Hematologic Observations on Patients with Sickle Cell Anemia Sustained at Normal Hemoglobin Levels by Multiple Transfusions

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Most of the knowledge concerning the lifespan of sickle cells has been derived from studies of the transfusion of sickle cells into normal recipients. The transfused sickle cells have been identified by the technics of differential agglutination or in vitro isotope tagging. However, there always exists some doubt as to the interpretation of the results so obtained. The circulatory environment in a normal recipient is not necessarily comparable to that in the sickle cell patient, and there is always the possibility that minor blood group incompatibilities will influence the rate of disappearance of the cells. Furthermore, it is not certain whether some damage may be done to sickle cells during the in vitro manipulations incident to these technics, particularly when isotope tagging is employed.

For these reasons it has been considered desirable to study the lifespan of sickle cells in the patient’s own circulation, omitting all in vitro manipulations. This has been attempted by an in vivo isotope technic employing N\textsuperscript{14}-labeled glycine. Although valuable information has been obtained by this method, interpretation of results is complicated during the initial days of the study by the simultaneous incorporation of the isotope into newly formed cells and its release due to red cell destruction.

One of the present authors (H. K.) undertook an investigation, which will be the subject of a subsequent report, to study the renal concentrating abnormality of sickle cell patients during a period of normal hemoglobin concentration sustained by frequent transfusions. It was realized that a unique opportunity was presented for the use of differential agglutination to study the behavior of the patient’s own cells in his own circulation in response to these unusual conditions. Because of the precision obtainable by the modified Ashby technic, greater accuracy could be expected than from reliance upon the sickling phenomenon as a means of identifying small numbers of the patient’s cells. It was hoped that

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The authors wish to thank Dr. Harvey A. Itano of the National Cancer Institute for determining the proportions of the S and F hemoglobin in the patients’ pretransfusion samples. We are also especially grateful to Dr. Marvin Schneiderman, of the National Cancer Institute, for his thoughtful statistical consideration of the sickle cell disappearance curves and his wise counsel in their interpretation. Our appreciation is also expressed to Drs. W. G. Workman and J. T. Tripp for their encouragement and support during the course of these studies.

Submitted Nov. 14, 1955; accepted for publication March 9, 1956.

the data obtained would contribute to a better understanding of the characteristics of sickle cells’ lifespan.

The present report concerns detailed hematologic studies on a type NN sickle cell patient who was sustained at normal hemoglobin levels for 4 months by means of repeated transfusions of fresh M-positive blood. Confirmatory observations on two additional patients are included. The value of this method for determining sickle cell lifespan will be discussed.

METHODS

Clinical Material: The admission data on the three patients are summarized in Table 1. Case 1, a ten year old female, had been transfused 50 or more times during her life for sickle cell anemia and sickle cell pains. Case 2, a one year old female, sister of Case 3, had one sickle cell crisis immediately prior to the present study evidenced by dactylitis and muscle pains. Case 3, a two year old male, had two attacks of sickle cell crisis, characterized by dactylitis and muscle pains, before the present study was undertaken. Symptoms of sickle cell disease, including anemia and icteric sclerae, were noted after the first year of life. All three patients tolerated the transfusions well. Case 1 gained 3 Kg. and clinically was markedly improved following the transfusions.

Donor Blood: All blood was from normal type MN or MM donors and was drawn into acid citrate dextrose (A.C.D.) preservative solution. In the management of Case 1, blood was drawn from the donors less than 6 hours before each transfusion. Cases 2 and 3 were transfused with blood stored between 24 and 48 hours at 4-6 C. All transfusions were administered as “concentrated cells,” following aspiration of approximately 75% of the plasma immediately prior to administration.

Red Cell Survival Studies: The pre-transfusion sickle cell count was determined employing calibrated 0.1 and 20.0 ml pipets to make a 1/201 dilution of the patient’s oxalated blood in saline containing 2 per cent of the patient’s own plasma. At least 4000 cells were counted. Subsequent sickle cell counts were obtained employing Dacie and Mollison’s modification of the Ashby method. Differential agglutination was performed by the addition of powdered anti-M globulin (Lederle Laboratories) to carefully prepared dilutions of the patient’s oxalated whole blood. At the beginning and at the end of the study, when a large number of sickle cells were present, a 1/200 dilution was employed; in the presence of fewer sickle cells,
Fig. 1.—Case 1. The upper diagram is a composite picture of the entire study. The lower diagram illustrates the latter part of the study on an expanded scale, to demonstrate the stabilization of the sickle cell count at the low level of 8000 cells/cu. mm., confirmed on 3 successive weeks. V.B. In figures 1, 3 and 5 the upper dotted line indicates the mean expected normal hemoglobin according to sex and age as described by Wintrobe. The hatched area represents the normal range (mean ± 2.0 Gm.%) a 1/100 and finally a 1/50 dilution was used. The anti-M “blank” count was assumed to be 5000 cells/cu. mm. throughout each study. At least 2000 unagglutinated cells were counted in determining all sickle cell counts above 50,000 cells/cu. mm. Eight hundred to 1300 cells were counted for results between 15-50,000 cells/cu. mm. The three lowest counts (Case 1) which revealed 8000 sickle cells/cu. mm. were based on counts of 400-800 cells.

