carrying tri-radial marks (Fig. 1). The graft, which was prepared as a cylinder of aortic root with the coronary ostia previously oversewn, was turned inside out (Suzuki and Kay, 1966). Three double-ended sutures were placed into the graft, one just above each commissure. The graft was then placed below the mitral annulus within the cavity of the left ventricle, inside out and upside down. Each pair of needles carrying these sutures were passed through the annulus from the ventricular to the atrial aspect at the points indicated by the tri-radial obturator. When these sutures were ligated, the graft was drawn up and secured beneath the annulus. Each single needle was then passed between the graft and the annulus away from its commissure to meet its fellow at the mid-commissural point, where the two approaching sutures were ligated. This method of suturing enabled the surgeon to visualize the cusp itself and to prevent piercing it with the suture. Thus, the first or annular suture-line was completed (Fig. 2). The graft was then pulled back through itself into

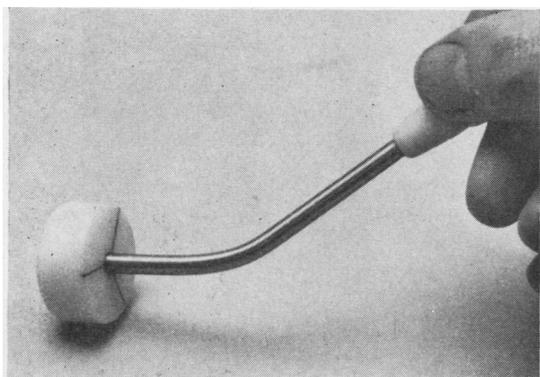


FIG. 1. *Obturator, 2.6 mm. diameter, carrying tri-radiate marks.*

the left atrium. Saline was injected from a syringe and needle into the cavity of the left ventricle. This displayed the orientation and competence of the graft cusps. The second 'atrial' row of fine interrupted silk sutures was then placed, taking care not to obstruct the outflow of the pulmonary veins (Fig. 3). The left atrium was then closed with a double layer of continuous suture material, leaving a small cannula in its cavity for post-operative pressure monitoring. Air

was removed from the heart. Heparin (given at a dosage of 3 mg./kg.) was neutralized with protamine sulphate in three divided doses. The heart was electrically defibrillated. The venous cannula was withdrawn and the right atrium was closed. The left femoral artery was sutured to maintain its patency, because we found that these animals became mobile more quickly post-operatively than those in which it had been ligated. Before the chest was closed with two drainage tubes, the simultaneous left ventricular and left atrial pressures were recorded to demonstrate the graft's function (Fig. 4). Both pleural cavities were drained for 12 hours post-operatively, because a considerable volume of blood was almost invariably found to be present within the right pleural sac. Once the chest had been closed, the animal was placed in a standard hospital cot with the endotracheal tube in situ, but positive pressure respiration was then discontinued. The endotracheal tube was withdrawn as soon as the animal began to gag on it. Continuous monitoring of the E.C.G. arterial and venous pressures and left atrial pressures was done. Blood-gas analyses were performed at intervals during the first 12 hours post-operatively. No post-operative sedation was required. Many of these animals operated on during the morning were drinking the same evening, the chest tube and pressure-recording cannulae could then be spigotted off, apart from intermittent readings of vital signs during the first night, and were all

