# The fate of preserved homograft pericardium and autogenous pericardium within the heart

C. P. CLARKE, B. G. BARRATT-BOYES, AND F. H. SIMS

*From the Cardiothoracic Surgical Unit, Green Lane Hospital, Auckland, New Zealand*

Animal experiments were performed to assess the usefulness of preserved homograft pericardium for intracardiac surgery, with particular reference to the problem of homograft mitral valve papillary muscle fixation. The fate of fresh autogenous pericardium was also studied. Both homograft and autogenous pericardium were destroyed and replaced by fibrous tissue. This reaction was faster when the pericardium was in direct contact with the blood stream and slower when it was buried within the myocardium. As homograft pericardium provided a more intense fibrous reaction than autogenous pericardium, it was considered to be very suitable for papillary muscle fixation and less suitable for other intracardiac procedures. The technique used for attaching the papillary muscle of a homograft mitral valve is briefly described.

Autogenous pericardium has been widely used in intracardiac surgery. It has proved an acceptable material for the repair of septal defects and in mitral valvuloplasty and as an interatrial baffle for the correction of transposition of the great vessels. At Green Lane Hospital, Auckland, New Zealand, strips of autogenous pericardium have been used successfully in patients to anchor the papillary muscles of a homograft mitral valve used to replace either the mitral or the tricuspid valves. The preparation of these strips during operation is, however, time-consuming, and it is more convenient to use homograft pericardial strips which have been attached to the papillary muscle of the valve graft before it is sterilized and stored.

There is little information on the behaviour of homograft pericardium in intracardiac procedures, and, in particular, it is not known whether it is destroyed or altered by the host. In this study animal experiments were performed to evaluate the suitability of preserved homograft pericardium in intracardiac surgery, with emphasis on the problem of papillary muscle attachment. Short-term studies on fresh autogenous pericardium were also performed to determine whether it survives or is retained only as a dead scaffold when placed within the heart, as there have been conflicting reports of its behaviour (Bailey, Jamison, Bakst, Bolton, Nichols, and Gemeinhardt, 1954 ; Sauvage, Wood, Bill, and Logan, 1962). In addition, the histological changes occurring in autogenous pericardium used as an atrial baffle in children

(Mustard, 1964) have been studied at 1, 2, and 28 days.

## METHODS

Adult mongrel dogs, varying in weight from 10 to 32 kg., were given methohexitone sodium intravenously in amounts just adequate to maintain light anaesthesia and were placed on a respirator with a 2/1 nitrous oxide/oxygen gas mixture. A right or left thoracotomy in the fifth interspace was performed and cardiopulmonary bypass was begun, using a standard low-prime disposable Rygg-Kyvsgaard bubble oxygenator (Frederiksen, Rosen, Rygg, Christensen, and Therkelsen, 1963 ; Arnfred, Rygg, Frederiksen, Engell, Poulsen, and Rosen, 1961). A mixture of uncrossmatched mongrel ACD blood, obtained 24 hours previously, and an equal amount of Ringer's lactate solution were used as the prime, and to each litre were added 20 ml. 7·5% sodium bicarbonate, 25 mg. heparin, and 4 ml. 10% calcium chloride. A flow of 100 ml./kg. body weight was used, and hypothermia to 30° C. was added in procedures involving the atrial septum, when the aorta was cross-clamped. The animal was given 2 mg./kg. of heparin intravenously before cannulation and the total dose of heparin used was later neutralized at the completion of bypass with twice the amount of protamine sulphate. Antibiotics (procaine penicillin, 1 million units, and streptomycin, 0·5 g.) were given for five days after surgery.

The following groups of experiments were performed.

GROUP 1    Through a right or left atriotomy a papillary muscle was divided through its base and reattached by means of a homograft pericardial sling. The pericardium was obtained from a donor animal not more than six weeks previously, sterilized by means of β-propiolactone, and kept in Hanks' solution after the manner described by Barratt-Boyes (1965). A strip of pericardium, 3 mm. wide, was threaded on a guide stitch of 000 silk and plicated through the base of the chordae of the divided papillary muscle and secured with several fine stitches (Fig. 1A). It was then threaded on a thick, round-bodied needle, and a generous bite was taken of the ventricular muscle at the base of the divided papillary muscle. The pericardial sling was drawn through and then tied and secured with several more fine stitches so that the papillary muscle was held firmly in its original position (Fig. 1B).

