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Research Article

RADIOACTIVITY OVER THE SPLEEN AND LIVER FOLLOWING THE TRANSFUSION OF CHROMIUM^{51}-LABELLED ERYTHROCYTES IN HEMOLYTIC ANEMIA[1]

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The beneficial effect of splenectomy in hereditary spherocytosis is well recognized. Selective retention of spherocytes in the spleens of patients with congenital hemolytic anemia was reported by Emerson, Shen, and Castle in 1946 (1). Subsequently, spherocyte "trapping" was demonstrated in spleens removed from patients without this disorder (2, 3). In addition, these abnormal red cells in the spleen have greater osmotic fragility than those in the peripheral blood (1). Ham and Castle first postulated that the spleen increased the osmotic fragility of red cells by intravascular stasis in a manner similar to that produced by sterile incubation of blood (4–7). Recently it has been suggested that the spleen "conditions" spherocytes for early osmotic hemolysis or increases their susceptibility to destruction by the mechanical forces of the circulation (8, 9). Although exact mechanisms of spherocyte destruction remain obscure, splenectomy in congenital hemolytic anemia alleviates the hemolytic process.

The role of the spleen in red cell destruction in acquired hemolytic anemia is less thoroughly understood. Splenic hemosiderosis, erythrophagocytosis, and congestion with red cells suggest the importance of red cell sequestration by this organ in the pathogenesis of many of the acquired hemolytic anemias. The first good response to splenectomy was reported by Micheli in 1911 (10). This operation has since been performed with unpredictable results in diseases having little in common other than hemolytic anemia. Criteria for predicting a favorable result from splenectomy in acquired, or autoimmune, hemolytic anemia have not been provided by conventional laboratory aids.

Determination of relative organ distribution of radioactive erythrocytes is feasible by external scintillation counting (11). Jandl, Greenberg, Yonemoto, and Castle have reported a method for determining organ sites of red cell sequestration using Cr^{51}-labelled erythrocytes (12, 13). They reported splenectomy was beneficial in one patient who had splenic accumulation of labelled red cells.

A method of determining organ localization of Cr^{51}-labelled red cells utilizing the ratio spleen: liver radioactivity as estimated by external scintillation counting was reported from this laboratory in 1955 (14). It was assumed that a high splenic uptake of radioactivity concomitant with an increased rate of Cr^{51} red cell disappearance from the peripheral blood was evidence for abnormal red cell accumulation in the spleen. The present report of red cell survivals and organ counting data was derived from 27 labelled red cell infusions. Splenic uptake of radioactivity in hemolytic anemia was correlated with response to splenectomy.

MATERIALS AND METHODS

Conventional laboratory data

In patients suspected of hemolytic anemia, determinations of hemoglobin, reticulocytes, hematocrit, red blood cell count, red cell fragilities (15), and serum bilirubin (16, 17) were performed by established techniques. The method of Dacie was used for the determination of autohemolysis of red cells (18). Erythroid: myeloid ratios were computed after counting 1,000 nucleated cells in the bone marrow smear. Average fecal urobilinogen in a four-day stool collection was determined by the method of Watson (19), using a Coleman junior colorimeter. One stool urobilinogen determination was semiquantitative (20). When these data showed evidence for hemolytic

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TABLE I

Initial evidence for hemolytic anemia in patients studied by Cr^{51} red cell survival and organ counting

Subject	Family history	Spleen size (*cm.* below costal margin)	Reticulocytes (%)	Hemoglobin (*Gm./100 ml.*)	Hematocrit (%)	No. red cells (*$\times 10^6$/mm.^{3}*)	Erythroid: Myeloid ratio	Mean osmotic fragility of red cells (% saline at 50% hemolysis)* Direct	Mean osmotic fragility of red cells (% saline at 50% hemolysis)* Incubated	48-hr. autohemolysis† (%)	Mechanical fragility‡ (%)	Indirect serum bilirubin (*mg./100 ml.*)	Fecal urobilinogen (*mg./24 hr.*)	Final diagnosis
N. H.	Present	0	7.1	13.0	36.5			0.44	0.58	8.0	9.4	1.1	154 204	Congenital hemolytic anemia
V. H.	Present	0	6.0	13.2	37.0	3.92		0.46	0.61		12.0	1.7	482	Congenital hemolytic anemia
E. Z.	Present	18	9.1	13.4	37.0	4.56		0.46	0.59	21.0	13.0	2.0	1,629	Congenital hemolytic anemia
L. R.	Absent	9	12.4	9.2				0.45	0.59	14.0	30.0	2.5	1,800	Congenital hemolytic anemia
A. K.	Absent	2	21.0	6.6	24.0	2.16	9.7	0.46	0.52	31.0	4.6	2.3	712 693	Paroxysmal nocturnal hemoglobinuria
P. P.	Absent	6	5.1	12.6	35.0	3.96	1.7	0.44	0.52	6.3	7.2	2.2	283	Acquired hemolytic anemia (idiopathic)
L. Y.	Absent	10	37.5	4.2	12.0	1.30	2.3	0.49	0.48	4.6	20.0	2.0	§	Acquired hemolytic anemia; chronic lymphocytic leukemia
L. H.	Absent	8	8.8	10.4	33.0	3.68	1.0			2.6	3.7	1.6	177	Acquired hemolytic anemia (idiopathic)
G. S.	Absent	27	4.7	11.7	38.5	4.20		0.52	0.32	2.7	5.1	1.4	431 400	Acquired hemolytic anemia; myelofibrosis
L. W.	Absent	3	16.4	5.1	16.5	1.83	1.4	0.37	0.51	1.4	3.9	2.0	728	Acquired hemolytic anemia; L.E.D.‖
W. M.	Absent	14	13.5	11.4	33.0	2.01	0.6					2.1	537 1,361 919	Acquired hemolytic anemia; chronic lymphocytic leukemia
N. A.	Absent	7	8.4	7.1	22.0	2.35	0.6	0.46	0.36	6.4	5.5	0.6	324 432	Acquired hemolytic anemia; myelofibrosis; acute leukemia
J. L.	Absent	11		9.6	27.0	3.34	1.8	0.42	0.54	5.6	6.7	1.3	1,578 1,200	Acquired hemolytic anemia (idiopathic)
J. T.	Absent	2	32.5	8.8	26.0		1.1	0.47			9.6	2.5	542	Acquired hemolytic anemia (idiopathic)

* Mean normal value in this laboratory: Direct, 0.43 ± 0.02 (1 S.D.); incubated, 0.52 ± 0.03 (1 S.D.).
† Mean normal value in this laboratory: 1.1 ± 0.5 per cent (1 S.D.).
‡ Mean normal value in this laboratory: 4.5 ± 1.3 per cent (1 S.D.).
§ Three thousand thirty Ehrlich units per 100 Gm. stool (markedly elevated).
‖ L.E.D. = Disseminated lupus erythematosus.

anemia, Cr^{51} red cell survival studies with organ counting were performed.

Technique of Cr^{51} red cell survival studies

A. Labelling and administration of erythrocytes. Sterile technique was used throughout. Seventy-five to 90 ml. of venous blood were collected by gravity flow into a 100-ml. siliconized bottle containing 10 ml. acid-citrate-dextrose[4] solution. Seventy-five to 100 microcuries Cr^{51} (specific activity range, 0.7 to 26.0 mc. per mg.) as sodium chromate diluted in 0.85 per cent NaCl were added at once. The mixture was incubated at 37°C. for 45 minutes with frequent gentle agitation. Following incubation, 200 mg. ascorbic acid were added to prevent further uptake of Cr^{51} by red cells (21). A single washing procedure, using cold sterile saline, was done as follows: The mixture was centrifuged and the supernatant plasma was removed. The red cells were washed once with saline and finally diluted with saline to a volume approximating that of the whole blood withdrawn. These infusions were designated "washed." The washing procedure removed Cr^{51} which was not bound to erythrocytes so that more than 97 per cent of Cr^{51} infused was red-cell bound. In some instances the blood was not washed and such infusions were designated "non-washed." Cr^{51}-labelled red cells were given intravenously through an 18-gauge needle by gravity drip from the collecting bottle or in 50-ml. volumes by calibrated syringe.

