Red Cell Life Span in Sickle Cell-Hemoglobin C Disease With a Note About Sickle Cell-Hemoglobin O\textsubscript{ARAB}

By Paul R. McCurdy, Laviza Mahmood, and Anita S. Sherman

Red cell survival was measured in ten subjects with S-C disease and one with S-O Arab (\(\alpha_2\beta^{121}\text{ Gly}^{98}\)) disease using both DP\textsuperscript{32P} and \(^{51}\)Cr as tags. Red cell volume was slightly reduced in most patients (87\% \pm 20\% of predicted normal). In nine SC patients, mean red cell life (DP\textsuperscript{32P}) was 28.9 \pm 4.0 days. For one SC subject it was significantly longer (47.9 days), as it was for the one with S-O Arab. The S-O Arab subject had irreversibly sickled cells in the peripheral blood, whereas those with SC had few (< 1/1000 red cells) or none. The S-O Arab hemolysate gelled at a hemoglobin concentration (16.2 g/100 ml) near that for sickle cell anemia hemolysates (15.9 \pm 1.0 g/100 ml; \(n = 8\)) but significantly lower than that for SC hemolysates (21.6 \pm 1.9 g/100 ml; \(n = 5\)). It seems likely that properties of S-C red cells other than their relative ease of sickling contribute significantly to their rate of hemolysis.

Patients with sickle cell-hemoglobin C (SC) disease have varying degrees of anemia in that it may be moderate in a few, while minimal in others. Some even have normal hematocrit and hemoglobin values. This anemia results from hemolysis with inadequate erythropoietic compensation, but information about erythrokinetics in SC disease is limited. Relatively few patients have had their red cell survival measured. The present work was designed to extend previous studies of red cell life span in patients with SC disease, to define anemia in these patients in terms of red cell mass, and to compare these data with similar studies in a patient with S-O Arab disease.

MATERIALS AND METHODS

Ten patients with SC disease and one with S-O Arab disease were included in the study. The diagnosis of SC disease was based upon a positive sickle cell solubility test\textsuperscript{11} and the results of hemoglobin electrophoresis on cellulose acetate (Tris-EDTA-borate buffer, pH 8.6) and on agar gel (citrate buffer, pH 6.2).\textsuperscript{12} The Hb O Arab (\(\alpha_2\beta^{121}\text{ Gly}^{98}\))\textsuperscript{13} was identified by standard fingerprint techniques.\textsuperscript{14}

CASE REPORT

Number 11, a 23-yr-old black woman, has had painful episodes every 1-3 mo since she was a young child. These commonly start in the knee joints and spread to involve the extremities, abdomen, and chest. Only recently was one severe enough to require hospitalization. The attacks last for a few days to 2 wk. She was admitted to the hospital at age 6 for pneumonia and empyema (drained) and again at age 17 for an acute urinary tract infection. Very recently she was admitted for a therapeutic abortion. She has never had a blood transfusion. Her sclerae are usually mildly

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Table 1. Laboratory Data

<table>
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<tr>
<th>Subject</th>
<th>Sex</th>
<th>Hematocrit (%)</th>
<th>RBC Volume (% N)</th>
<th>Reticulocytes (%)</th>
<th>t₁³¹Cr (days)</th>
<th>MCl DF³²P (days)</th>
<th>Ke</th>
<th>Indirect bilirubin (mg/dl)</th>
<th>Production Indices</th>
<th>Retic Without Shift</th>
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<th>Destruction Index DF³²P</th>
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<td>2.1</td>
<td>1.4</td>
<td>2.7</td>
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</tr>
</tbody>
</table>

* S-C?
† S-O Arab, ISC count, 7.1 ± 1.4.
SICKLE CELL–HEMOGLOBIN C DISEASE

SICKLE CELL–HEMOGLOBIN C DISEASE

SICKLE CELL–HEMOGLOBIN C DISEASE

icteric. Physical examination is normal except for mild icterus and a barely palpable spleen. Ocular
fundus are normal. Some laboratory values are summarized in Table 1. Additional data include di-
rect bilirubin, 0.3 mg/dl; urea nitrogen, 9 mg/dl; creatinine, 0.5 mg/dl; uric acid, 2.5 mg/dl;
GOT, 20–35 U; and LDH, 400–600 U (normal, 100–225). The hemolysate contained 56.3% Hb S,
41.3% Hb O Arab, and 2.4% Hb F (major Hbs quantitated after elution from cellulose acetate
strips). The patient has been working full time in a clerical position.