GROUP 2    Through a right atriotomy the interatrial septum was resected and a wedge was taken out of the limbus. The defect was repaired with a piece of fresh autogenous or preserved homograft pericardium measuring roughly $2 \times 1.5$ cm.

GROUP 3    Strips of fresh autogenous or preserved homograft pericardium were drawn through the myocardium on wide-bore, round-bodied needles. The strips were not threaded on a silk guide stitch in order to assess the reaction of pericardium alone.

The animals were sacrificed at various intervals and both a macroscopic and a microscopic assessment of the response to the pericardium was made. The sections were prepared with haematoxylin and eosin and van Gieson stains.

### RESULTS

GROUP 1    Eight animals were sacrificed from three to 26 weeks after papillary muscle reattachment using preserved homograft pericardium; five of these were tricuspid papillary muscles and three were mitral. In each case the detached papillary muscle had become fibrotic and incorporated in strong scar tissue which had undergone con-
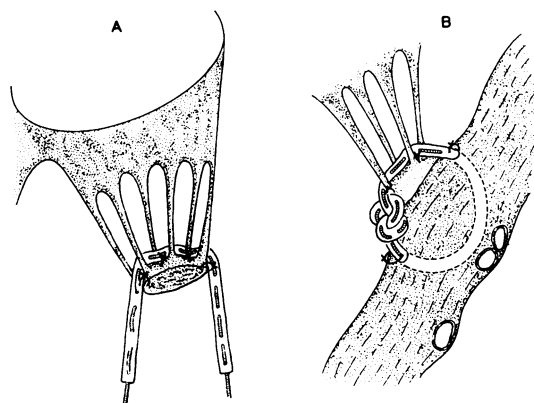


FIG. 1.   *Shows method of attachment of pericardial sling to papillary muscle and its placement in the ventricular wall. See text.*



FIG. 2.   *Mitral papillary muscle reattached with homograft pericardial sling 12 weeks after operation. Note surrounding fibrosis.*
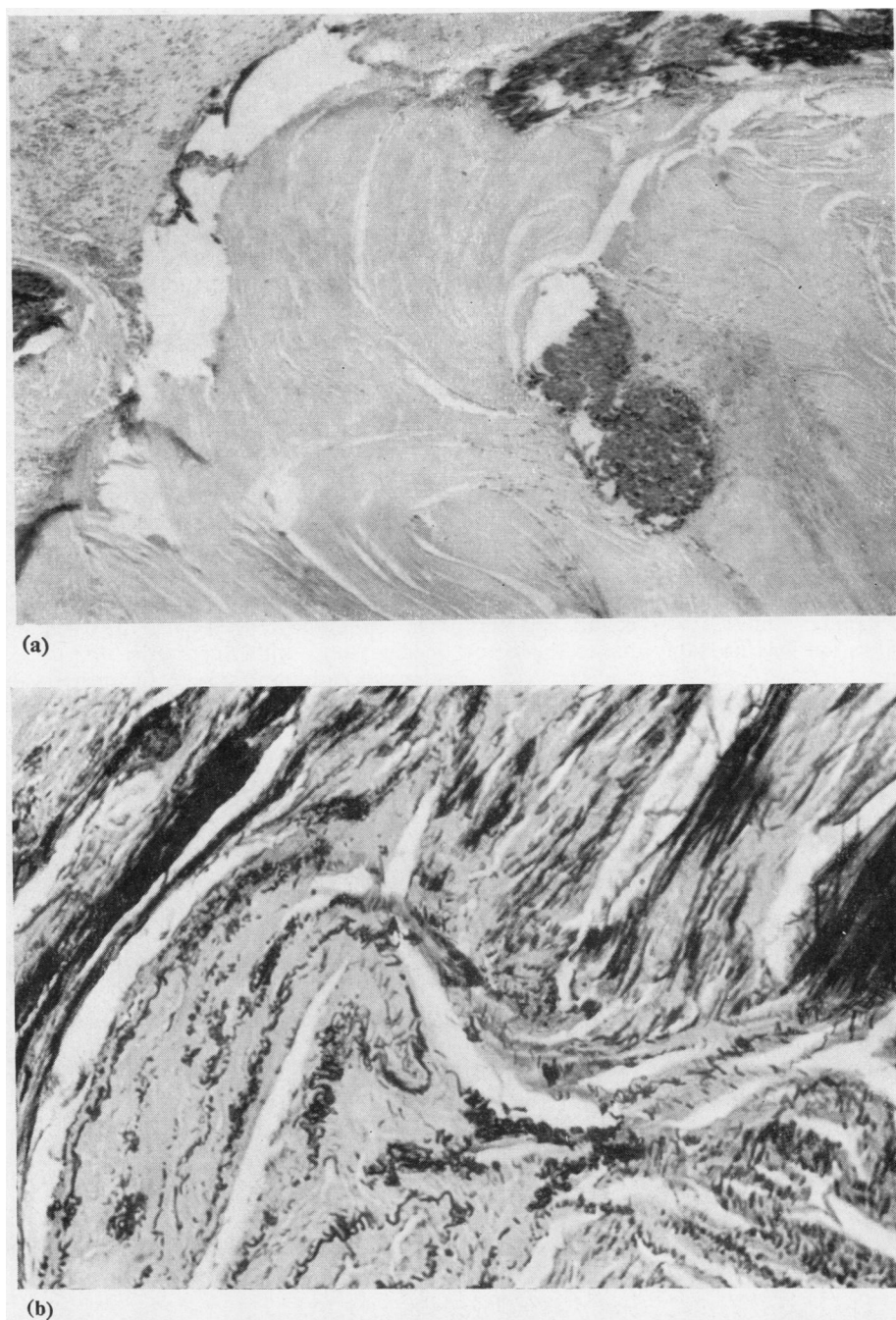
FIG. 3. *Photomicrographs of section through tricuspid papillary muscle reattached with homograft pericardial sling 12 weeks after operation. (a) Top left is the cellular connective tissue of the host, and lower right is the hyalinized graft tissue in the centre of which is a suture. (H. & E. ×40.) (b) Shows development of material staining with elastin stain. (E.V.G. ×250.)*

