Urinary iron excretion and other aspects of iron metabolism were studied in patients with valvular heart disease before and after valve replacement with heterografts or Starr-Edwards prostheses. Eighty-one per cent of preoperative patients had increased daily urinary iron excretion (0.14–2.2 mg./24 hours) and 61 per cent had a reduced ⁵¹Cr survival time. Serum iron levels were low in two patients but iron-deficiency anemia was not observed. Fifty-three per cent of bone marrow aspirates had reduced or absent storage iron. Patients with normally functioning heterografts had no hemolysis and urinary iron excretion decreased exponentially with time until normal values were reached in 6–10 months after surgery. Calculated iron loss over a 6-month postoperative period varied from 11 to 360 mg. Serum iron levels and results of ferrokinetic studies returned towards normal, as did marrow iron stores. Seven patients (78%) with Starr-Edwards valves had evidence of hemolysis by the ⁵¹chromium survival method and six were anemic. Urinary iron loss was abnormal in all nine patients (0.8–10.8 mg./24 hours) and iron deficiency was a significant factor in the anemia noted. Iron therapy raised hemoglobin values in the two patients to whom it was administered. Urinary iron excretion was found to be a sensitive index of intravascular hemolysis, particularly in the presence of an intermittent hemolytic process.

INTRAVASCULAR HEMOLYSIS after cardiac valve surgery is now a well-recognized phenomenon and a similar less severe process has been observed in patients with unoperated valvular heart disease.¹ ² Renal hemosiderosis³ and hemosiderinuria⁴ are common findings in patients with prosthetic valves and the resulting iron deficiency can be an important factor in the anemia so frequently noted in these patients.⁵

A previous report showed that hemolysis does not occur after heterograft valve replacement unless there is marked valve incompetence.⁶ The present investigation was undertaken to study the effect of heterograft or prosthetic valve replacement on iron metabolism and urinary iron loss in particular. Patients with valvular heart disease were studied preoperatively to allow evaluation of the changes produced by surgery.

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Table 1.—Hemolysis and Urinary Iron Loss in Patients with Unoperated Valvular Heart Disease

<table>
<thead>
<tr>
<th>Type of Valve Disease</th>
<th>All Patients</th>
<th>Patients with Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. % Per Cent with 51Cr T½ Below 25 Days</td>
<td>Per Cent with Excess Urinary Iron Loss No. Urinary Iron Loss* 52Cr (Days) Urinary Iron Loss (mg./24 Hours)</td>
</tr>
<tr>
<td>Aortic</td>
<td>14 78</td>
<td>84 11</td>
</tr>
<tr>
<td>Mitral and Aortic</td>
<td>7 56</td>
<td>70 4</td>
</tr>
<tr>
<td>Mitral</td>
<td>4 0</td>
<td>25 0</td>
</tr>
<tr>
<td></td>
<td>(1 only)</td>
<td></td>
</tr>
</tbody>
</table>

* Normal range 0.03–0.08 mg./24 hours.
† Normal range 25–33 days.

METHODS

A total of 53 patients with valvular heart disease were studied, 25 of these prior to operation. Thirty-two patients were investigated after heterograft valve replacement, including 13 of the patients studied preoperatively. The nine patients with Starr-Edwards valves were seen only after surgery but one of them was again studied after heterograft replacement of his prosthesis.

In postoperative cases, investigations were not begun until 8 weeks after surgery to avoid the immediate effects of extracorporeal perfusion. Red cell hemoglobin levels, reticulocyte counts, serum haptoglobin concentration and total plasma hemoglobin levels were performed by standard methods. 51Chromium red cell survival and 59Fe ferrokinetic studies were performed as described by Veall and Vetter. Serum iron levels were estimated by the method of Trinder’s modification was also used to measure urinary iron levels on the auto-analyzer. This technique yielded results closely correlated with those obtained by wet-ashing. Urine specimens were timed over two consecutive 12-hour periods corresponding to the times of daily activity and sleep, the sum representing 24-hour urinary iron excretion. They were collected and processed in iron-free materials. All urine specimens were screened for the presence of protein and hemoglobin. Chemical estimations of protein were performed on those showing proteinuria since heavy urinary protein loss may lead to the appearance of transferrin-bound iron in the urine. Proteinuria, however, was not significant in any patient. Iron in specimens positive for hemoglobin was estimated by wet-ashing since the auto-analyzer method does not measure heme-bound iron.

