Chronic Hemolysis Following Mitral Valve Replacement

A Comparison of the Frame-Mounted Aortic Homograft and the Composite Seat Starr-Edwards Prosthesis


SUMMARY
The incidence and severity of chronic intravascular hemolysis was evaluated in a total of 41 patients following mitral valve replacement. Valve replacement was with a gamma-radiated frame-mounted aortic homograft in 21 patients and with a composite seat Starr-Edwards prosthesis, model 6310 or 6320, in 20 patients. The parameters used to assess hemolysis were hemoglobin, hematocrit, reticulocyte count, red cell fragment count, serum haptoglobin, LDH and HBD, hemosiderin in the urine and red cell survival. The degree of hemolysis was classified as mild, moderate or severe. In the prosthetic valve group 85% showed evidence of chronic intravascular hemolysis, of which a third were mild and the rest moderate. The homograft patients did not show any comparable evidence of hemolysis. Statistical analysis of the comparative figures of the parameters used to assess hemolysis in the two groups of patients revealed a significant difference (P < 0.01) in hemoglobin and hematocrit and a highly significant difference (P < .001) in serum haptoglobins, hemosiderin in the urine, LDH, reticulocyte count, red cell fragment count and red cell survivals.

Additional Indexing Words:
Cardiac hemolytic anemia Frame-mounted homograft valves Red cell survival
Hemosiderinuria Haptoglobin Lactic dehydrogenase

Gamma-radiated homograft aortic valves, frame-mounted and tested have been used for mitral valve replacement at Killingbeck Hospital for the last three years. Clinical results have been satisfactory and in order to further evaluate this type of valve, studies were carried out to assess the incidence and severity of hemolysis in all the available patients. A comparison was then made with a similar group of patients in whom the mitral valve was replaced with a composite seat Starr-Edwards prosthesis.

Material and Methods
Studies of hemolysis were carried out on a total of 41 patients: 21 patients had a frame-mounted aortic homograft in the mitral position and 20 patients had a composite seat Starr-Edwards prosthesis, Model 6310 or 6320, size 2M or 3M. All valves were considered clinically to be functioning well. All investigations were carried out 4 months to 3 yr after operation in the homograft series, and 6 months to 5 yr postoperatively in the prosthetic series. The age distribution in the homograft group was between 33 and 59 years with a mean of 46.7 years and in the Starr-Edwards group between 30 and 60 years with a mean of 45 years. The sex distribution was similar in both groups.

Hemoglobin estimation was made by the routine cyanmethemoglobin method. The hematocrit was measured by micro-hematocrit using ultra centrifugation. The red cell fragment count was enumerated on 1,000 red cells from peripheral blood films, made from blood collected in ethylenediamine tetracetic acid (EDTA), fixed with methyl alcohol and stained with Leishmann’s stain. The results are an average of the counts by two experienced observers. A red cell was defined as a schistocyte (fragment) if it was small, irregularly shaped, or a piece of a cell. Reticulocyte count was made from blood collected in EDTA and stained with brilliant cresyl blue.

Serum haptoglobins, measured as hemoglobin binding capacity, was estimated by the method of Nyman. Serum lactic acid dehydrogenase (LDH) was estimated by the method of La Due and Wroblewski.

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Received April 9, 1973; accepted for publication May 17, 1973.

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hydroxybutyrate dehydrogenase (HBD) by the method of Wilkinson and Elliott⁴ and total serum bilirubin estimations were done by the hospital biochemistry laboratory. If the total bilirubin was elevated, then the conjugated and unconjugated components were determined. Table 1 lists the tests performed and the normal values for each parameter.