B. Determination of Cr^{51} red cell survival. All samples of peripheral blood were collected in balanced oxalate (15) in approximately 6-ml. quantities. Packed cell volumes were determined. Exactly 4 ml. were pipetted into counting tubes calibrated at 4 ml. Plasma was re-

[4] Abbott Laboratories, North Chicago, Illinois. The ACD solution had the following composition per 100 ml.: Dextrose, USP 132 mg.; sodium citrate, USP 250 mg.; citric acid, USP 80 mg.

TABLE II

Donors and recipients of Cr^{51}-labelled red cells

Study No.	Donor of Cr^{51} red cells		Recipient of Cr^{51} red cells				
	Subject	Diagnosis	Subject	Diagnosis	Spleen size (*cm.* below costal margin)	Cr^{51} red-cell half-life (*days*)	Average ratio spleen Cr^{51}: liver Cr^{51}
1	D. D.	Normal	D. D.	Normal	0	34	1.6
2	D. D.	Normal	J. H.	Normal	0	38	1.8
3	T. M.	Normal	T. M.	Normal	0	42	1.1
4	M. M.	Normal	M. M.	Normal	0	30	1.3
5	D. H.	Normal	D. H.	Normal	0	32	0.9
6	D. H.	Normal	L. R.	Congenital hemolytic anemia	9	30	1.9
7	J. K.	Polycythemia	J. K.	Polycythemia	8	27	2.8
8	L. K.	Myelofibrosis	L. K.	Myelofibrosis	17	27	1.8
9	L. R.	Congenital hemolytic anemia	D. H.	Normal	0	6	3.4
10	L. R.	Congenital hemolytic anemia	L. R.	Congenital hemolytic anemia	9	10	3.4
11		Normal	E. Z.	Congenital hemolytic anemia	18	22	2.4
12	E. Z.	Congenital hemolytic anemia	E. Z.	Congenital hemolytic anemia	18	11	3.3
13	V. H.	Congenital hemolytic anemia	V. H.	Congenital hemolytic anemia	0	11	1.4
14	V. H.	Congenital hemolytic anemia	V. H.	Congenital hemolytic anemia	0	29	(post-splenectomy)
15	N. H.	Congenital hemolytic anemia	N. H.	Congenital hemolytic anemia	0	17	2.4

TABLE II—*Continued*

Study No.	Donor of Cr^{51} red cells		Recipient of Cr^{51} red cells				
	Subject	Diagnosis	Subject	Diagnosis	Spleen size (*cm.* below costal margin)	Cr^{51} red-cell half-life (*days*)	Average ratio spleen Cr^{51}: liver Cr^{51}
16		Normal	A. K.	Paroxysmal nocturnal hemoglobinuria	2	30	1.8
17	A. K.	Paroxysmal nocturnal hemoglobinuria	A. K.	Paroxysmal nocturnal hemoglobinuria	2	6	1.5
18	L. H.	Idiopathic hemolytic anemia	L. H.	Idiopathic hemolytic anemia	8	16	3.0
19		Normal	P. P.	Idiopathic hemolytic anemia	6	17	4.2
20		Normal	N. A.	Myelofibrosis	7	12	3.4
21		Normal	W. M.	Chronic lymphocytic leukemia	14	6	3.5
22		Normal	L. W.	L.E.D.*	3	19	2.0
23	J. T.	Idiopathic hemolytic anemia	J. T.	Idiopathic hemolytic anemia	2	15	1.9
24	G. S.	Myelofibrosis	G. S.	Myelofibrosis	27	22	2.0
25		Normal	J. L.	Idiopathic hemolytic anemia	6–11†	30	2.4
26	J. L.	Idiopathic hemolytic anemia	J. L.	Idiopathic hemolytic anemia	11	8	3.4
27		Normal	L. Y.	Chronic lymphocytic leukemia	10	<1	6.4

* L.E.D. = Disseminated lupus erythematosus.

† The spleen enlarged by clinical estimate from 6 cm. below costal margin to 11 cm. below costal margin during the study.

moved after centrifugation and the red cells were hemolysed by the addition of distilled water to the 4-ml. mark. Radioactivity was measured in a well-type scintillation counter.

Blood samples were drawn in most instances at frequent intervals for 24 hours following infusion. The sample showing the most radioactivity during this period was chosen the "100 per cent sample of Red Cell Cr^{51}." In some, a single 2- or 24-hour sample served as the "100 per cent sample." Subsequent samples were collected at intervals of 1 to 14 days depending upon the rate of disappearance of radioactivity from the peripheral blood. Corrections for Cr^{51} decay were obviated by counting both the "100 per cent sample" and the interval samples on the same day. Corrections for variation in plasma volumes based on hematocrit determinations were not used, and no corrections were made for the elution of Cr^{51} from red cells. The per cent survival of Cr^{51} red cells at any time after infusion was given by:

$$\frac{\text{CPM per 4-ml. interval sample}}{\text{CPM per 4-ml. "100 per cent sample"}} \times 100$$

where CPM = net radioactivity in counts per minute. Studies were usually carried to 10 per cent survival of red cell Cr^{51} except when splenectomy terminated the study sooner.

External organ counting

Organ counting was done with an uncollimated scintillation probe at the time of sample collection for Cr^{51} red cell survival. With the patient supine, the approximate centers of both anterior and lateral skin projections of the liver and lateral projection only of the spleen were estimated by physical examination. The detector was

placed on the skin at these sites so the face of the crystal was approximately parallel to the body surface. When multiple determinations of radioactivity were made from a single organ at the same time, the results were averaged. A minimum of 5,000 counts over each organ site was obtained. Radioactivity values over either organ less than 1.5 times background were not used. The ratio net spleen counts: net liver counts was determined.

Selection of subjects for Cr^{51} studies

Twenty-one subjects were studied by Cr^{51}-labelled red cell survival and organ counting techniques. Five were healthy young men without splenomegaly. Studies were made on two patients who had splenomegaly without hemolytic anemia. Radioactivity studies were made in 14 patients who had classical evidence of hemolytic anemia.

With the exception of Patient N. A., all subjects with acquired hemolytic anemia were given adrenal steroids prior to splenectomy without significant benefit. We have hesitated to advise splenectomy for acquired hemolytic anemia when the process was controlled by steroids.

RESULTS

Initial data on patients with hemolytic anemia

Pertinent clinical features and laboratory data on the 14 patients showing evidence for hemolytic anemia are presented in Table I. The final diagnosis for each subject is entered in Table I for completion, but was not made in all patients prior to labelled erythrocyte studies. All subjects but N. H. and L. H. had increased (> 280 mg. per day) fecal urobilinogen. The osmotic fragility of red cells after incubation was abnormally increased in patients with hereditary spherocytosis. Increases in mean fragility upon incubation were not seen consistently in patients with acquired hemolytic anemia. Autohemolysis after 48 hours of sterile incubation was strikingly increased in hereditary spherocytosis and in the one subject (A. K.) with paroxysmal nocturnal hemoglobinuria. In acquired hemolytic anemia the degree of autohemolysis varied within wide limits from normal to abnormal. Mechanical fragility was regularly increased only in the hereditary spherocytosis group.

Cr^{51} red cell survival and organ counting

There were 26 simultaneous determinations of Cr^{51} red cell survival and spleen Cr^{51}: liver Cr^{51} ratios in the 21 subjects. Five of these subjects received labelled red cells from a suitable donor (homologous), and at another time an infusion of their own labelled red cells (autologous). An additional survival study was done in one subject (V. H.) after splenectomy. Pertinent data on donor red cells and recipients are shown in Table II.