traction. There was surrounding endocardial thickening and fibrosis roughly proportional to the length of time after operation. A representative example is shown in Figure 2.

Microscopic examination of sections taken through the area of reattachment at three weeks showed penetration of the pericardium by host fibroblasts and macrophages and ingrowth of blood vessels. Sections taken from later specimens showed acellular collagenous material surrounding the silk suture. There was a foreign body reaction to the suture but little reaction to the collagen, which in places had become hyalinized (Fig. 3a). Sections stained with Verhoeff's elastin-van Gieson stain showed increasing amounts of elastin-staining material being laid down (Fig. 3b). Examination of sections of normal dog pericardium showed no elastin-staining material.

One animal in this group died of acute mitral incompetence six weeks after surgery due to bacterial endocarditis of the septal leaflet; in this case there was a more marked cellular infiltration around the collagen material. The repair of the reattached papillary muscle was sound and this cellular infiltration was probably due to the nearby infection.

It was not clear in these experiments whether the collagenous material represented the remains of the homograft pericardium or was the result of a host fibrous reaction.

GROUP 2   Five animals were sacrificed six to 13 weeks after repair of an artificially created septal defect with homograft or autogenous pericardium. In each case all that remained of the original septal defect was a small, hard, fibrotic area. Microscopic examination showed an abrupt cessation of elastic tissue at the edge of the gap, which was filled with organized fibrous tissue. In places this had undergone hyalinization and calcification, but there was no recognizable evidence of the pericardial graft. There was little cellular infiltration except around the suture material.

