Sickle Cell–Hemoglobin C Disease

Quantitative Determination of Iron Kinetics and Hemoglobin Synthesis

By Eli R. Movitt, Jack F. Mangum and William R. Porter

The clinical syndrome associated with the combined presence of Hb-C and Hb-S was first described by Kaplan, Zuelzer and Neel. Later reports followed in prompt succession. River, Robbins and Schwartz recently published a comprehensive review of this subject.

The hemolytic feature of sickle cell-hemoglobin C disease was recognized early by the same authors, who originally described it as a clinical entity. Thus Kaplan, Zuelzer and Neel reported that the erythrocyte survival studies demonstrated a shortened life span. With the transfusion of the red blood cells of two patients into normal recipients, the half-life in one case was 12 days and 6 and 18 days respectively on separate occasions in the other. The survival of normal red blood cells transfused into both patients was normal.

Weinstein, Spurling, Klein and Necheles reported a study on a 30-year-old Negro woman with S-C hemoglobin disease. The Cr51 red cell survival determination demonstrated a half-life of 20 days. It is questionable whether radioactive chromium red cell survival data render themselves to extrapolation into terms of physiologic life span, but if one permits himself to do so, this patient's average red cell life span would be approximately 50 days, thus indicating only a mild hemolysis. The authors stated that the survival curve was clearly abnormal and decreased to about the same extent as in their patient with hemoglobin C disease. They also concluded that the patient with S-C hemoglobin disease studied with Cr51 exhibited erythrocyte destruction less rapid than that reported by Zuelzer and Neel, but here again it is questionable whether the data provided by two entirely different techniques are readily comparable.

In the previously mentioned review of the literature by Rivers, Robbins and Schwartz published in 1961, the authors remarked that "Information regarding red cell survival in S-C hemoglobin disease is meager. . . Further studies are obviously indicated." We decided to undertake this task with quantitative determinations of iron kinetics. The ferrokinetic study was carried out using the model of Pollycove and Mortimer. With this method the mean erythrocyte life span is determined through dividing the calculated
Table 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Weight in Kg</th>
<th>Hematocrit Volumes %</th>
<th>Hemoglobin Gm./100 ml</th>
<th>RBC Count mill./cu.mm</th>
<th>Reticulocyte Count %</th>
<th>Serum Iron ag./100 ml</th>
<th>Serum Iron Binding Capacity ag./100 ml</th>
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<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>39</td>
<td>59.5</td>
<td>38</td>
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<td>4.0</td>
<td>165</td>
<td>465</td>
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<td>34</td>
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<td>4.0</td>
<td>4.1</td>
<td>142</td>
<td>390</td>
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<td>3</td>
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<td>22</td>
<td>70.9</td>
<td>31</td>
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<tr>
<td>4</td>
<td>Female</td>
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<tr>
<td>5</td>
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<td>34</td>
<td>12.0</td>
<td>3.86</td>
<td>11.5</td>
<td>105</td>
<td>255</td>
</tr>
</tbody>
</table>

Results of Investigation

The routine hematologic data are summarized in Table 1. The anemia was mild in all patients but one, Case 3, with the hemoglobin of 10.1 Gm. The reticulocyte counts were only slightly elevated. Only a modest reticulocytosis in patients with Hb S-C disease had been noted by other authors. The serum iron was at the lower limits of normal in one patient (Case 3) and just below the normal range in another (Case 4). In all patients, hemoglobins S and C were present in nearly equal amounts and hemoglobin F was less than 2 per cent. In Case 3 there was also present hemoglobin A (20 per cent). Prior to the electrophoretic study this patient received blood transfusions following an abortion. The blood transfusions were given 3 months before the present study with radioiron.

The results of the red cell volume determinations with P32 and many of the data of the ferrokinetics are summarized in Table 2. All patients were in a steady state during the period of study.

In all patients but one (Case 1), the ferrokinetics showed a more rapid than normal (1 to 2 hours) half-time clearance of radioiron from the plasma during the initial period of 3 hours following the intravenous injection (Table 2; Fig. 1). In Case 1 it was 62 minutes, at the lowest limit of the normal range; in the remaining patients the half-time clearance varied between 32 and 48 minutes. This then represents a more rapid than normal rate of transfer of radioiron from the plasma into the labile erythropoietic iron pool in the bone marrow, as would be expected in the presence of enhanced erythropoiesis. It is well to remember that it is the degree of response on the part of the bone marrow to hemolysis, rather than the severity of the hemolytic process as such, that will serve as a determinant of the radioiron half-time clearance from the plasma during the initial period of study. The serum iron level is another factor influencing the rate of disappearance of radioiron from

*The existence of such a labile pool is consonant with recent electronmicroscopic observations of Bessis demonstrating large amounts of ferritin in the marrow reticulum cells "feeding" developing red blood cells.
the plasma. In the face of a low serum iron concentration the transfer of Fe$^{59}$ to the labile pool is likewise accelerated.

