Chronic Intravascular Hemolysis after Aortic Valve Replacement

Long-Term Study Comparing Different Types of Ball-Valve Prostheses

By Elaine Eyster, M.D., John Rothchild, M.D., and Olga Mychajliw, A.A.S.

SUMMARY
To assess the severity of hemolysis and to determine whether severity of hemolysis was related to type of prosthesis, hematocrit readings (Hct), schistocyte counts (S), reticulocyte counts (R), and determinations of haptoglobin (Hp), lactic acid dehydrogenase (LDH), bilirubin, urinary hemoglobin, and urinary hemosiderin (Hs) were performed on 54 patients who had three different types of aortic ball-valve prostheses. Hemolysis, as determined by absence of haptoglobins or hemosiderinuria or R > 3.5% in the absence of bleeding, was detected in 39 of the 54 patients (72%). S, R, LDH, and bilirubin were higher in patients without haptoglobins than in those with haptoglobins (P < 0.005). Hematocrits of less than 32% were found in 13 of the 34 patients (38%) with Starr-Edwards valves compared to none of the 11 with Magovern valves (P < 0.001) and one of the nine with Cutter (P < 0.05) valves. The combination of S > 10 per 1,000 cells, R > 5%, and LDH > 500 mU was observed only with Starr-Edwards valves (16 of the 34 patients) and was not seen in patients with Magovern or Cutter valves (P < 0.005). All patients with S > 10, R > 5%, and LDH > 500 mU had hemosiderinuria, and five had hemoglobinuria. Hemoglobinuria was always indicative of severe hemolysis. Hemosiderinuria was rarely detected less than 3 months after operation but was found in 32 of 47 patients so tested (68%) more than 3 months after operation. Hemosiderinuria was found in 25 of 30 patients with Starr-Edwards valves compared to two of the 11 with Magovern valves (P < 0.001). Hemosiderinuria was also detected in five of six patients with Cutter valves tested, but in three of these the test was only faintly positive. It is concluded that hemolysis is more severe in the presence of Starr-Edwards valves than Magovern or Cutter valves. Based on these observations, the following criteria for rapid clinical evaluation of cardiac hemolysis are suggested: Mild: Hs or absence of Hp but S < 10, R < 5, LDH < 500; moderate: Hs or absence of Hp but S > 10, R > 5, LDH > 500; severe: all of above plus hemoglobinuria.

Additional Indexing Words:
Hemolytic anemia Aortic valve prosthesis Ball-valve prostheses
Hematologic data Hemosiderinuria Hemoglobinuria

The subject of hemolysis following aortic valve replacement has been recently reviewed. While clinically significant hemolytic anemia has been reported in only 5

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Supported by grant PRA-35 from the American Cancer Society and the Krakower Fund.

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Received February 25, 1971; revision accepted for publication June 18, 1971.
an almost constant finding.\textsuperscript{5, 6} This variability in the reported incidence and severity of hemolysis may be related to the methods used to detect hemolysis as well as to the type of prosthesis inserted.

The purpose of this investigation was (1) to assess the severity of hemolysis in patients with aortic ball-valve prostheses and (2) to determine whether the severity of hemolysis differed among patients with Starr-Edwards, Magovern, and Cutter-Smelloff prostheses.

\section*{Methods}

The study was carried out at The New York Hospital-Cornell Medical Center on patients operated upon between 1964 and 1970 and involved 54 patients with aortic ball-valve prostheses. Starr-Edwards valves (SE) were used throughout the entire period; the 1000 series (bare metal cage with silicone rubber ball) was used from 1964 to 1966, the 1200 series modification from 1966 to 1968, the 2300 series (cloth-covered cage with Stellite ball) from 1968 to 1969, and the 2310 series modification from 1969 until the present. Magovern valves (M) were used from 1964 to 1966 and Cutter-Smelloff valves (C) from 1969 until the present. The majority of patients were investigated routinely 2 to 4 weeks after operation or during follow-up visits to the cardiac clinic. Six were referred for investigation of anemia.

The number of fragmented cells (schistocytes) was estimated from fixed smears of blood collected in ethylenediamine tetraacetate (EDTA) and stained with Wright's stain. In most instances, counts were done in duplicate, and the results averaged. A cell was considered a schistocyte if it was a small irregularly shaped cell or a piece of a cell. Using these criteria, two independent observers agreed within 1\% when the number of schistocytes was less than 25/1,000 cells, and within 0 to 2\% when the number was greater than 25/1,000 cells. Schistocyte counts on 40 consecutive hospitalized patients with normal hemoglobins and normal red cell indices serving as controls ranged from 0 to 5/1,000 cells.

