The Effect of Hemorrhage on Plasma Iron Turnover

By Eb C. Girvin, Seng Kah Ooi and Stanley Pi Shu Wong

It has long been recognized that erythropoiesis increases following hemorrhage. Bleeding has frequently been utilized to produce plasma containing an increased quantity of the "erythropoietic factor." Most studies of this type have been designed to produce anemia by removing blood on several successive days. The purpose of this investigation was to study the response of rats to a single hemorrhage.

Materials and Methods

We used male Holtzman rats ranging from 375 to 510 Gm. given Purina Laboratory Chow and tap water ad libitum. Fourteen rats were used as controls, and 49 experimental rats were bled by placing the tips of their cut tails in graduated centrifuge tubes containing three ml. warm sodium citrate solution. Severity of bleeding was determined by the increase in volume in the tubes and varied from 2.7 to 18 per cent of total blood volume. The per cent of total blood lost was calculated by using 8 per cent of the body weight as the blood volume. The hematocrit of all rats, which was determined by centrifuging the tubes, fell in the range of 40 to 50 per cent. At 12 and 22 hours and 1, 2, 4, 5, 7, and 10 days post hemorrhage, experimental and control rats, which had not been bled, were anesthetized and both femoral veins exposed. From 1 to 1.5 microcuries of Fe³⁰ as ferric citrate in 0.1 ml. of solution containing a maximum of 1.1 µg. of chemical iron was injected into one vein. Four 0.3 to 0.4 ml. blood samples were extracted from the other femoral vein at 15 minute intervals. These samples were centrifuged and 100 microliters of plasma pipetted into counting vials and the radioactivity determined. The data were plotted on semilog paper and time (T/2) for one half the radioactivity to disappear from the plasma was determined. Plasma iron determinations were made by the method of Schade et al. All measurements of plasma iron and plasma iron turnover rates were made between 1:30 and 3:30 P.M. to avoid diurnal variations.

Results

Average times required for one half injected radioactive iron to disappear from the plasma (T/2) at various post hemorrhage intervals and for the unbled rats are represented in figure 1. Increased erythropoiesis was detectable at 22 hours post hemorrhage. After loss of 2.7 to 18 per cent of total blood cells, iron utilization returned to normal by the seventh to tenth day. Maximum deviation around mean values was observed at two days, the time of maximum iron utilization, and again during the days when experimental animals approached normal iron utilization.

Discussion

The T/2 method used is suitable for observing the experimental animal's response to an erythropoietic stimulation and has frequently been employed...
Fig. 1. — The T \( \frac{1}{2} \) value (± S. D.) for the disappearance of Fe\(^{59} \) from the plasma of Holtzman rats at various times following hemorrhage. Numbers in parentheses indicate number of rats each point represents.

to reflect the rate of red blood cell production.\(^{6,8} \) It has been pointed out\(^{4} \) that fluctuations in size of plasma iron mass due to change in plasma iron concentration and plasma volume can also be reflected in the T \( \frac{1}{2} \) time. Chemical iron determinations on thirty rats were made which gave considerable variations, (ranging from 0.9 to 1.7 \( \mu \)g. per ml.). However, no pattern was detectable to indicate that the fluctuations in the experimental animals differed from the controls. Plasma volumes were calculated by extrapolation to zero time of the iron disappearance curves.\(^{9} \) Maximum variations obtained were 3.4 to 4.6 per cent of body weight with no apparent correlation with T \( \frac{1}{2} \) values. Some variation in T \( \frac{1}{2} \) values was due to differences in age as reflected in the weight range of 375–510 Gm.\(^{10} \) However, controls were run throughout the course of the experiment and had the same age range as the experimental animals. Thus it is felt that the erythropoietic activity was the major factor affecting the rates of plasma iron turnover in this experiment.

