Intraerythrocytic Hemoglobin Crystals in Sickle Cell-Hemoglobin C Disease

By L. W. Diggs and Ann Bell

On stained smears of patients with sickle cell-hemoglobin C disease the authors have observed intraerythrocytic hemoglobin crystals which produce changes in erythrocyte shapes that are intermediate between those found in homozygous hemoglobin S and homozygous hemoglobin C (fig. 1). The morphology of the misshapen red cell is considered characteristic of the hemoglobinopathy, and its recognition enables one to suspect sickle cell-hemoglobin C disease.

As first described in 1910 by Herrick,1 sickled red blood corpuscles in sickle cell anemia are thin, elongated, crescent-shaped and stained heavily. These red cells are pointed at each end and may assume "L," "V" and "S" shapes. In 1955, Diggs, Kraus, Morrison and Rudnicki2 reported that the smear of a patient with homozygous hemoglobin C disease showed intraerythrocytic crystals which were dark-hued, elongated, six-sided objects with blunt or pointed ends and this observation was substantiated by Wheby, Thorup and Leavell.3 To our knowledge, there has been no reference in the literature to a poikilocyte peculiar to hemoglobin S-C disease.

Morphology of Intraerythrocytic Crystals in Hemoglobin S-C Disease

A small number of erythrocytes in hemoglobin S-C disease contain a condensed hemoglobin crystal with parallel sides. This crystalline structure is dark, homogeneous and elongated and distorts the cell membrane (figs. 2, 3). The end of the protuberance tends to terminate as a pyramid but may be rounded (figs. 2, 3, 4). The base of the protruding structure may be flat, square or irregular (figs. 1–4).

Crystals are usually multiple in a given red cell (figs. 1, 4) and suggest the shape of a cluster of quartz crystals. Such protrusions inside the corpuscle may be parallel (figs. 3; 4E), may jut out at varying angles from a common matrix (figs. 2; 4B) or may be nonoriented in relation to each other (figs. 1; 4C). When multiple formations are present in a cell, they may vary in length and width from slender and rod-like to broad and stump-like and may be separated by light areas (fig. 1). Several structures in an erythrocyte may produce an overall contour that bends in a crescent shape or an "S" shape reminiscent of a sickled red cell (figs. 1; 2).

Rarely, there may be discerned within the red cell membrane a single, hexag-
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Fig. 1.—Painting of erythrocyte shapes in hemoglobin C-C, S-C and S-S diseases.

Fig. 2.—Painting of cell types found in a patient with hemoglobin S-C disease.
Fig. 3.—Photomicrograph of peripheral blood smear from patient with Hb S-C showing three distorted erythrocytes and numerous target cells. Red cell with one protuberance ending in blunt pyramid (left center). Erythrocyte with two elongated crystals of uneven length and width and with parallel sides (top center). Poikilocyte with two condensed hemoglobin masses protruding in different directions (bottom, right).

onial, intensely stained structure with little residual hemoglobin in the remainder of the cell. Another infrequent variation is an erythrocyte with a triangular-shaped area of condensed hemoglobin in one part of the cell and other areas with lesser amounts of hemoglobin (fig. 4A).

The morphologic abnormalities described are presumptive evidence of sickle cell-hemoglobin C disease. If the appearance of the erythrocytes is suggestive, electrophoretic confirmation should be obtained. Upon preliminary smear examinations from patients at the time of admission, before hematologic diagnosis was established, various members of our staff have observed intraerythrocytic crystals of the type found in hemoglobin S-C and have suggested the diagnosis (prior to electrophoresis). In each case of a tentative diagnosis based on erythrocyte morphology, hemoglobin S-C disease has been confirmed by paper-strip electrophoresis. Inability to demonstrate the morphologic variant does not exclude the possibility of hemoglobin S-C disease.

Intraerythrocytic hemoglobin crystals were not observed on the blood smears of 82 patients with sickle cell anemia, 21 patients with hemoglobin A-S disease and 2 patients with sickle cell-thalassemia disease.

