Biological (Homo-, Heterologous) Aortic Valve Replacement *

Yoshimasa Senoo

For the rehabilitation of a heart that has lost its function many surgical procedures are being tried. However, in the case of the valve whose damage is so marked that its functional recovery cannot be attained by palliative surgery, total valve replacement is desired. This has come to be possible only with the advent of prosthetic heart valve. For this mechanical prosthesis there are many kinds of them being used in clinics such as ball type, discoid type and some leaflet type, but as these are primarily prepared from the aspect of function, they have disadvantages as to incur; 1) thromboembolism, 2) hemodynamical and mechanical failures, 3) infection as well as postoperative complications after insertion of the valve. The degree of such complications tends to be severe with caged ball valve while it is light with leaflet valve, but these are the problems attendant upon the prosthetic heart valve. Recently, attempts are being made to eliminate these shortcomings and gradually better and improved ones have begun to appear. Yet, as the heart valve must perform its function constantly in vivo and it should be a passive one way check valve without space occupation, whatever prosthetic valve we have today is far from ideal one because of the limitation it would have to face as to its material and shape as well its function. In contrast, if we use a biological valves for the replacement, we can obtain the conditions identical with the natural valve as to its morphology and function and can eliminate such disadvantages as thromboembolism and hemodynamical failure as well as various other problems due to space occupation, which accompany the mechanical prosthetic valve and it approaches a fairly ideal valve. Nonetheless, it is true that biological valve tissue, because of its non-auto- genous protein nature, presents many formidable problems such as what would be its antigenicity, what effect histological changes over a long period of time would have on the valve function, what treatment would affect favorably on these two above-mentioned problems, and how to sterilize it. Should we have solved these problems, we have to face other formidable problems of difficult surgical procedures and procuring homologous valve as well as one with an appropriate size. The problem of the size becomes even more crucial in the case where a large ring has to be sutured because of valve insufficiency, but supposing we can solve the problems of antigenicity and histological change, it seems that we can employ heterologous valve.

Murray¹ has succeeded in homotransplantation by inserting the valve to the descending aorta, and Bigelow,¹ Ross, Barratt-Boyes⁵ have successfully transplanted it to the sub-coronary position, and a great contribution by these investigators⁶-⁷ has demonstrated the safety and utility of homotransplantation. With the consideration to the hemodynamic advantage of biological valve, the author studied the antigenicity, the representative sterilization methods, and influences of the valve preservation method on the antigenicity and the strength (elasticity) of valve tissue both of homologous and heterologous valves, and here presents clinical experiences and surgical procedures tested on the basis of the results of such a study.

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ANTIGENICITY OF TREATED HETEROLOGOUS VALVE

As most of heart valves are considered to be avascular, there are many reports stating that in the homotransplantation (allograft) there occurs hardly any reaction between the valve tissue and recipient because of antigenicity and especially the cusp of donor shows not any alteration over a long period of observations. In attempting the valve replacement with biological valve it is necessary to use such a valve of ample size because it would bring about attendant complications as compared with the prosthesis with rigid ring, hence for purposes to use heterologous valve the author studied differences in the antigenic reaction between homograft and heterograft.

By suturing fresh, untreated homologous and heterologous (pig) non-coronary cusps of aortic valve to the descending aorta of hybrid dogs in such a way as to have the blood circulate in opposite direction (the cusp is always kept in the diastolic phase), one month later histological observations were carried with the cusps and the tissues of host-graft junction by fixing these tissues with CARNOY’S solution and staining with methyl green pyronin stain. As a result it was found that the cusps in both homograft and heterograft transplantations were macroscopically normal, nor any change in the pliability. In some portion of the base of cusp in heterograft there could be seen a slight infiltration of round cells. Even in the tissue of host-graft junction of homograft similar round cell infiltration was observable, and among these cells were mixed very sparsely some pyroninophylic cells but all the cells being small lymphocytes, there was detected not any reaction suggestive of antigenic factor (Fig.1). In the case of heterograft a moderate cell infiltration can be seen on the side proximal to the graft, and among them are seen some pyroninophylic large lymphocytes, i.e. some immunoblast cells, as shown in Fig.2. These findings indicate the difference in the antigenicity between homograft valve and heterograft valve, but, as there often accompanies a severe infection at the suture site and in the thoracic cavity in such experiments, it is difficult to determine accurately the degree of antigenicity of the treated heterologous valve, the very purpose for which this experiment has been conducted. Therefore, as the direct method of determining the degree of antigenicity of valve tissue, we employed the reaction of lymph nodes against stimulation.