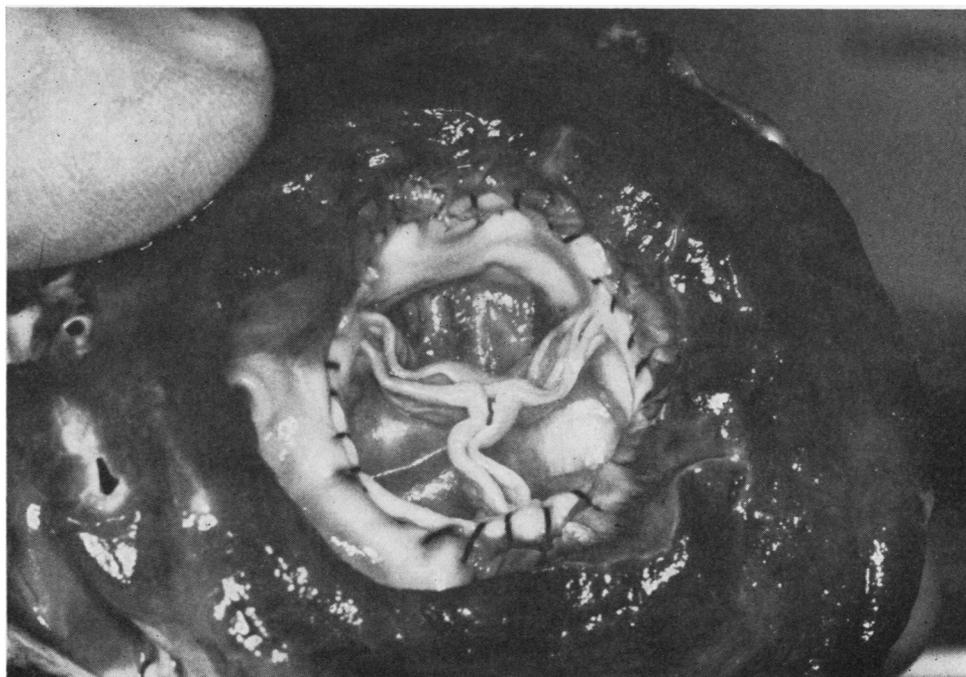


FIG. 2. *First suture line to mitral annulus seen from left ventricle. Aortic root on left.*

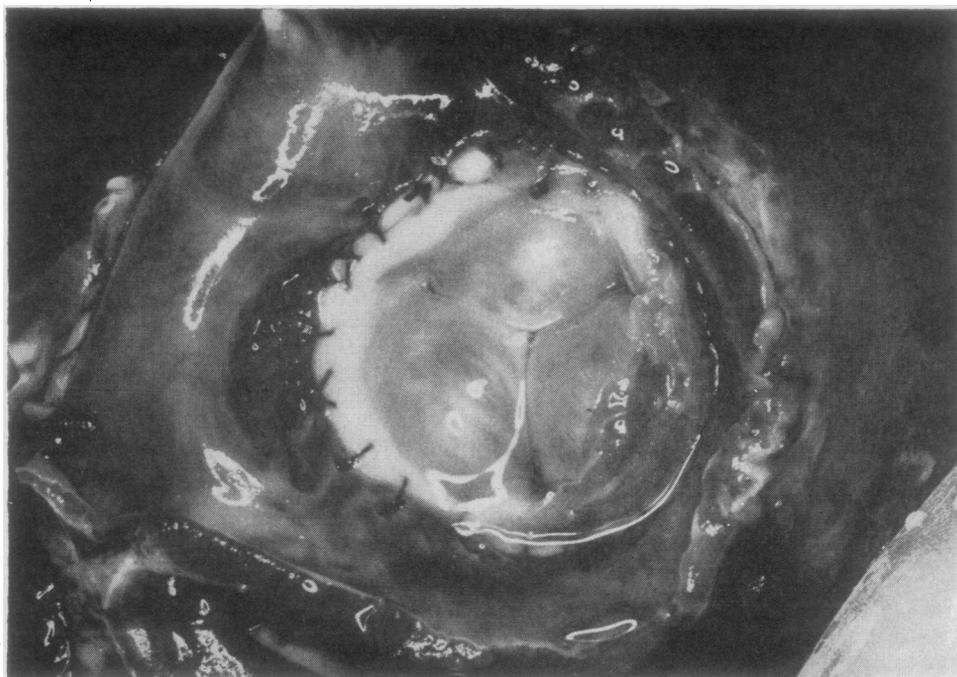


FIG. 3. *Second atrial suture line completed. Pulmonary vein opening on to graft on left side.*

removed next morning. Usually the animal was returned, often without help, to its kennel on the first post-operative day.

Enough encouragement was obtained from the first 14 dogs, including one four-week survivor, to warrant further studies. The main causes of death were due to haemothorax (the incidence of which was diminished on reducing the concentration of heparin in the flushing solution to 1,000 units per 500 ml. solution) and to mitral incompetence due to peripheral leaks developing alongside the graft where sutures had either cut out of the dog's soft mitral annulus or

away from the muscle left on the graft which had been sutured to the atrial wall, or both.