* Extensive experience with Lederle’s anti-M powder on the part of one of the authors (H. C.) has shown “blank” counts to fall within the range of 2000-8000 unagglutinated cells/cu. mm.
Fig. 2.—Case 1. The differential agglutination data fall along a straight line on a semi-log plot. Most of the early points lie to the left of the line and the later points to the right of the line.

Blood Volumes (Case 1) were based on measurements of plasma volume employing Evans Blue dye. In the calculation of total blood volume the observed venous hematocrit was corrected for trapped plasma and for the body/venous hematocrit ratio, as detailed by Chaplin, et al.6 Reticulocytes were counted by the method of Brecher and Schneiderman.7 Normal values by this method fall within the range 0.8-1.2/100 R.B.C. Total bilirubin was determined by a modification of the method of Malloy and Evelyn.8 Plasma hemoglobin was measured by Crosby’s modification of the benzidine method. Sickle and fetal hemoglobin were determined by moving boundary electrophoresis, as described by Wells and Itano.9 Serum iron was determined by a modification of the method of Ramsay.10 Radioactive iron (Fe59) utilization studies were as described by Huff et al.11 Sickling preparations (fig. 6) were made employing sodium bisulfite, as described by Daland and Castle.12

Results

Case 1

Disappearance of Sickle Cells from the Patient's Circulation: The upper diagram in figure 1 summarizes the entire study. It demonstrates a rapid curvilinear diminution in the number of the sickle cells, with elimination of 90 per cent of the original number of cells during the first 40 days. The lower diagram illustrates the lower segment of the curve, plotted on a larger scale. Here it is evident that the remaining cells were eliminated over the ensuing 50 days, and that a stable level was finally achieved, with counts of 8000 sickle cells/cu.mm. being obtained on three successive weekly determinations. This very low count indicated a marked depression of marrow output.

When the differential agglutination data are plotted on a semi-log scale (fig. 2), the points fall along a straight line. If these data were considered to represent
the lifespan of a representative population of the patient's cells, they would indicate equal susceptibility of all cells to a process of random destruction, with an approximate half-time of 11 days. The difficulty of interpreting the differential agglutination data will be reviewed in the discussion.

Reticulocytes: The upper diagram in figure 3 outlines the reticulocyte data throughout the study. The reticulocytes fell from an initial pretransfusion value of 22/100 R.B.C., to 19/100 R.B.C. on the second day, 7.4/100 R.B.C. on the fourth day, 2/100 R.B.C. on the seventh day, and 0.9/100 R.B.C. on the tenth day. During the ensuing 7 weeks, when the mean hemoglobin level was slightly below the expected mean normal value for a child of patient's sex and age, the

Fig. 3.—Case 1. The upper diagram illustrates the reticulocyte data throughout the entire study. The dark hatched area represents the normal range for the method employed (0.8-1.2/100 R.B.C.). The lower diagram illustrates on an expanded time scale the response of reticulocytes and sickle cells to anemia following cessation of transfusions.
mean reticulocyte count was 0.84/100 R.B.C.; during the next 7 week period, when the mean hemoglobin level was slightly above normal, the mean reticulocyte count was 0.38/100 R.B.C. At no time was the count less than 0.2/100 R.B.C.

Response to Developing Anemia: The lower diagram in figure 3 indicates that a demonstrable rise in reticulocytes and sickle cells occurred when the hemoglobin had fallen to approximately 11.0 Gm. per cent.

Bone Marrow: The pretransfusion marrow was hypercellular, with marked erythroid hyperplasia and many very young red cell forms. Repeat examinations at intervals throughout the ensuing 100 days showed regression to an essentially normal picture. Some observers considered that minimal erythrocytic hypoplasia was present during the last weeks of sustained normal hemoglobin concentrations.

Radioactive Iron Studies: Prior to transfusions, the serum iron was 58 μg/100 ml. The iron turnover rate was 2.45 mg./Kg./day, which is approximately 5 times normal. On the 100th day of the study, the serum iron had risen to 205 μg./100 ml. and the iron turnover rate was just above the upper limit of normal. Figure 4 illustrates the utilization of intravenously administered Fe⁵⁹ before transfusions and again after 100 days of sustained normal hemoglobin concentration. The upper curve is typical for a severe hemolytic anemia, the low peak and the decline apparent after the third day indicating that a proportion of newly formed cells is undergoing very early destruction. The lower curve is consistent with marked depression of red cell production. It is interesting that an early decline is again observed, indicating very early destruction of some of the newly formed cells.
Fig. 5.—Case 2 and 3. See legends to figures 1, 2 and 3. Note especially the simple exponential curves. Also, the reticulocyte response to mild anemia at the end of each study.

