

Red Cell Life Span after Splenectomy in Hereditary Spherocytosis

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ABSTRACT Despite the persistence of spherocytosis after splenectomy in hereditary spherocytosis, it has usually been assumed that red cell life span returns completely to normal after this treatment. Diisopropyl fluorophosphate, DF³²P, a non-eluting red cell label, was given intravenously to 11 patients in five unrelated families 2–27 yr after splenectomy for typical hereditary spherocytosis. Hemoglobin ranged from 14.0 to 19.8 g/100 ml in this group and reticulocytes from 1.1 to 2.9%, showing the excellent clinical response to splenectomy. Loss of red cell radioactivity corrected for radiophosphorus decay was linear with time during the 60–70 days of the study. Red cell survival as indicated by this rate of loss was 96 ± 13 days (range 76–118 days), significantly less than the 123 ± 14 days observed with the same method in 12 persons with normal red cells ($P < 0.0005$). I conclude that splenectomy does not eliminate the decreased red cell survival in hereditary spherocytosis. The residual 22% decrease in red cell survival is clinically unimportant, but it must be considered in evaluation of biochemical differences observed in hereditary spherocytic red cells.

INTRODUCTION

The disappearance of anemia, reticulocytosis, and hyperbilirubinemia after splenectomy for hereditary spherocytosis has led many to assume that red cell life span then returns completely to normal. Spherocytosis persists, however, raising doubts about this assumption (1, 2). The few reported measurements of hereditary spherocytic

red cell survival after splenectomy were made with methods not fully satisfactory for this purpose (3–7). These methods were either studying the behavior of the patient's red cells in the circulation of someone else or using a cell label, the loss of which from the circulation was complicated by "elution." Diisopropyl fluorophosphate, DF³²P, is a cell label well suited for the detection of slightly decreased red cell survival, for once attached to red cell cholinesterase it is lost only when the cell is lost. It has the further advantage of attaching to the red cell in vivo, thereby avoiding any harm to the red cells from in vitro handling (8). Measuring red cell survival with DF³²P in 11 patients with hereditary spherocytosis, I have found that splenectomy, despite the subsequent disappearance of anemia, reticulocytosis, and hyperbilirubinemia, does not return red cell life span fully to normal.

METHODS

Hematocrit was determined by the microhematocrit, hemoglobin concentration as cyanmethemoglobin (Acuglobin, Ortho Diagnostics, Raritan, N. J.), red cell count by an electronic counter (Coulter Electronics, Inc., Hialeah, Fla.), and reticulocytes in a methylene blue stained cover slip smear (9). After splenectomy many red cells in addition to reticulocytes contain distinct basophilic inclusions (10), some of which can be confused with the remnants of reticulum seen in mature reticulocytes. The number of red cells other than reticulocytes containing more than three such inclusions is quite small. For this reason I classed as reticulocytes only red cells containing over three basophilic inclusions and did all counts myself without knowledge of which subject was being counted. Red cell morphology was evaluated in Wright's stained blood smears.

Circulating red cells were labeled by injecting 0.5 ml of DF³²P (approximately 100 μ c/0.23 mg of DFP) in

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sterile propylene glycol¹ over a 3–5 min period into an antecubital vein. 7-ml blood samples were then drawn into disodium ethylenediaminetetraacetate (EDTA) Vacutainers (Becton, Dickinson & Co., Rutherford, N. J.) at weekly intervals for 5–6 wk and, thereafter, every other week for the next 5–6 wk for measurement of red cell radioactivity. Sufficient red cells remained in each sample for the above hematological studies. Red cells were prepared for counting by centrifugation at 4°C. To obtain good separation of white cells and platelets from the red cells, centrifugation was begun at 45 *g*. After 10 min the force was increased to 400 *g* for 5 min and then to 700 *g* for the final 5 min. The plasma and buffy layer were carefully removed by suction. After subsequent two-fold resuspensions in 10 volumes of 0.15 M NaCl, the packed red cells were suspended in 1 volume of distilled water, and the hemoglobin of this final suspension was measured in duplicate. After 1–2 hr at 4°C, at which time hemolysis appeared complete, the final suspension was mixed well and 2.0 ml of it was placed in each of two $\frac{5}{16}$ " × 1" diameter stainless steel planchets. These were dried at room temperature for at least 72 hr before counting in a model SC-100 1" diameter end window gas flow GM counter (Tracerlab, Inc., Waltham, Mass.).² Initial samples were counted with a standard error of less than 2%, but as radioactivity declined in the final two or three samples this increased to 3%. Radioactivity of each sample was expressed as net cpm/

¹ Obtained from the Radiochemical Centre, Amersham, England, through Nuclear-Chicago Corp., Des Plaines, Ill.

