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Preservation and function of heterologous aortic valves

An Experimental Study

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Heterologous aortic valves are used in many clinics as replacements for diseased human aortic and mitral valves. These valves possess all the advantages of homologous aortic valves and are more easily available to the surgeon. The heterologous valve also provides a greater choice of valve size than does the homograft; this can be of importance when replacing the mitral valve. Heterograft valves, like homografts, are usually preserved for periods ranging from a few days to a few months before insertion into a patient. Four methods of preservation, described, are currently in use. This study compares the effects of these four methods of preservation when pig valves are transplanted into the dog's aorta.

METHODS

Pig aortic valves were obtained from a local abattoir within 2 to 12 hours following slaughter.

The pig aorta was transected just distal to the sinus ridge. This ridge is a distinct anatomical feature arching between the highest attachment of the cusps. "It represents more than a slow curving shoulder naturally formed by the junction of the aorta and the sinus dilatation and is generally characteristically shaped and quite sharply angled." "The ridge is easily palpable if the moist vessel is allowed to slip between finger and thumb" (Bellhouse, Bellhouse, and Reid, 1968). A short skirt of Dacron tubing was stitched to this cut edge of the aorta. The left ventricular muscle was cut away from the proximal edge of the valve, leaving sufficient tissue to provide a sewing ring which would not interfere with valve function. A second "skirt" of Dacron was stitched to this edge. The valves were then preserved by one of the following methods:

GROSS SOLUTION (Gross, Bill, and Peirce, 1949). Half a gramme each of penicillin and streptomycin was added to 100 ml of solution. The valves were then stored in this solution for periods of two to seven days.

FORMALIN 10% pH 7·5–7·75 The valves were stored in this solution for two to seven days. The valves were soaked and washed in sterile saline for approximately one hour before insertion into a dog.

FREEZE DRIED The valves were first sterilized by ethylene oxide and then freeze dried. The valves were used before the 21st day following freeze drying. Reconstitution in warm saline was carried out immediately before their insertion into the dog.

IRRADIATION The valves were irradiated using gamma rays. The dose of radiation varied from 1 to 3 megarads. This was administered at a rate of 2 to 3 megarads per hour. The valves were irradiated in the dry state and the temperature was controlled at 4° C. The valves were stored at a temperature of 4° C after they had been removed from the source of irradiation. All valves were used before the 21st day following irradiation.

These pig valves were inserted into the dog's thoracic aorta just distal to the left subclavian artery. While the aorta was cross clamped, blood supply to the lower half of the body was maintained by left subclavian to left femoral artery bypass. None of the dogs received blood transfusions. All dogs had terramycin, 100 mg per day, for five days after surgery.

It had been our intention, using the technique of Duran, Manley, and Gunning (1965), to make the dog's own aortic valve incompetent. This procedure was carried out in the first 12 dogs of this series with high mortality and was abandoned. The work of Bellhouse *et al.* (1968) on closure of the aortic cusps made it clear to us that the sinus mechanism was operating in our transplanted valves, and that the cusps would open and close adequately and thus avoid the ill effects of immobility of the cusps described by Duran *et al.* (1965). The sinus mechanism consists of a vortex generated at peak systole which acts as a pump, drawing blood into the sinus and pushing the cusps closed (Bellhouse and Bellhouse, 1968a).

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Velocity flow measurements were made using a needle velocity flow probe described by Bellhouse and Bellhouse (1968b). These measurements were made in a series of acute experiments and velocity profiles were recorded from the aorta downstream from the transplanted valves.

RESULTS

A total of 92 dogs was used in this study. This number includes the first 12 dogs in the series where an attempt was made to induce aortic incompetence. A high mortality was associated with this procedure and the overall intraoperative mortality was 13%. The intraoperative mortality is 3% if we consider those animals (80) in whom the aortic valve was not made incompetent.

GROSS SOLUTION There were 29 animals in this group. Five died during or immediately after surgery. Three had irreversible ventricular fibrillation and two mitral incompetence. All were associated with attempts to produce aortic incompetence. Eleven animals died between two and five days after surgery. Three died because of infection. One died following rupture of the sinus of Valsalva of the transplanted valve. No cause of death was found in the remaining seven animals.

The remaining 13 animals died or were sacrificed between the 6th and 225th day following insertion of the valve. The causes of death and details of the macroscopic appearances of the transplanted cusps from these animals are given in Table I.