PREPARATION OF THE GRAFT At this time O'Brien (1967) published work on the establishment of an aortic valve heterograft bank using buffered acid formaldehyde. As the solution both stiffens and denatures the graft, it was thought that it might offer some advantages over β -propiolactone. The pig aortic heterograft was therefore modified and a standard type of graft was inserted in the following 15 experiments. Once this valve had been developed, normal haemodynamic traces were achieved across the valve after its insertion (Fig. 4). The preparation of the graft was extremely important, and this was time-consuming. The root of the aorta with the proximal $\frac{1}{4}$ in. of the coronary arteries and a collar of left ventricular outflow tract muscle, together with the aortic leaf of the mitral valve, was removed from the pig heart on the morning of slaughter (Fig. 5). The aorta was divided $\frac{1}{4}$ in. above the top of the commissures, to be trimmed accurately later. A 6 'O' Tevdek suture was passed through each lunule at the centre point of each of the three cusps, and this was lightly ligated. This suture was held long and supported the central point of cusp apposition during the stage of packing the cusps and sinuses of Valsalva with cotton-wool. This ensured accurate coaptation of the cusps and resulted in a competent graft during formaldehyde fixation.

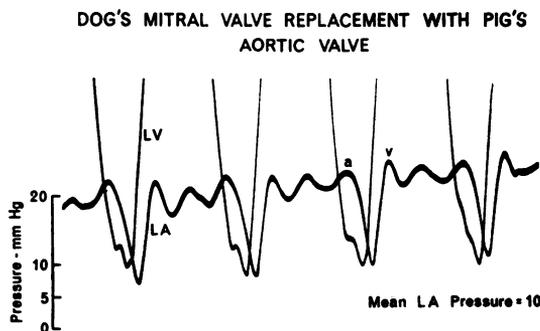


FIG. 4. *Simultaneous left atrial, left ventricular pressures.*



FIG. 5. Pig aortic root continuous with aortic leaf of mitral valve.

The whole was placed in buffered acid formaldehyde for one week. After this time the valve was washed in saline after the cotton-wool had been removed with the suture. This part of the preparation was performed in unsterile conditions. Using sharp, slightly curved scissors, with the index finger or thumb of the left hand within the sinus of Valsalva to stretch the cusp tissue taut, every piece of muscle was trimmed off the base of the cusps. This removed unwanted foreign tissue but also freed the base of the cusps, giving the valve a wider aperture and greater availability of functioning cusp tissue. The aortic leaf of the mitral valve was trimmed off, leaving a convenient portion of sub-aortic curtain available for suturing into the left atrium. The aorta was trimmed carefully to within 2 mm. of the tip of the commissures, making the graft as short in length as possible. To provide a strong suture ring for holding the second row of sutures to the left atrial wall, a piece of aortic wall or pulmonary artery wall (approximately $\frac{1}{4}$ in. square in cross section) was sutured to the graft wall with a horizontal mattress suture beneath the base of the cusps (Fig. 6). The graft circumference was measured on a tapered gauge and the details were entered in the graft bank log book. The valve was then returned to a new sterile jar containing fresh buffered form-

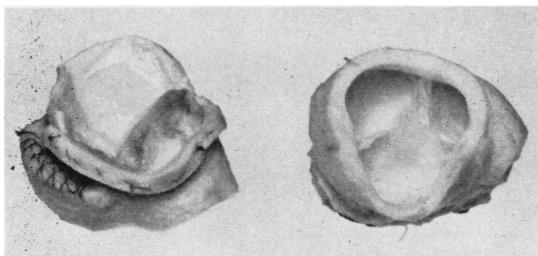


FIG. 6. Modified pig aortic valve ready for transplantation.

aldehyde solution and was labelled and placed ready for use in the valve bank.

RESULTS

The total series consisted of 27 valve replacements: 24 survived operation (89%); 9 survived one week or more (33%); and 5 survived four weeks or more (18.5%). Of the last 9 dogs which had satisfactory perfusions, 100% survived operation; 66% lived over one week; 44% lived over four weeks; and 22% survived up to four months.

The longer survivors were used for haemodynamic, catheter, and cine-angiographic studies. Many of these animals were well initially, and up to four months in two cases. The single frame taken from one cine-angiogram shows the width of the graft and its position in the left atrium (Fig. 7). The cusps can be visualized and these



FIG. 7. Cine-angiogram at $7\frac{1}{2}$ weeks. L.V. catheter passed down aorta sited below mitral annulus. Competent graft outlined with dome of aortic cusps demonstrated.

are mobile and competent. The right heart injection showed good opacification and emptying of the left atrium across the valve graft. This study was performed seven and a half weeks after operation. The macroscopic appearance of this graft soon after the cine-angiogram was taken was as shown in Figures 8 and 9. The cusps were thin and mobile and were competent to pressure within the left ventricle. Histological studies showed the absence both of infection and of foreign body reaction, or of rejection phenomena (Figs 10, 11, and 12).