Fractionation of urine collections in patients with high iron excretion was performed by the method described by Sears et al. to allow separate determination of hemosiderin, ferritin and hemoglobin. Iron stores in bone marrow aspirates and in renal tissue obtained at autopsy were assessed by Prussian blue staining.

RESULTS

Valvular Heart Disease

All patients in this study with heart valve lesions suffered from Grade III or IV exercise intolerance and were listed for valve replacement. Table 1 shows the extent of hemolysis and urinary iron loss in patients with valvular heart disease. The results suggest that aortic valve disease is more particularly associated with hemolysis and urinary iron loss than either mitral valve disease or mixed aortic and mitral disease.

Neither 51Cr T½ values, nor plasma hemoglobin levels correlated significantly with urinary iron excretion in individual patients. There was a marked variation between day and night time excretion of iron in patients with hemolysis which could not be accounted for by differences in urinary volume.
secreted during these periods (Fig. 1). Only two preoperative patients showed low serum iron levels but bone marrow iron stores were reduced in eight of 15 aspirates examined. Hemolytic or iron-deficiency anemia did not occur in the present series.

Ferrokinetic studies reflected the subclinical hemolytic process detected in these patients. The $^{59}$Fe $T_{1/2}$ values in six patients (56–76 minutes, mean 64 minutes) were marginally below the normal limits, 75–125 minutes in this laboratory, and plasma iron transport rates were only slightly elevated (0.56–1.18 mg./day/100 ml. of blood, mean 0.83 mg./day/100 ml., normal range 0.49–0.73 mg./day/100 ml.)

**Table 2.—Hemolysis and Urinary Iron Loss in Patients Studied Before and After Heterograft Valve Surgery**

<table>
<thead>
<tr>
<th>Case</th>
<th>Preoperative Hemolysis</th>
<th>Urinary Iron mg./Day</th>
<th>Postoperative Hemolysis</th>
<th>Urinary Iron mg./Day</th>
<th>Time Since Operation (Weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>+</td>
<td>0.41</td>
<td>0</td>
<td>0.25</td>
<td>12</td>
</tr>
<tr>
<td>30</td>
<td>+</td>
<td>0.25</td>
<td>0</td>
<td>0.22</td>
<td>13</td>
</tr>
<tr>
<td>27</td>
<td>0</td>
<td>0.32</td>
<td>0</td>
<td>0.15</td>
<td>16</td>
</tr>
<tr>
<td>23</td>
<td>+</td>
<td>0.34</td>
<td>0</td>
<td>0.16</td>
<td>16</td>
</tr>
<tr>
<td>29</td>
<td>0</td>
<td>0.17</td>
<td>0</td>
<td>0.14</td>
<td>18</td>
</tr>
<tr>
<td>21</td>
<td>+</td>
<td>2.12</td>
<td>0</td>
<td>0.28</td>
<td>18</td>
</tr>
<tr>
<td>26</td>
<td>+</td>
<td>0.26</td>
<td>0</td>
<td>0.13</td>
<td>20</td>
</tr>
<tr>
<td>22</td>
<td>+</td>
<td>0.25</td>
<td>0</td>
<td>0.06</td>
<td>22</td>
</tr>
<tr>
<td>33</td>
<td>+</td>
<td>0.14</td>
<td>0</td>
<td>0.02</td>
<td>28</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>0.39</td>
<td>0</td>
<td>0.04</td>
<td>36</td>
</tr>
<tr>
<td>25</td>
<td>+</td>
<td>0.81</td>
<td>0</td>
<td>0.04</td>
<td>40</td>
</tr>
<tr>
<td>28</td>
<td>+</td>
<td>0.15</td>
<td>+</td>
<td>0.51</td>
<td>16</td>
</tr>
<tr>
<td>31</td>
<td>+</td>
<td>1.48</td>
<td>+</td>
<td>0.19</td>
<td>20</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
<td>0.03–0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2.—Urinary iron excretion after heterograft valve replacement. Each point represents a patient with a normally functioning heterograft and no residual hemolysis. Since preoperative iron loss varied greatly among different patients, the correlation between falling urinary iron excretion and time since operation ($r = 0.90, p < 0.001$) was only evident, when postoperative iron excretion was expressed as a percentage of preoperative loss. The calculated regression line is represented by the equation $\log y = 5.63 - 0.108x$ . . .(1). If the $y$ values (iron excretion) are plotted on a linear scale, the regression line becomes $y = \frac{279.7}{0.108x}$.(2).