For this stimulation test, we prepare homogenates of valve, aorta and ventricular muscle from rabbits, and the suspension of homogenate (i.e. 250 mg homogenate suspended in 1 ml physiologic saline plus 1 ml. Freund’s complete adjuvant) is injected into the paw pad of rabbits, one week later the weight increase of the popliteal lymph nodes, the primary lymph nodes is compared with that of lymph nodes from the opposite limb (into which 2 ml physiologic saline solution was injected as the control) and also the amounts of the lymph liberated from the lymph nodes are measured. For weighing, the lymph nodes are stripped off of fatty and connective tissues as thoroughly as possible and weighed. For measuring liberated lymph, microcanule is inserted into the efferent lymph duct to allow lymph flow out freely and with the lymph thus collected the amount of lymph and the number of cells in it is counted. Next, the smear

Fig.1. Histological findings of host-graft junction in fresh homograft. methyl green pyronin stain X400

Fig.2. Histological findings of host-graft junction in fresh heterograft. methyl green pyronin stain X400

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specimens of lymph are prepared, fixed with Carnoy's fixative, stained with methyl green pyronin stain, and the number of medium, large lymphocytes as well as blast cells is counted (200 cells each of lymph node are classified, the average of 5 specimens are classified, and counts are taken, Fig. 3). As illustrated in Fig. 4, both fresh homologous valve and fresh homologous heart muscle show a slight increase in weight over that of the control (injected with 2 ml physiologic saline), but this is mainly due to the increase in the number of medium lymphocytes and a few large lymphocytes but no immuno-blast cells can be detected. In the case of fresh heterologous valve and fresh heterologous heart muscle, the weight increase of lymph node in each case proves to be 3.0 times and 4.5 times respectively, being a marked increase as compared in the case of homologous group and both show the blast cell formation. In addition, large and medium lymphocytes are increased in proportion to the increase in the weight of lymph nodes. Whether the antigenic reaction observable in this fresh heterologous valve is directly involved in the rejection is uncertain, these results parallel with those of the valve transplantation to the descending aorta mentioned in the foregoing. Consequently, it is reasonable to assume that, speaking of fresh valve, from the aspect of the ratio of the weight increase in lymph node or the ratio of blast cell formation between the heterograft and homograft, the former is more apt to induce the reaction derived from antigenicity. Even with such heterologous valve, when immersed in formalin solution (preserved for 4 weeks in 4% formalin solution buffered with acetic acid-sodium acetate, pH 5.6) or when freeze dried (frozen at −80°C and dried, kept for 4 weeks), after the same procedures as in the preceding cases, the ratio of the weight increase of both is not any appreciably different from that in the case of fresh homograft, no longer immunologic.

Fig. 3. Demonstration of various lymphocytes through efferent lymph.
- methyl green pyronin stain X400
- B: Blast cell
- L: Large lymphocyte
- M: Medium lymphocyte
- S: Small lymphocyte

Fig. 4. Various cell output through efferent lymph and ratio of increase in weight of lymph node after the stimulation.

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blast cells can be observed, and the number of large and medium lymphocytes decreases markedly. In other words, in studying the antigenicity of valvular tissue by utilizing the reaction of lymph node against the stimulation by antigenic substance, after the pretreatment of the valve (at present such a pretreatment is done mainly for the purpose of preservation), even the heterograft loses much of its antigenicity as compared with fresh one and its antigenicity becomes practically the same as that of fresh homograft.

Further, in the case of freeze drying processes, 1% beta-propiolactone (at 37°C for 2 hrs) is used for sterilization, but by this treatment alone, only a slight decrease can be observed in antigenicity.

**Influences of Treatment on the Fragility (Elasticity) of Valve**

The formalin-preserved or freeze-dried heterologous valve mentioned in the foregoing, from the aspect of antigenicity, can be handled practically the same as homograft, and the histological examinations of these treated valves, aside from vacuole formation in the endothelial cells or some exfoliation observable in the freeze-dried valve, show no other structural difference from untreated valves. However, as regards the fragility (elasticity) which is one of the important problems in biological valve, it would be desirous to study the fragility from the angle other than the change in the histological structure of the valve.

With substitute valve, especially with biological one which is liable to receive histological changes after transplantation, the appraisal of its fragility should be given a preference to the one which can function as a valve *in vivo* over a long time. In reality, however, it is very difficult to keep the animal surviving a long time after the transplantation of experimental homograft (conducted for the purpose to determine the fragility of the valve tissue). Therefore, in the present evaluation, the tensile strength of the valve immediately after the excision and that of the treated valve was compared. It is true that such a method can only manifest one aspect of the tensile strength, but it would determine at least whether or not there is a direct effect of treatment on the fragility of the valve.