² Saran-wrap (Dow Chemical Co., Midland, Mich.) was found to provide an inexpensive end window for this counter with little reduction in counting efficiency for the high energy ³²P radiation.

mg of Hb. Correction for radioactive decay and possible variations from day to day in counter efficiency was made by counting one of the original samples on each counting day and correcting all samples for the change in this sample's counting rate. The rate of decrease in red cell radioactivity was determined by calculation of linear regression, and statistical evaluation was done with Student's *t*-distribution for populations of equal variance (11).

RESULTS

The 11 subjects grouped by families are described in Table I. The relationships among the subjects within each of the five unrelated families are as follows: CG is the mother of PD, MG, and GH; DV is the sister of IG, and RG is their nephew; and BE is the father of JE. LG and LS are single representatives of the remaining two families. Typical hereditary spherocytosis was present in at least two consecutive generations of each family. Excellent clinical response to splenectomy had been obtained in all subjects and in all members of their families so treated. Increased numbers of spherocytes were present in all subjects, as well as the poikilocytosis and Howell-Jolly bodies anticipated after splenectomy. All subjects were in good health throughout the study. The hematological data shown in the table represent the mean from all seven or eight blood samples obtained from each subject. These values showed no significant change throughout the

TABLE I
Hematologic Values

Subject	Age	Sex	Yr since splenectomy	Hb	Reticulo-cytes	MCV	MCH	MCHC	Red cell life span
	<i>yr</i>			<i>g/100 ml</i>	<i>%</i>	μ^3	<i>pg</i>	<i>%</i>	<i>days</i>
CG	58	F	12	14.7	1.2	92.0	33.7	36.6	105
MG	27	F	7	14.1	2.4	89.8	32.2	35.9	104
PD	26	F	7	14.0	1.6	95.2	34.2	36.0	83
GH	22	F	12	14.6	1.1	81.6	29.2	35.8	109
DV	46	F	8	14.3	1.5	84.0	30.1	35.8	100
IG	52	M	2	17.8	1.7	87.1	31.8	36.5	118
RG	21	M	3	19.8	2.9	93.7	35.8	38.2	89
BE	65	M	27	16.0	1.5	94.3	35.2	37.3	96
JE	16	M	15	16.1	1.5	85.9	33.8	39.3	92
LG	27	M	6	18.3	2.0	90.3	35.4	39.2	80
LS	74	M	6	17.7	2.5	91.6	36.6	40.0	76
Normal ± 1 SD				16.4 ±1.1	1.0 ±0.4	92.3 ±3.4	32.4 ±1.1	35.1 ±0.9	123 ±14

study. For comparison, values obtained in six normal adults are shown. No correction has been made for the 3% greater plasma trapping in the hereditary spherocytic than in the normal control hematocrits (12). Only two subjects had mean red cell volumes (MCV) more than 2 sd below normal. Mean cellular hemoglobin content (MCH) and concentration (MCHC) were higher in the males than in the females, MCHC being normal in all females and above normal in all but one of the males. None of these subjects was anemic; and reticulocyte counts were above normal in only four of the 11. Bilirubin levels were less than 0.8 mg/100 ml in all but BE. He had values of 1.5 and 1.3 mg/100 ml, all but 0.1 and 0.2 mg/100 ml of which was free.

The rates of disappearance of radioactivity from the circulating blood are shown in Fig. 1 with subjects again grouped by families. Shown are the mean values for radioactivity per mg of Hb measured in duplicate planchets. With four exceptions in 84 determinations, duplicates were within $\pm 6.5\%$ of their mean value, and in 69 the duplicates were within $\pm 4\%$ of their means. Mean red cell life spans determined from the intercept of the regression line with the abscissa are indicated in the table. This varied from 76 to 118 days, showing little uniformity of values within individual families. The mean life span for red cells in all subjects was 96 ± 13 days, compared with a value of 123 ± 14 days in a group of 12 subjects with normal red cells studied in our

