Radioactive Sodium Chromate for the Study of Survival of Red Blood Cells

III. The Abnormal Hemoglobin Syndromes

By Irwin M. Weinstein, Carroll L. Spurling, Herman Klein and Thomas F. Necheles

In previous publications, we have reported that tagging human erythrocytes with radioactive chromium in the form of $\text{Na}_2\text{Cr}^{3+}\text{O}_4$ affords a simple, rapid and reproducible method for studying the survival of erythrocytes in normal subjects and patients with various hematologic disturbances. By this method the one-half survival time of $\text{Cr}^{3+}$ tagged red blood cells is $33.1 \pm 3.2$ days. This time refers to the apparent one-half survival, i.e., the curves are not corrected for chromium "leakage," a phenomenon which occurs at the rate of approximately one per cent of the remaining radioactivity per day, but which does not interfere with establishing an accurate quantitative relationship between normal and abnormal survival curves. Of the various abnormal red cells tested thus far, "leakage" occurs at the same rate with abnormal as with normal red cells.

In this paper we are reporting the hematologic findings including $\text{Cr}^{3+}$ red cell survival studies in a group of patients with abnormal hemoglobin syndromes. Although a few reports on the survival time of erythrocytes containing one or a combination of the recently identified abnormal hemoglobins have appeared in the literature to date, to our knowledge this is the first such report using the $\text{Cr}^{3+}$ technique.

The Abnormal Hemoglobin Syndromes

Interest in hemolytic syndromes associated with an abnormal hemoglobin was stimulated by Pauling's demonstration in 1949 that hemoglobin from patients with sickle cell anemia had an abnormal electrophoretic mobility as compared with normal adult hemoglobin. Singer et al., utilizing earlier observations by von Körber and von Krüger, established a simple technic for quantitating fetal hemoglobin. They reported elevated values particularly in sickle cell anemia and severe cases of the Mediterranean syndrome. Itano and Neel as well as Kaplan, Zuelzer, and Neel described a third type of abnormal hemoglobin (hemoglobin C) occurring in American Negroes, as demonstrated by electrophoretic studies. Finally, Itano discovered a fourth abnormal hemoglobin (hemoglobin D) occurring in one Negro family and associated with sickle hemoglobin. This hemoglobin was identified by its specific solubility characteristics which differ from other known hemoglobin types. It has the electrophoretic mobility of sickle hemoglobin though it does not produce sickling. Table 1 lists the various combinations of normal and abnormal hemoglobins that have been identified at the time of this publication.

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METHODS AND MATERIALS

The technic for tagging the erythrocyte with \( \text{Cr}^{51} \) in the form of \( \text{Na}_2\text{CrO}_4 \) has been outlined in detail previously.\(^1\) All studies were carried out by reinjecting the patient's own blood, after labeling, with approximately 200 microcuries of \( \text{Cr}^{51}. \)** Figure 1 demonstrates normal survival curves by this method.

Standard hematologic procedures were used for red cell counts and the determination of hemoglobin and hematocrit. Reticulocytes were counted on blood films stained with brilliant cresyl blue. Sickling tests were performed using the sodium metabisulfite technic.\(^3\) Electrophoretic mobility of hemoglobin solutions was measured by the filter paper method.\(^4\) Figure 2 illustrates the electrophoretic patterns of hemoglobins which can be identified by this method. Alkali-resistant hemoglobin was determined according to Singer's technic.\(^5\) Osmotic fragility tests were performed on freshly-drawn venous blood.\(^6\) Mechanical fragility was estimated by the method of Shen, Castle and Fleming.\(^7\) The excretion of fecal urobilinogen was determined on pooled three-day collections of stools, using the semiquantitative method of Watson.\(^8\) Results are expressed in Ehrlich units.

All the patients in this report are Negroes.

RESULTS

I. Sickle Cell Anemia

The hematologic data of four patients with sickle cell anemia are tabulated in table 2 and the survival curves are shown in figure 3.