FREEZE DRIED There were 24 animals in this group. Three died during the surgical procedure. Two had irreversible ventricular fibrillation and one mitral incompetence. These were associated with the attempts to produce aortic incompetence. Eight dogs died between one and five days after surgery. The sinus of Valsalva of the transplanted valve had ruptured in two of these animals. Three died from infection, while in the remaining three no cause of death was discovered.

Thirteen dogs survived for periods ranging from 6 to 150 days. The causes of death and details of the macroscopic appearances of the valves from these animals are given in Table II.

TABLE I

VALVES PRESERVED IN GROSS SOLUTION

Survival (days)	Cause of Death	Macroscopic State of Cusps					State of Sinus of Valsalva	
		Preservation	Clean	Mobile	Retraction + to +++	Perforation	Thrombus + to +++	Calcification
6	Rupture of sinus of Valsalva	Good	Yes	Yes	—	—	—	—
6	Rupture of sinus of Valsalva	Good	Yes	Yes	—	—	—	—
9	Rupture of sinus of Valsalva	Good	Yes	Yes	—	—	—	—
10	Rupture of sinus of Valsalva	Good	Yes	Yes	—	—	—	—
10	Unknown	Good	Yes	Yes	—	—	—	—
15	Rupture of sinus of Valsalva	Good	Yes	Yes	—	—	—	—
21	Rupture of sinus of Valsalva	Good	Yes	Yes	—	—	—	—
28	Sacrifice	Good	Yes	Yes	—	—	—	—
35	Unknown	Good	Yes	Yes	—	—	—	—
56	Sacrifice	Good	—	Yes	+	—	+	—
68	Sacrifice	Good	—	Yes	+	One	+	—
100	Sacrifice	Good	Yes	Yes	—	—	—	—
225	Sacrifice	Good	Yes	Yes	+	—	—	Yes

TABLE II

VALVES PRESERVED BY FREEZE DRYING

Survival (days)	Cause of Death	Macroscopic State of Cusps					State of Sinus of Valsalva	
		Preservation	Clean	Mobile	Retraction + to +++	Perforation	Thrombus + to +++	Calcification
8	Rupture of sinus of Valsalva	Good	Yes	Yes	—	—	—	—
9	Unknown	Good	—	Yes	+	—	+	—
10	Rupture of sinus of Valsalva	Good	Yes	Yes	—	—	—	—
10	Rupture of sinus of Valsalva	Good	Yes	Yes	—	—	—	—
16	Rupture of sinus of Valsalva	Good	—	Yes	—	—	+	—
19	Rupture of sinus of Valsalva	Good	Yes	Yes	—	—	—	—
21	Rupture of sinus of Valsalva	Good	Yes	Yes	—	One	—	—
24	Rupture of sinus of Valsalva	Good	Yes	Yes	+	—	—	—
24	Unknown	Good	Yes	Yes	—	—	—	—
35	Rupture of sinus of Valsalva	Good	—	Yes	—	—	+	—
80	Sacrifice	Good	Yes	Yes	+	—	—	—
100	Sacrifice	Good	—	Yes	—	—	+++	—
150	Sacrifice	Good	—	Yes	+	—	+	—

TABLE III

VALVES PRESERVED IN FORMALIN 10% pH 7.5 TO 7.75

Survival (days)	Cause of Death	Macroscopic State of Cusps					State of Sinus of Valsalva	
		Preservation	Clean	Mobile	Retraction + to +++	Perforation	Thrombus + to +++	Calcification
8	Unknown	Good	—	Yes	—	—	+	—
9	Rupture of sinus of Valsalva	Good	Yes	Yes	—	—	—	—
11	Rupture of sinus of Valsalva	Good	—	Yes	—	—	+	—
11	Rupture of sinus of Valsalva	Good	—	Yes	+	—	+++	—
16	Rupture of sinus of Valsalva	Good	—	Yes	—	—	+++	—
18	Rupture of sinus of Valsalva	Good	—	Yes	+	—	+++	—
20	Rupture of sinus of Valsalva	Good	—	Yes	—	—	+++	—
29	Sacrifice	Good	—	Yes	—	—	+++	—
29	Sacrifice	Good	—	Yes	—	—	+++	—
42	Unknown	Good	—	Yes	—	-	++	—
49	Sacrifice	Good	Yes	Yes	—	—	—	—
75	Sacrifice	Good	—	Yes	++	—	++	—
100	Sacrifice	Good	—	Yes	+	—	++	—
265	Sacrifice	Good	Yes	Yes	—	—	—	Yes

