ALTERATIONS IN OSMOTIC AND MECHANICAL FRAGILITY RELATED TO *IN VIVO* ERYTHROCYTE AGING AND SPLENIC SEQUESTRATION IN HEREDITARY SPHEROCYTOSIS *†

By ROBERT C. GRIGGS,‡ RUSSELL WEISMAN, JR. AND JOHN W. HARRIS §

(From the Department of Medicine, Western Reserve University School of Medicine at Cleveland Metropolitan General Hospital, and University Hospitals of Cleveland, Ohio)

(Submitted for publication July 20, 1959; accepted August 20, 1959)

Increased osmotic and mechanical fragility of the erythrocytes is a consistent finding in patients with hereditary spherocytosis, although in a few individuals it is necessary to incubate the cells at 37° C. for 24 hours before the otherwise latent abnormality becomes demonstrable. Splenectomy will correct the hemolytic process of hereditary spherocytosis despite the continuing production of abnormally fragile cells by the patient. The spleen is, therefore, essential in the destruction of the intrinsically defective erythrocytes. In the peripheral blood of some individuals with this disease a "double population" of cells exists. One component consists of more markedly fragile cells that are not present after splenectomy and must, therefore, have been related to the presence of the spleen.

The present study was undertaken to investigate the changes in osmotic and mechanical fragility accompanying *in vivo* erythrocyte aging and splenic sequestration and to elucidate the source and fate of the more markedly fragile group of red cells found in some patients.

Advantage was taken of the increased erythropoiesis in patients with hereditary spherocytosis prior to splenectomy to label *in vivo* with Fe^{59} the new red cells produced during a short time span, thereby obtaining an identifiable group of cells of known age. Using techniques that will be described, it was possible to differentiate these isotopically-labeled cells from the other cells in the peripheral circulation and to compare their char-

acteristics to those of the general population. The changes in osmotic and mechanical fragility occurring in these labeled red cells during *in vivo* aging and splenic sequestration were followed. In addition, erythrocytes were recovered from the spleen at the time of splenectomy and reinjected into the patient's peripheral circulation. The post-splenectomy survival and changes in osmotic and mechanical fragility of these cells, labeled with Cr^{51} , were followed and distinguished from the cells of the general population.

METHODS

Routine hematologic studies were performed by standard methods (1). Autohemolysis was determined by the method of Selwyn and Dacie (2) as modified by Young, Izzo, Altman and Swisher (3). Erythrocyte osmotic fragility tests were done as described by Emerson and associates (4) and the osmotic fragility of the labeled cells was distinguished from that of the general population by the following modification of this method. The volume of each saline solution was increased to 3 ml., and 0.3 ml. of defibrinated whole blood was added to each tube. These were then centrifuged, decanted and the hemoglobin content of 0.5 ml. of the supernatant solution determined in a Beckman (Model B) spectrophotometer. The remainder of the supernatant was transferred to a calibrated isotope counting tube and the radioactivity determined in a low background, well-type scintillation counter (Tracerlab). Sufficient counts were obtained to give a statistical error of ± 3 per cent or less. As a blank, 0.3 ml. of the defibrinated whole blood was suspended in hypertonic saline (1.25 Gm. NaCl per 100 ml.) and the radioactivity found in this supernatant after centrifugation was subtracted from that obtained for the other samples. The value for radioactivity thus obtained represented isotope released by the red cells lysed at each concentration of sodium chloride. The amount of radioactivity in each sample was calculated as a percentage of that released into the supernatant when 0.3 ml. of the defibrinated whole blood had undergone complete hemolysis by addition to 3 ml. distilled water. The results were plotted as two curves, one showing the percentage of hemolysis measured as hemoglobin (the osmotic fragility of all the cells in the sample) and the

* A preliminary report of this work appeared in abstract form (J. clin. Invest. 1958, 37, 899).

† This study was supported by a grant from the Webster-Underhill Fund and Public Health Service Grant No. A-745.

‡ Webster-Underhill Fellow, Western Reserve University School of Medicine.

§ Markle Scholar in Medical Science, Western Reserve University School of Medicine.

other curve representing the percentage of radioactivity released at each concentration of sodium chloride (the osmotic fragility of only the isotopically labeled cells). Control studies demonstrated that no appreciable Fe^{59} was present in the stroma of the lysed cells and that osmotic fragility curves obtained by this isotope method on our patients after general distribution of the Fe^{59} through the red cell population were identical with those obtained by the usual method in which hemoglobin from lysed red cells is measured.

