

# Storage of heart valve allografts in glycerol with subsequent antibiotic sterilisation

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**Parker, R, Randev, R, Wain, W H, and Ross, D N (1978).** *Thorax*, 33, 638–645. **Storage of heart valve allografts in glycerol with subsequent antibiotic sterilisation.** Fresh allograft valves stored in a nutrient medium at +4°C have a limited storage time of eight weeks. Dura mater has been stored in glycerol for longer periods, and this paper presents work on the glycerol storage of allograft heart valves.

The elastic properties of the valve cusps showed a fall during storage in glycerol that was associated with an altered histological appearance of the cusp tissue. The loss in nuclearity in the histological sections of stored tissue was partially responsible for the observed decrease in viability during storage.

All these changes during storage in glycerol, or glycerol and subsequent antibiotic treatment, were similar to the changes seen in valves stored in a nutrient medium. Glycerol therefore offers an alternative storage system to the cold nutrient medium but has no practical advantages. Glycerol alone will not sterilise the allograft tissue, and a post-storage treatment with antibiotics is essential.

The storage of human heart valve allografts in a nutrient medium in the presence of a sterilising mixture of antibiotics has been a routine procedure at this hospital for 1700 valves during the past six years. The introduction of nutrient medium (Lockey *et al*, 1972) was shown to enhance the viability of the allograft fibroblasts (Al-Janabi and Ross, 1973). The slow decline in viability (Al-Janabi and Ross, 1973) and in elastic properties (Ng and Wright, 1975) during storage in nutrient medium at +4°C has restricted the practical storage time to eight weeks after preparation.

Previous attempts to overcome this problem of storage and preservation have included freeze drying (Longmore *et al*, 1966) and flash freezing (Moore *et al*, 1975). Between 1963 and 1967, 165 freeze dried valves were inserted and resulted in 56 valve failures up to 1974 (Moore *et al*, 1975). Most of the frozen valves were sterilised by ethylene oxide or by gamma radiation, which killed the allograft cells, in contrast to the retention of viability during the antibiotic-treatment of allografts. An alternative approach for long-term storage of biological tissue is preservation in glycerol, and this has been applied to dura mater used to form the cusps of stented valves (Puig *et al*, 1974).

Pigossi (1967) claimed that glycerol acts as a sterilising agent.

We have therefore examined the effects of glycerol storage followed by antibiotic sterilisation of heart valve allografts. The effects of these treatments have been examined by assessing viability, testing elastic properties, and studying the tissue architecture of the valve allografts.

## Materials and methods

Aortic and pulmonary valves were dissected under clean but not sterile conditions. They were from routine post-mortem material taken within 48 hours of death. The dissected valve was separated into three along the line of the commissure, keeping each intact cusp attached to the appropriate sinus of Valsalva. One of these cusps was used as the control. The cusps from any one valve were used either for viability studies or for measurements of elastic properties or for histological examination. The two experimental cusps from any one valve were stored for different periods in either glycerol alone or in glycerol followed by nutrient medium. The prepared experimental cusps were placed in sterile glycerol for 10 minutes

and then transferred to a fresh container of sterile glycerol for up to 12 weeks. Some cusps were transferred from the glycerol to a nutrient medium containing the antibiotic mixture "C" of Waterworth *et al* (1974). The period in glycerol and in nutrient medium was identical for any single cusp. On removal from the storage medium the cusp was washed in sterile physiological saline for one hour before applying the appropriate test.

Viability assays were carried out on all three cusps of the valve at selected intervals using autoradiography of incorporated tritiated thymidine (Al-Janabi *et al*, 1972). A modification (Parker *et al*, 1977) of the technique of Wright and Ng (1974) was used to measure the elastic properties of the cusps. The pressure-volume curves were measured in three ranges to correspond to the components of the cusps as follows: 0–12 mmHg (0–1.6 kPa) probably due to elastin; 88–100 mmHg (11.7–13.3 kPa) probably due to collagen; and 0–100 mmHg (0–13.3 kPa) for the overall value (Hallock and Benson, 1937). A histological examination was performed on 7  $\mu$ m paraffin sections after Bouin's fixation. The sections were stained with either haematoxylin and eosin or elastic Van Gieson stain.