Discussion. The impairment of erythrocyte survival time in these cases as demonstrated by the \( \text{Cr}^{51} \) technic is of the same order as that demonstrated by the more conventional differential agglutination (Ashby) technic. Although patients with sickle cell anemia have very active erythroblastic bone marrows, anemia invariably occurs because these patients cannot maintain a normal red cell mass in the face of erythrocyte destruction of this magnitude.\(^9\) It will be

\* Procured from the Radioactive Pharmaceuticals Division of the Abbott Laboratories under allocation from the Isotopes Division, U. S. Atomic Energy Commission.
ELECTROPHORETIC DETERMINATION OF HEMOGLOBIN TYPES

Table 1.—Abnormal Hemoglobin Syndromes

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Hemoglobin Type†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle cell trait*</td>
<td>A + S</td>
</tr>
<tr>
<td>Sickle cell disease*</td>
<td>S + F</td>
</tr>
<tr>
<td>Hemoglobin C trait*</td>
<td>A + C</td>
</tr>
<tr>
<td>Hemoglobin C disease*</td>
<td>C</td>
</tr>
<tr>
<td>Sickle cell—Hemoglobin C disease*</td>
<td>C + S (+F)</td>
</tr>
<tr>
<td>Hemoglobin D trait</td>
<td>A + D</td>
</tr>
<tr>
<td>Sickle cell—Hemoglobin D disease</td>
<td>S + D</td>
</tr>
<tr>
<td>Thalassemia Major</td>
<td>A + F</td>
</tr>
<tr>
<td>Sickle cell—Thalassemia</td>
<td>S + A + F</td>
</tr>
<tr>
<td>Hemoglobin C—Thalassemia†</td>
<td>C + A (+F)</td>
</tr>
</tbody>
</table>

* Included in the present study.
† Personal communication with Dr. Karl Singer.
‡ The designation of hemoglobins in this paper conforms with the recommendations of the Symposium of the Hematology Study Section of the Division of Research Grants of the National Institutes of Health (Blood 8: 386, 1953).

noted that patient M. W. has a survival curve with two components, a very rapid initial component and a second less rapid but still abnormal curve. This type of erythrocyte survival curve has been noted previously in some patients with sickle cell anemia, using the Ashby technic, but the meaning of such a curve is not completely understood. Singer has demonstrated an uneven distribution of S and F hemoglobin within the cells. We feel that this may in part explain a two-component curve in this disease. However, the lack of correlation between the amount of fetal hemoglobin and the severity of the disease and the fact that indisputable one-component curves have been reported such,
SICKLE-CELL ANEMIA

4 6 8 10 14 18 22 26 30 34 38

DAY S

FIG. 3.—Cr\textsuperscript{41} red cell survival curves in four patients with sickle cell anemia.

TABLE 2.—Hematologic Data on Sickle Cell Anemia Patients

<table>
<thead>
<tr>
<th></th>
<th>T. P.</th>
<th>M. W.</th>
<th>G. L.</th>
<th>L. A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (Gm./100 cc.)</td>
<td>8.3</td>
<td>5.5</td>
<td>7.7</td>
<td>6.2</td>
</tr>
<tr>
<td>Red blood count (X 10\textsuperscript{6})</td>
<td>2.72</td>
<td>1.48</td>
<td>1.98</td>
<td>1.70</td>
</tr>
<tr>
<td>White blood count</td>
<td>7700</td>
<td>13,100</td>
<td>12,000</td>
<td>11,600</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>9.0</td>
<td>21.0</td>
<td>16.6</td>
<td>11.5</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>—</td>
<td>16</td>
<td>—</td>
<td>17</td>
</tr>
<tr>
<td>Sickle preparation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkali resistant Hgb (%)</td>
<td>4.5</td>
<td>2.6</td>
<td>9.7</td>
<td>7.0</td>
</tr>
<tr>
<td>Electrophoretic pattern</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

as patient T. P., make it seem very likely that other factors influence the shape of the survival curve. Singer postulates that stromal factors may play an important role. Eadie and Brown\textsuperscript{20} have shown that continuing random destruction plus senescence of the sickle cells may account for a two-component curve, assuming equal distribution of the abnormal hemoglobins. Our one case presented here sheds no light on this interesting problem. Unfortunately patients G. L. and L. A. were not studied long enough to say whether or not two-component curves were present.