Blood Volume: Based on Evans Blue plasma volume measurements, the pre-transfusion total blood volume was 2680 ml., plasma volume 2190 ml., and red cell volume 490 ml. On the 94th day of the study, when the hematologic values were all normal, and only 8000 sickle cells/cu.mm. could be demonstrated, the total blood volume was slightly lower (2580 ml.), with the red cell volume increased to 1050 ml. and compensatory shrinkage of the plasma volume to 1530 ml. During the interval between the two observations the patient had gained 3 Kg.

Other Hematologic Observations: The elevated pretransfusion values for total bilirubin and plasma hemoglobin declined to normal levels during the transfusion period.

Cases 2 and 3

Figure 5 summarizes the sickle cell studies on the two siblings. Curvilinear diminutions in the numbers of the patients' cells were again obtained, which fell along straight lines on a semi-log plot. The half-times of 25 and 23 days are more than double that observed in Case 1. It is not certain whether this is in some way related to the presence of only 77–78 per cent S-hemoglobin in these very young patients, as compared to 95 per cent S-hemoglobin in Case 1.

Again, the pretransfusion reticulocyte counts were considerably increased
Fig. 6.—Case 3. Comparison of sickling characteristics of patient's cells prior to transfusion (A), and on the 90th day of the study (B), recovered by differential agglutination. Note marked similarity between the two preparations. Numerous platelets are present in the background in A. Daland and Castle's sodium bisulfite method. Photographs are of unstained wet preparations under the phase contrast microscope. Magnification 1000 X.
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above normal. During the period of sustained high normal or supernormal hemoglobin concentration, the reticulocytes in Case 2 were 0.1–0.3 per cent, and in Case 3, 0.0–0.1 per cent. In both patients a rise in reticulocytes was demonstrable as the hemoglobin declined to approximately 11.0 Gm. per cent following cessation of transfusions.

The patients' own cells, recovered by differential agglutination on the 90th day of the study, demonstrated sickling indistinguishable from that observed in the pretransfusion sample (see figure 6).

DISCUSSION

The present studies have demonstrated several consistent findings. When each of the sickle cell patients was transfused to a normal hemoglobin level, there was a prompt decline in the reticulocyte count, reaching normal values in 5–7 days. This is in agreement with the observations reported originally by Singer and more recently by Donegan. When the hemoglobin level was maintained just at or slightly below the mean normal value, the reticulocyte count stabilized at the lower level of normal range. Maintenance of the hemoglobin at the upper limits of normal resulted in suppression of the number of reticulocytes below the normal range but in only one of the three patients (Case 3) were any preparations obtained in which no reticulocytes could be seen. Following cessation of transfusions, a clearly defined reticulocyte response was evident in each patient when the hemoglobin level fell to approximately 11.0 Gm. per cent. The latter observation indicates that the marrow of patients with this disease is capable of responding to the stimulus of mild anemia, and confirms the similar findings by Donegan.

Several difficulties are encountered in attempting to define the sickle cells' lifespan on the basis of the differential agglutination data shown in figures 1, 2 and 5. Other authors studying the lifespan of sickle cells transfused into normal recipients, have described curves of elimination which departed significantly from simple exponentials. This has been ascribed to the presence of populations of sickle cells of differing susceptibilities to destruction. By contrast, the data from Cases 1, 2 and 3 in this report could be fitted by a simple exponential curve. A likely explanation for the apparent discrepancy between these results and those reported by the other authors lies in the continuing, albeit diminishing, output of considerable numbers of new sickle cells throughout the initial 5–7 days of the present studies.

It is not known to what extent the patients' reticulocyte counts on each succeeding day represented new cells formed since the preceding day. Little is known about the persistence in the peripheral blood of the reticulocyte form in the hyperplastic response to severe hemolytic anemia or even in the normal subject. Lacking this knowledge, it is impossible accurately to predict the effect upon the differential agglutination counts of the sickle cells newly formed during the early days of these studies. In any event, the addition of newly formed sickle cells should have produced an upward distortion of the differential agglutination curves. Failure to observe this alteration in the shape of the curves implies the existence of a proportion of sickle cells having a lifespan considerably shorter than the apparently uniform population of cells present after the first week of
the study. The elimination of the short-lived cells has apparently been balanced by the production of new cells during the same period.