Five animals were sacrificed for short-term study at intervals of 1, 2, 3, 5, and 7 days after repair of an artificially created atrial septal defect with fresh autogenous pericardium. After one day the pericardium was covered with fibrin and cells from the blood stream. The cells of the pericardium showed degenerative changes, and host cells had started to penetrate the graft (Figs 4a, b, and c). On subsequent days the pericardium became oedematous with separation of the connective tissue bundles and progressive penetration by host cells. After seven days the collagenous fibrous tissue bundles of the pericardium were well on
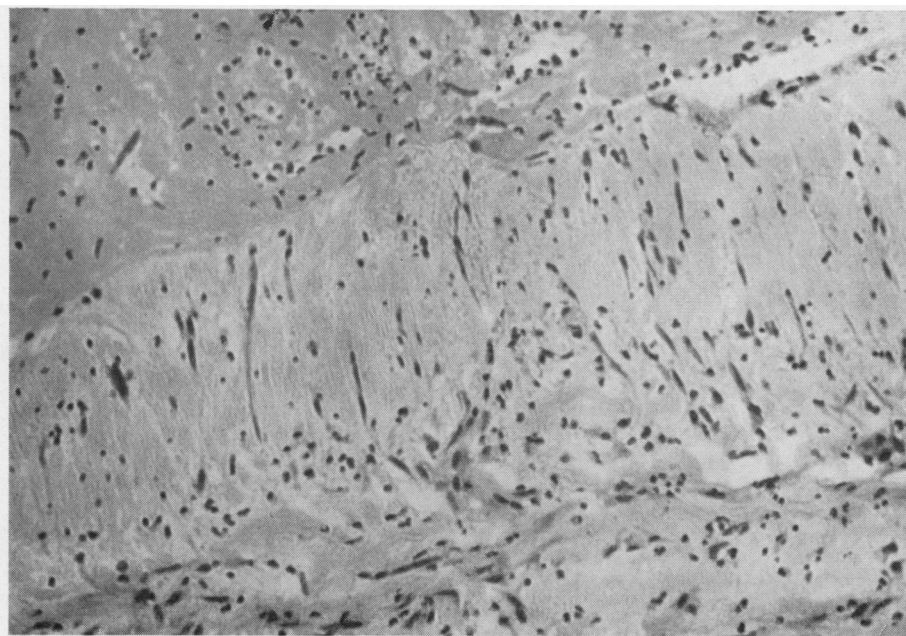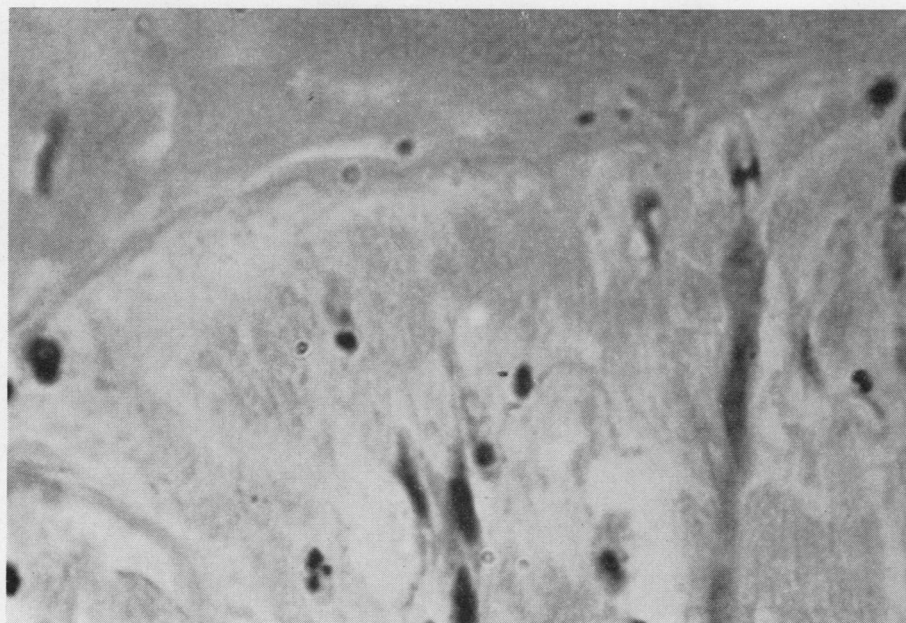