Red cell volume and autorsurvival were determined with 51Cr and DF32P. Normal erythrocyte
mass was estimated from both body weight and height.3 Red cell destruction and production
indices were calculated.4 The destruction index is DI-DF32P = 110/MCL, where 110 is the normal
red cell life span and MCL is the observed mean cell life measured with DF32P. The production
index is

\[
\text{PI-DF}^{32}\text{P} = \frac{110 \times \text{RBC volume (} % \text{ normal)}}{\text{MCL}}.
\]

Reticulocytes were also used to estimate red cell production (PI-retic) after appropriate cor-
rections for hematocrit and marrow to peripheral blood shift were made.19 All subjects were in a
steady state during the period of the study. Informed consent was obtained from each individual in
the study which was approved by appropriate institutional review committees.

Irreversibly sickled cells (ISCs) were counted on cover glass blood smears made from native
venous blood during the red cell survival study.17 A Miller optical disc was used to speed the
procedure.18 Nine slides from patients with sickle cell anemia (unpublished observations) were
exchanged with Dr. G.R. Serjeant; the correlation between the ISC counts done in the two labora-
tories was excellent (r = +0.97).

The minimum concentration of hemolysate hemoglobin required to gel when deoxygenated was
determined by the method of Bookchin and Nagel,19 modified to be done at 37°C. Eight subjects
with sickle cell anemia, one with S-D Punjab disease,20 and five with S-trait were studied for
comparison. The subjects with S-trait had Hb S proportions of 36%–43% except for one with
probable α-thalassemia trait too (Hb S, 30.1%; microcytosis; reduced α-chain synthesis in
vitro).

RESULTS

The clinical and erythrokinetic data are summarized in Table 1. Nine of the
ten patients with SC disease were similar in all parameters studied and are
analyzed together. For this group the hematocrit value was 34.4 ± 3.0 (mean ±
SD). As a per cent of normal, the hematocrit value was 78.0 ± 7.4 (normal for
males, 47%; females, 42%). The red cell mass was 86.8% ± 19.7% of the pre-
dicted normal value. Nevertheless, all but one fell within the predicted normal
range; subject 4 was below. There was good correlation between the hemat-
ocrit and the red cell mass (r = +0.71; p < 0.05). The t½ 51Cr was 15.7 days ±
2.7 days. The DF32P red cell survival curves all fit exponential equations better
than linear ones, but in only a few was the difference statistically significant.
The exponential line was used. The average MCL was 28.9 days ± 4.0 days.
The calculated hemolytic rate (DI-DF32P) was 3.9 ± 0.6 times normal; this red
cell destruction was countered by erythropoiesis (PI-DF32P) which was 3.3 ±
0.7 times normal. The rate of red cell production estimated from the reticulo-
cyte counts (PI-retic) with all suggested corrections16 was 2.1 ± 0.6 times nor-
mal and seemed to underestimate production as calculated from the red cell life
span. When corrections for “shift” were omitted, the values were closer (3.1 ±
1.0). The elution rate (Ke) for 51Cr was 1.02 ± 0.45 (for patients with normal
Hb, 1.3 ± 0.3).3

For one other patient (No. 10) whose hemoglobin appeared to be SC, all
Table 2. Minimum Gelling Concentrations of Hemolysates in Various Disorders