In Figures 1 to 9 data on Cr^{51} red cell survival and organ counting for each recipient are plotted

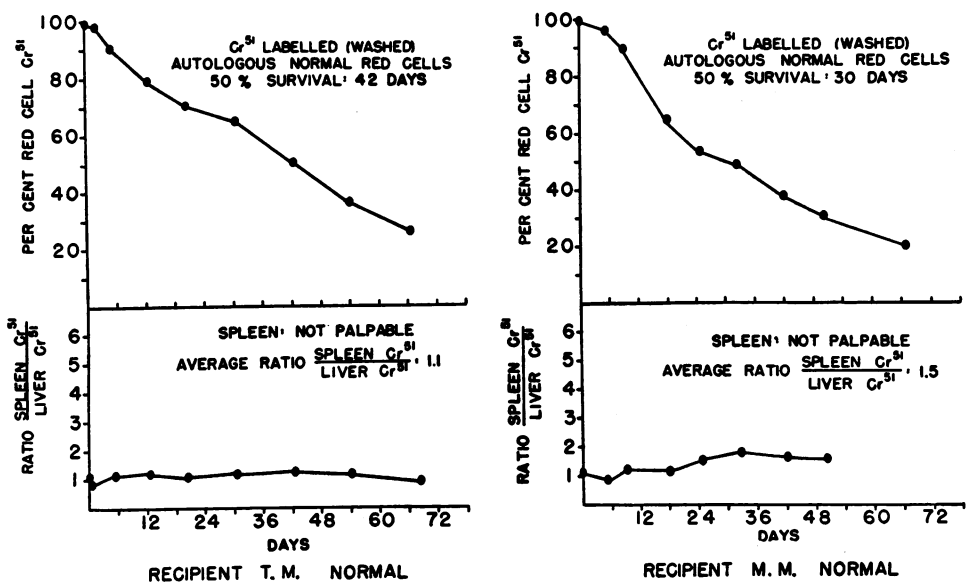

FIG. 1. NORMAL RED CELLS INFUSED INTO NORMAL RECIPIENTS
Recipients T. M. and M. M. (Studies No. 3 and 4—Table II).

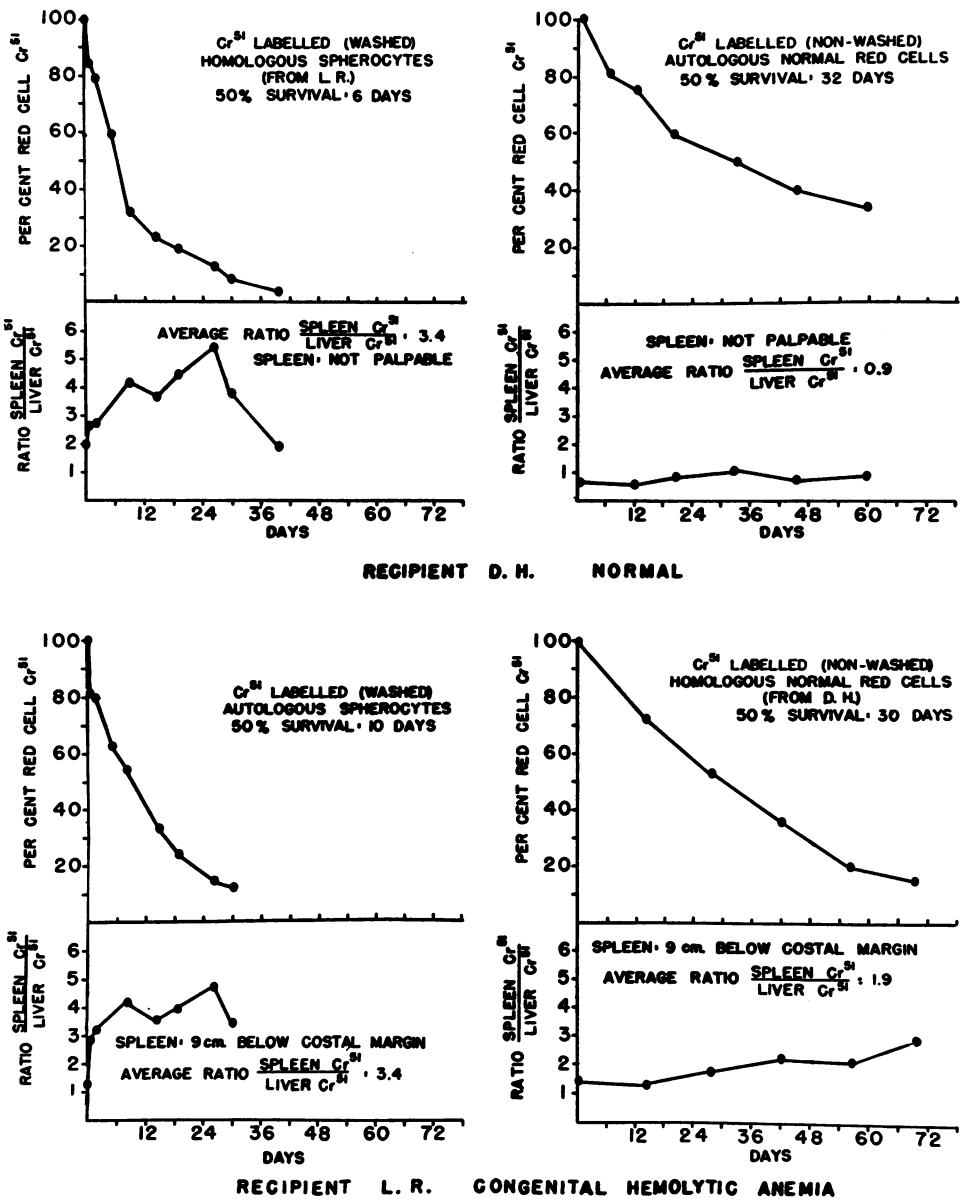

FIG. 2. A MODEL OF RED CELL SEQUESTRATION

Recipients D. H. and L. R. (Studies No. 5, 6, 9, and 10—Table II). Patient L. R. served as a spherocyte donor in the upper and lower left studies which were carried out simultaneously. There was a rapid accumulation of splenic radioactivity as evidenced by rising spleen Cr^{51}: liver Cr^{51} ratios concomitant with a grossly reduced red cell survival in both subjects. On the right are presented the data on the infusion of Cr^{51} red cells from normal Subject D. H. into each subject simultaneously. In contrast, the red cell survival times were in the normal range and the spleen Cr^{51}: liver Cr^{51} ratios did not rise appreciably relative to changes which occurred after infusion of spherocytes.

on arithmetical coordinates. Figures 1 to 7 have the same scale to facilitate comparison of studies. The data in two subjects with severe hemolysis are shown on expanded scales in Figures 8 and 9.

A. Normal studies. Individual studies in normal subjects are depicted in Figures 1 and 2. In the five studies of normal donors and recipients, the Cr^{51} red cell half-life varied from 30 to 42 days,