Patients With Heterograft Valves

Table 2 gives comparative data of the 13 patients studied before and after surgery. In all cases which ceased to hemolyze after surgery there was a fall in urinary iron loss which was progressive with time until normal levels were reached. The logarithmic relation between decreasing urinary iron excretion and time elapsed since operation is demonstrated graphically in Fig. 2. The exponential fall-off in urinary iron values observed applied not only to the group as a whole but was also observed in three individual patients in whom serial urinary iron measurements were possible.

The area under the exponential curve obtained by integrating expression (2) in Fig. 2 signified total urinary iron loss between two arbitrary points of time. On this basis, urinary iron excretion in individual patients from the second to the eighth postoperative month varied from 11 to 360 mg. (mean 83 mg., estimated normal losses 5–13 mg). Other aspects of iron metabolism in patients with heterografts showed a return towards normal after surgery, except where there was evidence of continuing hemolysis. Plasma iron transport rates were in the upper normal range (0.67–0.88 mg./day/100 ml., mean 0.77 mg./day/100 ml. of blood) and bone marrow iron stores were normal in all but two specimens examined.
Table 3.—Fractionation of Urinary Iron in Patients with Starr-Edwards Prostheses

<table>
<thead>
<tr>
<th>Case</th>
<th>$^{51}$Cr T$^{1/2}$ Days</th>
<th>Plasma Hb mg./100 ml.</th>
<th>Urinary Iron mg./Day</th>
<th>Ferritin (Per Cent)</th>
<th>Hemosiderin (Per Cent)</th>
<th>Hemoglobin (Per Cent)</th>
<th>&quot;Supernatant Iron&quot; (Per Cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>10</td>
<td>67.0</td>
<td>10.8</td>
<td>14</td>
<td>38</td>
<td>6</td>
<td>42</td>
</tr>
<tr>
<td>50</td>
<td>10.5</td>
<td>37.6</td>
<td>5.6</td>
<td>3</td>
<td>60</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
<td>52</td>
<td>10</td>
<td>21.5</td>
<td>8.8</td>
<td>7.5</td>
<td>72</td>
<td>0</td>
<td>20.5</td>
</tr>
<tr>
<td>53$^*$</td>
<td>—</td>
<td>3.0</td>
<td>5.75</td>
<td>4.5</td>
<td>82</td>
<td>0</td>
<td>13.5</td>
</tr>
</tbody>
</table>

* After heterograft replacement of defective Starr-Edwards aortic valve.

**Patients With Starr-Edwards Prosthesis**

Patients with Starr-Edwards prostheses had considerable iron losses (0.8-10.8 mg./24 hours, mean 5.7 mg./24 hours), and a marked diurnal variation in iron excretion (Fig. 1). Low serum iron values were common, transferrin saturation was below 15 per cent in three patients and all specimens of bone marrow examined had reduced or absent iron stores.

In two patients, oral iron therapy resulted in a rise in red cell hemoglobin levels of 2.8 and 3.6 Gm./100 ml. and produced a state of compensated hemolysis. Ferrokinetic studies showed accelerated iron clearance (30-60 minutes, mean 46 minutes) and elevated plasma iron transport rates (0.92-2.9 mg./day/100 ml. blood, mean 1.4 mg./day/100 ml.), consistent with the increased marrow activity observed morphologically.

Further analysis of urinary iron loss produced the pattern shown in Table 3. Hemoglobinuria, though prominent clinically, made only a small contribution to total urinary iron loss. The "supernatant fraction" of iron was freely dialyzable and readily chelated by transferrin.

All patients with hemoglobinuria had very low or absent serum haptoglobin levels and plasma hemoglobin levels in excess of 26 mg./100 ml. This renal threshold phenomenon is well known.