Serum haptoglobin determinations were performed by the method of Javid and Horowitz.\textsuperscript{7} Normal values ranged from 70 to 150 mg/100 ml. Serum lactic acid dehydrogenase (LDH) and total bilirubin determinations were performed by the hospital chemistry laboratory on the SMA 12/60. When total bilirubin was elevated, fractionation was done. Normal values were 90 to 200 mU/ml for LDH and 0.1 to 0.8 mg/100 ml for total bilirubin. Coombs' tests with a broad spectrum antiserum, hemoglobin electrophoresis, glucose-6-phosphate dehydrogenase determinations, and sucrose hemolysis tests for paroxysmal nocturnal hemoglobinuria were performed to exclude other causes of intravascular hemolysis.

Urine was centrifuged and examined microscopically for red blood cells before testing the supernate for free hemoglobin using Hemastix.* The sediment was fixed with methyl alcohol, stained with acidified potassium ferrocyanide, and examined microscopically. Slides were considered faintly positive if only a few granules could be detected under the oil immersion objective, positive if granules could be seen readily on scanning with a 43x objective, and markedly positive if the stained sediment appeared blue to the naked eye.

The level of significance (P) of the difference between two averages was calculated from the standard error (sx) of each mean, using Student's t-test. The level of significance of the difference between two proportions was obtained similarly from the standard error of each proportion. The correlation matrix was obtained on an IBM 360/67 computer at Pennsylvania State University using a Biomedical Computer Program Package.

\section*{Results}

A total of 54 patients who had aortic prostheses, three of whom also had mitral prostheses, were studied. Twenty-six were evaluated on two or more occasions. Anemia as determined by a hematocrit of less than 40% for males and less than 37% for females was found in 50% of the patients. Hemolysis as determined by the absence of serum haptoglobins, the presence of hemosiderinuria, or a reticulocyte count of 3.5\% or greater in the absence of bleeding was present in 39 of the 54 patients (72\%). Hemolysis was detected in 66\% of those studied from 2 to 4 weeks postoperatively, and 72\% of those studied 2 or more months postoperatively.

Five of the 54 patients had other possible causes for hemolysis. One patient had a weakly positive Coombs' test, two had elevated A\textsubscript{2} hemoglobinns, one had sickle trait, and one had deficiency of glucose-6-phosphate dehydrogenase. The patient with this deficiency had an acute episode of hemolysis.

\*Hemastix—Ames Laboratories, Elkhart, Indiana.
CHRONIC INTRAVASCULAR HEMOLYSIS

triggered by sulfonamide therapy. In the remaining patients hemolysis was attributed solely to valvular disease.

Serum haptoglobins were absent in 30 patients (55%), reduced in 21 (38%), and normal in three. Schistocyte counts, reticulocyte counts, serum LDH and bilirubin determinations were significantly higher ($P < 0.005$) in patients without haptoglobins (fig. 1, group A) than in patients with haptoglobins (fig. 1, group B). Excellent correlation was noted between schistocyte and reticulocyte counts, schistocyte counts and LDH levels, schistocyte counts and serum bilirubin levels, and LDH and serum bilirubin levels ($r > 0.60$, $P < 0.005$) (table 1).

Hemosiderinuria was detected in 23 of 28 patients (82%) without haptoglobins as well as in nine of 19 patients (47%) with haptoglobins studied 3 or more months postoperatively. Hemosiderin was rarely detected less than 3 months postoperatively, and when present during this time, was only weakly positive

<table>
<thead>
<tr>
<th>Schistocytes (per 1,000 cells)</th>
<th>Reticulocytes (per cent)</th>
<th>LDH (mU)</th>
<th>Bilirubin (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
</tbody>
</table>

**Figure 1**
Comparison of schistocyte counts, reticulocyte counts, serum LDH and bilirubin determinations (A) in the patients without haptoglobin and (B) in patients with haptoglobin. The most recent representative set of values is plotted for patients studied more than once. Normal ranges are shown by shaded areas. Key: ● = Starr-Edwards valve; ▲ = Magovern valve; ■ = Cutter valve.

**Table 1**

<table>
<thead>
<tr>
<th>Correlation Matrix*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
</tr>
<tr>
<td>1*</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

Abbreviations: In heading and first column: 1 = schistocyte counts; 2 = reticulocyte counts; 3 = lactic acid dehydrogenase; 4 = total bilirubin.

†$P < 0.005$ for all values except those marked.
Urine hemosiderin determinations on individual patients are connected by a vertical line. Key ○ = Starr-Edwards valve; △ = Magovern valve; □ = Cutter valve; ● = positive; ○ = negative; ● = faintly positive; * = months following reoperation for ball variance.