The mechanical fragility of the red cells was determined by a modification of the method of Shen, Castle and Fleming (5). The hematocrit of a sample of defibrinated venous blood was adjusted to 35 per cent and 0.5 ml. subjected to the standardized trauma produced by rotating it for 90 minutes in a 50 ml. Erlenmeyer flask at 30 rpm with 10 uniform 4 mm. selected glass beads. To obtain an adequate sample for the isotope counting procedures, two flasks were used for each determination and combined after rotation. The results were corrected by a blank consisting of a similar blood sample in hypertonic saline (1.25 Gm. NaCl per 100 ml.) and expressed as percentage of hemolysis compared to hemolysis in distilled water.

Ten $\mu\text{c.}$ of Fe^{59} in the form of ferric chloride (Abbott) (3 to 10 $\mu\text{g.}$ of elemental iron) was incubated with 20 ml. of normal compatible heparinized plasma and administered intravenously to each patient. Since patients with hemolytic anemias have increased saturation of their iron-binding protein, normal compatible plasma was used instead of the patient's. The plasma Fe^{59} clearance and the red cell Fe^{59} utilization studies were performed according to the method of Huff and associates (6, 7).

Red cell survival was determined by the method of Ebaugh, Emerson and Ross (8) using 120 $\mu\text{c.}$ of Cr^{51} . In each case the patient's own red cells, obtained from either peripheral vein or splenic pulp, were used. Intact red cells were recovered from the spleen at operation. Using sterile technique, this was accomplished by making a number of large incisions into the surgical specimen immediately after removal and allowing the formerly entrapped blood to flow into a glass flask where it was defibrinated by rotating with glass beads.

Scintillation counting over the body surface was done by the methods described by Huff and co-workers (7) and Jandl, Greenberg, Yonemoto and Castle (9). The counting rates over the liver and spleen are plotted as ratios relative to the counting rate over the precordium.

RESULTS

Three unrelated adults who were referred to this hospital for investigation of anemia or jaundice were studied. All had splenomegaly and showed spherocytosis on peripheral blood smears; appropriate laboratory tests confirmed the diagnosis of hereditary spherocytosis. Family history in all three was negative for anemia, jaundice or splenomegaly. The mother of Case 2 was the only relative available for examination and her blood studies, including osmotic and mechanical fragility, were normal. Identifying data and laboratory findings are recorded in Table I. Presplenectomy studies with Fe^{59} were performed on Cases 1 and 2 and post-splenectomy studies with Cr^{51} on Cases 2 and 3.

Presplenectomy studies

Case 1 showed evidence of a marked hemolytic process. A Cr^{51} autosurvival had been performed in our laboratory on this individual one year prior to the present study and demonstrated a red cell half-life of eight days compared to a normal of at least 28 days for this method. During the present study he showed a marked hyperbilirubinemia (indirect reacting) and sustained reticulocytosis (Table I). Both the spleen and liver were enlarged. The administered Fe^{59} was rapidly cleared from the plasma, with a half-time of 15 minutes, contrasted to a normal of 60 to 120 minutes. The utilization of Fe^{59} for red cell formation and the

TABLE I
Laboratory data on patients in study

Case	Age Race Sex	Hgb.*	Hct.	Retics.	Bilirubin direct total	Erythrocyte osmotic fragility	Erythrocyte mechanical fragility		% Autohemol- ysis 48 hours		Plasma clearance Fe^{59} half-time	Erythro- cyte survival Cr^{51} half- time
							0 hrs.	24 hrs.	No glucose	With glucose		
Normal range		Gm.%	%	%	mg./100 ml.		% 1-5	% 9-15	0-3	0-1	min. 60-120	days 28+
1	68 W M	10	30	15	1.4	5.1	Increased	22	37		15	8
2	32 N F	9.5	32	4	0.7	1.1	Increased	18	37	12.4	8.3	31
3	54 W M	9.5	29	3	0.5	1.0	Increased	7	17	5.7	1.5	16

* Hgb. = hemoglobin; Hct. = hematocrit; Retics. = reticulocytes.