The direct anti-human globulin test was performed with a broad spectrum Coombs' reagent. Glucose-6 phosphate dehydrogenase was determined to exclude any intrinsic abnormality of the red cell, osmotic fragility studies were performed on fresh heparinised blood and Ham's test (acidified-serum test) was carried out to exclude paroxysmal nocturnal hemoglobinuria (all tests done according to the standard methods of Dacie and Lewis).⁵

Freshly voided urine was centrifuged and examined microscopically for red cells; if none were found a 'hemostix' was used to test for hemoglobinuria. A 20 ml sample of urine was centrifuged at 5,000 rev/min and some of the deposit spread on two glass slides. The smears were heat fixed and then alcohol fixed for 10 min. They were then stained for 25–30 min with a mixture of equal volumes of 20% v/v hydrochloric acid and 10% w/v freshly prepared potassium ferrocyanide. After this the slides were washed in water for 15–20 min to remove excess of acid and counter-stained with Neutral red (0.1%) for 30–60 sec. Iron containing pigment appeared as isolated or grouped, blue staining granules. These were examined using a microscope objective 43X under oil immersion and a scale of scoring adopted for hemosiderinuria which ranged from 0–4 plusses.

Red cell survival studies using radioactive ⁵¹Cr were carried out with autologous cells in 10 patients with homografts and 10 with Starr-Edwards prosthesis. All patients were tested on more than one occasion.

The level of significance (P) of the difference between two means was obtained from the standard error of each mean, using the Student t-test. A similar method, using the standard error of each proportion was employed to obtain the level of significance of the difference between the proportions.

### Table 1

**Investigations and the Normal Values**

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/100 ml)</td>
<td>Males: 13.5 - 18</td>
</tr>
<tr>
<td></td>
<td>Females: 11.5 - 16.5</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>Males: 40 - 54</td>
</tr>
<tr>
<td></td>
<td>Females: 35 - 47</td>
</tr>
<tr>
<td>Reticulocyte Count (%)</td>
<td>0.2 - 2</td>
</tr>
<tr>
<td>Red Cell Fragment Count</td>
<td>&lt; 1.0%</td>
</tr>
<tr>
<td>Serum Haptoglobin</td>
<td>70 - 150 mg/100 ml</td>
</tr>
<tr>
<td>Serum Laetie</td>
<td>50 - 200 I.U./L at 25°C</td>
</tr>
<tr>
<td>Dehydrogenase</td>
<td>55 - 150 I.U./L at 25°C</td>
</tr>
<tr>
<td>Red Cell Survival</td>
<td>T½ 27 ± 3 days</td>
</tr>
</tbody>
</table>

### Results

None of the patients showed any abnormality in the direct anti-human globulin, glucose-6 phosphate dehydrogenase screening, osmotic fragility and Ham's tests, and any intravascular hemolysis found was always wholly attributable to the prosthesis. The five lowest hemoglobin estimations (fig. 1) in the 21 patients with a homograft were between 12 and 13 g/100 ml; the remainder were above 13 g/100 ml. Eleven of twenty patients with a Starr valve had a hemoglobin of less than 13 g/100 ml, in five of whom it lay between 10 and 12 g/100 ml and in five between 12 and 13 g/100 ml. The mean hemoglobin of the homograft group was 14.1 g/100 ml ± sd 1.25. The mean hemoglobin of the Starr

![Figure 1](http://circ.ahajournals.org/)

**Distribution of hemoglobin levels (g/100 ml) among 21 homograft (H) and 20 Starr (S) valve patients.**

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Red cell fragment count (schistocyte count) was less than 10 cells/1,000 red cells in all patients with a homograft valve and was more than 10 cells/1,000 red cells in 9 patients (45%) with a prosthetic valve (fig. 3).

All patients with homografts showed normal serum haptoglobin levels (fig. 4). Haptoglobins were not detectable or were very low in 19 patients (95%) with Starr valves, and normal in one.

Serum LDH was more than 200 I.U. in three (15%) of the cases with homografts, whereas all patients (100%) with Starr valves had more than that figure. The mean value for LDH in the homografts was 189 I.U. and in the Starr valves was 450 I.U. (fig. 5). The mean value for HBD in the homografts was 178 and in the Starr valve group was 370.