TABLE IV

VALVES PRESERVED BY IRRADIATION

Survival (days)	Radiation Dose (megarad)	Cause of Death	Macroscopic State of Cusps					State of Sinus of Valsalva	
			Preservation	Clean	Mobile	Retraction + to +++	Perforation	Thrombus + to +++	Calcification
8	1	Ruptured sinus of Valsalva	Poor	—	—	+++	—	+++	—
8	2	Ruptured sinus of Valsalva	Poor	—	Yes	+	—	+	—
8	3	Ruptured sinus of Valsalva	Poor	—	Yes	+	—	+	—
13	2	Ruptured sinus of Valsalva	Poor	—	—	+++	—	+++	—
15	1	Ruptured sinus of Valsalva	Poor	—	—	+	—	+++	—
18	3	Ruptured sinus of Valsalva	Poor	—	Yes	+	—	+	—
30	3	Sacrifice	Poor	—	Yes	+	—	+	—
42	2	Ruptured sinus of Valsalva	Poor	—	—	+++	—	++	—
51	2	Sacrifice	Poor	—	Yes	+	—	+	—
52	2	Sacrifice	Poor	—	—	+++	—	++	—
152	3	Sacrifice	Poor	—	2 cusps	++	—	+	Yes

FORMALIN 10%, pH 7·5–7·75. There were 28 dogs in this group. Four animals died during surgery. Three had irreversible ventricular fibrillation and one animal had an uncontrollable haemorrhage. All four were associated with the attempts to produce aortic incompetence.

Ten animals died between one and five days after surgery. No causes of death could be found in any of these dogs. Fourteen animals survived for periods ranging from 6 to 265 days. The causes of death and details of the macroscopic appearances of the transplanted valves from these animals are given in Table III.

IRRADIATION There were 11 animals in this group. All survived for longer than five days. The causes of death and details of the macroscopic appearances of the transplanted cusps from these animals are given in Table IV. The differing doses of radiation which were given to these valves are also set out in Table IV.

Tracings of velocity flow profiles, obtained in acute experiments from dogs with heterologous valves preserved in Gross solution, by freeze drying, and in formalin are shown in Figures 1, 2, and 3 respectively. All these heterologous valves have been submitted to histological examination and will be reported in a later communication.

DISCUSSION

The macroscopic appearances of these transplanted heterologous aortic valves and simple manipulative testing of the mobility of their cusps indicate a poor state of preservation and inefficient function of those valves preserved by gamma irradiation. This statement can apply only to valves irradiated under precisely the same conditions as are set out in this paper. The possibility that irradiation of valves in the "wet" state, or storing them at lower temperatures, might improve the results of using gamma ray preservation needs further investigation. Valves preserved in Gross solution functioned better than those preserved by freeze drying or formalin. This was most evident up to a period of one month. The clinical results reported by Gibbens and Allendine (1967), Malm, Bowman, Harris,

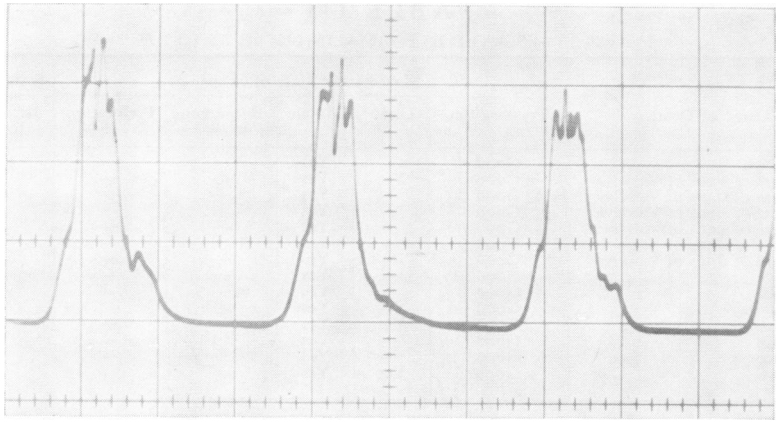

FIG. 1. *Velocity flow trace of heterologous aortic valve stored in Gross solution (see text).*