As the amount of blood removed from each experimental rat varied rather widely from 2.7 to 18 per cent, it is not surprising that their response was somewhat different. However, the same range of response was seen in rats which had lost no more than 6 per cent of their blood volume as in those having lost 10 per cent or more. Table 1 shows the T \( \frac{1}{2} \) values of rats selected for slight and severe hemorrhage at two, seven, and ten days post hemorrhage. Some T \( \frac{1}{2} \) times were normal in seven days while some were still shorter than normal at ten days regardless of severity of hemorrhage. Also at two days post hemorrhage a rat having lost 4 per cent of his blood had as short or shorter T \( \frac{1}{2} \)
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Table 1.—$T \frac{1}{2}$ Values Selected to Show Variation with Regard to Per Cent of RBC Removed from Rats. Average $T \frac{1}{2}$ for 14 Control Rats is 68 Minutes

<table>
<thead>
<tr>
<th>Time Post Hemorrhage</th>
<th>% of Total RBC Removed</th>
<th>$T \frac{1}{2}$ in Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 days</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>2 days</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>2 days</td>
<td>17</td>
<td>31</td>
</tr>
<tr>
<td>7 days</td>
<td>16</td>
<td>45</td>
</tr>
<tr>
<td>7 days</td>
<td>11</td>
<td>53</td>
</tr>
<tr>
<td>7 days</td>
<td>13</td>
<td>86</td>
</tr>
<tr>
<td>10 days</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>10 days</td>
<td>3</td>
<td>73</td>
</tr>
<tr>
<td>10 days</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>10 days</td>
<td>12</td>
<td>68</td>
</tr>
</tbody>
</table>

value as rats having lost 17 and 18 per cent of their blood. It is suspected that individual variations as well as the amount of red blood cells removed accounts for the maximum rate of iron utilization attained in the first two days and also the length of time required for return to normal. As the rats were sacrificed when their $T \frac{1}{2}$ time was determined, this method cannot be used to establish whether an individual which showed a marked shortening of $T \frac{1}{2}$ value at two days would also show a correspondingly rapid return to normal. The difference in individual response is inferred by the greater variation about the mean value for recovering rats than for those tested within the first 24 hours after hemorrhage or for controls.

SUMMARY

The plasma iron turnover rate in rats following a single hemorrhage reaches a maximum in about 48 hours and returns to normal between the seventh and tenth day. There is considerable variation in individual rats in both the maximum rate attained and the time required for recovery. No significant difference in response was observed due to the severity of hemorrhage upon removal of 2.7 to 18 per cent of total red cells.

SUMMARIO IN INTERLINGUA

Le transition de ferro in le plasma de rattos post unic hemorrhagias attinge su maximo de intensitate in circa 48 horas e retorna al norma inter le septime e le decime die. Il existe considerabile variationes in rattos individual tanto conrespecto al attingimento del maximo como etiam con respecto al tempore requirite pro le renormalisation. Nulle significative differentias in le responsa eseva observate in correlasion con le grado de severitate del hemorrhagias que resultava in extractiones de inter 2,7 e 18 pro cento del erythrocytos total.

REFERENCES


STUDIES IN ANAEMIA OF INFECTION. PART IV. MECHANISM OF HYPOFERREMIA.


In human infection, iron absorption was impaired and intravenously injected iron was rapidly removed from the plasma. In experimental infections, increased amounts of iron were found to accumulate in liver, spleen and bone marrow.—J. B. C.


In 19 cases of lead poisoning erythrocyte survival was studied by means of differential agglutination, by using Cr51 and a reticulocyte method. The daily destruction of hemoglobin and erythrocyte regeneration was also investigated. Lead is able to produce hyperhemolysis even in the absence of anemia. In some cases the destruction of hemoglobin may be four times greater than normal. It seems likely that lead is bound to erythrocytes or produces permanent damage and subsequent hemolysis, even in a normal environment. The erythropoietic activity of the bone marrow is not decreased.—P. d. N.
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