DISTRIBUTION OF Fe^{59} IN HEREDITARY SPHEROCYTOSIS FIG. 1. CASE 1: UTILIZATION OF Fe^{59} FOR RED CELL FORMATION AND *IN VIVO* DISTRIBUTION

distribution of body surface counts are represented in Figure 1. There was rapid incorporation of Fe^{59} into new red cells as might be anticipated in a patient with hemolytic anemia. This reached a maximum of 65 per cent on Day 6. Body surface counting demonstrated early and rapid accumulation of isotope in the spleen after the expected initial rise and fall in counting rate over the sacral bone marrow.

Peripheral blood samples were studied at frequent intervals for changes in erythrocyte fragility by the osmotic and mechanical fragility methods described above. There was not sufficient radioactivity present in the red cells prior to the third day to give statistically valid counting rates. The results of the studies of the osmotic fragility on Days 3, 7, 10, 13, 24 and 30 are presented in Figure 2. The osmotic fragility of the patient's total peripheral red cell population is increased. The

lower portion of the fragility curve shows some asymmetry with approximately 20 per cent of the cells being more markedly fragile than the other red cells in the peripheral blood. The broken lines represent the osmotic fragility of the Fe^{59} -labeled red cells. Since the labeling of the red cells is occurring as they are formed *in vivo*, on Day 3 all cells represented by this curve must be no more than three days old. The fragility curves of these new cells on Days 3 and 7 show some increase in fragility as compared to normal red cells, but do not have the asymmetrical curve of the total peripheral cell population and none of the cells are as osmotically fragile as some of the red cells in the peripheral blood. It is not until Day 10 and subsequently that some of the labeled cells show fragility changes comparable to that of the general cell population.

During the early phases of this study, observa-

OSMOTIC FRAGILITY OF TOTAL CELL POPULATION AND ERYTHROCYTES TAGGED WITH Fe^{59}

FIG. 2. CASE 1: OSMOTIC FRAGILITY OF THE Fe^{59} -LABELED RED CELLS CONTRASTED WITH THAT OF THE TOTAL PERIPHERAL CELL POPULATION

tions were also made on cells after sterile incubation for 24 hours. However, no difference was noted between the fragility of the labeled cells and the general peripheral population, and these studies were not continued. All observations reported are on fresh, unincubated whole blood.

The concurrent changes in mechanical fragility of red cells of Case 1 are depicted on the lower half of Figure 3. The mechanical fragility of this patient's peripheral red cells varied from 18 to 27 per cent compared to a normal of 1 to 3 per cent

by this method. On Day 3 the mechanical fragility of the new red cells as measured by the Fe^{59} was 10 per cent. This gradually increased until it was equal to that of the total cell population (24 per cent) on Day 13. Subsequently, the labeled cells exhibited a mechanical fragility slightly increased above that of the general population.

A similar study was performed on Case 2 and the results are outlined in Figures 3 through 5. This individual showed evidence of a less severe hemolytic process, a reticulocytosis of 4 per cent

and no hyperbilirubinemia (Table I). The half-time for plasma clearance of Fe^{59} was 31 minutes with rapid reappearance of the isotope in the peripheral red cells. This reached a maximum recorded utilization of 80 per cent on Day 10. There was evidence by body surface counting of gradual accumulation of Fe^{59} in the spleen (Figure 4). Studies of osmotic fragility similar to those described above were performed on this patient and the results are presented in Figure 5. In an effort to diminish the reutilization of Fe^{59} from destroyed red cells in further red cell production, a relatively large dose of nonisotopic iron was administered beginning on Day 11 of the study. From Day 11 to Day 28, 950 mg. of iron was administered in divided doses intramuscularly in the form of Imferon®. Approximately 15 per cent of this patient's peripheral red cells showed a marked increase in osmotic fragility as represented by the lower portion of the curve (solid lines, Figure 5). On Day 4 the new Fe^{59} -labeled red cells, represented by the broken line, did not

show any cells with this marked increase in fragility. By Day 11 the proportion of fragile cells had increased and by Day 17 the proportion had increased above that of the general peripheral cell population and about 20 per cent of the cells showed a marked increase in fragility. These very fragile cells then apparently disappeared from the peripheral circulation within several days, for the fragility curve of the labeled cells on Day 24 is similar to that seen on Day 4. Subsequent studies, not shown in the figures, demonstrated that a portion of the labeled cells again became more fragile and both curves were essentially the same after Day 31.