FIG. 4a

FIG. 4b



FIG. 4c

FIG. 4.    *Photomicrographs of autogenous pericardial patch in atrial septum of dog* 3 *days after operation.* (a) *The pericardium, which is oedematous, lies between thick layers of fibrinous material heavily infiltrated with inflammatory cells.* (*H. & E.*  ×40.) (b) *Higher magnification shows penetration of fibroblasts and inflammatory cells into the graft.* (*H. & E.*  ×250.) (c) *Cellular detail of* (b). (*H. & E.*  ×400.)
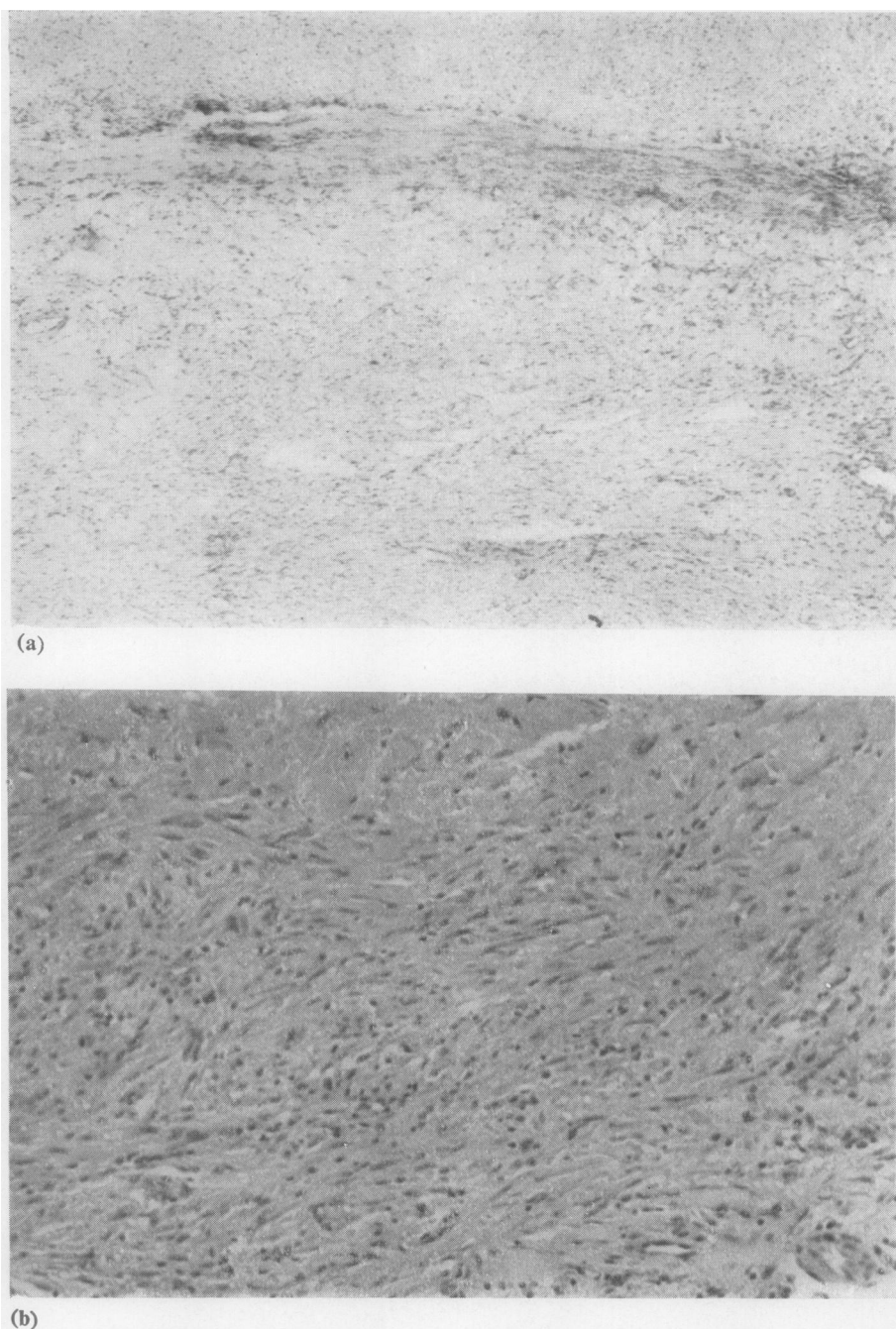
FIG. 5. *Photomicrographs of section through autogenous pericardial patch in atrial septum 7 days after insertion. (a) The pericardium is oedematous and has been broken down and penetrated by host tissue. There is a thick layer of organizing fibrous tissue on either side. (H. & E. ×40.) (b) Higher magnification of lower portion of this graft, showing intense fibrosis of the fibrin deposit. (H. & E. ×250.)*

the way towards resorption and there was evidence of organization of new fibrous tissue produced by the host (Figs 5a and b).

Five animals were similarly studied after repair of an artificially created atrial septal defect with preserved homograft pericardium. The response was similar, except that the resorption and organization of the homograft pericardium occurred more slowly than in the autogenous graft and was more pronounced.

GROUP 3 Ten animals were sacrificed for study one to 15 days after strips of fresh autogenous and preserved homograft pericardium had been threaded through the myocardium. The histological changes were similar to those seen with pericardium placed in atrial septal defects, but the host response was not so rapid. The autogenous pericardium did not survive, and with both materials the graft became surrounded by host fibroblasts and inflammatory cells. These penetrated and broke down the pericardial graft (Fig. 6), but this process was clearly taking a much longer time than when the pericardium was in direct contact with the blood stream. There was no evidence of haematoma formation around the graft.

