

Figure 1. Aortic valve dissected from a heart for preservation.

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## Current Methods

# Preserving and Sterilizing the Aortic Valve

Surgeons today have made it possible to replace the diseased aortic valve with a homograft<sup>1, 2, 3, 4, 5, 6</sup> or heterograft<sup>7</sup> preserved valve. The purpose of this communication is to outline current methods of preservation and sterilization.

### Preservation and Sterilization by 4% Formaldehyde

In 1908 Ward<sup>8</sup> and Guthrie, 1919,<sup>9</sup> used formaldehyde as a preservative for aortic segments. Interest in formaldehyde preservation returned in 1949 with Gross and others<sup>10</sup> who reported that formaldehyde buffered to an acid pH and was a better preservation method than any that had been used up to that time. Since then 4% formaldehyde has been used successfully both as a preservative and sterilization agent.<sup>7, 11</sup>

### Technique

Aortic valves were removed by a clean but not sterile technique from

cadavers or dead animals a few hours after death. The valves are excised in a tissue block having approximately one to two inches of proximal ascending aorta and a small rim of myocardial muscle and anterior mitral valve leaflet below. (Fig. 1)

Prior to immersion, the three aortic sinuses of each valve are tightly packed with formaldehyde-soaked swabs or cotton wool. This is to insure perfect apposition of the three leaflets. Valves that are not packed in this manner prior to preservation often lose their shape and the base and side of the sinuses become distorted. After 3-4 weeks in formaldehyde 4%, the valves retain this shape and the wool or swabs can be removed.

The valve is stored in 4% formaldehyde which is made up as follows:

*Dilute one part of 40% formaldehyde in nine parts of acetic acid-sodium acetate buffer. The acetic acid-sodium acetate buffer*

*is obtained by mixing 9.5 ml. of 0.2 m acetic acid with 90.5 ml. of 0.2 sodium acetate.*

After one week in this solution the pH is checked; if it has drifted above a pH of 5.6 it is corrected. Little change will be noted after the first two weeks as the solution stabilizes quickly and thereafter remains relatively constant.

### Measurement of Valve Size

After the valve has been in the solution for a minimum of three weeks it is removed from the jar, trimmed to the surgeon's own specifications and measured. For measuring the valve size, Starr-Edwards aortic obstetator cups, or one of a series of measuring obstetators ranging in size from 1.5 cm. to 3.75 cm. can be used. The valves are then stored in 4% formaldehyde at room temperature awaiting use.

In our laboratory, valves stored in 4% formaldehyde were sterile after 4 years. Other authors<sup>7, 11</sup> reported the same after putting valves stored

in formaldehyde under severe bacteriology testing.

On the morning of the operation when a valve replacement is anticipated, the valves are taken to the operating room. The valve which has been selected for use is removed from the jar with plain forceps. The valve is washed in either Ringer's solution or normal saline for a minimum of two hours. The solution chosen for washing the valves is changed as many times as possible during this period.

### Preservation by Freeze Drying

Preservation of the aortic valve by freeze drying is a well-established technique in many centers around the world.<sup>1, 12, 13, 14, 15, 16</sup> It enables tissues that can be transplanted to be stored for many years. The method necessitates building a vacuum system and glass circuit to achieve drying. The wide use of this method of preservation has brought about sophistication in the type of machinery used today.<sup>17</sup>

### Technique

On immediate receipt of the donor heart specimen, using an aseptic technique, the aortic valves are dissected out from the heart. The diameters of the valves' rings are then measured with one of a series of measuring obturators, sizing from 1.5 cm. to 3.75 cm., thus insuring a wide range of sizes in the valve bank.

The newly dissected valves are then placed in a solution of 100 ml. Aqua. Dist. + 500,000 units of penicillin + 0.5 grams of streptomycin. The valves in this antibiotic solution are then kept at 4°C. for 24 hours. The treatment of the valve tissues with antibiotics is aimed chiefly at impregnating the valves with antibiotic resistance required during the immediate postoperative stage.