Seventeen (85%) of the prosthetic valve group showed hemosiderinuria (ranging from + to +++)

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group was $12.8 \text{ g/100 ml} \pm 1.78$. Hematocrit estimation showed a mean of 42.43% with a standard deviation $\pm 4.27$ in the homografts and a mean value of 40.88% with a standard deviation $\pm 4.37$ in the Starr valve group.

The reticulocyte count was greater than 2% in 1 of 21 patients (4.8%) with homograft valves and 13 of 20 patients (65%) with prosthetic valves. The mean reticulocyte count of patients with a homograft was 1.8% and with a Starr valve was 3.6% (fig. 2).

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**Figure 2**
Distribution of reticulocyte count (%) among homograft (H) and Starr (S) valve patients.

*Figure 3*
Distribution of schistocyte count (%) among homograft (H) and Starr (S) valve patients.
but none of the homograft patients did. None of the patients in either group showed hemoglobinuria at any time during the study (fig. 6).

Erythrocyte survival was measured in twenty patients: in ten with the homografts the $T_3$ survival of $^{51}$Cr labelled autologous red cells varied between 25 and 29 days, with a mean of 26.9 days; in ten with Starr valve survival times varied between 15 days and 25.5 days, with a mean of 20.4 days. The statistical difference between the two groups is highly significant ($P < 0.001$) (fig. 7).

**Discussion**

The occurrence of chronic intravascular hemolysis following valve replacement with a mechanical prosthesis has been repeatedly demonstrated\textsuperscript{6-10} and has been reviewed by Marsh and Lewis.\textsuperscript{11}

Although intravascular hemolysis appears to be related to the presence of an intracardiac prosthesis, the precise mechanism remains controversial. Severe hemolysis has been observed in patients with a hemodynamically defective valve and has also been reported in patients with bare prosthetic material which has failed to become covered with endothelium.\textsuperscript{12} It has been suggested that extreme cardiac turbulence can lead to hemolysis particularly within an induced cavity or sac.\textsuperscript{13} More recently Nevaril et al.\textsuperscript{14} have estimated that a shearing stress in excess of 3,000 dynes/cm², which is sufficient to cause hemolysis in vitro, can readily develop from the increased diastolic pressure gradient caused by
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incompetence around an aortic valve prosthesis or when, during systole, the lumen of a prosthetic valve is small relative to the stroke volume, or when a ball in a ball valve is large relative to the diameter of the aorta.

Pirofsky et al. suggested that mechanical destruction is not the whole explanation and that sometimes an autoimmune mechanism may be partly responsible for the hemolysis. They postulated that damage to the red cells might either unmask or modify surface antigens, with the resultant formation of autoantibodies against them.

All the patients in this study have been carefully assessed clinically on several occasions to eliminate possible contributory causes of hemolysis. We found no clinical evidence of valve dysfunction in any of them, no electrocardiographic evidence of recent myocardial damage, nor did they have any detectable red cell antibodies by direct and indirect anti-human globulin tests.

We have based our hematologic evaluation of the incidence and severity of hemolysis on the following modification of a classification formulated by Eyster, Rothchild and Mychajliw.9

Mild: Hemosiderinuria, low or absent haptoglobins, but schistocytes < 10/1,000 red cells, reticulocytes < 3.5%, LDH < 400 I.U./L at 25°C.

Moderate: Hemosiderinuria, low or absent haptoglobins, but schistocytes > 10/1,000 red cells, reticulocytes > 3.5%, LDH > 400 I.U./L at 25°C.

Severe: All the above but hemoglobinuria also present.

In the present study, 85% of patients with a Starr-Edwards mitral valve replacement showed evidence of chronic intravascular hemolysis, which was mild in 35% and moderate in 65%. None of the patients with a homograft showed any comparable evidence of hemolysis.