The results of the mechanical fragility studies on this patient are presented in the upper half of Figure 3. As in the previous study, the mechanical fragility of the labeled cells was initially low and then gradually increased, eventually becoming slightly greater than that of the general cell population.

FIG. 3. MECHANICAL FRAGILITY OF THE Fe^{59} -LABELED CELLS CONTRASTED WITH THAT OF THE TOTAL PERIPHERAL CELL POPULATION IN CASES 1 (W. B.) AND 2 (A. D.)

DISTRIBUTION OF Fe^{59} IN HEREDITARY SPHEROCYTOSIS FIG. 4. CASE 2: UTILIZATION OF Fe^{59} FOR RED CELL FORMATION AND *IN VIVO* DISTRIBUTION*Splenectomy and post-splenectomy studies*

Cases 2 and 3 were studied during and after elective splenectomy for correction of the hemolytic process. In Case 2, intact red cells were recovered from the splenic pulp at the time of splenectomy. The osmotic fragility of these cells is shown in the upper left-hand graph of Figure 6. As described previously by Emerson and associates (4), these cells were considerably more osmotically fragile than those found in the peripheral blood represented by the solid lines in Figure 5. Approximately 25 per cent of the cells showed a marked increase in fragility. These spleen-drip cells were labeled with Cr^{51} and at the conclusion of the operative procedure, reinjected into a peripheral vein after suitable filtration. Differential osmotic and mechanical fragility studies of a small sample of these cells indicated that the curve

for Cr^{51} release on hemolysis of the cells was identical with the curve for hemoglobin liberation. The studies of Gray and Sterling (10) showed that 95 per cent of the red cell Cr^{51} was bound to the hemoglobin; Necheles, Weinstein and Leroy (11) found only 78 per cent with the hemoglobin fraction.

Starting one hour after reinjection peripheral blood samples were taken at frequent intervals. The survival of the chromium-labeled cells was followed and, in addition, the changes in osmotic and mechanical fragilities of the labeled cells were studied in a manner similar to that used for the Fe^{59} -labeled cells in the previous experiments. These results are presented in Figures 6 through 8. In Figure 6 the changes in osmotic fragility are recorded. The broken lines represent the isotope-labeled, spleen-drip cells and the solid lines

OSMOTIC FRAGILITY OF TOTAL CELL POPULATION AND ERYTHROCYTES TAGGED WITH Fe^{59}

FIG. 5. CASE 2: OSMOTIC FRAGILITY OF THE Fe^{59} -LABELED RED CELLS CONTRASTED WITH THAT OF THE TOTAL PERIPHERAL CELL POPULATION

represent the osmotic fragility of the peripheral cell population. Even one hour after reinjection of the labeled cells, there has been some change in the osmotic curve with a decrease in the number of markedly fragile cells. In the subsequent seven days there were further decreases in the fragile cells, until by Day 7 the two curves were essentially the same. There also has been a decrease in the number of markedly fragile cells in the peripheral blood as a whole, so that the curve of hemolysis

has become more symmetrical than before splenectomy.

The concurrent changes in mechanical fragility are seen in Figure 7. Twenty-seven per cent of the spleen-drip cells and 20 per cent of the peripheral cells were destroyed by the standard trauma. Both gradually showed less marked fragility, the labeled cells showing a more rapid change, both mechanical fragilities being essentially the same by Day 13.

CHANGES IN OSMOTIC FRAGILITY - POST-SPLENECTOMY

FIG. 6. CASE 2: CHANGES IN OSMOTIC FRAGILITY OF SPLEEN-DRIP RED CELLS LABELED WITH CR⁵¹ AND RETRANSFUSED INTO THE PATIENT POST-SPLENECTOMY CONTRASTED WITH THE OSMOTIC FRAGILITY OF THE TOTAL PERIPHERAL RED CELL POPULATION

The survival of these Cr⁵¹-labeled, spleen-drip red cells is presented in Figure 8. The one hour postinjection sample, the first one obtained, was plotted as 100 per cent. During the first 48 hours there was a rapid loss of approximately 20 per cent of the labeled cells. The remainder of the cells showed a normal length of survival.