HUMAN MATERIAL Human autogenous pericardium used as an atrial baffle in Mustard's operation was examined in three cases. In two, who died one and two days after operation, the autogenous pericardial graft was oedematous and contained groups of cells which appeared viable. There was a layer of fibrin on both surfaces and this contained acute inflammatory cells. At this stage there was no evidence of organization of the graft material. In the third specimen, however, obtained four weeks after the operation, there was clear evidence of penetration of the graft by fibroblasts and early replacement fibrosis of the graft collagenous tissue (Figs 7a and b).

DISCUSSION

From these results it appears that in the dog the reaction to both autogenous and homograft pericardium is similar, except that there is a more pronounced fibrous tissue response to homograft pericardium. Preserved homograft pericardium is already dead tissue, and autogenous pericardium does not survive even when placed in direct contact with the blood stream. In both instances the pericardium becomes oedematous and is destroyed by infiltrating macrophages. Blood vessels grow
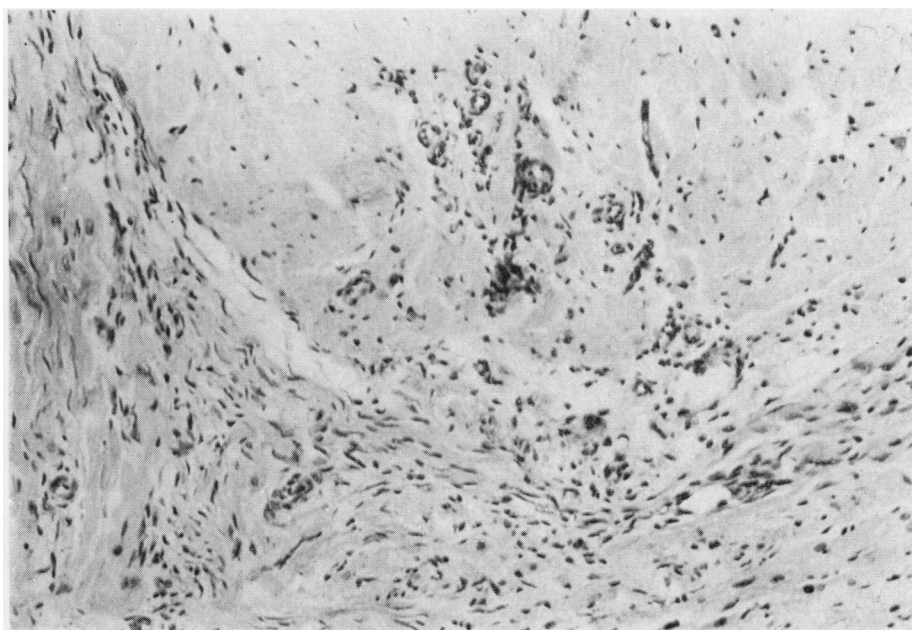