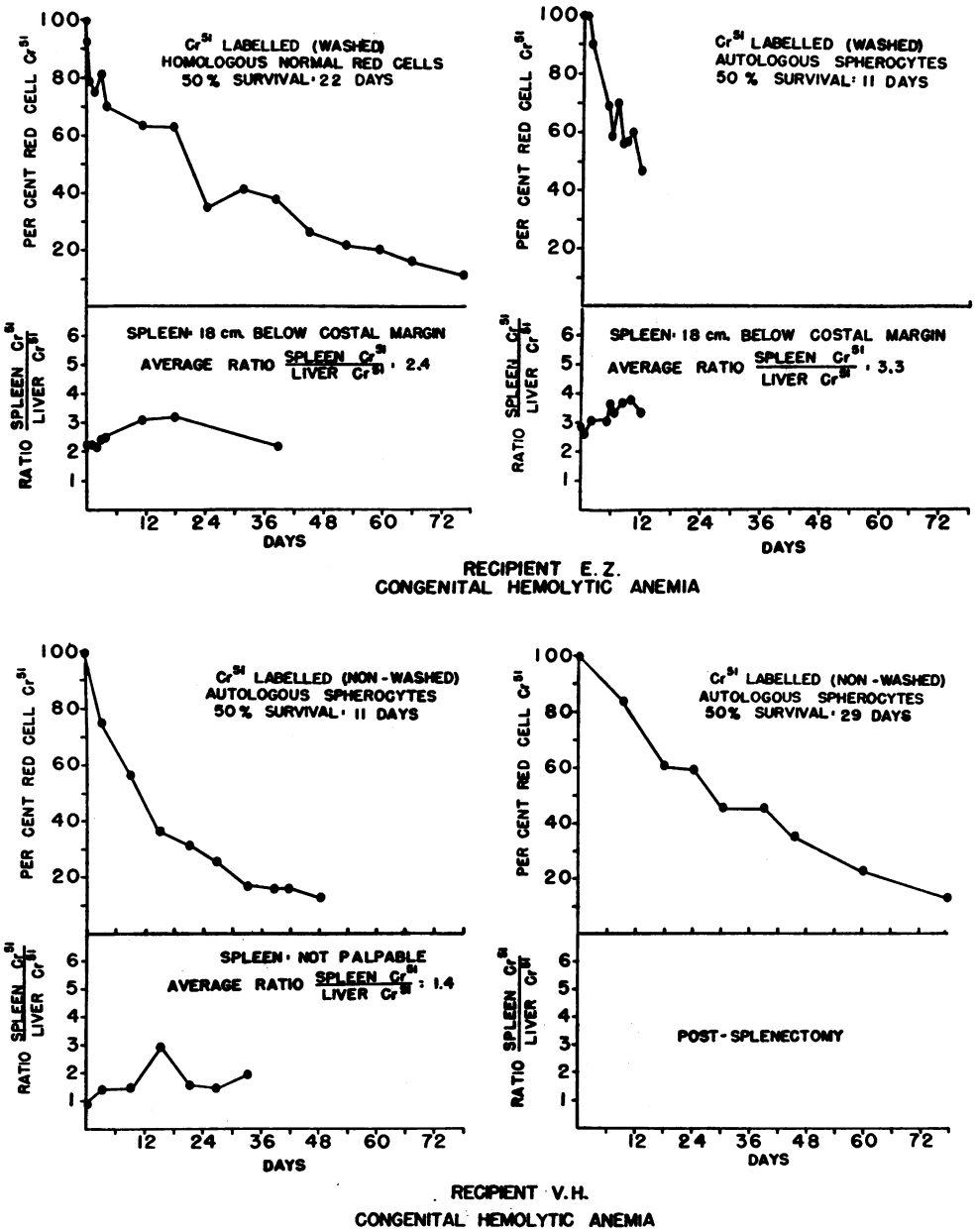

FIG. 3. CONGENITAL HEMOLYTIC ANEMIA

Recipient E. Z. (Studies No. 11 and 12—Table II). Homologous normal and autologous (spherocyte) studies are shown in the upper plots. The homologous normal red cell data is shown on the left. The patient suffered an infection between the 18th and 25th days of this study coincident with a fall in hematocrit. The drop in the red cell survival curve at this time produced a red cell half-life slightly less than normal (22 days). After recovery the curve became normal. On the right is shown the autologous (spherocyte) plot. The red cell half-life was grossly reduced concomitant with a rapid accumulation of splenic radioactivity. This study was terminated by splenectomy on the 12th day after infusion.

Recipient V. H. (Studies No. 13 and 14—Table II). Autologous studies before and after splenectomy are shown in the lower plots. The pre-splenectomy study plotted on the left demonstrates only a slight early rise in the spleen: liver radioactivity ratio. The average ratio was not high. The post-splenectomy survival of red cells (spherocytes) shown on the right was normal. The spleen weighed 200 Gm.

and average spleen: liver radioactivity ratios from 0.9 to 1.8 (Table II—Studies 1 to 5).

B. Splenomegaly without hemolytic anemia. These studies are illustrated in Figure 4. In recipient J. K. (polycythemia vera in remission), the Cr^{51} red cell half-life was 27 days, and the average spleen: liver radioactivity ratio was 2.8. Patient L. K. had myelofibrosis without anemia. The Cr^{51} red cell half-life was 27 days, and the average spleen: liver radioactivity ratio was 1.8. A very slight increase in ratios occurred concomitant with the disappearance of labelled red cells from the peripheral blood in both of these subjects.

C. Congenital hemolytic anemia. For the observations shown in Figure 2, Subjects L. R. (congenital hemolytic anemia) and D. H. (normal) served a dual role, *i.e.*, as autologous and reciprocal homologous recipients of Cr^{51} red cells (Table II—Studies 5, 6, 9, 10). Labelled spherocytes infused into a normal person or back into the donor were rapidly eliminated from the peripheral circulation, and the spleen Cr^{51}: liver Cr^{51} ratios increased to abnormally high levels. In contrast were the normal survival of normal red cells in the same subjects.

Three other subjects with congenital hemolytic anemia were studied (E. Z., V. H., N. H.—Tables I and II, Figures 3 and 3a).

D. Paroxysmal nocturnal hemoglobinuria. One patient with paroxysmal nocturnal hemoglobinuria was studied. Initial data (Subject A. K., Table I) indicated a moderately severe hemolytic anemia. There was a mild thrombopenia and leukopenia. There has been no gross hemoglobinuria. Studies with radioactive red cells are shown in Figure 5. The diagnosis of paroxysmal nocturnal hemoglobinuria was not considered until a normal red cell survival was obtained after the infusion of labelled cells from a normal donor. Survival of the patient's own labelled red cells was markedly reduced suggesting an intracorpuscular defect. Previous data did not suggest hereditary spherocytosis (Table I). The spleen Cr^{51}: liver Cr^{51} ratios did not indicate selective splenic sequestration of red cells in either study. The acid hemolysis test of Ham (15) was positive.

E. Acquired hemolytic anemia. Nine patients with acquired hemolytic anemia were studied with radioactive red cell techniques (Table II—Studies 18 to 27). The data are shown in Figures 6 to 9.

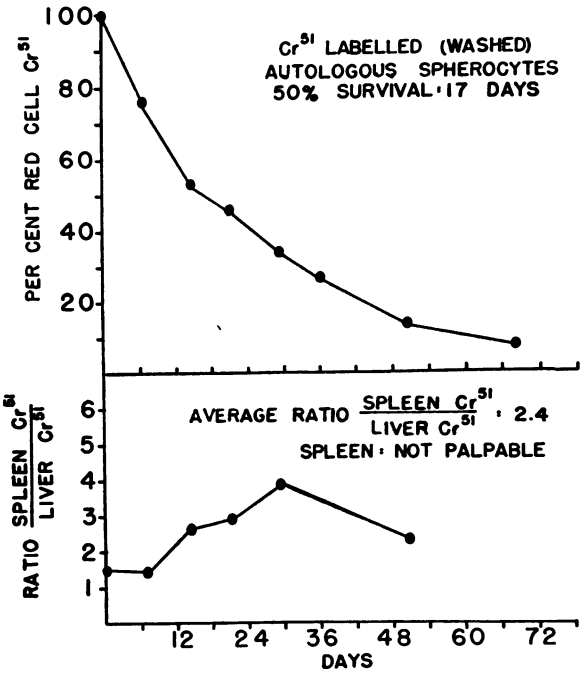

FIG. 3a. CONGENITAL HEMOLYTIC ANEMIA

Recipient N. H. (Study No. 15—Table II). Patient N. H. was the son of V. H. He was asymptomatic with initial evidence for minimal hemolytic anemia. The autologous red cell survival was moderately reduced and there was a prominent rise in spleen Cr^{51}: liver Cr^{51} ratios.

Patient L. Y., with chronic lymphocytic leukemia, had the most severe hemolytic anemia (Table I). Daily blood transfusions were necessary to maintain a stable hematocrit. Labelled red cell studies are shown in Figure 9. The scale used in this graph is enlarged so that the rapid changes may be clearly seen. The 50 per cent survival of Cr^{51}-labelled red cells from a normal donor was less than one day. With the rapid decline in the peripheral blood radioactivity there was an approximately reciprocal rise in the spleen Cr^{51}: liver Cr^{51} ratio. The average ratio of spleen to liver radioactivity was 6.4.