A similar study of the postsplenectomy survival of spleen-drip red cells was performed on Case 3 and the findings recorded in Figure 9. The

solid line represents the Cr⁵¹ autosurvival of this patient's red cells done in our laboratory several months prior to splenectomy. A half-life of 16 days was obtained. A sample of cells obtained from the spleen at the time of splenectomy was labeled with Cr⁵¹ and reinjected into the peripheral circulation postoperatively. About 30 per cent of these cells showed a marked increase in osmotic fragility. The survival curve (broken line, Figure 9) reveals that 50 per cent of the autotrans-

FIG. 7. CASE 2: CHANGES IN MECHANICAL FRAGILITY OF SPLEEN-DRIP RED CELLS LABELED WITH Cr^{51} AND RETRANSFUSED INTO THE PATIENT POST-SPLENECTOMY CONTRASTED WITH THE MECHANICAL FRAGILITY OF THE TOTAL PERIPHERAL RED CELL POPULATION

FIG. 8. CASE 2: SURVIVAL OF SPLEEN-DRIP RED CELLS LABELED WITH Cr^{51} AND RETRANSFUSED INTO THE PATIENT POST-SPLENECTOMY

Data are not corrected for chromium elution.

FIG. 9. CASE 3: SURVIVAL OF SPLEEN-DRIP RED CELLS LABELED WITH Cr^{51} AND RETRANSFUSED INTO THE PATIENT POST-SPLENECTOMY

Data are not corrected for chromium elution.

fused red cells were removed from the circulation within 48 hours; the remaining cells had a normal life span of 100 to 120 days. Neither of these patients showed any significant blood loss postoperatively and on follow-up studies over a period of months, both patients have shown the anticipated good clinical response.

DISCUSSION

The clinical picture and pathologic physiology of hereditary spherocytosis have been reviewed recently in some detail by Dacie (12) and Young (13). Only a brief outline will be presented here of the erythrocyte abnormalities and of the role of the spleen in the hemolytic process. The relationship of these two factors, the abnormal red cell and the spleen, has been most concisely demonstrated in a study of red cell survival by Emerson (14). He showed that normal red cells had

a normal survival in a patient with hereditary spherocytosis, but that the patient's red cells were incapable of normal survival in a recipient *except* in the absence of a spleen. Although patients with hereditary spherocytosis characteristically have an enlarged spleen, there is no primary abnormality of the spleen, since it has been amply demonstrated both *in vivo* and *in vitro* that a spleen from a patient with hereditary spherocytosis and one from a normal individual will both selectively remove perfused spherocytic red cells but will not selectively remove normal red cells. Although maturing erythrocytes in the patient's bone marrow may show some minor shape changes, they evidently become more abnormal in shape shortly after release into the peripheral circulation (12). Abnormalities in glucose and phosphate metabolism have been demonstrated in these cells (15); but probably related to the abnormal thickness of the cells and their inability to escape easily through

slit-like openings in the venous sinusoids (12), the spheroidal cells are trapped in the spleen. The entrapped cells are evidently in a deleterious environment, which perhaps, in combination with the deficiencies of metabolism that these spherocytes demonstrate, eventually results in alterations in the cells. These changes have been designated by the term "conditioning," and are recognized by abnormally increased osmotic and mechanical fragility. These "conditioned" cells may then either be destroyed in the spleen as evidenced by the elevated bilirubin level in splenic vein blood (12), or, escaping into the peripheral circulation, be identified as the markedly fragile portion of the osmotic curve. These markedly fragile cells are not found in the peripheral circulation of all individuals with hereditary spherocytosis, but their relationship to the spleen has been well established by the experiments of Young, Platzer, Ervin and Izzo (16), Dacie (12), Emerson and associates (4), and Weisman, Ham, Hinz and Harris (17), who have shown that fragile cells of this type are found in the spleen pulp and they are not present in the peripheral blood of patients after splenectomy.