The hemoglobin level is the least significant index of hemolysis since some patients can maintain a

\[
p < 0.001
\]

\[\begin{array}{c}
\text{Normal Range} \\
\text{T} \frac{1}{2} \text{Cr in days}
\end{array}\]

\[\begin{array}{c}
15 \\
20 \\
25 \\
30
\end{array}\]

\[p < 0.001\]

Distribution of hemosiderinuria among homograft (H) and Starr (S) valve patients.

Distribution of red cell survival times among homograft (H) and Starr (S) valve patients.
normal hemoglobin level by increasing erythropoietic activity by up to eightfold.16 The schistocyte and reticulocyte counts were significantly raised in five patients with Starr valves with near normal hemoglobin levels indicating hemolysis with compensatory erythropoietic activity.

Red cell fragmentation liberates free hemoglobin into the plasma. This free hemoglobin dissociates into half molecules, which become bound to plasma haptoglobin. On exceeding the binding capacity of haptoglobin, hemoglobin filters in the glomerulus, is absorbed in the proximal tubule, catabolised to hemosiderin, ferritin and other iron compounds and shed into the urine when the cells are desquamated. This may explain the time lag of approximately twelve weeks before hemosiderinuria is detected. In severe hemolysis the haptoglobins are saturated by the free hemoglobin and excess hemoglobin is filtered in the glomerulus, exceeds the transport mechanism of the proximal tubule and appears as hemoglobinuria. Thus hemosiderinuria is a good indication of chronic intravascular hemolysis while hemoglobinuria denotes acute massive hemolysis. Seventeen of the twenty patients (85%) in the Starr-Edwards group had hemosiderin in the urine and this was found to be a good indicator of the incidence of hemolysis. In many of these patients, it was found that specimens of urine examined less than four weeks after operation were negative for hemosiderin while they were persistently positive when examined three months or more postoperatively. None of the group of patients with homograft valves showed hemosiderin in the urine and none of the patients in either group had hemoglobinuria.

The estimation of nonhemoglobin bound haptoglobins or the hemoglobin binding capacity is a sensitive test for hemolysis especially at subclinical levels. In the group of patients with Starr-Edwards valve 85% had very low or absent haptoglobins, while those with homograft valves had normal haptoglobin concentrations.

The level of activity of the enzymes serum LDH and HBD correlated significantly with the hemosiderinuria and haptoglobin pattern in these two groups of patients. Thus, all patients with Starr-Edwards valves had LDH levels above the normal range, with a mean of 450 I.U./L. All patients with homograft valves had LDH levels within normal limits.

Total LDH activity gives a reliable indication of erythrocyte survival time.17,18 There is no doubt that the best method for estimating the degree of hemolysis is the erythrocyte survival time using $\text{Cr}^{51}$. We utilized both these parameters besides others to assess the incidence and degree of hemolysis and have shown that the homograft group with normal LDH activity had a mean $\text{Cr}^{51}$ % of 26.9% in contrast to the prosthetic valve group which had a mean LDH activity of 450 I.U. and a mean $\text{Cr}^{51}$ % of 20.4% days. Red cell survival figures in the mitral prosthetic valve group match the results of Andersen, Gabrielli and Zizzi20 and as far as we are aware, there are no published survival figures for patients with homografts in the mitral position.

While confirming previous findings by others that chronic hemolysis is common in patients with Starr-Edwards valve replacement in the mitral position, the results of this study have shown clearly that mounted aortic homograft valves in the mitral position have not caused any comparable degree of hemolysis in patients with up to three years postoperative follow-up.

Acknowledgment

We wish to thank Mr. Alan Steel and Mrs. Mary Greenwood for carrying out the biochemical and hematological investigations respectively; Mr. Malcolm Pepper for help with the red cell survival studies and Mr. William K. Haggas for the statistical analysis of the figures. We also wish to thank Miss Janette Thornton for secretarial assistance and Mr. William Irvine of Leeds Grammar School for assistance in collating the results.

References

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Circulation. 1973;48:823-829
doi: 10.1161/01.CIR.48.4.823

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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