Method of appraisal: In this instance, non-coronary cusp of pig aortic valve attached only with the aortic wall of the commissure serves as the cusp. By holding the aortic wall in hand, the load is added to the valve at a given speed, and the lengthening of the valve tissue is recorded and continuing this loading until the tensile strength of the tissue is lost, the load required for this is measured with Tensilon (a product of Toyo Precision Machine Mfg. K.K.). It is true that with small pieces of the cusp more simple values can be obtained, but there is no denying that the integrated tensile strength of entire fibers cannot be attained with sections of valve tissue and also by this method some mechanical factors will intervene. Therefore, usually measure the tensile strength of one whole cusp. In this procedure, while no problem in recording that of the stretching phase, but encounter complex problem in the disruptive phase. To overcome this difficulty, measured both the load required to induce a local cleavage (primary cleavage of the shortest portion of valve) and that required to cause total cleavage of the entire tissue, taking care to avoid error in the calculation.

The results are as shown in Table I. The load required for the primary cleavage is 490–550 g (the minimum threshold) and 875–1,070 g (the maximum threshold), in either case that of formalin-preserved valve being somewhat higher, but otherwise values are about equal. By the average value (that of 10 specimens tested) the load value with formalin-treated valve is higher than that of untreated valve, while the values of the preserved valve (in lactate Ringer solution at 4°C, with PC. SM. and the freeze-dried valve) are 20–14% lower than that of the untreated. As for the actual load required for the total cleavage, one of the valves preserved in the lactate Ringer solution is 50% of the load required for untreated one, and after the cleavage its minimum threshold is 25% less than that of freeze-dried valve, otherwise the average load tends to be about equal to that required for the primary cleavage. The tensile strength was studied with valves preserved in the lactate Ringer solution for 4 and 6 weeks, and with freeze dried valves preserved in the formalin solution for 4, 6 and 24 weeks, but the length of preservations time made no significant difference in the tensile strength measured.

There are some valves in those preserved in the lactate Ringer solution and in those freeze-dried ones whose average load required for the primary cleavage and that for the total cleavage become less (namely, those that show the load required for the total cleavage close to that for the primary cleavage), but this seems to be due to autolysis in the Former group and to the vacuolization of the tissue during the freezing process in the latter.

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TABLE I  TENSILE STRENGTH OF VARIOUS VALVE

<table>
<thead>
<tr>
<th>Kind of Valve</th>
<th>Load for primary cleavage</th>
<th>Load for total cleavage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Actual value (g)</td>
<td>Average (g)</td>
</tr>
<tr>
<td>fresh</td>
<td>490 – 890</td>
<td>880</td>
</tr>
<tr>
<td>in Lactate Ringer solution</td>
<td>550 – 875</td>
<td>708</td>
</tr>
<tr>
<td>4% formalin preserved</td>
<td>500 – 1,070</td>
<td>948</td>
</tr>
<tr>
<td>freeze-dried</td>
<td>510 – 925</td>
<td>753</td>
</tr>
</tbody>
</table>

However, such defects can be detected at the time of trimming (to be explained later) so that careful observation would avoid these undesirable defects.

STUDY ON THE SURGICAL TECHNICS

Biological valve is an excellent substitute valve from the hemodynamical standpoint, but when treated variously as aforementioned, it becomes as a non-viable one and serves only as a biological prosthetic material. However, the reason why mechanical prosthesis is still extensively used today in clinics lies mainly in the fact that surgical procedures for the total valve replacement with biological valve are extremely complicated and require utmost precaution so that it takes a long time to complete "bypass". For purposes to master such complicated technics of total replacement with biological valve as well as to establish the procedures as simple and reliable as possible, the author performed homo- and heterograft replacements of aortic valve with dogs and calves as the test animals.

In Series 1 with mongrel dogs (15–20 kg), under extracorporeal circulation and selective cardiac cooling, a fresh homologous valve was fixed by interrupted single layer suture. However, due to complications accompanying the extracorporeal circulation and the selective cardiac cooling, as well as injury to aorta and coronary ostia, suture insufficiency and surgical technical errors, long survival could not be attained in any animal.