In the present study an identifiable group of spherocytic red cells was produced in patients with hereditary spherocytosis by *in vivo* labeling with Fe^{59} . It is well established that iron administered in this fashion is incorporated only into new red cells and not into older cells. However, when the red cell containing Fe^{59} is destroyed, the iron is reutilized for new cell production. In an individual with normal red cell survival and normal iron metabolism, no reutilization would occur for 100 to 120 days. Red cells were rapidly being destroyed in the patients in the present study so that Fe^{59} was, to some degree, being incorporated repeatedly into new cells. This fact limits the usefulness of this technique for studying the cells for a prolonged period of time. Despite this objection it was possible to demonstrate progressive and significant changes in fragility of the new red cells and to follow them for approximately 30 days. Estimation of Fe^{59} incorporation into peripheral red cells by the usual techniques indicated a utilization of 65 per cent in Case 1 and 80 per cent in Case 2. This undoubtedly does not represent the true maximum utilization of the isotope for hemoglobin labeling since red cells con-

taining Fe^{59} were being continuously sequestered in the spleen and therefore, removed from the peripheral circulation.

The osmotic fragility of the new red cells was found to be similar to the fragility of the majority of the cells in the peripheral blood. Of particular interest is the fact that none of the new erythrocytes was as osmotically fragile as some of the "conditioned" cells in the patient's peripheral blood. Thus, it is possible from this study to get some idea of the time required for the conditioning process. This would appear to be at least 10 days. From the present evidence it is impossible to say whether the cells are trapped in the spleen for the full period or the conditioning process is the result of recirculation or repeated short episodes of stagnation in the spleen.

Other investigators have studied the changes in red cell fragilities of dogs and humans without hematologic disease. Cruz, Hahn, Bale and Balfour (18), and later, Stewart, Stewart, Izzo and Young (19) using dog erythrocytes labeled *in vivo* with radioactive iron, found that newly formed cells were more osmotically fragile and less mechanically fragile than older cells. As they aged, they became more mechanically fragile, but the osmotic fragility was not significantly altered. In contrast, recent studies in normal man by Simon and Topper (20) and Marks and Johnson (21) have shown that normal young erythrocytes are more resistant to osmotic lysis than older cells.

The observations made after splenectomy on the survival and changes in osmotic and mechanical fragility of the spleen-drip red cells demonstrate that cells with the most marked abnormalities have a short survival and disappear from the circulation within 48 hours. The curves for osmotic fragility changes found in the spleen-drip red cells presented in Figure 6 suggest that there may have been some decrease in osmotic fragility between the time of reinjection of the labeled blood and the first sample studied one hour later. However, it seems more likely that some of the most fragile cells have already been eliminated from the peripheral circulation. Motulsky and co-workers (22) have suggested that the marked increase in osmotic fragility may revert toward normal if the cells are placed in a circulation without a spleen. He transfused red cells labeled with Cr^{51} from a patient with hereditary spherocytosis, who had not

undergone splenectomy and had a cell population consisting of approximately 30 per cent very fragile cells, to the patient's splenectomized brother whose peripheral blood had no very fragile cells. The labeled donor cells showed a gradual decrease in osmotic fragility over a six day period. Since information on survival of these transfused cells is not presented, the observed changes could be due either to a reversible process in the very fragile red cells or to destruction and removal of these cells from the circulation. There is the possibility that the spleen-drip red cells studied in the present experiments had irreversible changes in contrast to the peripheral blood cells studied by Motulsky. He also performed the reverse experiment and transfused labeled red cells from the patient without a spleen into the brother with a spleen. Approximately six days were required for these red cells to show the marked increase in fragility seen in the recipient's own cell population.

The data from the present study demonstrate that in some patients with hereditary spherocytosis there is a progressive increase in osmotic and mechanical fragility of a portion of the red cell population, and that a period of approximately 10 days is required for these changes to reach their maximum from the time the cell first appears in the peripheral circulation. Previous information and the present study certainly implicate the spleen as the source of these very fragile red cells, since they can be found in the splenic pulp and are no longer seen in the peripheral blood after removal of the spleen. From information available at the present time it is impossible to say just how these red cells are conditioned in the spleen. Our experiments on the survival of red cells obtained from the spleen indicate that these very fragile cells have a short life span in the peripheral circulation, less than 48 hours. There was no evidence that the osmotic fragility of these cells reverted toward normal in the absence of the spleen. Therefore, it seems likely that in some patients with hereditary spherocytosis a significant proportion of the red cells is conditioned in the spleen over a period of days and escapes into the peripheral circulation, there undergoing rapid destruction either by the forces of mechanical trauma, osmotic lysis, or undetermined mechanisms.