Splenectomy

The effect of splenectomy upon the hemolytic process in eight patients is given in Table III. The two patients with congenital hemolytic anemia, V. H. and E. Z., had the anticipated good response.

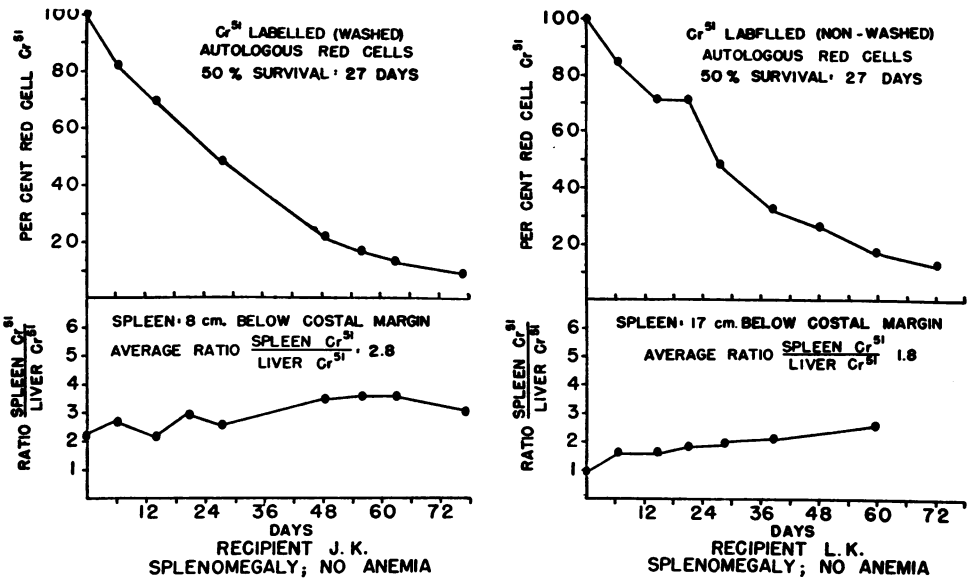

FIG. 4. SPLENOMEGALIC SUBJECTS WITHOUT HEMOLYTIC ANEMIA: AUTOLOGOUS Cr^{51} RED CELL INFUSIONS

Recipient J. K. and L. K. (Studies No. 7 and 8—Table II). These studies serve to illustrate the effect of splenomegaly without hemolytic anemia on the spleen Cr^{51}: liver Cr^{51} ratios. Subject J. K. had polycythemia vera in remission, and L. K. had myelofibrosis.

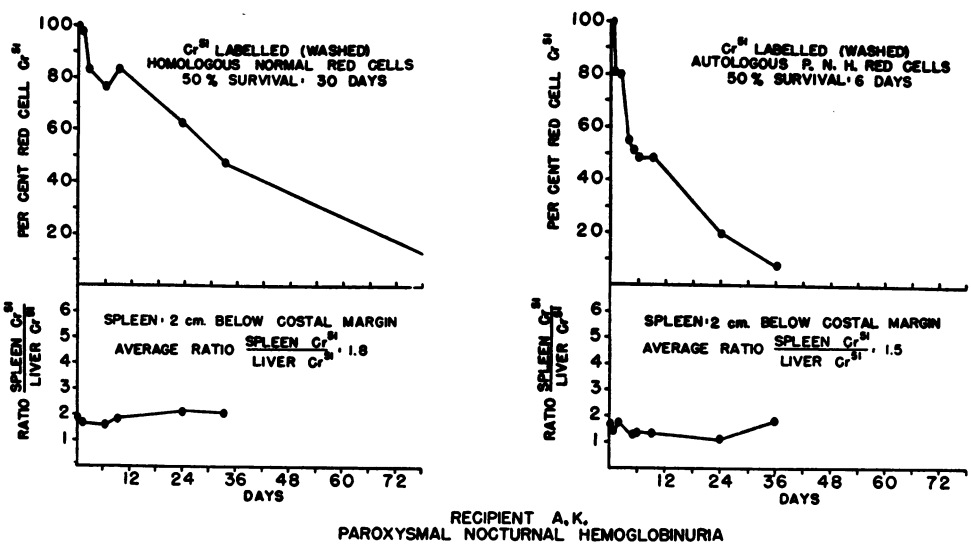

FIG. 5. PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: HOMOLOGOUS NORMAL AND AUTOLOGOUS Cr^{51} RED CELL INFUSIONS

Recipient A. K. (Studies No. 16 and 17—Table II). The homologous infusion data are shown on the left, and the survival of these cells was normal. On the right, the autologous infusion data are given. Note the rapid rate of destruction of the patient's own labelled cells suggesting an intracorpuscular defect. There was no selective splenic uptake of Cr^{51} in either study as determined by spleen Cr^{51}: liver Cr^{51} ratios.

TABLE III

Effect of splenectomy in eight patients with hemolytic anemia

Patient	Diagnosis	Cr^{51}-labelled red-cell infusion	Cr^{51} red-cell half-life (*days*)	Average ratio spleen Cr^{51}: liver Cr^{51}	Spleen wt. (*Gm.*)	Result
V. H.	Congenital hemolytic anemia	Autologous	11	1.4	200	Good
E. Z.*	Congenital hemolytic anemia	Autologous	11	3.3	1,000	Good
L. H.*	Idiopathic hemolytic anemia	Autologous	16	3.0	958	Good
J. L.*	Idiopathic hemolytic anemia	Autologous	9	3.3	1,380	Fair
J. T.	Idiopathic hemolytic anemia	Autologous	15	1.9	323	Poor
N. A.	Myelofibrosis	Homologous normal	12	3.4	518	Good
W. M.	Chronic lymphocytic leukemia	Homologous normal	6	3.5	1,860	Good
L. Y.	Chronic lymphocytic leukemia	Homologous normal	<1	6.4	1,045	Good

* Cr^{51}-labelled red cell survival with organ counting was interrupted by splenectomy at 40 to 50 per cent survival of labelled erythrocytes.

Patient V. H. (Table II—Study 14, and Figure 3) had a normal autologous Cr^{51} red cell survival after splenectomy.

Each of the five patients with acquired hemolytic anemia benefitting from splenectomy had an average spleen: liver radioactivity ratio of 3.0 or higher following the infusion of Cr^{51}-labelled red cells. Probably more important than the average ratio is evidence for a rising ratio of spleen: liver radioactivity, suggesting selective splenic trapping of red cells. Data from Patients N. A. (Figure 6), J. L. (Figure 8), and L. Y. (Figure 9) show striking evidence of increasing splenic radioactivity as compared to the liver during the period of rapid removal of labelled red cells from the circulation. Studies on W. M. (Figure 6) show a high initial spleen: liver radioactivity ratio, but this point was obtained 12 hours after the infusion of cells and may not be representative of earlier splenic radioactivity. The data from Patient L. H. (Figure 6) are suggestive of splenic accumulation of trapped red cells, but we lack data for the first several days of the study. In contrast, Patient J. T. (Figure 7) had an average ratio of 1.9 and was not improved by splenectomy. There was no evidence of a rising spleen: liver radioactivity ratio in this patient.

The results given in Table III represent the effect of splenectomy upon the hemolytic anemia and not the primary disease. Following splenectomy, Patients L. H. and W. M. have had sustained remissions for 20 and 30 months, respectively. Patient J. L., who in the six months prior to splenectomy required at least 19 blood transfusions, did not require transfusions for three months post-operatively. He expired at home four and one-half months following splenectomy. A primary diagnosis was not established. Patient N. A., who had developed myelofibrosis and hemolytic anemia following polycythemia vera, received dramatic relief of anemia for at least 12 months post-splenectomy. Fourteen months after splenectomy this patient had developed leukemia without evidence of hemolytic anemia. He died two months later in the hospital with severe anemia and blasts of undetermined type in the peripheral blood and bone marrow. Patient L. Y. (chronic lymphatic leukemia), whose hemolysis was so severe as to require daily blood transfusions, was dramatically relieved by splenectomy. There was no evidence for hemolytic anemia when this patient was seen in the hospital a few days prior to his death three and one-half months post-operatively.