In the healthy state there is a constant exponential rate of radioiron decrease in the plasma after the first 2 days of study due to the feedback of radioactivity from the marrow labile pool back to the plasma. Figure 2 demonstrates that instead of such a constant exponential there is a quick equilibration of the plasma iron due to reentry into the blood of radioactivity from the prematurely lysed red blood cells. In Case 2 there is also seen an early and characteristic “dip” of the plasma radioiron curve observed sometimes in a hemolytic process, the “dip” being followed by a prompt rise due to the return of iron to the plasma from destroyed erythrocytes.

The more rapid than normal iron turnover in the plasma in practically all patients (being slowest in Case 4) is more evidence pointing to accelerated transit of iron from the plasma to the marrow and hence increased erythropoiesis. The normal range is between 0.22 mg. and 0.40 mg. of iron per liter of blood. The range in our patients was from 0.42 mg. to as high as 0.9 mg. The half-time for incorporation of Fe$^{59}$ into the circulating red blood cells, normally 4 to 4.5 days, was shortened in all our patients, varying from 2 to
<table>
<thead>
<tr>
<th>Case</th>
<th>Red Cell Volume (F^2) ml.</th>
<th>Plasma Volume (Fe) ml.</th>
<th>Total Body Hemoglobin Gm.</th>
<th>Total Plasma Iron mg.</th>
<th>Erythropoietic Iron Pool mg.</th>
<th>Fe^{2+} Clearance Half-time (hours) (normal 1-2)</th>
<th>mg./hr./L. of blood (normal, 0.22-0.40)</th>
<th>Max. Net RBC Incorporation of Fe^{2+} %</th>
<th>Daily Iron for Hemoglobin Synthesis mg.</th>
<th>Daily Hemoglobin Synthesis Gm./L. of blood (normal, 1.0-1.6)</th>
<th>Mean Erythrocyte Life Span (days) (normal, 110-130)</th>
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</thead>
<tbody>
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<td>0.71</td>
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<td>0.53</td>
<td>2.8</td>
<td>0.90</td>
<td>80</td>
<td>69.7</td>
<td>20.5</td>
</tr>
</tbody>
</table>

Table 2

Iron Turnover in Plasma

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3 days, as would be expected in a hemolytic process with enhanced erythropoiesis. The maximal net red cell incorporation of radioiron varied from 60 per cent to 90 per cent, being lowest in Case 3. This patient had more marked anemia than others (fig. 3). The patient with 90 per cent incorporation of Fe\textsuperscript{59} into circulating red blood cells was also the one with mildest anemia in this group of cases—hemoglobin of 13 Gm. per cent, and the mean erythrocyte life span approximately half of the normal survival.

The daily hemoglobin synthesis was increased in all patients, only slightly so in Case 4 (8 Gm.), considerably more in other patients, with the highest value of 29.5 Gm. For valid comparison, instead of the total daily hemoglobin synthesis, the figures relating hemoglobin production to blood volume are more significant. In health the amount of hemoglobin produced daily varies from 1 Gm. to 1.6 Gm. per liter of blood. In our patients the lowest value was 2.2 Gm. (Case 4) and the highest 6.6 Gm. (Case 5), i.e., practically a five-fold increase over the mean normal value.

Of interest are the data of the in vivo counting over the sacrum. Normally the curve reaches the base line, as the marrow empties itself of the radioiron which then reappears in the circulating erythrocytes, but it remains at varying levels above the base line in a hemolytic process due to more rapid rate of return of iron from prematurely lyzed red blood cells back to
the marrow. The shorter the red blood cell survival, the more rapidly will the radioiron return to the marrow from destroyed erythrocytes and consequently that much higher above the base line will the sacral curve be sustained. Another process operating in the same direction is the intramedullary hemolysis. In this event there is a continuous recirculation of iron within the marrow itself where the lysis of cells is taking place; thus this results in ineffective erythropoiesis. The relatively high position of the sacral curve in Case 2 (fig. 4a), although not anywhere as striking as in cases with predominantly intramedullary hemolysis, still suggests that in this patient the hemolytic process in part is occurring within the marrow. That this may well be so is further suggested by somewhat prolonged mean effective erythrocyte hemoglobinization time (MEEHT) of 2.5 days (normal 1 to 1.8 days).

The erythrocyte life span (normal 110–130 days) varied in our patients from 18 days to 56 days. It was rather markedly shortened in two cases (18 and 20 days), moderately so in one case (29 days) and only relatively slightly in the remaining two cases (46 and 56 days), indicating only mild hemolysis.