In Series 2 similarly dogs were used. In this series the cardiac arrest was induced by the surface cooling (esophageal temperature, 20–18°C) and the injection of Young's solution into the coronary arteries, and the homograft was sutured by the same surgical technic as in Series 1. As a result it was quite difficult to elicit heart beats probably due to a long time required for the suture. Therefore, in order to lessen the operation time (i.e. the time to elicit cardiac arrest) a metal ring, as shown in Fig.5, was prepared, and after suturing the homologous valve to the ring, it was fixed to the host by the procedures identical with those used in mechanical prosthesis. By this method resuscitation of heart became possible so that hemodynamical examinations measuring of intracardiac pressure of the aorta and left ventricle were done. As a result a faint systolic thrill of the heart became palpable and there was hardly any systolic pressure gradient of the aorta and left ventricle, suggesting that hemodynamically aortic stenosis could be ignored.

Series 3. In the experiments with dogs the study on surgical technic could not be carried out sufficiently because of extremely small field of the surgery. Therefore, in this series calves (80–120 kg) were used. In this instance, fresh homograft and heterograft (of pig) were replaced under extracorporeal circulation and selective cardiac cooling or continuous coronary perfusion. However, being hampered by complications such as postoperative respiratory disturbances and bleeding, it was not possible to achieve long survival. Nonetheless, in this series of experiments it has been found that the continuous single layer suture and an additional mattress suture (to be explained later) at the lower end of commissure would ensure a sufficient fixation, and the formalin pre-

Fig.5. Supporting metal ring for experimental use.
served valve is generally more rigid than the fresh one or the freeze-dried valve, and it is easier to manipulate (as illustrated in Fig.6). Further, this rigidity has no effect on hemodynamics.

CLINICAL EXPERIENCES

On the basis of the results in the experimental basic studies as well as the favorable clinical results of ROSS, BARRATT-BOYES and others, homologous or heterologous aortic valve replacement was tried on 10 cases of aortic valvular disease. The first case was operated on September 7th, 1967 which proved to be the first case of biological valve ever performed in Japan. The patients were consisted of 7 males and 3 females in the age ranging from 13 years to 39 years old. Four cases were congenital origin, others were rheumatic origin in thirties excepting one 22-year old case.

The etiology of valve disease was found to be as follows: Congenital cases were composed of 2 cases with aortic incompetence accompanying ventricular septal defect, one case of annuloaortic ectasia complicated with pyoderma gangrenosum, and one with bicuspid aortic stenosis to the total of 4 cases. Acquired cases were 6 in number, all of which had dominantly aortic incompetence, while 4 out of the 6 had compation of aortic stenosis, and isolated aortic regeneration was found only in two of them. Further, 2 cases of mitral valve disease were included in the six, but there was none that required surgical repair on the tricuspid valve. In these cases of acquired valve disease were 3 cases having definite history of previous rheumatic fever, and the other three were proven to be also of rheumatic origin on the operation, but there was none of bacterial endocarditis or syphilitic origin in this series.

of previous rheumatic origin on the operation, but there was none of bacterial endocarditis or syphilitic origin in this series.

Cardiac symptoms are usually classified according to the classification of New York Heart Association, but as the author considered the classification of BARRATT-BOYES to be more suitable to aortic valvular disease. The latter was used.

Grade I: Asymptomatic
Grade II: Effort dyspnea only
Grade III: Effort dyspnea, angina of effort, and syncope
Grade IV: Congestive heart failure, paroxysmal nocturnal dyspnea, orthopnea, or angina decubitus

Three patients were of Grade II, all of them had congenital aortic lesion (2 cases with VSD, AI, one case with bicuspid AS). Three other cases were of Grade III and the remaining four were of Grade IV.

Findings of electrocardiogram are indicated by the following criteria:

LV+ : $SV_1 + RV_5 > 35 \text{ mm}$
LV++ : low-voltage $T \pm ST$ depression in left ventricular surface leads or equivalents
LV+++ : T inversion + ST depression in left ventricular surface leads or equivalents

Block 1st d. : 1st degree of heart block

Electrocardiogram revealed LV++ in five out of the ten cases, and the remaining five were LV++. Of them 8 cases showed sinus rhythm and the rest two, atrial fibrillation. In addition, there were two cases showing the first degree of heart block.

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Of the preoperative intracardiac pressure the values that represent directly the degree of lesions and the severity of aortic regurgitation as determined by aortography are as shown in Table 1.

The severity of aortic regurgitation is illustrated in the following manner according to Lillehei:

I Evidence of a jet of regurgitant contrast material without opacification of the left ventricle
II Evidence of a regurgitant jet with faint opacification of the left ventricle
III Dense opacification of the left ventricle and no distinct jet is usually visible
IV The left ventricle is opacified more densely than the aorta

Excepting the case of bicuspid aortic stenosis complicated with a faint regurgitation and a case of VSD·AI, showing 2nd degree regurgitation, all others revealed the severity of regurgitation of III-IV degrees. As for the tenth patient who was admitted to the hospital with subjective symptoms of frequent anginal pain in the state of congestive heart failure, intensive medical treatment slightly alleviated congestive heart failure but anginal pain could not be controlled, the operation was performed only on the diagnosis of physical signs without any special examinations like cardiac catheterization.