DISCUSSION

The technique of red cell labelling and estimating erythrocyte survival with radioactive chromium used in this study is similar to that employed by Ebaugh, Emerson and Ross (22). Red cell survival data reported here are based upon the radioactivity in red cells per unit volume of whole blood

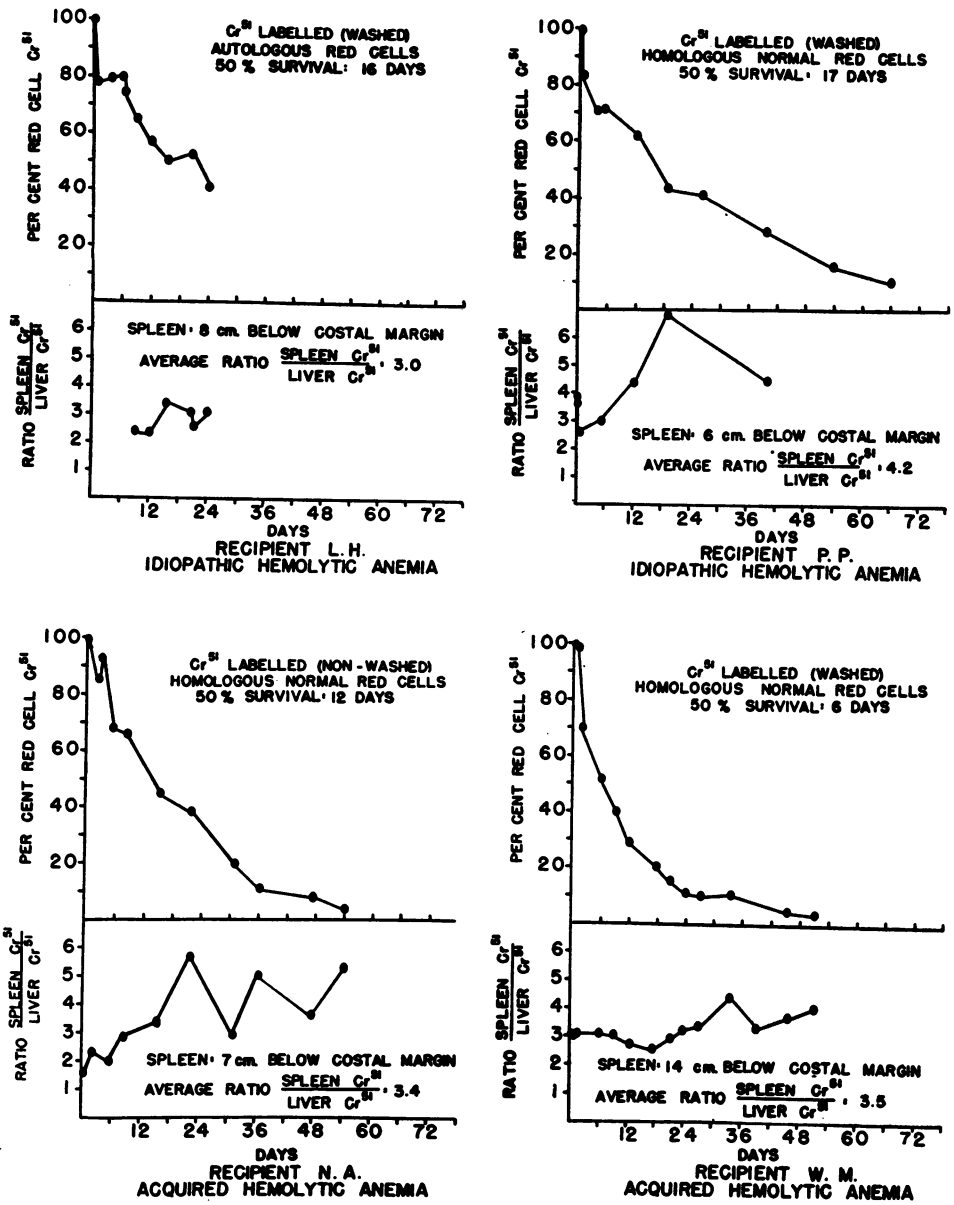

FIG. 6. ACQUIRED HEMOLYTIC ANEMIA

Recipient L. H. (Study No. 18—Table II). Patient L. H. had splenomegaly, thrombopenic purpura and mild hemolytic anemia of unknown cause. Autologous infusion data are given in the upper left graph. The patient's own labelled cells were eliminated at a moderately increased rate (50 per cent survival in 16 days). The average spleen Cr^{51}: liver Cr^{51} ratio was 3.0. This study was terminated by splenectomy on the 25th day after infusion. There was a good response to splenectomy.

Recipient P. P. (Study No. 19—Table II). On the upper right homologous infusion data for Patient P. P. are shown. The patient had minimal anemia. The survival of homologous Cr^{51}-labelled red cells substantiated a moderate hemolytic process (50 per cent survival in 17 days). The rise in spleen Cr^{51}: liver Cr^{51} ratios, however, is striking and approximates in a reciprocal manner the fall in peripheral blood red cell Cr^{51}. The average spleen Cr^{51}: liver Cr^{51} ratio was 4.2. Splenectomy was not performed.

Recipient N. A. (Study No. 20—Table II). This patient had myelofibrosis and acquired hemolytic anemia following polycythemia vera. He was given Cr^{51}-labelled red cells from a

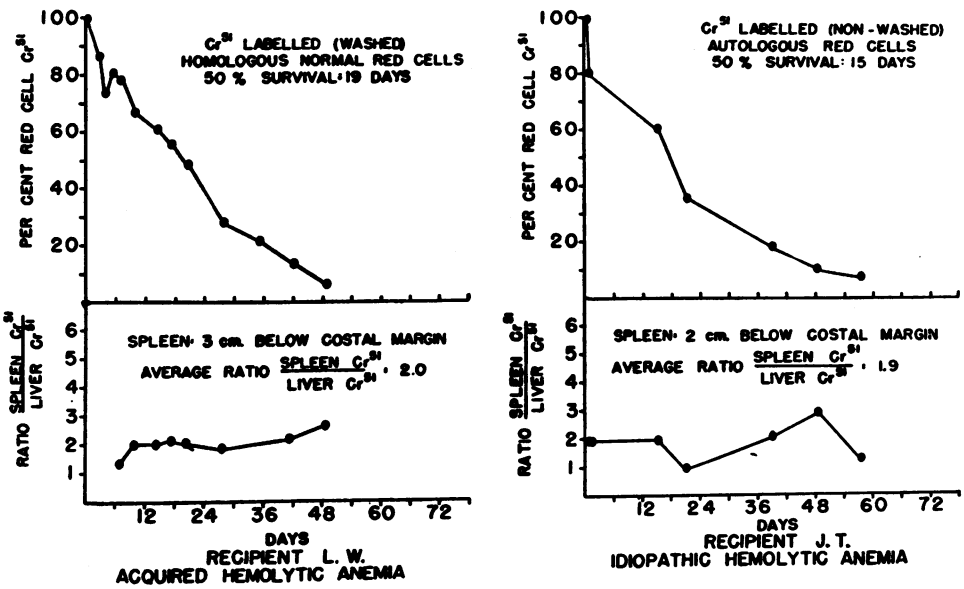

FIG. 7. ACQUIRED HEMOLYTIC ANEMIA

Recipient L. W. (Study No. 22—Table II). Patient L. W. had a positive "LE test." The labelled red cell data in this subject are shown on the left. The survival of normal donor's cells was moderately reduced (half-life, 19 days) and there was no evidence for selective sequestration of labelled erythrocytes in the spleen by external counting (average spleen Cr^{51}: liver Cr^{51} ratio, 2.0). Splenectomy was not done.