### Sterilization

At the completion of the first 24 hour stage the valves are then transferred into the pyrex glass drying tube. (These drying tubes are shown suspended in Fig. 2.) Sterilization of the valve to gaseous ethylene-oxide.<sup>18</sup> A 10% in 90% CO<sub>2</sub> mixture of ethylene-oxide is used. The valves are left in the gaseous ethylene-oxide for a further 24 hours at 4°C. At the completion of this stage a section of aortic wall is removed aseptically for bacteriological investigation.

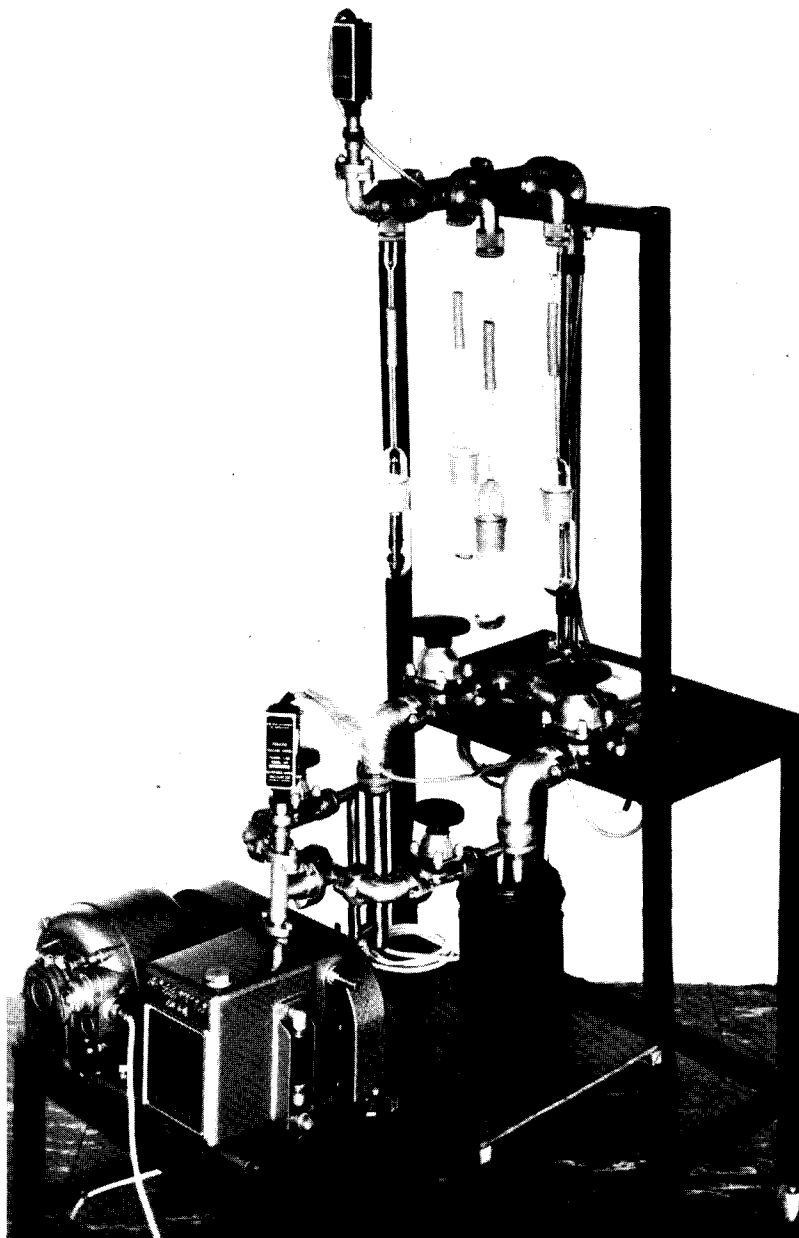


Figure 2. Tissue freeze drier used in aortic preservation (designed at Guy's Hospital, London.)