Method of the valve preparation

Homologous aortic valves were obtained at the autopsy of legal medicine from donors (20–35 years old) without cardiovascular disease (no calcification or atheromatous change as the local findings). Aortic heterografts were from pigs weighing 70–120 kg. For the case No. 5 because of markedly large aortic ring the graft was taken.
from a calf. All of them are excised under unsterile conditions without separating from the heart (for 24 hours) and after measuring the size with Starr's obturator, each valve is separated from the heart, washed several times with physiological saline containing penicillin and streptomycin kept at 4°C. For the formalin preservation cotton is packed in the Valsalva sinus to keep the valve in diastolic phase, and immersed in the 4% formalin solution buffered with acetic acid-sodium acetate (pH 5.6). Thereafter until the use, the formalin solution is exchanged with fresh one once every week. For the freeze-dried valves, each valve is chemically sterilized with 1%-beta propyolactone for 2 hours at 37°C, then frozen at -80°C, dried and kept in a vacuum vessel at 4°C\(^\text{22}\).

Just before the use each valve is given final trimming, its size is measured, the conditions of the valve are checked and also a small piece of it is cultured under bacterial aerobic and anaerobic conditions to confirm the sterility. By removing thoroughly ventricular muscle, fatty and connective tissues the final trimming is completed, but the valve is measured once more to check whether or not its change has occurred during the preservation period\(^\text{17}\).

During the period between the final trimming and the use the formalin preserved valve is again kept in 4% formalin solution and the freeze-dried

Fig. 8. 
Top: Before operation-diseased aortic valve. 
Bottom: After operation-heterograft in aortic position.

Fig. 9. Aortography of case 8. 
Left: Preoperative 
Right: Postoperative

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<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Valve lesions</th>
<th>Symptoms.model (Grade)</th>
<th>L.V.H. in E.C.G.</th>
<th>Preoperative pressures (mmHg)</th>
<th>Aortic regurgitation</th>
<th>Valve preparation</th>
<th>Coronary perfusion</th>
<th>Associated surgery</th>
<th>Postoperative pressures (mmHg)</th>
<th>Postop. murmurs and results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>♂</td>
<td>Aortic regurgitation due to VSD</td>
<td>II</td>
<td>Sinus rhythm</td>
<td>LV²₀₀ 200 100</td>
<td>Heterograft Frozen</td>
<td>Selective cardiac cooling</td>
<td>Patch closure of VSD</td>
<td>LV²₁₀ 125 75 100</td>
<td>120/80 80</td>
<td>replaced to ball valve</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>♂</td>
<td>Aortic regurgitation due to VSD</td>
<td>II</td>
<td>Sinus rhythm</td>
<td>LV²₁₀ 125 75 100</td>
<td>Heterograft Frozen</td>
<td>Selective cardiac cooling</td>
<td>Patch closure of VSD</td>
<td>LV²₁₀ 125 75 100</td>
<td>120/80 80</td>
<td>replaced to ball valve Postop. 131 d.</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>♂</td>
<td>Aortic regurgitation and mitral stenosis</td>
<td>IV</td>
<td>Sinus rhythm block 1st d.</td>
<td>LV²₁₀ 125 75</td>
<td>Heterograft Frozen</td>
<td>Selective cardiac cooling</td>
<td>Mitral commissurotomy</td>
<td>LV²₁₀ 125 75 100</td>
<td>120/80 80</td>
<td>Postop. 4 d. died Cerebral damage</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>♂</td>
<td>Aortic stenosis, regurgitation</td>
<td>IV</td>
<td>Atrial fibrillation</td>
<td>LV²₁₀ 125 75</td>
<td>Heterograft Frozen</td>
<td>Continuous perfusion (both)</td>
<td>Mitral commissurotomy</td>
<td>LV²₁₀ 125 75 100</td>
<td>120/80 80</td>
<td>Postop. 10 d. dist. m 1/6</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>♂</td>
<td>Annulo-aortic ectasia</td>
<td>IV</td>
<td>Sinus rhythm block 1st d.</td>
<td>LV²₀₀ 200 100</td>
<td>Heterograft Frozen</td>
<td>Continuous perfusion (both)</td>
<td>Mitral commissurotomy</td>
<td>LV²₁₀ 125 75 100</td>
<td>120/80 80</td>
<td>Postop. 7 d. dist. m 2/6</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>♂</td>
<td>Bicuspid aortic stenosis</td>
<td>II</td>
<td>Sinus rhythm</td>
<td>LV²₀₀ 200 100</td>
<td>Heterograft Frozen</td>
<td>Continuous perfusion (both)</td>
<td>Mitral commissurotomy</td>
<td>LV²₁₀ 125 75 100</td>
<td>120/80 80</td>
<td>replaced to ball valve</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>♂</td>
<td>Aortic stenosis, regurgitation</td>
<td>III</td>
<td>Sinus rhythm</td>
<td>LV²₀₀ 200 100</td>
<td>Heterograft Frozen</td>
<td>Continuous perfusion (both)</td>
<td>Mitral commissurotomy</td>
<td>LV²₁₀ 125 75 100</td>
<td>120/80 80</td>
<td>No distal m.</td>
</tr>
<tr>
<td>8</td>
<td>31</td>
<td>♂</td>
<td>Aortic stenosis, regurgitation, mitral stenosis</td>
<td>III</td>
<td>Atrial fibrillation</td>
<td>LV²₀₀ 200 100</td>
<td>Heterograft Frozen</td>
<td>Continuous perfusion (both)</td>
<td>Mitral commissurotomy</td>
<td>LV²₁₀ 125 75 100</td>
<td>120/80 80</td>
<td>No distal m. Apex: syst. m. (Mitral origin?)</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>♂</td>
<td>Aortic regurgitation</td>
<td>III</td>
<td>Sinus rhythm</td>
<td>LV²₀₀ 200 100</td>
<td>Heterograft Frozen</td>
<td>Continuous perfusion (both)</td>
<td>Mitral commissurotomy</td>
<td>LV²₁₀ 125 75 100</td>
<td>120/80 80</td>
<td>Postop. 13 d. died sepsis No distal m.</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>♂</td>
<td>Aortic stenosis, regurgitation</td>
<td>IV</td>
<td>Sinus rhythm</td>
<td>LV²₀₀ 200 100</td>
<td>Heterograft Frozen</td>
<td>Continuous perfusion (both)</td>
<td>Mitral commissurotomy</td>
<td>LV²₁₀ 125 75 100</td>
<td>120/80 80</td>
<td>No distal m.</td>
</tr>
</tbody>
</table>