Thus, in the light of the reticulocyte data, the simple exponential curves in figures 2 and 5 cannot be considered true lifespan curves. Paradoxically, they actually constitute indirect evidence in favor of the existence of at least two populations of sickle cells of different susceptibilities to destruction. The half-times obtained from the simple exponential curves must thus be considered misleadingly long with respect to each patient's composite sickle cell population. That the half-times are indeed falsely long is further suggested by the high levels of the pretransfusion reticulocyte counts. For example, employing the 11 day half-time obtained for Case 1, it can be calculated that in order to maintain the patient's pretransfusion red cell count, red cell production would need to be increased by three to four times normal. However, the pretransfusion reticulocyte count was 22/100 R.B.C. as compared to the normal value of 1/100 R.B.C. Similar discrepancies were observed in Cases 2 and 3.

An apparent excess of reticulocytes can be accounted for by (a) the presence of a proportion of cells which are very rapidly destroyed, (b) a prolongation of the time during which red cells remain in the reticulocyte form in the peripheral circulation, or (c) a combination of both factors. With regard to the second alternative (b) it can be calculated that if in Case 1 the red cells newly released into the circulation remained in the reticulocyte form for 3-4 days, a half-time of 11 days for the total red cell population would result in the observed pretransfusion steady state of 2.5 million sickle cells per cu.mm. of blood. Alternatively, it would be possible to achieve stability according to (c) by postulating, for example, a two day existence as reticulocytes and a proportion of cells showing a lifespan with a half-time shorter than 11 days. It is apparent that an infinite number of combinations could be hypothesized.

The present data do not define the relative importance of the various mechanisms for maintaining the steady hematologic state of patients with sickle cell anemia. However, the data do support the concept advanced by previous authors of a proportion of red cells which are especially rapidly destroyed. Singer7 has reported a patient in whom 50 per cent of the sickle cells were destroyed during the first 3 days of observation with a slower destruction of the remaining cells over the ensuing 25 days. The failure of the present authors to observe an upward distortion of the differential agglutination curves has already been mentioned. Additional evidence of multiple cell populations may be derived from the pretransfusion radioactive iron utilization curve (fig. 4). The slope of the curve between days 4 and 8 is considerably steeper than can be accounted for by a process of random destruction with an 11 day half-time. In fact, the iron data strongly support the existence of a proportion of cells with a distinctly shorter half-time. It is possible that many of the cells with the very short lifespan are destroyed while still in the reticulocyte phase.

Since the present differential agglutination data do not permit quantitative evaluation of red cell destruction during the important first few days of observation, it may be concluded that the design of these experiments does not constitute the method of choice for defining the over-all pattern of the sickle cells' lifespan. The chief value of the study has been to provide quantitative measure-
ments of the numbers of sickle cells present in the circulation of patients sustained at normal hemoglobin levels by transfusion, and to illustrate the attendant depression of marrow output and its subsequent response to anemia.

Summary

(1) Three sickle cell patients were sustained at normal hemoglobin levels for 3–4 months by means of repeated transfusions of fresh blood.

(2) In response to transfusions, there was a decline in reticulocytes to normal levels during the first 5–7 days of observation. During periods in which the hemoglobin was maintained at high-normal or super-normal levels, the reticulocyte values were depressed below the normal range. A distinct reticulocyte response was observed when the hemoglobin declined to approximately 11.0 Gm. per cent following cessation of transfusions.

(3) Employing anti-M differential agglutination, a simple exponential decline was observed in the number of sickle cells in each patient’s circulation during the period of sustained normal hemoglobin concentration.

(4) The continued production of new sickle cells during the first week of observation complicated the interpretation of the differential agglutination data, but provided indirect evidence for the presence of an especially short-lived proportion of the patient’s cells. Support for this concept was derived from the radioactive iron utilization studies performed in Case 1.

Summario in Interlingua

1. In tres patientes con morbo de cellulas falciforme, le hemoglobina esseva mantenite a nivellos normal durante periodos de 3 a 4 menses per medio de repetitive transfusiones de sanguine fresce.

2. In responsa al transfusiones il habeva un reduction a nivellos normal in le reticulocytos durante le prime 5 o 7 dies de observation. Durante periodos in que le hemoglobina esseva mantenite a nivellos alte-normal o supra-normal, le valores del reticulocytos esseva deprimite infra le nivello normal. Un distincte responsa del reticulocytos esseva observate quando le hemoglobina declinava a approximativemente 11,0 g pro cento post le cessation de transfusiones.

3. Quando le agglutination differential anti-M esseva empleate, un declino exponential simple esseva observate in le numeros de cellulas falciforme in le circulation de omne patiente durante le periodo de sustenite concentrationes de hemoglobina normal.

4. Le production continue de nove cellulas falciforme durante le prime septimana del observation complicava le interpretation del datos de agglutination differential, sed illo provideva un prova indirecte del presentia de un typo ephemerissime inter le cellulas del patiente. Iste concepto esseva appoiate per studios in re le utilisation de ferro radioactive in le prime de iste tres casos.

REFERENCES


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