Recipient J. T. (Study No. 23—Table II). Patient J. T. received his own labelled red cells and is illustrated on the right. The red cell survival was reduced (half-life, 15 days), and there was no indication from spleen Cr^{51}: liver Cr^{51} ratios for splenic sequestration of labelled erythrocytes. This patient did not benefit from splenectomy.

which assumes a constant whole blood volume. It is believed this method gives a better estimation of Cr^{51} red cell survival than techniques based on measurements of radioactivity per unit volume of packed cells. The latter method assumes a constant red cell volume, an assumption which may not be correct in hemolytic states (23).

There are many errors involved in the estimation of relative organ radioactivity by external monitoring of gamma emissions. Variations in counter positioning, counter-to-source distance, organ size, and radioactivity in adjacent viscera all affect the results. We have estimated the anatomic center of the surface projection of the liver and spleen by percussion, and, by using external landmarks, repositioned the probe at the same area in subsequent observations. Particular care must be employed in positioning the detector over the smaller organ (*e.g.*, small spleen). If the approximate center is not located or there are moderate variations in day-to-day positioning, relatively large errors may be made in evaluating organ radioactivity. There are geometric limits of radioactivity accepted by the scintillator at close range. Consequently, less radioactivity is detectable over the large organ as compared to the small one, even though both contain equal quantities of isotope. This problem can theoretically be solved by

normal donor. The data are shown in the lower left graph. There was a rapidly rising spleen Cr^{51}: liver Cr^{51} ratio concomitant with a rapid rate of Cr^{51} red cell removal from the peripheral blood. This patient responded well to splenectomy but 14 months later the clinical features of acute leukemia led to his demise.

Recipient W. M. (Study No. 21—Table II). This patient had chronic lymphatic leukemia with an acquired hemolytic anemia. The survival of Cr^{51}-labelled red cells from a normal donor was grossly reduced (half-life, 6 days). There was an associated rise in spleen Cr^{51}: liver Cr^{51} ratio (average ratio, 3.5). This patient had an excellent response to splenectomy. He has had no treatment or anemia during the two and one-half years since.

increasing the counter to source distance; however, without elaborate shielding apparatus, specificity of organ radioactivity is lost. Organ areas have been counted with an unshielded probe touching the skin, assuming this procedure would give valid data.

There is evidence that not all of the Cr^{51} found in the spleen after the infusion of labelled red cells is hemoglobin bound (24). It is considered unlikely, however, that such radioactivity is derived from sources other than Cr^{51}-labelled red cells destroyed in the spleen. $Cr^{51}Cl_3$ (13, 25) and Cr^{51}-labelled hemoglobin infusions (13) showing low flat curves of splenic radioactivity support this conjecture.

In Figure 10, average spleen to liver radioactivity ratios in acquired hemolytic anemia patients were grouped according to splenectomy response and compared with the ratios obtained in normal and splenomegalic subjects not demonstrating hemolytic mechanisms. The latter group noted by an asterisk in Figure 10 includes average organ radioactivity ratios from Studies 6 to 8, 11 and 25, Table II. Average ratios of spleen: liver radioactivity in the five normal subjects receiving labelled normal red cells ranged from 0.9 to 1.8. The higher ratios of spleen: liver radioactivity in splenomegalic subjects without hemolytic anemia are presumptive evidence for a greater labelled red cell volume "seen" by the detector over the larger organ. These studies provided control observations. From these data it appears that patients with reduced Cr^{51} red cell survival and a rising or high spleen: liver radioactivity ratio will have a favorable response to splenectomy. Obviously many more patients will have to be studied by

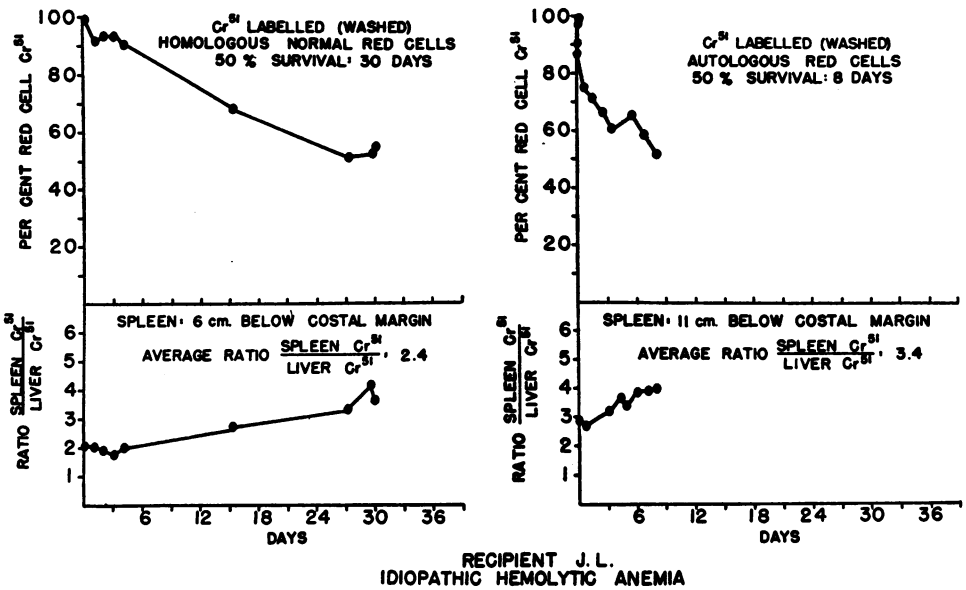

FIG. 8. ACQUIRED HEMOLYTIC ANEMIA

(Note change in scale of abscissa.)

Recipient J. L. (Studies No. 25 and 26—Table II). Patient J. L. had severe hemolytic disease. The survival of compatible labelled donor red cells ("homologous"), given on the left, was normal. During this study the spleen increased in size from 6 cm. below costal margin to 11 cm. below costal margin. The average spleen Cr^{51}: liver Cr^{51} ratio was 2.4 prior to interruption of the study at 30 days for blood transfusions.

The autologous study given on the right showed a grossly reduced red cell half-life of the patient's cells, and concomitantly a rapid selective accumulation of radioactivity in the spleen. The spleen Cr^{51}: liver Cr^{51} ratios averaged 3.4 when the study was terminated by splenectomy on the ninth day.

Subsequent detailed study of the Rh genotype of the patient and compatible donor revealed a difference. The patient was found to be CDE/CDe and the donor CDe/Cde. These findings are compatible with, though not diagnostic of, an anti-Rh type specific hemolytic anemia. The acid hemolysis test of Ham was negative.

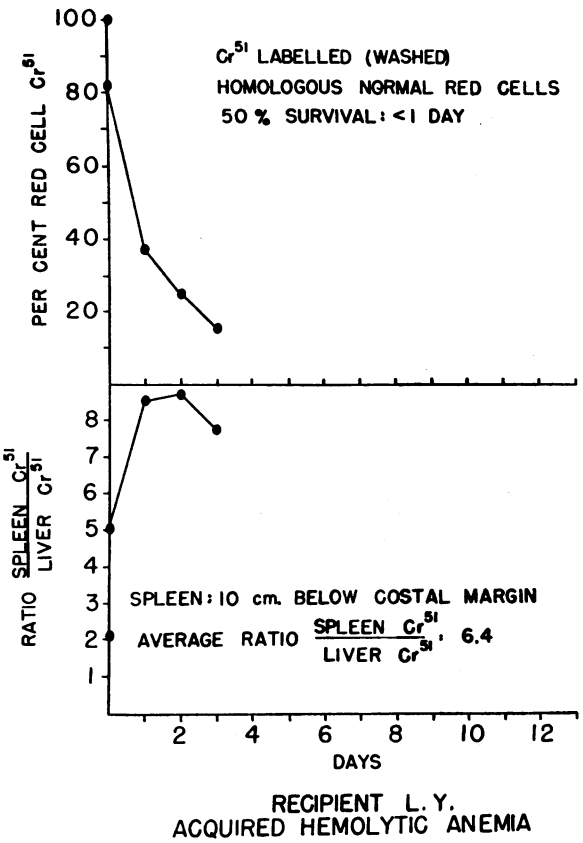

FIG. 9. ACQUIRED HEMOLYTIC ANEMIA

(Note change in scale of abscissa.)