**DISCUSSION**

The renal handling of iron derived from catabolism of hemoglobin is central to studies of iron metabolism in intravascular hemolysis. It has been demonstrated by a variety of techniques that proximal tubular cells have the capacity to absorb filtered free hemoglobin and metabolize it. The ferritin particles rapidly produced by this process are electron dense and are readily identified by electron microscopy. Hemosiderin clumps later become evident under the light microscope and are demonstrated by Prussian blue staining. Both of these iron-protein complexes have been observed in the urine of patients with mechanical hemolysis, and in severe cases, hemoglobinuria may be present in addition. Hemosiderin is found in the urinary sediment both within cast-off renal tubular cells and as free iron granules, while ferritin is mostly in solution. It is evident, both from the reports by Sears et al. and the findings of this investigation, that a high proportion of urinary iron derived from hemoglobin catabolism is not in complex with proteins and is possibly in ionic form.

The results in Table 3 suggest that the proportion of hemosiderin and
"supernatant iron," which appear in the urine may be related to the rate of plasma hemoglobin influx into the kidney, and to its capacity to resynthesize ferritin and hemosiderin. Thus, after cessation of hemolysis, virtually all of the urinary iron excretion is in the form of hemosiderin, while high plasma hemoglobin levels are associated with an elevated "supernatant fraction."

Diurnal variation in iron excretion is probably related to differences in exercise during the collection periods. Sears and Crosby showed that exercise increased red cell trauma and raised plasma hemoglobin levels in patients with mechanical hemolysis. The greater inflow of free hemoglobin into the renal tubules is thus reflected in a higher urinary iron concentration.

Both experimental and clinical observations suggest that mobilization of renal iron into plasma is a slow and limited process and represents a progressively smaller proportion of the iron derived from heme catabolism as renal filtration of hemoglobin increases.

The association of severe iron-deficiency anemia and massive renal hemosiderosis observed in one of our patients at autopsy further supports the concept that renal iron deposits are not available for hemoglobin synthesis or marrow storage repletion.

The evidence therefore points to the urine as the only major pathway by which the kidneys discharge their iron load; and the exponential nature of the decreasing iron loss over 6-10 postoperative months implies a concentration-dependent leaching of renal deposits which proceeds until all excess iron has been excreted.

In consequence of the above considerations, it becomes evident that total postoperative iron loss represents an approximate measure of renal iron deposits at the time of surgery. The predictable course of the iron loss allows calculation of the amounts involved and, on this basis, total iron excretion for patients with heterografts ranged up to 800 mg. with a mean of 185 mg.

The quantities of iron involved in renal deposits provide an explanation for the reduced marrow stores observed. Although normal iron absorption could readily compensate for the daily urinary iron losses observed in heart valve disease, there remains a net transfer of a significant fraction of storage iron to the kidney, where it becomes unavailable for recirculation.

Diagnostically, the presence of renal hemosiderosis and hemosiderinuria are important indices of chronic intravascular hemolysis, and in the presence of an intermittent hemolytic process, constitute more reliable evidence than the serum haptoglobin and plasma hemoglobin levels. Leonardi and Rual in fact proposed renal biopsy as a valuable aid in diagnosing intravascular hemolysis in doubtful cases. However, Roberts found stainable renal iron in only 3 per cent of 132 patients with Grade III or IV valvular heart disease, while the present investigation revealed increased urinary iron loss in 81 per cent of patients with comparable severity of disease. It is therefore evident that abnormal iron excretion may occur in the absence of visible renal deposits and that the measurement of 24-hour urinary iron loss represents a more sensitive method of detecting intravascular hemolysis.

Diagnostic difficulty may arise in patients with iron storage disease since hemosiderinuria has been described in hemochromatosis. However, this
URINARY IRON EXCRETION IN VALVULAR HEART DISEASE

diagnosis can usually be made on the clinical findings, and laboratory procedures related to the amount and site of body iron stores.

Other aspects of iron metabolism studied in the three groups of patients largely reflect the presence and degree of hemolysis, iron depletion and bone marrow activity. They thus confirmed our previous finding that hemolysis ceases upon replacement of diseased heart valves with competent heterografts and that iron stores then return toward normal.

The value of iron therapy for patients with hemolytic anemia due to malfunctioning ball valves was again exemplified in this series, in that both patients so treated required no further transfusions and were symptomatically improved.

ACKNOWLEDGMENT

This work was supported by a grant from the National Health and Medical Research Council of Australia. We wish to thank Dr. M. F. O'Brien, Dr. G. M. Neilson, and Dr. E. C. Galea for allowing us to study patients under their care and Mr. N. Keats and the Photographic Section of the University of Queensland for preparing the figures.

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Urinary Iron Excretion in Valvular Heart Disease and After Heart Valve Replacement

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