## Results

### ELASTIC PROPERTIES

There was an average initial overall value of  $23.93 \pm 5.81$  mm<sup>3</sup> per 100 mmHg and an average initial ratio between 0–12 : 88–100 mmHg (0–1.6 : 11.7–13.3 kPa) (that is, elastin : collagen) of  $3.23 \pm 1.1$  (table 1). These initial results are comparable with other experiments using mixed aortic and pulmonary artery valve collections. Figure 1 shows the fall in elastic properties during storage in glycerol and in glycerol and nutrient medium. The third curve in fig 1 was constructed by extrapolation from fig 4 of Ng and Wright (1975) and shows

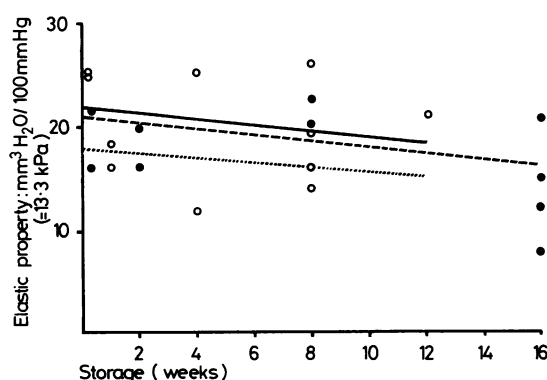


Fig 1 Changes in elastic properties of valve cusps stored in glycerol and nutrient medium. Stress-strain (compliance) values over pressure range 0–100 mm Hg (0–13.3 kPa) were measured at intervals during storage. Glycerol or nutrient medium was washed out with physiological saline for 60 minutes before testing. Control values at zero time were made on fresh tissue. Nutrient medium "C" was that described by Waterworth *et al* (1974). Curve for nutrient medium "B" was derived by extrapolation from fig 4 of Ng and Wright (1975) and is represented only by the calculated correlation curve. ○ glycerol; ● glycerol + nutrient medium "C", — glycerol, - - - glycerol + nutrient medium "C", and ···· nutrient medium "B".

the fall in elastic properties in nutrient medium over a 12-week period and is included for comparison. It can be seen that there was a similar fall in overall elastic properties during all three treatments over a 12-week period. Table 1 shows that the valves stored in glycerol and nutrient medium had a 29% fall over 24 weeks in the 0–12 mmHg (elastin) component, whereas the valves stored in glycerol alone exhibited little change in this component. The collagen component (88–100 mmHg) (11.7–13.3 kPa) (table 1) showed a fall of 30% both in glycerol and in glycerol followed by nutrient medium. These changes are reflected in the ratio elastin : collagen, which remained constant for the valves stored in glycerol and nutrient medium but increased for the valves stored in glycerol.