The main cause of death in these later experiments was thrombus formation in and around the

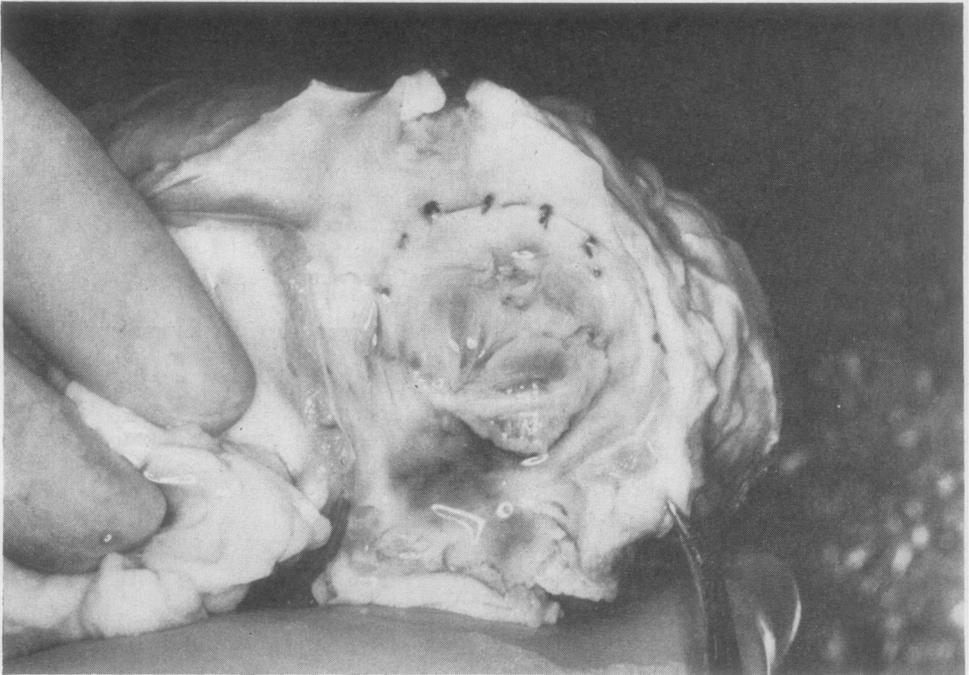


FIG. 8. *Appearance of graft from left atrium at 7½ weeks.*

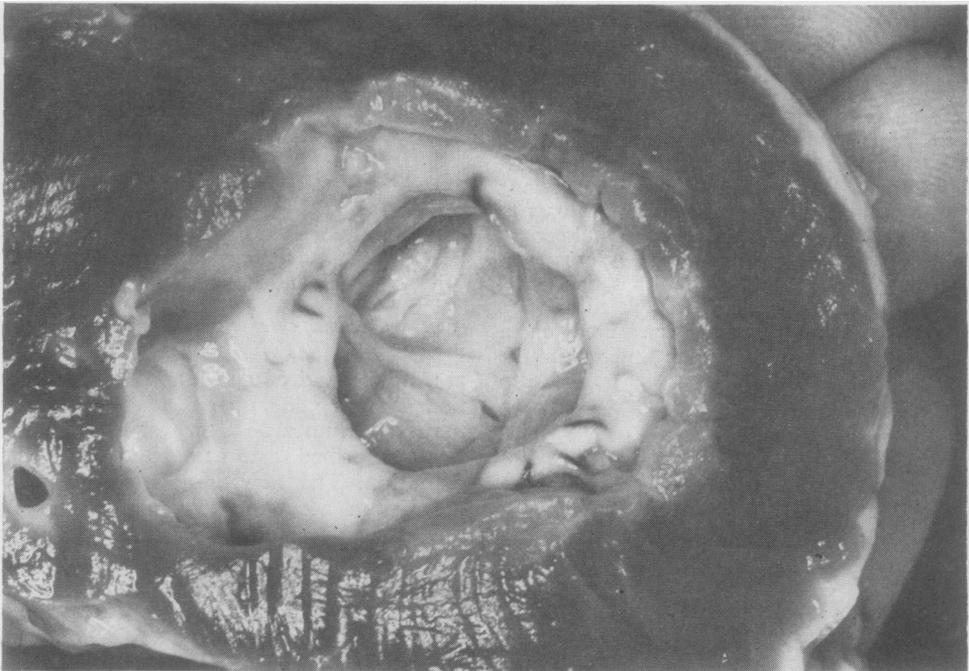


FIG. 9. *Appearance of graft from left ventricle at 7½ weeks. Most of the suture line is covered, with no evidence of thrombus formation.*