<table>
<thead>
<tr>
<th></th>
<th>MGC (g/dl)</th>
<th>ISC (%)</th>
<th>RBC-MCL (days)</th>
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<tr>
<td>SC</td>
<td>21.6 ± 1.9 (5)*</td>
<td>0</td>
<td>28.9 ± 4.0 (10)</td>
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<tr>
<td>S-O Arab</td>
<td>16.2</td>
<td>7.1</td>
<td>44.6</td>
</tr>
<tr>
<td>SS</td>
<td>15.9 ± 1.0 (8)</td>
<td>9.3 ± 5.5 (20)†</td>
<td>17.7 ± 4.6 (20)†</td>
</tr>
<tr>
<td>AS</td>
<td>23.5 ± 1.3 (4)</td>
<td>0</td>
<td>&quot;Normal&quot; ‡</td>
</tr>
<tr>
<td>S-D Punjab</td>
<td>12.6</td>
<td>6.6</td>
<td>23¶</td>
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</table>

*Mean ± SD (n).
†Unpublished observations.
‡Reference 33.
§S-trait and probably α-thalassemia trait—see text.
¶Calculated from 1/2 51Cr (12 days) assuming a normal 51Cr elution rate; reference 20.

Values are similar to those reported above except the red cell life span, which was much greater (DF32P – MCL = 47.9 days). He is a 32-yr-old black man who was referred to the Hematology Clinic from the Eye Clinic because of "typical SC retinopathy." He had often had leg pains as a child but the frequency had decreased to about once yearly during adult years. He had gross hematuria for 1 wk at age 31. He had been taking methadone for 2 yr after a period of 6 yr of intravenous drug abuse. Except for the ocular fundi, faint icterus, and scars of old leg ulcers, the physical examination was normal. The spleen was not palpable. Some laboratory data are found in Table 1. Other data include direct bilirubin, 0.3 mg/dl; urea nitrogen, 7 mg/dl; GOT, 88; LDH, 265. The hemolysate contained 57.8% Hb S, 41.2% Hb C, and 1.0% Hb F. The main bands migrated similarly to Hbs S and C on agar gel at an acid pH. No ISCs were found in the peripheral blood.

During this study, a patient proved to have S-O Arab disease was detected (see above) and included. Again, all parameters were similar to the group with SC disease except the red cell life span, which was significantly longer—44.6 days. This patient had ISCs readily detectable in her peripheral blood (7.1%). None of the SC patients had more than one such cell per 1000 red cells.

Minimum gelling concentration (MGC) at 37°C of hemoglobin from the hemolysate of five of the subjects with SC disease was 21.6 g/dl ± 1.9 g/dl (Table 2). For the S-O Arab patient the value was 16.2 g/dl. For comparison, the MGC for eight homozygous S patients was 15.9 g/dl ± 1.03 g/dl; for four subjects with S-trait (Hb S, 36%–43%), it was 23.5 g/dl ± 1.3 g/dl; for the one with S-trait and probable α-thalassemia (Hb S, 30.1%) it was 27.5 g/dl; and for one patient with S-D Punjab it was 12.6 g/dl.

DISCUSSION

These data extend those from previous studies of erythrokinetics in SC disease. Most of our patients form a homogeneous group. The hematocrit and red cell mass are about 80%–85% of normal, but many are actually within the normal range. Red cell life span is about 25% of normal, and erythropoiesis nearly keeps pace. Despite slightly incomplete compensation for hemolysis, the bone marrow is working at about four times the normal rate. This is less than
the six to eight times normal supposedly within the capability of a normal marrow.\textsuperscript{16} This minimal decompensation in SC disease is unexplained. Although it has been suggested that oxygen transport may not be normal,\textsuperscript{22} affected by such parameters as the oxygen dissociation curve. No abnormalities of hemolysate oxygen affinity have been described in SC disease. On the other hand, a recently described variation of the oxygen affinity of Hb S with concentration and upon association with other hemoglobins may be pertinent.\textsuperscript{23}

There is a poor correlation between the red cell production indices calculated from $\text{DF}_{32}^\text{P}$ life span measurements and those calculated from the reticulocyte count, when all suggested corrections are applied to the latter.\textsuperscript{16} When the shift factor is omitted from the reticulocyte calculation, the correlation becomes good. This also occurs in patients with sickle cell anemia (unpublished observations). In patients with chronic hemolysis the sojourn of reticulocytes in the peripheral blood may not be prolonged beyond normal.