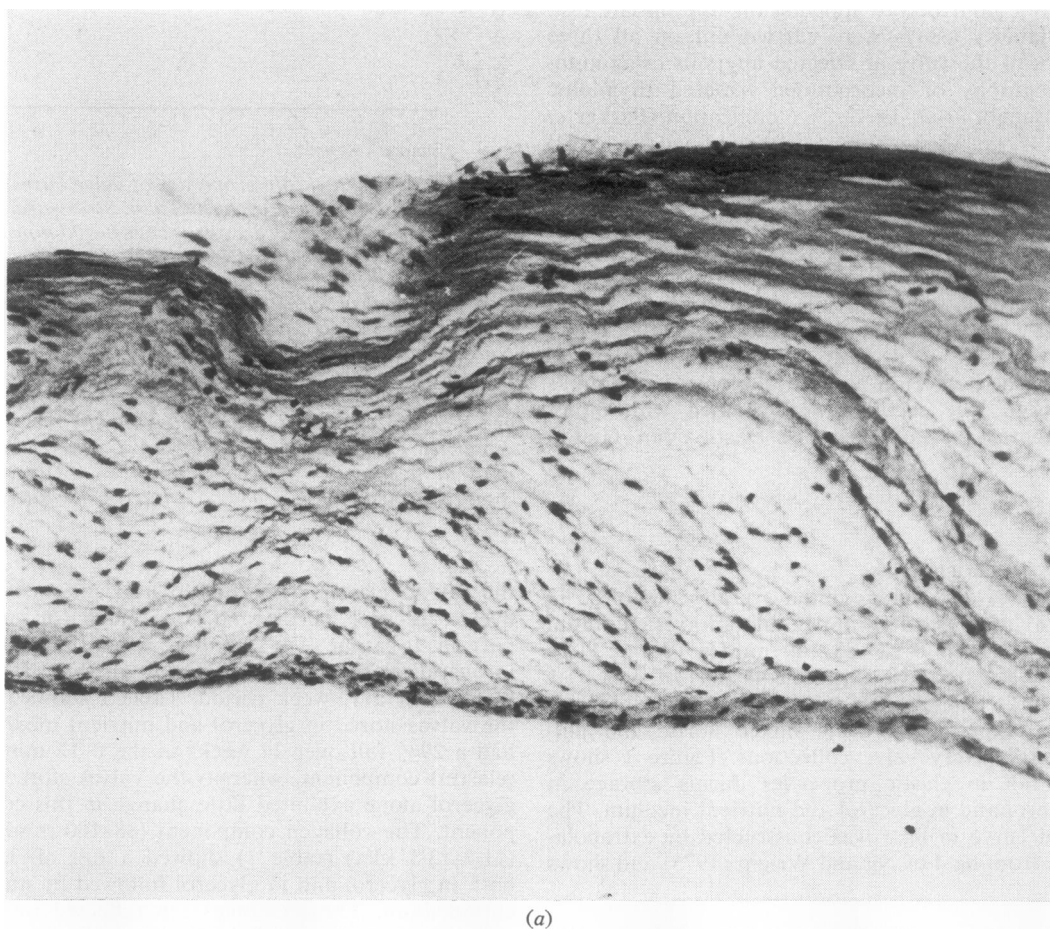
### HISTOLOGY

Generally, the numbers of nuclei during storage in glycerol decreased and this was reflected in the viability assays. Valves stored in glycerol also developed a patchy distribution of the collagen, which had a loose, disorganised arrangement when compared with the fresh control tissue (fig 2b cf 2a). When the valves were treated in the antibiotic

Table 1 Changes in elastic properties of allograft valve cusps during storage in glycerol and in glycerol followed by nutrient medium