Ao = Aorta; B.A. = brachial artery; dist. - syst. m. = diastolic - systolic murmur; LA = left atrium;
LV = left ventricle; PA = pulmonary artery; VSD = Ventricular septal defect; * calf

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Operative Procedures

Under anesthesia by inhalation of Fluothane, every case is subjected to median sternotomy, and the intracardiac pressure is measured to confirm diagnosis. Total body perfusion is performed with Pemco oxygenator and transverse or oblique aortotomy is conducted. In every case coronary perfusion is carried out by cannulating coronary arteries, but for the first three cases cardiac cooling was concurrently done to have cold arrest for the operation and with the rest 7 cases normothermic perfusion (6–8 cc/min/kg) was done as much as bilaterally but in case not possible only on the left side. Diseased aortic valve is excised leaving about 2 mm of it and the aortic ring size is measured with STARR's obturator. For the biological valve to be used in replacement one whose inner diameter is slightly smaller than that measured at the final trimming is selected. The method of valve suture to the aortic annulus is as shown in Fig.7. Namely, at first the aortic wall at the lower end of the donor valve is fixed onto the left ventricular wall of the recipient by mattress suture. Only near His' bundle thread is inserted into the membranous septum to avoid injury to His' bundle. Next, continuous suture is done by adapting the remnant valve of recipient with the aortic wall (2–3 mm) of donor well enough to ensure no space between them. Fig.8 shows the conditions before and after the valve replacement in Case 8. In the case where cardiac cooling is applied after the closure of aortotomy, ventricular fibrillation is removed with D-C defibrillator. Further, in the case with complication of VSD, aortic valve surgery is performed after patch closure, while in the case with mitral valve disease its repair is done after the aortic valve replacement. At the termination of surgery the record is take of postoperative intracardiac pressure.

Results

The postoperative blood pressure of those successful cases in biological valve replacement is shown in Table II and all of these cases showed a marked improvement in aortic incompetence. Fig.9 illustrates the aortography of Case 8. In every case fine systolic thrill could be palpated at the aortic root, but 2 cases out of the five that used formalin preserved valve showed systolic gradient across the aortic valve of 15–20 mmHg, the others had not problem.