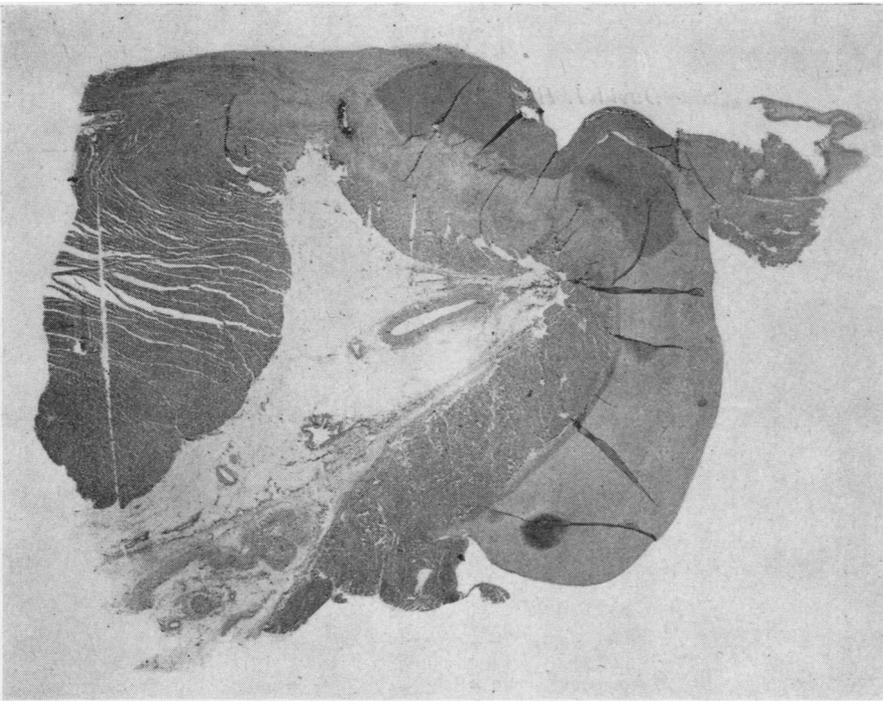


FIG. 10. *General appearance of graft applied to mitral annulus and left atrial wall. Cusp on upper right.*