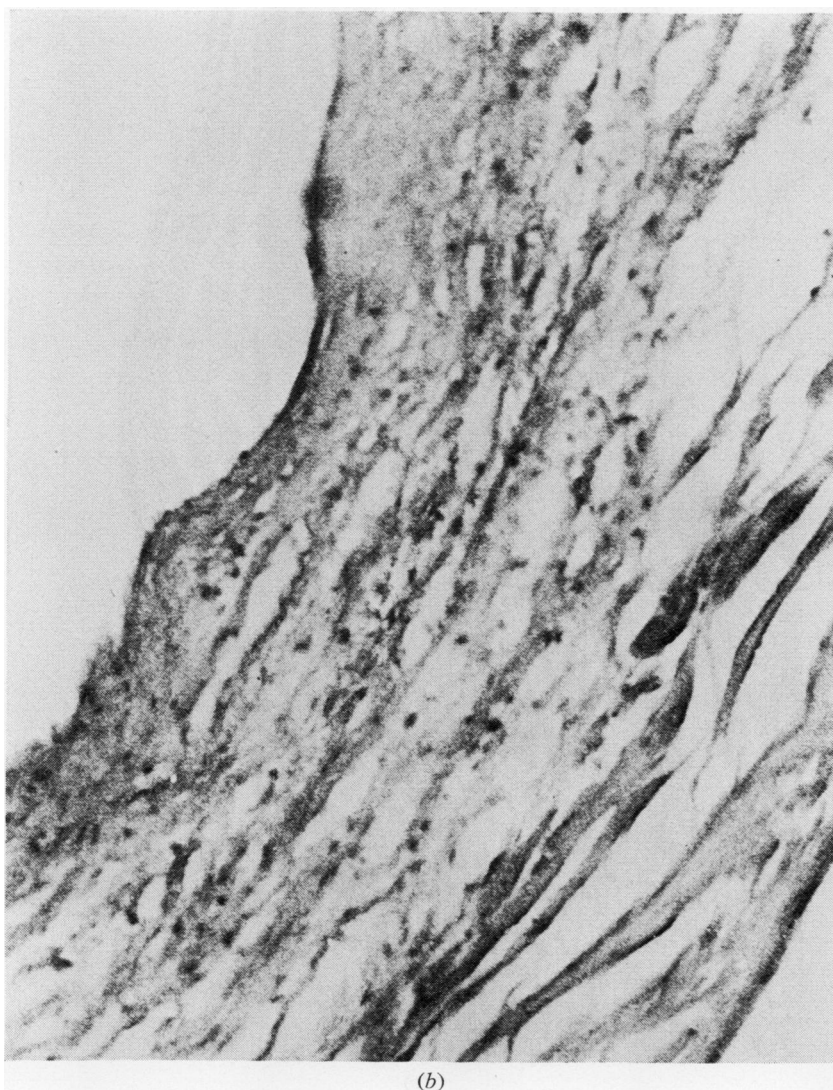
Pressure range	Interpretation	0 weeks (control)	12 weeks glycerol	12 weeks glycerol + 12 weeks nutrient medium
0–12 mmHg (0–1.6 kPa)	'Elastin'	5.09*	5.00	3.50
88–100 mmHg (11.7–13.3 kPa)	'Collagen'	1.68	1.21	1.10
	Ratio	3.23	4.05	3.27

\*Stress-strain (elasticity) in mm<sup>3</sup>.