There are at least two other hemoglobins found in American blacks which have electrophoretic mobilities at an alkaline pH similar to hemoglobin C: hemoglobins $\text{O Arab}^{17}$ and $\text{E}$.\textsuperscript{24} Even though they can be distinguished from hemoglobin C by electrophoresis on agar gel (citrate buffer, pH 6.2),\textsuperscript{25} $\text{S-O Arab}$ and $\text{S-E}$ diseases may be confused with SC disease. Clinically, $\text{S-O Arab}$ disease seems to be more severe than SC disease, and $\text{S-E}$ disease seems to be similar to it.\textsuperscript{26}

The concentration at which SC hemolysates gel when deoxygenated is greater than that of SS hemolysates and close to but lower than that of $\text{S-trait}$ (Table 2). The $\text{S-O Arab}$ hemolysate gels at a lower concentration than the SC ones, nearly as low as do SS ones, and the S-D Punjab combination gels at an even lower concentration. Irreversibly sickled cells may occur after repeated sickle-un sickle cycles\textsuperscript{27} and hence are related to the ease with which sickling occurs. Clinical findings of pain and organ damage correlate in part with these data and probably result from oxygen-linked sickling. The most seriously ill patients are those with SS; S-D Punjab subjects seem to have more crises and other problems than do those with SC disease. Too few $\text{S-O Arab}$ patients have been reported\textsuperscript{25,28-30} to be certain of their clinical course, but they appear to be as ill as many SS patients. Sickle cell trait subjects are essentially asymptomatic.

The hemolytic rate also correlates positively, albeit imperfectly, with the propensity to sickle (proportion of ISC's), both within the spectrum of sickle cell anemia (reference 17 and unpublished observations) and between patients with the sickling diseases (Table 2). For example, one subject with S-D Punjab\textsuperscript{20} had a $\text{t}^{\text{31}}\text{Cr}$ similar to those found in SS individuals, and his ISC count (6.6\%) was similar also. Since the blood of our $\text{S-O Arab}$ patient seemed to sickle as readily as did that from individuals with SS and S-D Punjab, it was rather surprising that her red cell life span was prolonged, not only beyond that of those with SS or S-D Punjab but also beyond that of those with SC, who have few or no ISC's. In the only other report of red cell survival studies in a patient with $\text{S-O Arab}$,\textsuperscript{25} $\text{t}^{\text{5}}\text{s}$ $\text{31Cr}$ of 5 and 6 days were found on two occasions in an infant who had both marked splenomegaly and G-6-PD deficiency. His reticulocyte count was higher than that of other older subjects with the S-O Arab combination. It is possible that his hemolytic rate was greater than is commonly the case in this disease.
Hemoglobin C does not polymerize as hemoglobin S does, but it has reduced solubility and crystallizes within the red cells under certain conditions. This crystallization, which is not oxygen linked (the sickling phenomenon is), may produce membrane damage. Red cells from subjects with homozygous hemoglobin C disease (CC) do not deform or pass Millipore filters as well as do normal ones. These features probably contribute to the reduced red cell life span in subjects with the hemoglobin C diseases (CC, SC, C-β-thalassemia). They may explain why the SC patients have a shorter red cell survival than the ones with S-O Arab, even though the latter tends to sickle more easily. Unfortunately, no studies of red cell deformability or of the tendency of the hemoglobin to crystallize have been done in subjects with S-O Arab disease. There appears to be a dichotomy between the oxygen-linked sickle-dependent manifestations of these diseases (crisis, organ infarct) and hemolysis, which is governed not only by sickling but also by membrane phenomena and perhaps other factors.

ACKNOWLEDGMENT
We are grateful to Dr. R. Casey and Dr. H. Lehmann, Cambridge, England, for the identification of hemoglobin O Arab.

REFERENCES
17. Serjeant GR, Serjeant BE, Milner PFA:
SICKLE CELL–HEMOGLOBIN C DISEASE

Red cell life span in sickle cell-hemoglobin C disease with a note about sickle cell-hemoglobin O ARAB

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