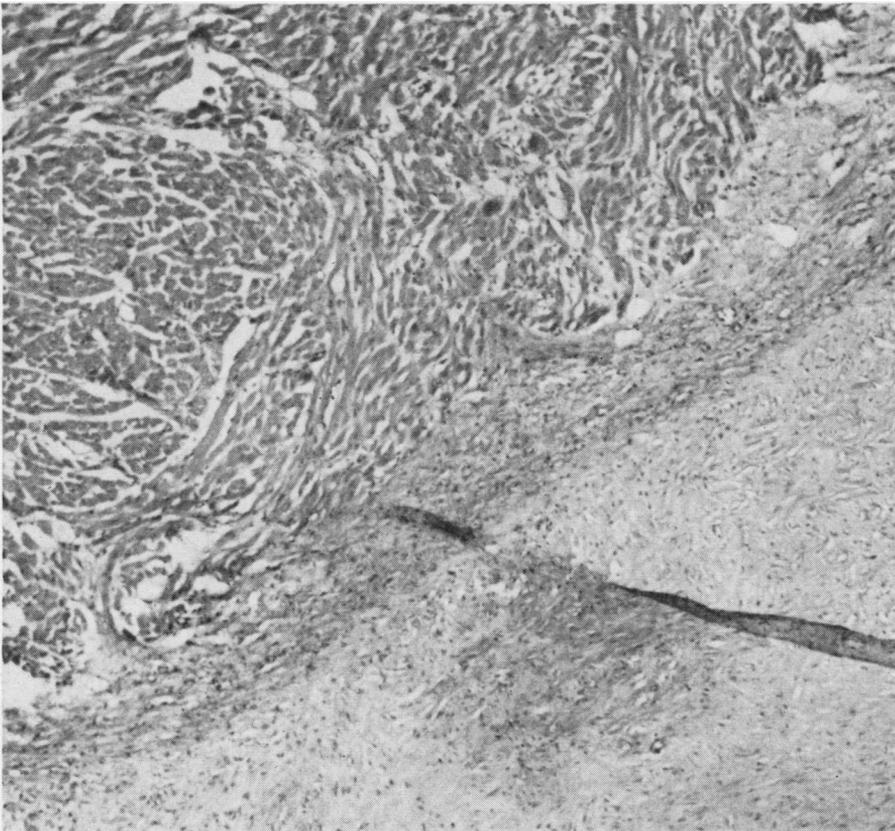


FIG. 11. *Graft junction with cardiac muscle above and left.*

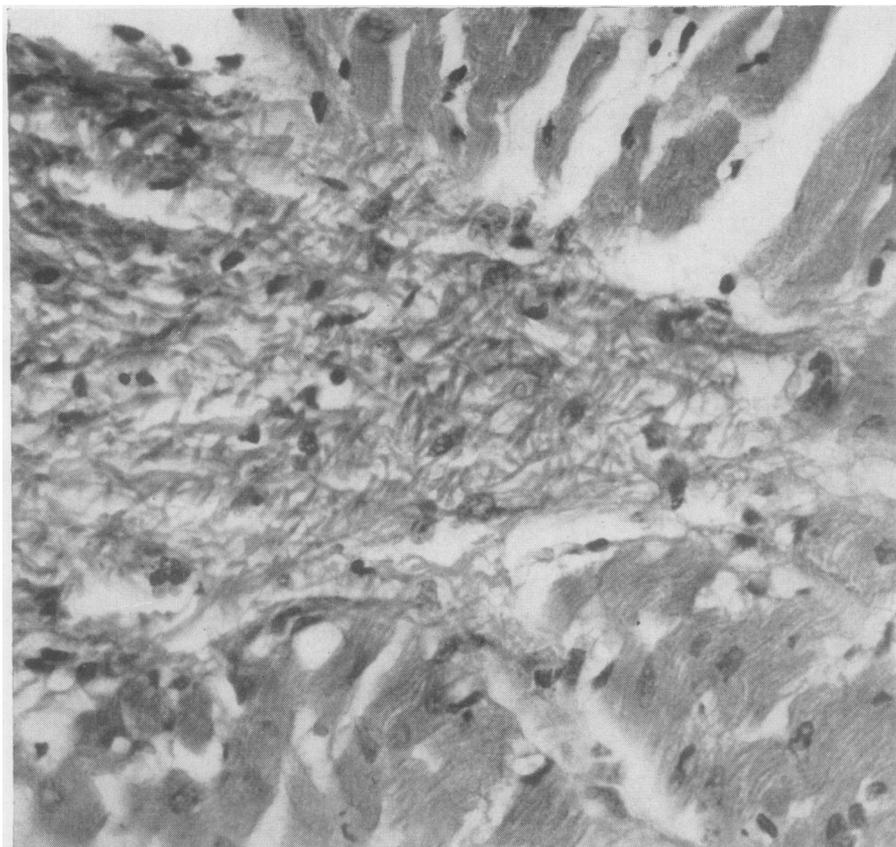


FIG. 12. *High-power view of junctional zone between cardiac muscle above and below graft tissue.*

grafts. Some of these thrombi appeared to start in the crypts left when the coronary arteries were oversewn, even though this amounted to no more than a shallow slit. Thrombus, once established here, had propagated sufficiently to obstruct the valve orifice. Other thrombus had formed on sutures in the left atrium.

DISCUSSION

This study in itself does not offer any valuable information on the longer-term fate of the heterograft. It does suggest, however, that the aortic valve (and possibly also the pulmonic valve) can be suitably adapted for replacing the mitral valve and that normal haemodynamics can be achieved across such grafts. The larger size of the heterograft annulus necessary in the mitral area, compared with the host's aortic root, is shown in Figure 13. Those who remember the fate of using

formaldehyde-prepared free aortic grafts for replacing resected aneurysms in the past, some of which developed calcification, speak with foreboding of the likely fate of these heterografts now under study. We believe that the method of fixation of these valves (formaldehyde) turns the cusps into non-viable membranes and that the possibility of rejection may therefore be reduced. Others advocate that the advantages of the tissue valve be amalgamated with the facility with which the prosthetic valve may now be inserted, using a single row of sutures to the mitral annulus.