FIG. 6. *Photomicrograph of section through edge of homograft sling in myocardium 15 days after operation. The pericardium is uppermost and is penetrated by host cells and blood vessels. (H. & E. ×250.)*
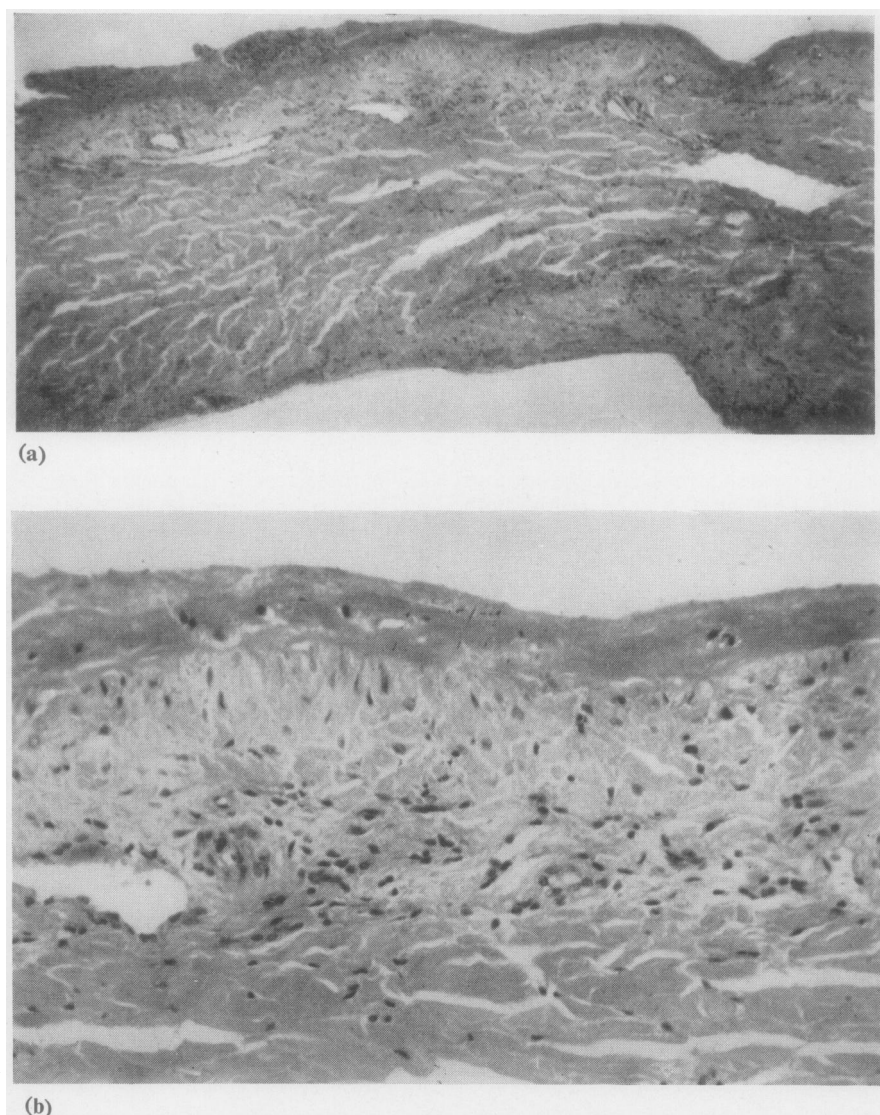
FIG. 7. *Photomicrographs of human autogenous pericardial graft 4 weeks after operation. (a) Compared with Fig. 4a, the surface layers of fibrin are thinner. The pericardium shows degeneration of intrinsic cells and penetration of fibroblasts and inflammatory cells from the surface. (H. & E. ×40.) (b) Higher magnification of the upper surface showing cellular detail. (H. & E. ×250.)*

into the pericardium and there is a fibrous tissue response by the host. This matrix becomes organized by fibroblasts into connective tissue, which matures and contracts in the normal manner. When the pericardium is covered by blood clot these changes happen in a matter of days, but they take a considerably longer period when the pericardium is buried within the myocardium.

The autogenous pericardium used as an atrial baffle within the human atria had clearly not survived but had undergone similar, although less marked, changes than those seen in dog pericardium.

The results of the experimental studies of Frater, Berghuis, Brown, and Ellis (1965) with autogenous pericardium in dogs were similar to ours, in that the grafted areas became thickened with contraction and loss of pliability. More favourable results were reported by Sauvage *et al.* (1962) and Longmore, Cook, Jepson, Curran, and Kinmonth (1964). Lepley, Hausmann, and Weisel (1959), taking advantage of the vascular arcades within the pericardium, placed pedicle grafts in the hearts of dogs to patch artificially created atrial and ventricular septal defects. Only those within the atria survived and they attributed the non-survival of those within the ventricles to the high pressure interfering with their vascular supply. Both Sauvage *et al.* (1962) and Mustard (1964) considered that autogenous pericardium placed within the blood stream remained alive, citing experiments with dogs and piglets respectively.

For most purposes autogenous pericardium would seem preferable to homograft pericardium when used inside the heart, because of the less intense fibrous response. An exception occurs, however, in fixation of the papillary muscles of mitral homografts, for in this procedure some contraction to counterbalance the papillary muscle necrosis is advantageous, and the increased fibrous reaction gives greater ultimate strength. As the pericardium is only broken down slowly when in the myocardium, it provides fixation of the papillary muscle until it is well incorporated in mature fibrous tissue: if a guide suture of strong silk is also used this gives added strength. The problem of papillary muscle fixation, however, may not be strictly comparable in dogs and humans, as simpler methods of papillary muscle attachment have proved adequate in dogs (O'Brien and Gerbode, 1964), at least when fresh grafts are used.