Postoperatively, ejection type systolic murmur was heard over the aortic area in every case, and though there was no diastolic murmur immediately after the operation, in Cases 2, 4 and

Fig.10. Ruptured aortic valve (case 2).

Fig.11. Histological findings of the ruptured cusp. X100

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5 it was heard on postoperative day 7, 10, and 7 respectively. In Cases 4 and 5, 1/6 and 2/6 degree respectively remained unchanged and the diastolic pressure decreased only by 10–15 mmHg, but in Case 2 it gradually increased and by about postoperative day 60 the diastolic pressure fell to 40 mmHg. In this case on postoperative day 131 the replaced biological valve was exchanged for STARR prosthesis. The valve taken out at the reoperation had a large rupture mainly in the noncoronary cusp as shown in Fig. 10. However, the suture region is completely covered with epithelium, and there is no marked change though it looks somewhat edematous. Histologically, there can be observed exfoliation of only a portion of epithelial cells but the principal structure remains intact (Fig. 11).

Cases 1 and 6 are the ones to whom mechanical prosthesis was placed immediately after biological valve replacement. Case I was reoperated because of a marked enlargement of the left ventricle due to the operation being performed under ventricular fibrillation by cardiac cooling so that the left ventricular vent had not sufficiently functioned, but the case seems to have required no reoperation had its vent been sufficiently effective and had defibrillation been done. Case 6 is of congenital bicuspid aortic valve, and due to errors in the fixation of commissure there appeared regurgitation immediately after the operation so that the valve was replaced with prosthesis.

Cases 3, 7 and 9 died on postoperative day 4, 7 and 10 respectively due to cerebral damage, respiratory disturbances and acute endocarditis. However, even in these cases no diastolic murmur was audible nor any sign of left ventricular failure was observed.

**DISCUSSION**

Mechanical prosthesis centering around STARR-EDWARDS ball valve has greatly contributed to the surgical treatment of severe valvular disease. Nonetheless, even these prosthetic valves that are being widely used in the field of surgery still have many problems to be solved. Because of low incidence of thromboembolism, mechanical or functional failure, the prosthetic valves do not present fatal problem like the hardening of previous prosthetic leaflet valves, but on account of the construction of prosthesis these accidents often bring about severe results. Indeed, a truly ideal substitute valve would be the passive one-way check valve without space-occupation, and biological valve, when several of its problems are solved, would satisfactorily meet these conditions.

In view of this, recent studies on the replacement surgery with biological valve have gained inpetus. Experimental study on this field had its beginning with LAM24 and a successful valve replacement to the descending aorta was reported by MURRAY (1956). The valve transplantation to the sub-coronary position was started with technical studies of DURAN25 and its chemical application by BIGFLOW through many clinical experiences ROSS2 and BARRATT-BOYES obtained favorable results similar to those by the predecessors who attempted the ball valve replacement. What is noteworthy is the fact that, excepting a few cases of rupture of cusp or leakage, there had been no case whose valvular function has aggravated. Among the cases encountered by these two surgeons histological examinations of the transplanted valve obtained at reoperation or at autopsy tell us the fate of the preserved homologous valve. HUDSON reports that with biological valve there can be observed replacement by the host tissue as seen in the case of fresh acellular valve, the collagen and elastic fibers all maintain their structure well, and the cusp is pliable and no deposit of fibrin or calcium can be observed.

BARRATT-BOYES and SMITH29 state that by the nutrition from circulating blood the fiber construction is maintained well and there can be seen newly regenerated elastic fibers and endothelial cells. In other words, the opinions by these two investigators do not necessarily agree with each other. This mild chronic inflammation might prove to be the cause of malfunction of replaced valve in future. However, the progress of this mild inflammation is far slower than the speed with which cells of the host are replaced as seen in fresh valve so that this would rather mean less hardening-contraction than in the case of fresh valve. Actually in the results obtained by ROSS, BARRATT-BOYES and other surgeons there has never been any malfunction of the valve. In addition, the elimination of thromboembolism and acute mechanical or functional failure as observable in prosthetic valves as well as the excellent hemodynamics attainable can more than cover up various apprehensions attendant to such a valve replacement.

There are problems that cannot lightly be ignored such as the difficulty in procuring homograft, it is often too small for the enlarged annulus in the case of valve insufficiency, a difficulty in obtaining the graft of ample size as well as aging changes of the valve tissue.20,31
There are some attempts being made at the solution of the size problem by the surgical means applied to the non-coronary cusp of aorta in the recipient. However, the utilization of heterologous valve may serve as a solution of these problems presented by homograft. Many experimental and clinical data have demonstrated that there is no need of taking the immunologic reaction into consideration in the case of homograft with preserved valve as well as with fresh valve, but in the case of heterologous valve which is not so avascular as with human valve it is necessary to study its antigenicity.