Urine hemosiderin determinations after operation. Serial determinations on individual patients were connected by a vertical line. Key ○ = Starr-Edwards valve; △ = Magovern valve; □ = Cutter valve; ● = positive; ○ = negative; ● = faintly positive; * = months following reoperation for ball variance.

Hemosiderinuria was detected in 27 of 33 patients (82%) studied 3 to 25 months postoperatively and was persistent in all but one patient in whom subsequent determinations were done. Fifteen patients were observed more than 25 months postoperatively. Ten of these had negative determinations, suggesting that conversion was not likely to occur after 25 months. Blood urea nitrogen determinations were normal for 27 of 29 patients with hemosiderinuria.

Hematocrits of less than 32% were found in 13 of the 34 patients (38%) with SE valves compared to none of the 11 with Magovern (P < 0.001) and one of the nine with Cutter valves (P < 0.05) (table 2). The combination of schistocyte counts of 10/1,000 cells or greater, reticulocyte counts of 5.0% or greater, and serum LDH values over 500 mU were seen only with SE valves (primarily the 2300 series) and were not seen with Magovern or Cutter valves (P < 0.005). These valves all had orifices of 1.8 cm² or less (sizes 8–10), but the majority of SE valves in this study were of similar sizes. Total bilirubin levels above 0.8 mg/100 ml were seen with SE (series 1200 and 2300) and with M valves, but not with C valves (P < 0.05).
Table 2
Hematologic Evaluation of 54 Patients with Ball-Valve Replacements of Aortic Valve

<table>
<thead>
<tr>
<th>Type of aortic valve</th>
<th>Total patients</th>
<th>Het &lt; 32%</th>
<th>S &gt; 10/LDH &gt; 500 mU</th>
<th>Bilirubin &gt; 0.8 mg/100 ml</th>
<th>Hemoglobinuria</th>
<th>Hemosiderinuria‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starr-Edwards, 1000, sizes 10 - 12</td>
<td>3</td>
<td>0 (3)*</td>
<td>0 (3)</td>
<td>0 (3)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Starr-Edwards, 1200, sizes 8 - 10</td>
<td>4</td>
<td>0 (4)</td>
<td>1 (4)</td>
<td>2 (4)</td>
<td>0 (4)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Starr-Edwards, 2300, sizes 8 - 10</td>
<td>20</td>
<td>0 (4)</td>
<td>13 (19)</td>
<td>10 (18)</td>
<td>4 (18)</td>
<td>18 (18)</td>
</tr>
<tr>
<td>Starr-Edwards, 2310, sizes 8 - 10</td>
<td>4</td>
<td>3 (4)</td>
<td>1 (2)</td>
<td>1 (3)</td>
<td>0 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Starr-Edwards, 2310, ? type</td>
<td>3</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Magovern</td>
<td>11</td>
<td>0 (11)</td>
<td>0 (11)</td>
<td>3 (11)</td>
<td>0 (10)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Sizes 2 - 4</td>
<td>5</td>
<td>mean 41%†</td>
<td>1 (5)</td>
<td>2 (5)</td>
<td>0 (5)</td>
<td></td>
</tr>
<tr>
<td>Sizes 5 - 7</td>
<td>5</td>
<td>mean 41%†</td>
<td>2 (5)</td>
<td>0 (1)</td>
<td>0 (1)</td>
<td></td>
</tr>
<tr>
<td>Size ?</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutter</td>
<td>9</td>
<td>1 (9)</td>
<td>0 (7)</td>
<td>0 (7)</td>
<td>0 (5)</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Size 2 - 3</td>
<td>3</td>
<td>mean 36%†</td>
<td>0 (7)</td>
<td>0 (7)</td>
<td>0 (5)</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Size 4 - 5</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size ?</td>
<td>2</td>
<td>1 (2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Het = hematocrit (%); S = schistocyte count (/1000 cells); R = reticulocyte count (%); LDH = lactic acid dehydrogenase (mU/ml).
*Numbers in parentheses are the number of patients undergoing the test in each group.
†Mean hematocrit for group.
‡3 or more months after operation.

Hemoglobinuria was present in five patients; all had SE valves. Each of these patients had reticulocyte counts above 7% and LDH levels above 800 mU. Four of the five had schistocyte counts above 40, and three had hematocrits below 30% even though taking iron orally. One with severe anemia requiring numerous transfusions had repeat surgery with replacement of a cloth-covered cage which had failed to endothelialize. In this patient and in another with ball variance whose case was previously reported,8 hemoglobinuria ceased following reoperation. One other patient with hemoglobinuria had bacterial endocarditis. The three remaining patients with hemoglobinuria had no detectable valve dysfunction 16, 20, and 22 months, respectively, after surgery. All five patients with hemoglobinuria had urine that was markedly positive for hemosiderin.