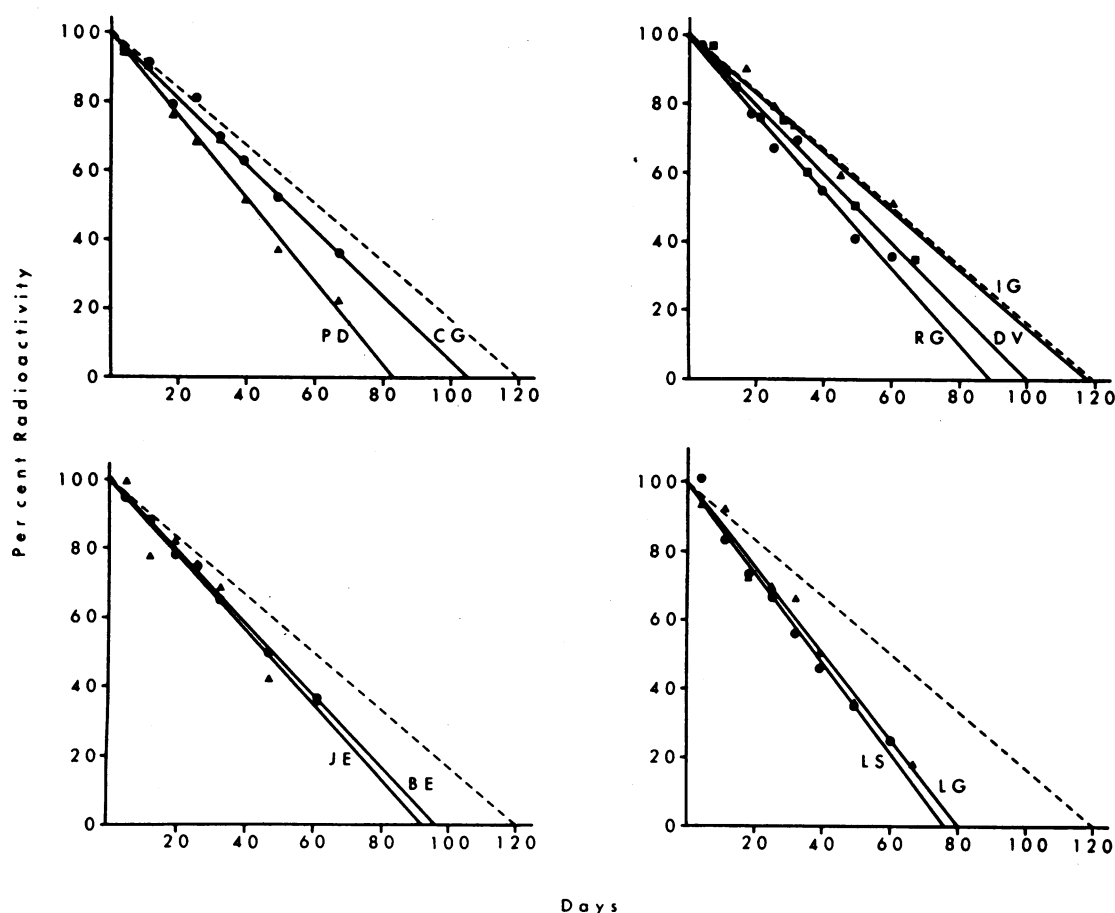


FIGURE 1 Red cell survival postsplenectomy in hereditary spherocytosis. The survival of red cells was measured with $DF^{32}P$ and the solid lines obtained by calculation of linear regression. The dashed lines show the slope for a normal red cell survival of 120 days. Circles are the values in CG, RG, BE, and LS. Triangles are the values in PD, IG, JE, and LG. Squares are the values in DV. Results for MG and GH, not shown, were very similar to those in CG.

laboratories.³ This difference is highly significant ($P < 0.0005$).

DISCUSSION

These results show that splenectomy does not completely correct red cell life span in this disease, confirming the suggestions of others (1, 2). Furthermore, unlike the usual curvilinear rate of red cell loss observed with $DF^{32}P$ before splenectomy (13), the rate of cell loss appears to be quite linear and therefore age-dependent (14) after splenectomy. Standard clinical laboratory tests failed to indicate this continuing increased red cell loss.