Paton et al. have carried out histological studies in their valve replacement experiments to the descending aorta both with fresh homograft and heterograft and report that there occurs a fairly strong rejection with the fresh homograft but this can be inhibited by some immuno-suppressive agents like azathioprine. In the replacement experiment of one cusp to the descending aorta the author has observed the infiltration of a considerable number of round cells at the host-graft junction in the case of fresh heterograft to the host, and among these infiltrating cells there were some pyroninophilic large lymphocytes (immunoblast cells). This phenomenon seems to be somehow involved in the future rejection. However, a marked decrease in the antigenicity of the valve can be attained by the routine treatments such as with formalin solution or freeze-drying as applied for the preservation of heterologous valve. In the study conducted by utilizing the primary reaction of lymph node against antigenic stimulation, the author observed blast-cell formation in the case of fresh heterograft but it could not be seen with preserved heterograft, and lymphocytes other than blast cells showed the reaction only in the degree practically the same as with fresh homograft. In other words, it is known with homograft but in the case of heterograft it can be said that when it is made non-viable, it becomes a biologic prosthetic material, and weakens its antigenicity.

What changes are brought about on the mechanical fragility (elasticity) of valve tissue due to various treatments given for the purpose of preservation, lessening of antigenicity or sterilization are usually determined by tensile strength or pressure tests of the valve. The author has applied the test (which differs from the routine method) to see the fragility of preserved heterologous valves, by checking the tensile strength of one whole cusp, but has observed no marked changes as compared with fresh valve, and with the load of 350g (the standard set by Ross) the author has observed not any change in every instance.

One of the other important problems in biological valve replacement is central or peripheral leakage or postoperative incompetence due to the twisting of the transplanted valve. For the central leakage generally the tailoring of host aorta is done, while for the peripheral leakage or twisting Ross has improved the surgical technic. Most of the other surgeons perform the double layer suture just as Ross, Barrat-Boyce, Kirklin, and J. H. Kay do. However, O'Brien use single layer suture, and E. B. Kay and Suzuki only the suture of cusp. Further, there are some who prepare the prosthetic biological valve with supporting ring taking advantages of both excellent hemodynamics of leaflet valve coupled with the simplicity of operative technic of prosthesis. All these procedures have advantage and disadvantage in that the double layer fixation utilizes fully the excellent features of biological valve but it takes a long time for operation; E. B. Kay and Suzuki's method has limited indication, and the method using supporring ring is simple in its operative technic but it eliminates the elasticity of annulus.

In contrast, the author uses the valves of ample size including heterologous valve for the central leakage, and for the peripheral leakage the host remnant valve and the aortic wall of graft are made in about the same width and these are adapted well to each other as described in the foregoing, and by single layer suture coupled with mattress suture of the lower end of commissure the time required for the operation is shortened.

There is no other method of saving the life of patients with severe valvular disease other than the valve replacement. As to the substitute valve to be used, the most suitable one will be a prosthesis which remains intact in vivo for indefinite period of time and shows natural hemodynamics, but having no such ideal prosthesis at present, the role of biological valve as a passive one-way check valve seems to be indeed great.

Summary

Fresh heterologous aortic valve has a stronger antigenicity than fresh homologous valve, but preserved heterologous valve shows antigenicity only in about the same degree as fresh homologous valve. The tensile strength of such a preserved valve differs not much from that of fresh

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valve. On the basis of these findings, the replacement surgery was performed on 10 patients with aortic valvular disease using preserved homograft or preserved heterograft. For operative procedures continuous single layer suture coupled with mattress suture of the lower end of commissure were employed, but no leakage was observed due to this method. Excepting the two cases which required the change to prosthesis immediately after the operation, two cases of the 8 showed postoperative systolic gradient across the aortic valve of 15–20 mmHg. Three cases died due to the cerebral damage, postoperative respiratory disturbances and acute bacterial endocarditis resulting from the heart-lung bypass, and one case required reoperation due to aortic incompetence by the rupture of cuff.

Those given homograft amount to 4 cases, those receiving heterograft 6 cases; and the formalin preserved valves were given to 5 cases and the freeze-dried valves to 5 cases, but there could be observed no prominent difference in these cases.

While the number of cases handled is small and the results are also not quite so satisfactory, it has been demonstrated that biologic valves including preserved heterologous valves are excellent for the substitute valve. Therefore, the results are present as a preliminary report of the study in this line.

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