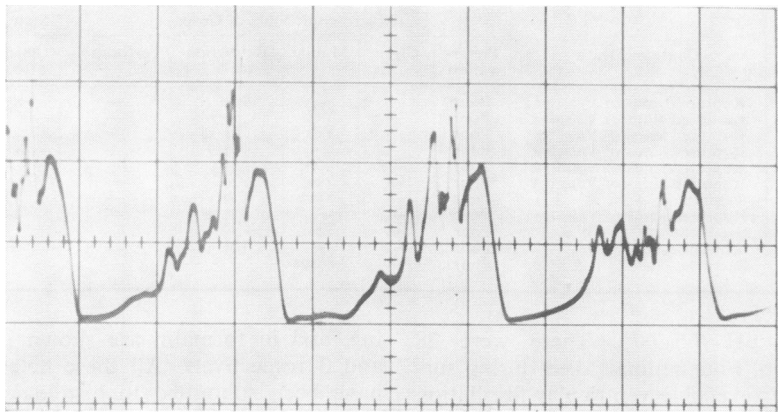

FIG. 2. *Velocity flow trace of heterologous aortic valve preserved by freeze drying (see text).*

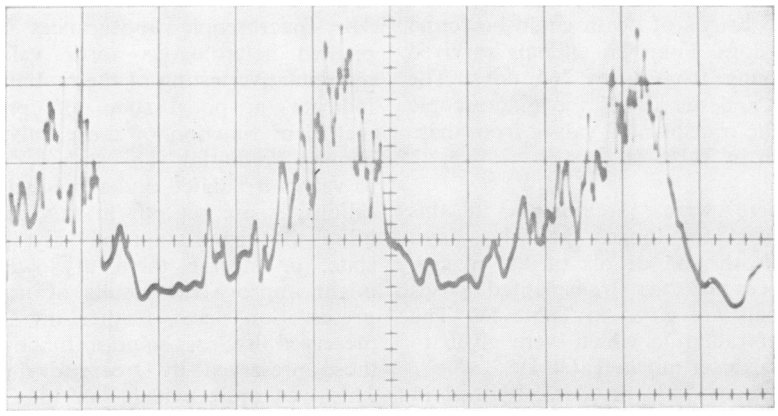

FIG. 3. *Velocity flow trace of heterologous aortic valve preserved in formalin (see text).*

and Kowalik (1967), and Karp and Kirklin (1969) suggest that good, relatively long-term (2–4 years) results can be obtained with valves sterilized by electron beam energy and stored.

It is difficult to see any difference between those valves preserved by freeze drying and those in formalin. The valves preserved in formalin showed a greater tendency to thrombus formation in the sinuses of Valsalva.

The velocity flow profiles show clear differences between the three non-irradiation methods of preservation. The tracings from valves preserved in Gross solution show a pattern approaching laminar flow (Fig. 1). The tracing from the formalin valves show a turbulent flow pattern (Fig. 3). The freeze dried valve lies between the two (Fig. 2). We believe that the turbulence is due to stiffness of the preserved cusps. The fact that the cusps of a valve preserved in formalin are stiffer than those of a valve preserved in Gross solution is evident to the naked eye. We have considered whether the mechanical performance of formalin-preserved valves might be improved by improving our technique of washing the formalin from the valve before insertion, and at this time we are investigating this possibility in a series of experiments. These experiments have shown that the degree of washing makes little or no difference to the mechanical performance. It may, however, be that the stiffness is due to chemical changes in the cusp and these would not be altered by the physical processes of washing in saline.

The velocity studies suggest that the better results of valves in Gross solution in their early transplanted life may be due to the fact that mechanically they more nearly simulate the normal valve. We feel that once the valve cusps are thickened or in any way changed as the result of preservation then turbulence ensues, and that if turbulent flow is present then the natural deterioration that occurs in preserved valve cusps may well be accelerated.

The high incidence of thrombus formation in the sinuses of Valsalva of those valves preserved in formalin and by freeze drying is probably caused by inadequate sinus lavage which is also the result of poor cusp mobility and turbulence. Thrombus in the sinus will organize and may calcify, thereby adding futher to malfunction of the valve.

Heavy calcification of the sinus of Valsalva occurred before macroscopic evidence of calcification of the cusps. This would seem to indicate the desirability of removing as much as possible of the aortic wall when using biological valves in the human being.

This experimental method provides a simple way of comparing the effects of preservation of heterologous aortic valves during the early period of their transplantation.

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