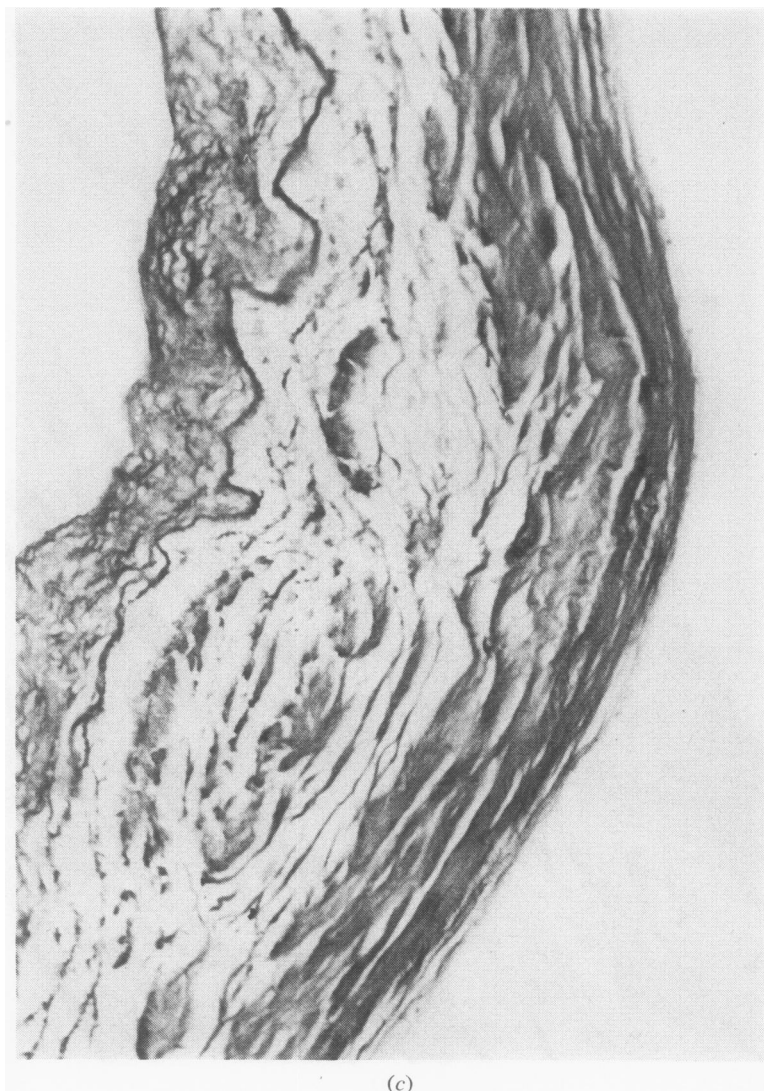


(a)

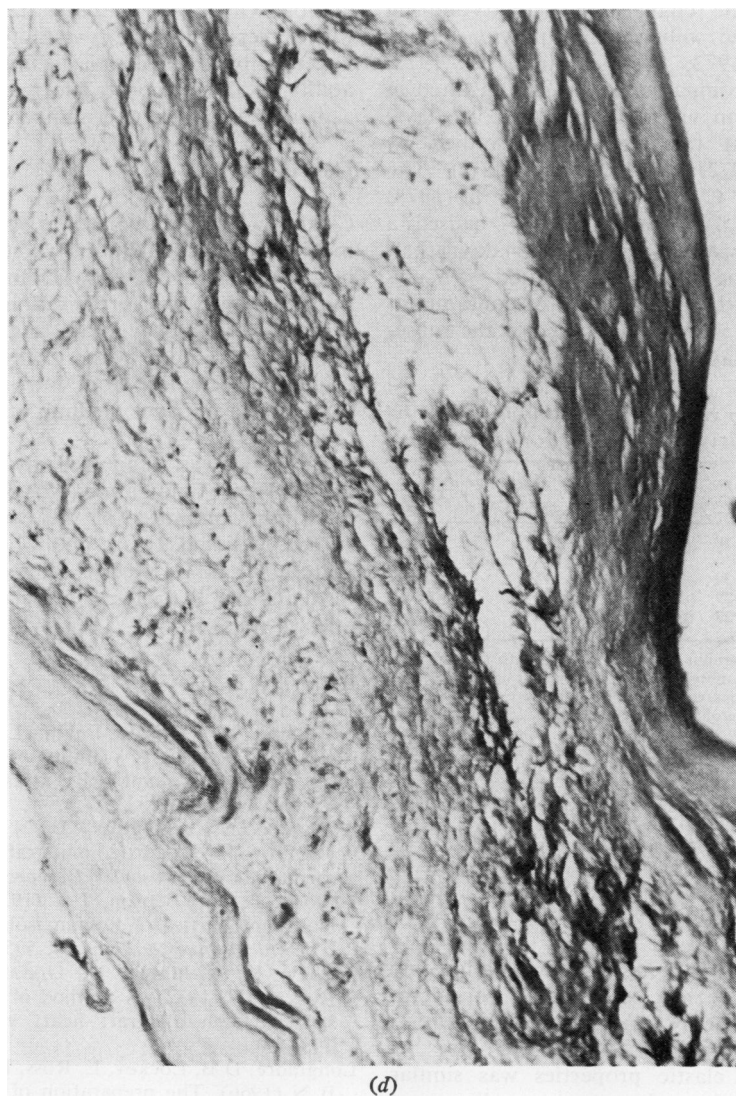
**Fig 2** Sections of pulmonary valve cusps after storage in glycerol or in glycerol and nutrient medium "C" of Waterworth *et al* (1974). (a) Control (Haematoxylin and eosin  $\times 200$ ). (b) 84 days in glycerol (H and E  $\times 183$ ). (c) Control (elastic Van Gieson  $\times 157$ ). (d) 84 days in glycerol followed by 84 days in nutrient medium "C" (elastic Van Gieson  $\times 163$ ).