The few results others have reported for post-splenectomy red cell survival in hereditary spherocytosis agree with mine when one considers the less accurate methods they used. Using the Ashby technique of differential agglutination, Schrupf in an abstract reported the detection of hereditary spherocytic red cells up to 111 days after transfusion into one recipient (7). Emerson, Shen, Ham, Fleming, and Castle, using the Ashby method, showed greatly improved survival of hereditary spherocytic red cells in a splenectomized recipient, but the survival curve was still not completely normal (3). With ^{51}Cr labeling, Read, Wilson, and Gardner observed normal survival of hereditary spherocytic red cells in four patients during studies conducted 10 days after splenectomy (5). Schmidt and Keiderling, using the ^{51}Cr method for studies performed in six patients with hereditary spherocytosis 5–330 days after splenectomy found normal survival in four, two of the latter being studied within 10 days of splenectomy (6). Goldberg, Hutchison, and MacDonald observed a normal ^{51}Cr -labeled red cell half-life in one patient 3 months after splenectomy for hereditary spherocytosis (4). No reports of red cell survival measured with $DF^{32}P$ after splenectomy have been found.

If red cell survival returns to normal after splenectomy, the survival of randomly labeled red cells in the period immediately after splenectomy should be longer than normal because of the youthful bias in cell age at that time. This bias results from the erythropoietic response to the greatly increased red cell loss present in these

patients up to the time of splenectomy. With this consideration in mind, only two or three of the above 11 patients studied with ^{51}Cr had normal red cell survival after splenectomy; and because of possible minor variations in elution rate for ^{51}Cr , we cannot even be sure these two or three were truly normal. Interpretation of results obtained with the Ashby method is uncertain because red cell survival had to be measured in someone other than the patient.

The measurements of red cell survival I report avoid these causes of inaccuracy. Adequate time had elapsed since splenectomy for resumption of the new steady state in which there was an equal distribution of red cells of different ages; and a noneluting red cell label was used to follow the subject's red cell survival in the subject himself. Furthermore this label was applied in vivo with no handling whatever of the labeled red cells. Radioactive phosphorus does have a short half-life of 14 days compared with the normal red cell half-life of 60 days in the human, but with a safe dose of $100 \mu c$ (15), disappearance of labeled cells could be followed accurately for 60 days. This was long enough to establish the linearity and rate of red cell loss in each subject.

A normal control group of splenectomized subjects was not available for comparison with the splenectomized patients in this study. Studies by others have failed, however, to demonstrate that splenectomy alters in any significant way the survival of red cells in the normal human adult (3, 16). Comparison of results in the patients with results in a group of nonsplenectomized subjects with normal red cells is therefore reasonable. Such a comparison shows that mean red cell life span in hereditary spherocytosis after splenectomy was still below normal by 22%. This mild decrease in red cell survival was not reflected by an elevation of free bilirubin. The elevated free bilirubin in BE must be explained by unknown factors since five others with shorter red cell survivals than his had normal levels. The reticulocyte counts did show a partial correspondence to the amount of compensatory erythropoiesis known to be present in these subjects, but counts more than 2 sd above the normal mean occurred in only four. The highest reticulocyte count was 2.9%, a value in itself not unusual after splenectomy for any reason (17). One therefore cannot rely on normal

³ The data in normal subjects were obtained by Dr. Roger Hamstra.

levels of free bilirubin or reticulocytes to exclude persistent hemolysis and consequent unusual red cell age distribution after splenectomy for hereditary spherocytosis.

Cell size and lipid content as well as various enzyme activities change with red cell age (18, 19). A decrease of 22% in mean cell age such as was found in these subjects will produce significant changes in the rate of glycolysis as well as in the activity of such enzymes as hexokinase and aldolase in normal red cells (20). One cannot therefore compare such values in red cells from patients with hereditary spherocytosis directly with the same values in normal red cells even after splenectomy has eliminated clinical evidence of hemolysis.

Hereditary spherocytosis shows rather little uniformity of expression even within the same family, for relatives of a patient with rather severe hemolysis may have quite mild hemolysis. The post-splenectomy red cell survival data presented here show this same variability, with a range from 83 to 109 days in one family and from 89 to 118 days in another. Nevertheless, the failure of splenectomy to correct red cell life span in this disease was found in all five unrelated families, which strongly suggests that this observation is not unique to certain families with hereditary spherocytosis but applies to all families with the typical features of this disease.

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