Recipient L. Y. (Study No. 27—Table II). Patient L. Y. had chronic lymphatic leukemia with severe hemolytic anemia. The accumulation of radioactivity in the spleen is striking and the plot of spleen Cr^{51}: liver Cr^{51} ratios is approximately reciprocal to the plot of disappearance of Cr^{51}-labelled erythrocytes from the peripheral blood. The average spleen Cr^{51}: liver Cr^{51} ratio was 6.4. This patient had a good response to splenectomy.

these techniques before one can say that they provide valid criteria for preoperative prediction of the results of splenectomy. In a correlation of splenic sequestration with results of splenectomy the following combinations are possible:

	Evidence for sequestration	Benefit from splenectomy
1)	+	+
2)	+	−
3)	−	+
4)	−	−

Our data from patients with acquired hemolytic anemia show five examples of the first combination and one example of the fourth. We have not observed No. 2 or 3. Thus it cannot be claimed that the data provide evidence enabling one to predict a failure to benefit from removal of the spleen. The data are considered evidence in favor of the theory that splenectomy will be beneficial in those patients demonstrating selective splenic sequestration of erythrocytes.

We have no data from patients with acquired hemolytic anemia without detectable splenomegaly (Table II), and have studied only the one patient (J. T.—Table III) with minimal splenomegaly who had splenectomy. However, it is likely that patients with acquired hemolytic anemia, red cell sequestration in the spleen and normal spleen size (should these features co-exist) would show spleen: liver radioactivity ratios similar to that in subjects without splenomegaly infused with labelled spherocytes (Figures 2 and 3a). We are unable to explain the failure to demonstrate Cr^{51}-labelled spherocyte accumulation in the spleen of the patient with hemolytic anemia without splenomegaly (V. H.—Figure 3).

Cr^{51}-labelled erythrocyte studies, in addition to the estimation of organ accumulation of red cells, may occasionally suggest mechanisms of hemolytic disease previously unsuspected. Clinical evaluation of Patient A. K. had indicated a brisk hemolytic anemia, mild thrombopenia and leukopenia. Splenectomy might have been advised for "hypersplenism" had not erythrocyte survival studies indicated an intracorpuscular defect (Figure 5). There was no evidence for congenital hemolytic anemia (Table I), and the acid hemolysis test was unequivocally positive establishing the diagnosis of paroxysmal nocturnal hemoglobinuria. Organ radioactivity ratios in this patient did not suggest splenic accumulation of autologous or homologous erythrocytes. These studies are in accord with the usual observation that patients with this disorder are not helped by splenectomy (18). On the other hand, Jandl and his colleagues have reported that splenectomy was beneficial in a patient with this disorder in whom splenic sequestration of autologous Cr^{51}-labelled red cells had been demonstrated (13).

Studies with radioactive red cells in Patient J. L. were of particular interest. This patient had severe hemolytic anemia of unknown type. The survival of "homologous" normal Cr^{51}-labelled red cells was normal. The relatively marked rise of the spleen: liver radioactivity ratio during this study (Figure 8) was considered secondary to an

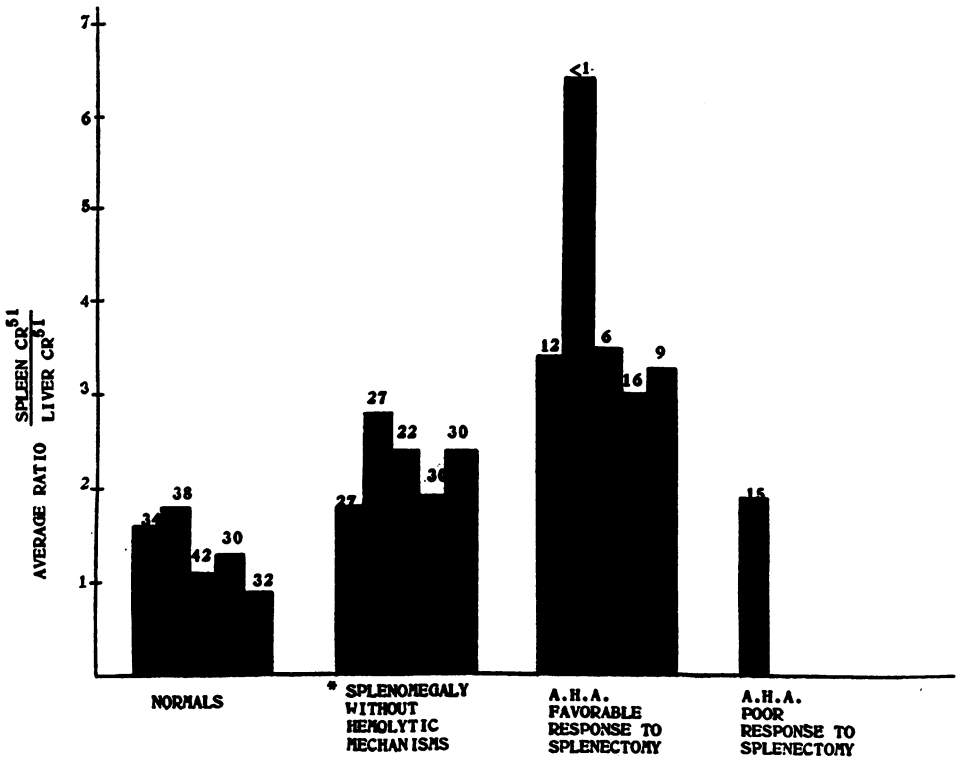

FIG. 10. CORRELATION OF RESPONSE TO SPLENECTOMY WITH AVERAGE SPLEEN: LIVER RADIOACTIVITY RATIOS IN ACQUIRED HEMOLYTIC ANEMIA

Each bar represents the average spleen: liver radioactivity ratio for a single study. The number at the top of each bar represents the Cr^{51} red cell half-life in days.

A.H.A. = Acquired hemolytic anemia.

* See text.

expanding spleen red cell volume in an enlarging spleen. An even greater accumulation of splenic radioactivity with a grossly reduced red cell survival occurred after infusion of the patient's own labelled red cells. A negative family history and conventional laboratory data excluded congenital hemolytic anemia (Table I). The acid hemolysis test for paroxysmal nocturnal hemoglobinuria was negative. Further typing of the donor's and the patient's red cells, however, revealed a difference in Rh genotypes. We were unable to repeat a study using truly homologous erythrocytes, but the observations made suggested an Rh type-specific hemolytic anemia.

Prior to labelled red cell studies, our laboratory data in acquired hemolytic anemia did not provide criteria for predicting response to splenectomy. This observation is in accord with that recently reported by Chertkow and Dacie (26). Gross splenomegaly, however, was common to all patients with acquired hemolytic anemia showing evidence for splenic accumulation of Cr^{51}-labelled red cells. Patients with acquired hemolytic anemia and splenic sequestration of labelled red cells did not have spherocytosis in the peripheral blood as judged by osmotic fragility studies.

SUMMARY

The ratio spleen: liver radioactivity as determined by external scintillation counting after the infusion of Cr^{51}-labelled erythrocytes has been used to determine organ localization of red cells in seven control subjects and 14 patients with hemolytic anemia. As anticipated, the spleen accumulated radioactivity rapidly following the transfusion of labelled spherocytes. Some patients with acquired hemolytic anemia showed gross evidence of trapping of radioactive red cells in their spleen. Removal of the spleen in five such patients was beneficial in each. One patient who showed no evidence for splenic trapping of red cells was splenectomized without beneficial effects on the

hemolytic process. The method described is considered a useful aid in the selection of patients whose hemolytic process will be benefitted by splenectomy.

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