THE MOUNTED GRAFT We have therefore studied methods of mounting heterografts supported by a stainless steel ring completely covered with Teflon or Dacron velour, which is moulded into a suture flange. These materials encourage cellular infiltration. We have inserted such valves in patients using a similar technique to that recently

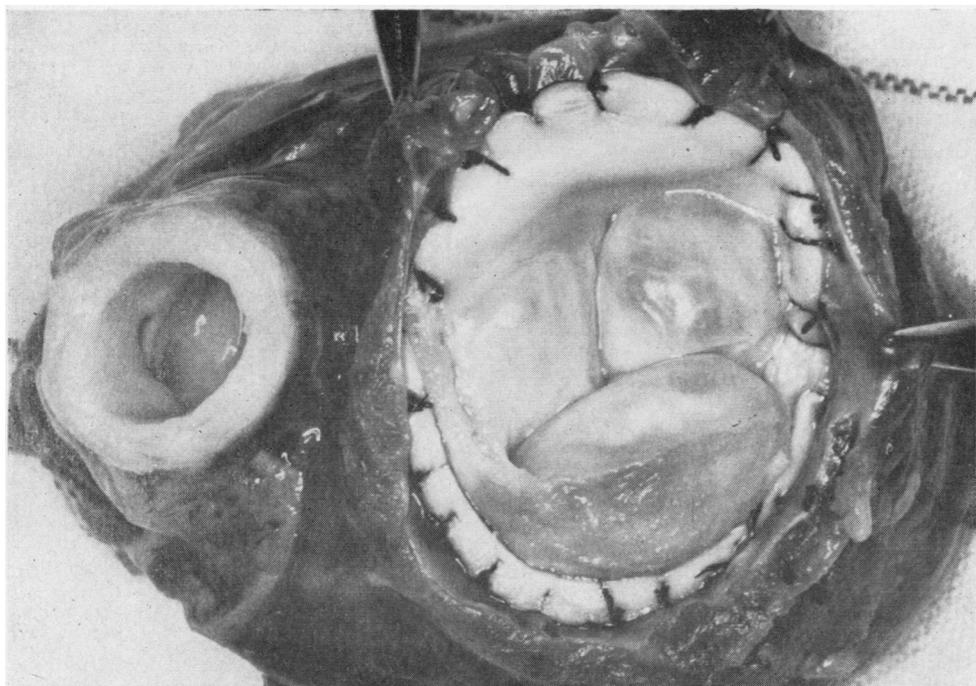


FIG. 13. Comparative size of aortic heterograft in mitral annulus against the normal dog's aortic valve.

described by Ionescu and Wooler in Leeds (Ionescu, Wooler, Smith, and Grimshaw, 1967). Further laboratory trials are at present in progress. One such patient has died of a myocardial infarction six months post-operatively. This was apparently unrelated to the 'prosthesis', which was well incorporated and was free of thrombus. The valve cusps showed good pliability with no stiffening and no calcification. We are impressed by the rapid pick-up of the cardiac action in these patients on discontinuing bypass, and this may be due to the minimal resistance to flow through the graft and to the unobstructed left ventricle, which is free to deliver an adequate stroke volume through the aortic valve at each beat. The present titanium, Dacron velour-covered frame in use in Leeds offers the advantage that all of the aortic wall tissue comprising the sinuses of Valsalva and the coronary ostia are trimmed away to within 2 mm. of the cusp tissue. Once mounted, these grafts consist of cusp tissue and very little other donor tissue. The flange suture ring enables the valve to be inserted in precisely the same manner as any other prosthetic valve, using a single row of interrupted sutures. The present clinical series

already reported in the world literature (O'Brien *et al.*, 1967; Ionescu *et al.*, 1967) and our own observations lead us to consider that there is promising evidence for continuing investigations in this field. Much work remains to be done in studying the best method of mounting the aortic heterograft (or homograft) so that the frame surrounding it is well incorporated with the tissues of the heart. The other question requiring further elucidation is which is the best way of sterilizing and storing tissue grafts for transplantation.

This study was made in the Laboratory of the Institute of Medical Sciences, Presbyterian Medical Center, San Francisco, California, U.S.A., whilst one of us (D. I. H.) was holder of the Comyns Berkeley Travelling Fellowship awarded by Caius College, Cambridge, and the Middlesex Hospital, London. We acknowledge the support of the International Cardiology Foundation, Boston, U.S.A., Grant No. 66IF53.

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