Hemosiderinuria was detected in 25 of 30 patients (83%) with SE valves compared to two of 11 with M valves (P < 0.001) studied 3 or more months postoperatively. Markedly positive determinations were associated with all types of SE valves, and all patients with schistocyte counts greater than 10, reticulocyte counts greater than 5%, and serum LDH levels greater than 500 mU had hemosiderinuria. Hemosiderinuria was also detected in five of six patients with C valves, but in three of these the test was only faintly positive. Of interest was the finding that two of the Cutter valves and the two Magovern valves in patients with hemosiderinuria were valves of the smallest size.

Discussion
The red cell has a critical tolerance to shearing stress.9 High shearing stresses cause stretching of the membrane with tearing and cell fragmentation.10,11 Fragmented cells or schistocytes are not specific for heart valve hemolysis since fragmentation is an important final common pathway for red cell destruction in a variety of hemolytic states.12,13 However,
the number of fragmented cells in the peripheral blood of patients with artificial heart valves appears to bear a direct relationship to the severity of the hemolysis.\textsuperscript{14}

In the present study schistocyte counts were significantly higher in patients without haptoglobins than in those with haptoglobins. Moreover, the magnitude of the counts correlated well with other measurements of red cell destruction such as reticulocyte counts, serum LDH and bilirubin (primarily unconjugated) determinations.

The red cell is rich in LDH fractions 1 and 2.\textsuperscript{15} Hemolysis results in elevated serum levels,\textsuperscript{16} and increased concentrations of both total LDH and the isoenzyme LDH 1 have been reported in patients with aortic prostheses.\textsuperscript{17} In the present study elevation of total LDH was a constant finding, and good correlation was noted between LDH levels, schistocyte counts, reticulocyte counts, and serum bilirubin levels (table 1). The significantly higher levels found in the group without haptoglobins compared to the group with haptoglobins were due largely to more severe hemolysis in the former group. Extremely high values for LDH above 800 mU were seen only in those patients with hemoglobinuria.

Red cell fragmentation results in loss of a piece of membrane which may or may not contain hemoglobin.\textsuperscript{12} Free hemoglobin dissociates into half molecules which are rapidly bound to plasma haptoglobin.\textsuperscript{18} The hemooglobin-haptoglobin complex is too large to pass through the glomerulus and is cleared from the circulation by the reticuloendothelial system.\textsuperscript{19} After plasma haptoglobin has been depleted, the half molecules (molecular weight, 34,000) pass through the glomerular filter into the proximal tubule, and the free hemoglobin remaining in the circulation is oxidized to methemoglobin. When large quantities of hemoglobin are presented to the tubule, the transport mechanism is exceeded and hemoglobinuria occurs. When smaller quantities are filtered, hemoglobin is absorbed by the cells of the proximal tubule where it is converted to ferritin and hemosiderin. Later when the tubule cells desquamate, hemosiderin granules may be seen by light microscopy in the sediment stained for iron. Thus hemoglobinuria occurs acutely with massive hemolysis while hemosiderinuria is found in chronic intravascular hemolysis.\textsuperscript{20, 21}

After intravenous infusion of 19 g of hemoglobin into a normal human volunteer, Sears and associates\textsuperscript{21} found that essentially all the urinary iron was accounted for by hemoglobin itself. No hemosiderin was detected in the urine sediment up to 48 hours after the infusion of this large amount of hemoglobin. After infusion of 2 mg of \textsuperscript{59}Fe hemoglobin to haptoglobin depleted rats, Bunn and Jandl\textsuperscript{22} showed that the labeled iron disappeared very slowly from the kidney with half the activity remaining after 37 days. Urinary excretion of radioiron amounted to only 1.5% in 25 days, implying that most of the label was probably mobilized from the kidney into the body pool. No appreciable hemoglobin was detected in the urine after the infusion of this small amount of hemoglobin.

In the present study, hemoglobinuria was observed only in those patients with the most severe hemolysis. Hemosiderinuria was frequently seen 3 months or more postoperatively but was rarely observed less than 4 weeks postoperatively. This lag in excretion is presumably due to the time required for the iron to be metabolized by the proximal tubule cells and for the tubule cells to desquamate. We have no data on the amount of iron excreted compared to the amount mobilized into the body pool. However, the large amounts of nonhemoglobin iron, presumably hemosiderin and ferritin, previously quantitated in the urines of these and other patients\textsuperscript{23, 24} suggest that the amount excreted may be proportional to the amount absorbed by the tubule.