### Freeze Drying

The apparatus employed, shown in Fig. 2, consists of a two stage ballast pump which draws a continuous vacuum to a point of 0.02 torr. The drying circuit is made up of a manifold and coupling system. The sterile valves in the drying tube are first "flash frozen" immediately prior to freeze-drying. The method employed is to immerse the drying tube into a mixture of solid

CO<sub>2</sub> and absolute methylated alcohol, which maintains a temperature of -79°C. The valves are kept at this reduced temperature for a period of 30 minutes so that solid state freezing of the valves takes place. This protects the valve tissue's cellular structure from the immediate onset of high vacuum.

With special pyrex adaptors the drying tubes are connected to the vacuum system. A water trap, as a part of the

drying circuit, collects the water sublimated from the valve. The valve is left in the vacuum drying system for a period of 24 hours. After this length of drying time the micro-moisture content for each valve is 1.7%. At the completion of this final stage the drying tube is cut down from the vacuum system. This is achieved by heating the fine glass tube connecting the drying tube to the system. At a maximum temperature this tube collapses under the heat sealing the valves under vacuum.

The valves in their sealed tubes are stored in a rack at room temperature and may be kept indefinitely. Each tube is labeled with a reference number and size of the valve therein. A filing system is kept which records all details of stored valves.

### Reconstitution

When a valve is required a selection of valve sizes is made available in the operating room. When the required valve size is determined, the appropriate donor valve is reconstituted by removing the valve from its storage tube and immersing the valve in sterile water at 37° C. Reconstitution takes approximately 10 to 15 minutes.

### Conclusion

Methods of aortic valve preservation and sterilization have been discussed. The techniques involved are 4% formaldehyde and freeze drying together with ethylene-oxide sterilization. It is the authors' experience that these techniques have to be carried out with precision. Failure to do so will render the valve unusable when it has been reconstituted and ready for implantation.

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## The Cost of Living . . .

(Continued from Page 12)

private insurers have been reluctant to enter the field.

Persons covered by the Federal Medicare program for the elderly receive little aid for dialysis. Medicaid, the Federally assisted program adopted by some states to help low-income people pay medical expenses, provides more aid—\$25 for each in-hospital dialysis treatment—but still leaves substantial bills.

What's left for some kidney disease sufferers, then, is charity. While or-

ganized support for kidney care has been slow in coming, instances abound of local largess in individual cases. Last Christmas, for example, residents of Whitesville, Ky., a town of fewer than 1,000, raised \$26,000 in four days for Roscoe French, a 33-year-old carpenter for whom machine dialysis represented the only chance at life.

Even well-off victims may end up needing charity. "If you aren't indigent when you start dialysis, you soon will be," says one physician.

## CAUSE AND EFFECT

*The cardiac monitor unit had been given only superficial cleaning for some time which irked the house cardiologist no small amount. In an effort to put an end to such conditions, he phoned the administrative assistant in charge at his home one evening. The conversation went something like this:*

*"Mr. Jones, a puddle of scrub water was allowed to remain on the floor in the cardiac unit for a rather extended period this evening. During that time, the patient pulled loose a monitoring electrode, setting off the alarm. In her dash to answer the alarm, the nurse slipped on the wet floor, knocked against the ceiling-mounted I.V. standard, and smashed the bottle of solution against the electronic monitor."*

*"The contents of the bottle flooded the monitor causing an immediate short circuit. Since the patient was receiving oxygen, the shorted monitor erupted into flames. This fire caused a flash-back through the oxygen lines, exploding the oxygen storage bottles outside."*

*"Because of the magnitude of the explosion and the amount of high-flying debris, Air Force observers monitored the blast as an invasion and fired all of the retaliatory ICBM rockets! And all because of your darn scrub water!"*