Incidence of Various Erythrocyte Shapes

Wright's stained blood smears from 60 patients having a moist preparation positive for sickling and an electrophoretic mobility pattern of hemoglobin S-C, as determined by paper-strip electrophoresis, were examined under oil im-
Fig. 4.—Photomicrograph of misshapen erythrocytes in blood smears of patients with Hb S-C. A. Red cell with dark, blunt protuberance (top right). Erythrocyte containing a three-sided structure having blunt tip and chromophobic separation of hemoglobin (bottom left). B. Crescent-shaped erythrocyte with three deep-hued crystals (center left). Two bizarre condensed hemoglobin masses in a red blood cell (bottom right). C. Elongated red corpuscle with concentration of hemoglobin at each end and hemoglobin-free central area (center). D. Red cell with two parallel, dark, crystal-like structures of different lengths, terminating in a pyramid tip (center). E. Erythrocyte with two parallel formations separated by a clear area (top right). Red cell with one elongated mass (bottom left). F. Erythrocyte with densely stained hemoglobin masses (top right). Red cell with one dark, elongated, rounded bulge and one small triangular hemoglobin mass, leaving two areas relatively free of hemoglobin (bottom left).
Table 1.—Distribution of Erythrocyte Shapes in Blood Smears from 60 Patients with Hemoglobin S-C Disease

<table>
<thead>
<tr>
<th>Red Cell Shape</th>
<th>Average Number Per 1000 Red Cells</th>
<th>Range Per 1000 Red Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elongated cells with point at each end (sickled red cells)</td>
<td>0.6</td>
<td>0–4</td>
</tr>
<tr>
<td>Intraerythrocytic hemoglobin crystals</td>
<td>3.2</td>
<td>0–24</td>
</tr>
<tr>
<td>Target cells (bull’s eye)</td>
<td>199.1</td>
<td>18–617</td>
</tr>
<tr>
<td>Other poikilocytes (spherical, oval, folded, single-pointed and bizarre cells and cells with hemoglobin at opposite poles)</td>
<td>45.1</td>
<td>2–184</td>
</tr>
<tr>
<td>Normal red blood cells</td>
<td>752.0</td>
<td>219–958</td>
</tr>
</tbody>
</table>

The incidence of various erythrocyte shapes in 1000 to 3000 red blood cells was determined on each smear. Erythrocyte shapes were tabulated as follows: (1) elongated cells with a point at each end (sickled red cells); (2) cells with intraerythrocytic hemoglobin crystals; (3) target cells; (4) other poikilocytes (spherical, oval, folded, single-pointed and bizarre cells and cells with condensation of hemoglobin at opposite poles) and (5) normal red blood cells. Erythrocytes designated as target cells do not include those with slight irregularities in the inner surface or those with peninsular-like extensions, but only those erythrocytes with a “bull’s eye” area. The distribution of the number of cells according to shape is given in table 1. It is to be noted that the number of intraerythrocytic crystals of the hemoglobin S-C type (3.2/1000 red cells) is greater than the number of classical sickled red blood cells (0.6/1000 erythrocytes).

Forty-two patients (or 70 per cent) revealed varying numbers of intraerythrocytic crystals characteristic of sickle cell-hemoglobin C disease. No missshapen cells having the afore-mentioned morphology were encountered in a differential count of various shapes on smears from 18 cases (or 30 per cent). A frequency distribution curve showing the number of intraerythrocytic structures in 60 patients is given in figure 5 and reveals that 20 patients have less than 4 per 1000 red blood cells, 15 patients have from 4 to 12/1000, and 7 patients showed 12 to 24/1000 erythrocytes.

**SUMMARY**

On 70 per cent of the blood smears from 60 cases of electrophoretically proven sickle cell-hemoglobin C disease, there is observed a misshapen erythrocyte that contains condensed hemoglobin crystals which are dark-hued, homogeneous and elongated and which have parallel sides with one end terminating in a pyramid or rounded shape. A red blood corpuscle may have multiple protuberances at varying angles to each other. The incidence of intracellular hemoglobin crystals was found to be 0–24 per 1000 red blood cells with an average of 3.2/1000. Recognition of this unusual morphology is presumptive evidence of sickle cell-hemoglobin C and warrants examination by electrophoretic procedures.

**SUMMARIO IN INTERLINGUA**

In 70 pro cento del frottis sanguine ab 60 patientes con electrophoreticamente demonstrate morbo a cellulas falciforme con hemoglobina C, le autores
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Fig. 5.—Frequency distribution of number of intraerythrocytic structures in red cells in smears of patients with hemoglobin S-C disease.

ha observate malformate erythrocytos que contineva un condensate crystallo de hemoglobina, de color obscuer, de consistentia homogene, e de conformation elongate, con lateres parallel e un termino cappate de un structura pyramidal o ronde. Tal erythrocytos habeava a vices multiple protuberantias, positionate de manera que illos formava varie angulos le unes con le alteres. Le incidentia de intraerythrocytic crystallos de hemoglobina esseva inter 0 e 24 per 1000 cellulas, con un valor medie de 3,2. Le constatation de iste inusual morphologia es evidentia presumptive de morbo a cellulas falciforme con hemoglobina C e suggere le initiation de un studio electrophoretic.

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REFERENCES


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