Hemosiderinuria was observed in 81% of patients without haptoglobin and in 47% of patients with haptoglobin studied 3 or more months postoperatively. Since haptoglobin can be regenerated within several days following cessation of hemolysis while iron appears in the urine at a very slow rate,\textsuperscript{25} the
simultaneous presence of haptoglobin and hemosiderin is probably indicative of intermittent rather than continuous hemolysis. Thus, a previous hemolytic episode could explain why hemosiderinuria was detected at a time when haptoglobin was present.

In most reported cases of chronic intravascular hemolysis renal function has not been significantly impaired. In this study renal function was normal, even in those patients with hemoglobinuria as well as hemosiderinuria.

Hemolysis has been previously reported in association with the cloth-covered 2300 series SE valve, and serum LDH levels have been shown to be significantly higher with this prosthesis than with the 1200 series. The 2300 series valve has a Dacron velour-covered cage with a hollow Stellite ball. It was developed in an attempt to reduce thrombus formation on the bare metal cage and prevent structural changes which occurred in the silicone rubber ball of earlier models. Because of the high systolic gradients observed with this model, and 2310 modification was developed.

Nevaril and associates have demonstrated that a shearing stress of 3,000 dynes/cm² is sufficient to cause hemolysis in vitro. Stresses of this magnitude can develop from turbulent jets and from increased diastolic or systolic gradients in vivo. Stasis and turbulence are greater with the SE than with the C valves. Moreover systolic gradients of 20 to 50 mm Hg have been observed with the cloth-covered SE valves while significant supravalvular gradients have not been found with M valves.

In the present study, the combination of schistocyte counts above 10, reticulocyte counts of 5.0% or greater, and serum LDH values over 500 mU were seen only with SE valves (primarily the 2300 series) and were not seen with M or C valves. Hemosiderinuria was more frequent with all types of SE valves, including the 2310 series, than with M valves. Hemoglobinuria was seen only with SE valves. This suggests that higher gradients and more turbulence with the SE valves may be associated with greater amounts of hemolysis, presumably in association with greater shearing stress.

Based on these observations, the following approach to the hematologic evaluation and management of patients with aortic ball-valve prostheses has been formulated. It is proposed that cardiac hemolysis be defined as mild, moderate or severe according to the following criteria:

Mild: Hemosiderinuria or absence of haptoglobins but S < 10/1,000 cells, R < 5%, LDH < 500 mU/ml.

Moderate: Hemosiderinuria or absence of haptoglobins but S > 10/1,000 cells, R > 5%, LDH > 500 mU/ml.

Severe: All of above plus hemoglobinuria.

It is suggested that haptoglobin levels be obtained during the first 3 to 6 months following surgery. Virtually all patients have decreased haptoglobins, but patients with significant degrees of hemolysis almost always have no haptoglobins. Schistocyte counts, reticulocyte counts, and LDH and bilirubin determinations are helpful in estimating the severity of hemolysis in patients without haptoglobins.

Urines should be routinely evaluated for the presence of hemosiderin at 6-month intervals following surgery. As soon as hemosiderin is detected, the patient should be given iron by mouth. Iron therapy should be continued indefinitely since hemosiderinuria is usually persistent and may lead eventually to iron deficiency. One cannot rely on the absence of anemia to exclude hemolysis since many patients with significant hemolysis manage to maintain normal hematocrits by increasing red cell production as much as eightfold. This increased rate of production can be sustained only as long as adequate supply of iron is available. Therefore, we feel that iron supplements should be given as soon as hemosiderinuria is detected, even in the absence of anemia.

In our experience most patients with hemosiderinuria can be managed satisfactorily.

Circulation, Volume XLIV, October 1971
with iron therapy as long as hemoglobinuria is not present. Hemoglobinuria is indicative of massive hemolysis, and although restriction of activity is usually helpful, parenteral injections of iron or transfusions of red cells may be required. The rare patient with hemoglobinuria requiring frequent blood transfusions often requires surgery for the replacement of a defective valve.

Acknowledgments

We wish to thank Drs. J. Bloch and J. R. Rees for their many valuable suggestions, the special hematology laboratory of the New York Hospital for the serum haptoglobin determinations, Mr. Richard Lemon for technical assistance, and Miss Judith Lunger for secretarial assistance.

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CHRONIC INTRAVASCULAR HEMOLYSIS


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Circulation. 1971;44:657-665
doi: 10.1161/01.CIR.44.4.657

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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