The provision of adequate fixation of the papillary muscles proved to be the main problem in our attempts at human homograft mitral valve replacement, using grafts either stored in Hanks' solution or freeze-dried. Fixation with silk sutures alone was inadequate as the papillary muscle broke free. The only successful cases were in the group where strips of autogenous pericardium were used to provide fixation of the papillary muscles, in a manner similar to that described in these experiments.

In these patients the pericardial strips were threaded on a silk guide suture, for ease of handling, and fixed to the papillary muscle of the homograft as shown in Fig. 1, working through a left atrial approach from the right chest. The
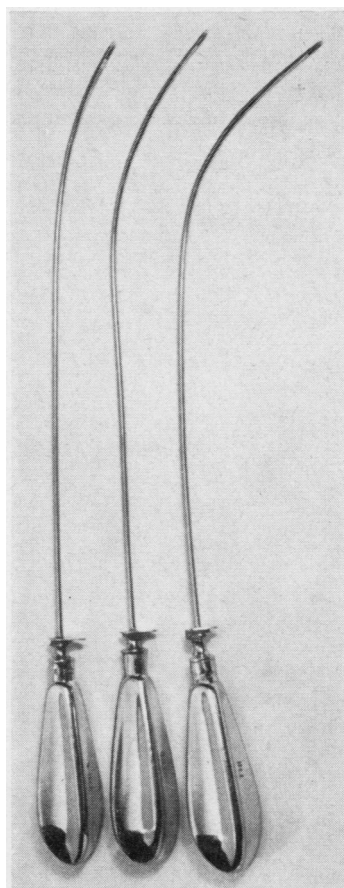


FIG. 8. *Special probes for passing slings through the ventricular wall from within.*

pericardial sling was drawn through the myocardium at the base of the excised host papillary muscle on a thick, round-bodied needle or special probe (Fig. 8), so that it passed through about three-quarters of the thickness of the ventricular wall. It was found that if it was passed through the whole thickness of the ventricle there was not only danger of damaging one of the major coronary vessels, which run just under the epicardium, but there was also troublesome bleeding from the puncture sites. The pericardial slings were tied and fixed to the heart with additional silk sutures, to prevent any see-saw action which might cut the pericardium free. We hope in future to use homograft valves prepared beforehand with homograft pericardial strips.

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### REFERENCES

Arnfred, E., Rygg, I. H., Frederiksen, T., Engell, H. C., Poulsen, T., and Rosen, J. (1961). A clinical evaluation of the Rygg-Kyvsgaard III and the Mark III pump oxygenators. *Thorax*, **16**, 309.

Bailey, C. P., Jamison, W. L., Bakst, A. E., Bolton, H. E., Nichols, H. T., and Gemeinhardt, T. W. (1954). The surgical correction of mitral insufficiency by the use of pericardial grafts. *J. thorac. Surg.*, **28**, 551.

Barratt-Boyes, B. G. (1965). A method for preparing and inserting a homograft aortic valve. *Brit. J. Surg.*, **52**, 847.

Frater, R. W. M., Berghuis, J., Brown, A. L., Jr., and Ellis, F. H., Jr. (1965). The experimental and clinical use of autogenous pericardium for the replacement and extension of mitral and tricuspid valve cusps and chordae. *J. cardiovasc. Surg.* (Torino), **6**, 214.

Frederiksen, T., Rosen, J., Rygg, I. H., Christensen, E., and Therkelsen, F. (1963). Prolonged extracorporeal circulation. *Thorax*, **18**, 158.

Lepley, D., Jr., Hausmann, P. F., and Weisel, W. (1959) The fate of pericardial pedicle grafts used in a fixed position in various chambers of the dog heart. *J. thorac. Surg.*, **37**, 711.

Longmore, D. B., Cook, W. A., Jepson, B. A., Curran, R., and Kinmonth, J. B. (1964). Comparison of materials for repair of cardiac defects. *Thorax*, **19**, 454.

Mustard, W. T. (1964). Successful two-stage correction of transposition of the great vessels. *Surgery*, **55**, 469.

O'Brien, M. F., and Gerbode, F. (1964). Homotransplantation of the mitral valve. Preliminary experimental report and review of literature. *Aust. N.Z. J. Surg.*, **34**, 81.

Sauvage, L. R., Wood, S. J., Bill, A. H., Jr., and Logan, G. A. (1962). Pericardial autografts in the mitral valve. A preliminary report. *J. thorac. cardiovasc. Surg.*, **44**, 67.