II. Sickle Cell Trait

The hematologic findings and red cell survival curves in a father and son with sickle cell trait are illustrated in table 3 and figure 4.

Discussion. Normal survival of erythrocytes from patients with sickle cell trait has been described previously by other investigators.\textsuperscript{21, 22} Our studies
merely confirm this finding, using a different technic. The half-life survival time in patient E. R. is not a statistically significant variation from our normals.

III. Hemoglobin C Disease

Because of current interest in the clinical syndrome of hemoglobin C disease and the paucity of published reports, it seems appropriate to present a case history of our patient with this disorder.

D. W., a thirty-five year old Negro male, was admitted to the University of Chicago Clinics with a two year history of periodic dizzy spells and weakness lasting 5 to 10 minutes. Systemic inquiry was essentially negative. His health previously had been good. There was no history of jaundice, crises, bone pain or leg ulcers. Family history was not remarkable. There were six siblings and two daughters.

On physical examination he was found to be a well-developed slender male, not apparently ill. The only significant finding was an enlarged spleen palpable 12 cm. below the left costal margin.

Pertinent laboratory data included the following studies: 13.5 Gm. hemoglobin, 4.56 million red blood cells, 9350 white blood cells with normal differential, hematocrit of 39, and 1.5 per cent reticulocytes. Approximately 25 per cent target cells were seen on smear. No sickling was present after 24 hours. An osmotic fragility test showed beginning hemolysis at 0.42 per cent sodium chloride solution (control 0.46) and complete hemolysis at 0.20 (control 0.36). Mechanical fragility showed hemolysis of 4.2 per cent patient's cells with a control of 2.9 per cent. A moderate erythroid hyperplasia was found on bone marrow examina-
HOMOZYGOUS HGB C DISEASE

Fig. 5.—Cr¹⁹ red cell survival curve in a patient with hemoglobin C disease.

Discussion. Our patient presents a clinical syndrome of a mild compensated hemolytic disease, splenomegaly, and increased numbers of target cells occurring in a Negro without a known family history of anemia. The diagnosis is substantiated by the electrophoretic analysis of his hemoglobin.

This case is quite similar to the ones reported by Spaet¹ and Ranney et al.²³ A minor difference is the absence of leukocytosis in our patient. The red cell survival time shown here is of the same order of magnitude as that described by Spaet in his case, using the Ashby technic.

IV. Hemoglobin C Trait

The hematologic data of two subjects with hemoglobin C trait are given in table 4 and the red cell survival curves in figure 6. The first patient (upper curve) is the wife of sickle-cell-trait subject E. R. These parents have a daughter with a moderate hemolytic anemia, whose electrophoretic pattern reveals the presence of C, S, and a faint band of A hemoglobin, the latter resulting from a recent blood transfusion given at another hospital. The second patient (lower curve) is the mother of a subject with sickle cell–hemoglobin C disease who is being reported here (vide infra).

Discussion. The only hematologic abnormality noted in these two cases is the presence of many target cells. Apparently the presence of the C trait is a perfectly benign situation, comparable to the subjects with sickle trait. Erythrocyte survival time is within the normal range, as would be anticipated in view of normal clinical and laboratory findings. In the only other known reports of
survival time in the hemoglobin C trait, Kaplan, Zuelzer, and Neel in their first publication found markedly abnormal survival time of donor cells with this abnormality transfused into normal recipients, using the Ashby technic. However, in a recent publication these authors found that the survival of hemoglobin C trait erythrocytes was probably within normal limits, although the rate of disappearance was more rapid than that of control normal erythrocytes transfused at the same time. Our results agree with the latter paper from this group.

V. Sickle Cell–Hemoglobin C Disease

Table 5 presents the pertinent laboratory findings of patient M. S. whose electrophoretic analysis demonstrates the presence of both C and S hemoglobin. This patient, a thirty-year-old Negro woman, had had about fifteen crises of abdominal and bone pain in the last twelve years. The erythrocyte survival time is shown in figure 7.