Of practical interest are the results of in vivo organ counts in Case 3 (fig. 4b). The analysis of the splenic curve indicates a significant and rather marked degree of sequestration of red blood cells in the spleen, with the radioactivity

*The mean effective erythrocyte hemoglobinization time (MEEHT) is the period elapsing between irreversible fixation of iron by maturing erythrocytes and their emergence from the marrow into the circulation. By the same token MEEHT is shortened in extramedullary hemopoiesis when a significant number of immature cells escape into circulation.
SICKLE CELL--HEMOGLOBIN C DISEASE

over that organ approaching 27 per cent of the total injected radioiron. This patient had more marked anemia than others, with hemoglobin of 10.1 Gm. per cent and mean erythrocyte survival time of 29 days. It is entirely possible that splenectomy may lead to more prolonged erythron life span, perhaps up to 50 days or more; this may in turn result in the hemoglobin concentration of between 12 and 13 Gm. per cent. In Case 4 there was also a significant degree of sequestration of red blood cells in the spleen (fig. 4c) although not as marked as in Case 3, representing only 14 per cent of the total injected radioactivity. This, along with the mildness of the anemia (hemoglobin of 12.2 Gm. per cent), and the erythrocyte life span of 56 days would argue against the advisability of splenectomy. In still another patient (Case 5) the splenic trapping of red blood cells was still less, only 7 per cent. In the remaining two patients (Cases 1 and 2) there was no sequestration of erythrocytes in the spleen (figs. 4a and 4d).

Chernoff, Ruchnagel and Jim9 transfused the Cr\textsuperscript{59}-tagged red blood cells of three patients with sickle cell-hemoglobin C disease into three normal subjects with and without the spleen and found that the erythrocyte survival time was approximately 50 days in all three cases. They concluded that the spleen did not appear to have a critical role in the hemolysis in this disease. However, these authors admitted that, on clinical grounds, there were reasons to believe that the spleen did play some role in red blood cell destruction or sequestration, at least during crisis.

**SUMMARY**

Quantitative determinations of iron kinetics and hemoglobin synthesis were made on five patients with sickle cell-hemoglobin C disease. The anemia was mild in all patients but one who had the hemoglobin of 10.1 Gm. per cent. All patients were in a steady state during the period of this study.

The ferrokinetic determinations demonstrated a hemolytic process in all cases. The mean erythrocyte life span in these patients was 18, 20, 29, 46 and 56 days respectively (normal range, 110–130 days). The hemoglobin synthesis was increased in all. Reduced to terms of daily hemoglobin production per liter of blood, the values were 2.2 Gm., 2.9 Gm., 3.6 Gm., 5.8 Gm. and 6.6 Gm. The latter figure represents a five fold increase over the normal mean value of daily hemoglobin synthesis of 1.3 Gm. (normal range, 1.0 to 1.6 Gm. of hemoglobin per liter of blood per day).

The results of the in vivo organ counts demonstrated a significant degree of sequestration of red blood cells in the spleen of two patients. The question of advisability of splenectomy in such patients was discussed.

**SUMMARIO IN INTERLINGUA**

Determinaciones quantitative del ferrocinetica e del synthese de hemoglobina esseva effectuate in cinque patientes con morbo de hemoglobina de cellula falciforme e de hemoglobina C. Le anemia esseva leve in omne le patientes con un exception. In iste ultime le hemoglobina mesurava 10,1 g per 100 ml.
Figs. 4a (above) and 4b (below).—See explanation in the text.
Figs. 4c (above) and 4d (below).—See explanation in the text.
Omne le patientes esseva in un stato stabile durante le periodo del presente studio. Le determinationes ferrocineti c demonstrava un processo hemolytic in omne le casos. Le longevitate medie del erythrocytos in iste patientes esseva 18, 20, 29, 46, e 56 dies, respectivemente. (Le region normal se extende ab 110 ad 130 dies). Le synthese de hemoglobina esseva augmentate in omnes. Calculate como production diurne de hemoglobina per litro de sanguine, le valores esseva 2,2, 2,9, 3,6, 5,8, e 6,6 g. Iste ultime representa un quintuplication del normal diurne synthese medie de hemoglobina per litro de sanguine, i.e. 1,3 ± 0,3 g.

Le resultatos del numerations per organos in vivo demonstrava un significa-tive grado de sequestration de erythrocytos in le splen de duo patientes. Le possibile utilitate de splenectomia in tal patientes es discutite.

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REFERENCES

6. —, and —: The quantitative determina-
SICKLE CELL–HEMOGLOBIN C DISEASE

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Sickle Cell—Hemoglobin C Disease: Quantitative Determination of Iron Kinetics and Hemoglobin Synthesis

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