(b)



(c)



mixture in nutrient medium after storage in glycerol there was no further change in the nuclear content of the tissue. Nevertheless, the collagen degenerated in many foci, and in larger areas as the storage time increased (fig 2d cf 2c).

#### VIABILITY

Table 2 shows the changes in viability during storage. Calculated values from previous work (Al-Janabi *et al*, 1972; Al-Janabi and Ross, 1973) are included for comparison. Glycerol resulted in a greater change in viability (−81% in 84 days) than the nutrient medium "B" described by Lockey *et al* (1972) (−29% in 84 days). The nutrient medium "C" of Waterworth *et al* (1974) used after glycerol storage, however, caused a smaller change in viability (−59% in 84 days) than glycerol alone. One problem associated with glycerol storage was the preservation of contaminating fungi with the valve, which affected the subsequent viability assays.

Table 2 Changes in viability of valve cusps during 84 days storage in glycerol and nutrient medium

	No of valves	Control viability	84-day viability	% Change
Glycerol	30	27%	5%	−81
Glycerol and nutrient medium "C"	30	27%	11%	−59
Hanks's "B"	29	68%	0%	−100
Nutrient medium "B"	28	68%	48%	−29

Viability from autoradiographs of nuclei labelled with tritiated thymidine was measured at intervals during storage. Results at 84 days were calculated from the regression line for all storage times. Storage in glycerol, and in glycerol followed by nutrient medium "C" of Waterworth *et al* (1974) was contrasted with storage in Hanks's balanced salt solution "B" (from Al-Janabi *et al*, 1972) and in nutrient medium "B" (from Al-Janabi and Ross, 1973). The difference in viabilities between these control viabilities and the control values calculated from the results of Al-Janabi and Ross (1973) was reflected in the viabilities of implanted allograft valves during 1972–5. This is ascribed to changes in the source of routine post-mortem material between 1972 and 1975.

#### Discussion

The preservation of contaminating pathogenic fungi in glycerol precludes the serious consideration of glycerol alone as a method of preservation for heart valve allografts.

The decline in elastic properties was similar during storage in glycerol, nutrient medium and glycerol followed by nutrient medium. This fall of 30% in the elastic properties during 24 weeks of storage was reflected in the histological changes seen in the valve cusp tissue. The elastin : collagen ratio (table 1) did not change appreciably during storage in glycerol followed by nutrient medium, which suggested that the higher retention of vi-

ability in this method may have permitted some tissue repair. This was at variance with the histology of the stored valves in which the collagen was seen to degenerate to a greater extent after transfer from the glycerol to the nutrient medium.

The viability changes were partially reflected in the anucleate tissues seen on histological examination. Obviously if there are no demonstrable nuclei there will be zero viability, but low viability is generally ascribed to a decline in the functional ability of the demonstrable nuclei rather than to a mere decrease in the numbers of nuclei.

#### Conclusion

Clearly a combination of glycerol and nutrient medium offers an alternative storage method for heart valves, but the only advantage appears to be the avoidance of refrigeration for the storage period. We do not feel that this method offers any practical advantage at the moment, and we do not propose to change from our present system of storage in a nutrient medium at +4°C.

This work was supported by grants from the British Heart Foundation (No 456) and the Clinical Research Committee of the Board of Governors of the National Heart and Chest Hospitals. We gratefully acknowledge receipt of the grants, and we thank Mr B Richards for preparing the photomicrographs.

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