Discussion. As can be seen from the above data, this patient has only a mild hemolytic process as demonstrated by the usual clinical and laboratory means, yet her survival curve is clearly abnormal and decreased to about the same extent as the subject with hemoglobin C disease. Erythrocyte destruction in
FIG. 7.—Cr$^{31}$ red cell survival curve in a patient with sickle cell-hemoglobin C disease.

**TABLE 5.**—Hematologic Data on Patient M. S. with Sickle Cell-Hemoglobin C Disease

<table>
<thead>
<tr>
<th>Hemoglobin (Gm./100 cc.)</th>
<th>11.7</th>
<th>Osmotic fragility (% saline)</th>
<th>3.87</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood count (× 10$^6$)</td>
<td>3.87</td>
<td>Initial hemolysis (control 0.46)</td>
<td>0.38</td>
</tr>
<tr>
<td>White blood count</td>
<td>5500</td>
<td>Complete hemolysis (control 0.32)</td>
<td>0.20</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>3.0</td>
<td>Serum bilirubin (mg.)</td>
<td>3.0</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>37</td>
<td>Direct</td>
<td>0.4</td>
</tr>
<tr>
<td>Target cells (%)</td>
<td>40</td>
<td>Total</td>
<td>0.6</td>
</tr>
<tr>
<td>Sickle preparation</td>
<td>+</td>
<td>Sternal marrow: Mod. erythroid hyperplasia</td>
<td></td>
</tr>
<tr>
<td>Fecal urobilinogen (Ehrlich units/24 hr.)</td>
<td>208</td>
<td>Alkali resistant hemoglobin (%)</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Electrophoretic pattern</td>
<td>C + S</td>
</tr>
</tbody>
</table>

this patient (M. S.) as determined by the Cr$^{31}$ technic is less rapid than in those reported by Kaplan, Zuelzer, and Neel.$^4$ It seems important to emphasize that this interesting combination of C and S hemoglobin should be suspected in Negroes with sickling whose hemolytic manifestations are milder than would be expected in classical sickle cell disease, in whom splenomegaly persists well into adult life, and in whom increased numbers of target cells are demonstrated in the peripheral blood smear.

**SUMMARY AND CONCLUSIONS**

Cr$^{31}$ erythrocyte survival times are reported in a group of patients with a variety of abnormal hemoglobin syndromes. Marked decreases in survival time are demonstrated in pure sickle cell anemia. Shortened survival times are reported in one case each of hemoglobin C disease and sickle cell–hemoglobin C disease with compensated hemolysis. Normal survival times are reported in sickle cell trait and hemoglobin C trait.

Red cell life span as measured by the Cr$^{31}$ technic agrees well with most published reports of survival times in these disorders in cases performed with the Ashby technic. The Cr$^{31}$ method appears to be as useful in measuring the survival of erythrocytes containing abnormal hemoglobins as it has been shown
to be in other hemolytic disorders as well as in normals. Its decided advantages are its simplicity, adaptability, and reliability.

**SUMMARIO E CONCLUSIONES IN INTERLINGUA**

Es reportate le superviventia del erythrocytos determinate per medio del technica a Cr⁶¹ in un gruppo de patientes con un varietate de syndromes a hemoglobina anormal. Marcate reductiones del superviventia es demonstrate in pur anemia a cellulas falciforme. Reducte superviventia es reportate etiam in un caso de morbo a hemoglobina C e in un caso de morbo a cellulas falciforme plus morbo a hemoglobina C con hemolyse compensate. Superviventia normal es reportate in casos de tracto a cellulas falciforme e a hemoglobina C.

Le superviventia erythrocytic mesurate per medio del technica a Cr⁶¹ concorda ben con le majoritate del publicate reportos de superviventia in tal disordines investigate per medio del technica Ashby. Le methodo a Cr⁶¹ pare tanto utile in mesurar le superviventia de erythrocytos que contine hemoglobinas anormal como illo se ha provate in alte disordines hemolytic e in casos normal. Su grande avantages es su simplicitate, su adaptabilitate, e le facto que illo es altemente digne de confidentia.

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