SUMMARY

Changes in erythrocyte osmotic and mechanical fragility associated with aging and splenic sequestration were studied in two patients with hereditary spherocytosis by the use of Fe^{59} . New red cells showed some increase in fragility, but after approximately 10 days, conditioned cells with a marked increase in osmotic and mechanical fragility appeared in the peripheral circulation. At the time of splenectomy, red cells were recovered from the spleen, labeled with Cr^{51} and reinjected into the patient's peripheral circulation. The conditioned, most fragile cells disappeared rapidly, surviving less than two days; the other red cells, less osmotically and mechanically fragile, had a normal length of survival.

REFERENCES

1. Ham, T. H. *A Syllabus of Laboratory Examinations in Clinical Diagnosis*. Cambridge, Mass., Harvard University Press, 1950.
2. Selwyn, J. G., and Dacie, J. V. Autohemolysis and other changes resulting from the incubation in vitro of red cells from patients with congenital hemolytic anemia. *Blood* 1954, 9, 414.
3. Young, L. E., Izzo, M. J., Altman, K. I., and Swisher, S. N. Studies on spontaneous in vitro autohemolysis in hemolytic disorders. *Blood* 1956, 11, 977.
4. Emerson, C. P., Jr., Shen, S. C., Ham, T. H., Fleming, E. M., and Castle, W. B. Studies on the destruction of red blood cells. IX. Quantitative methods for determining the osmotic and mechanical fragility of red cells in the peripheral blood and splenic pulp; the mechanism of increased hemolysis in hereditary spherocytosis (congenital hemolytic jaundice) as related to the functions of the spleen. *A.M.A. Arch. intern. Med.* 1956, 97, 1.
5. Shen, S. C., Castle, W. B., and Fleming, E. M. Experimental and clinical observations on increased mechanical fragility of erythrocytes. *Science* 1944, 100, 387.
6. Huff, R. L., Hennessy, T. G., Austin, R. E., Garcia, J. F., Roberts, B. M., and Lawrence, J. H. Plasma and red cell iron turnover in normal subjects and in patients having various hematopoietic disorders. *J. clin. Invest.* 1950, 29, 1041.
7. Huff, R. L., Elmlinger, P. J., Garcia, J. F., Oda, J. M., Cockrell, M. C., and Lawrence, J. H. Ferrokinetics in normal persons and in patients having various erythropoietic disorders. *J. clin. Invest.* 1951, 30, 1512.
8. Ebaugh, F. G., Jr., Emerson, C. P., and Ross, J. F. The use of radioactive chromium 51 as an erythrocyte tagging agent for determination of red cell survival in vivo. *J. clin. Invest.* 1953, 32, 1260.

9. Jandi, J. H., Greenberg, M. S., Yonemoto, R. H., and Castle, W. B. Clinical determination of the sites of red cell sequestration in hemolytic anemias. *J. clin. Invest.* 1956, 35, 842.
10. Gray, S. J., and Sterling, K. The tagging of red cells and plasma proteins with radioactive chromium. *J. clin. Invest.* 1950, 29, 1604.
11. Necheles, T. F., Weinstein, I. M., and Leroy, G. V. Radioactive sodium chromate for the study of survival of red blood cells. I. The effect of radioactive sodium chromate on red cells. *J. Lab. clin. Med.* 1953, 42, 358.
12. Dacie, J. V. *The Haemolytic Anaemias; Congenital and Acquired.* New York, Grune and Stratton, 1954.
13. Young, L. E. Hereditary spherocytosis. *Amer. J. Med.* 1955, 18, 486.
14. Emerson, C. P. The influence of the spleen on the osmotic behavior and the longevity of red cells in hereditary spherocytosis (congenital hemolytic jaundice), a case study. *Boston Med. Quart.* 1954, 5, 65.
15. Pranker, T. A. J., Altman, K. I., and Young, L. E. Abnormalities of carbohydrate metabolism of red cells in hereditary spherocytosis (abstract). *J. clin. Invest.* 1954, 33, 957.
16. Young, L. E., Platzer, R. F., Ervin, D. M., and Izzo, M. J. Hereditary spherocytosis. II. Observations on the role of the spleen. *Blood* 1951, 6, 1099.
17. Weisman, R., Jr., Ham, T. H., Hinz, C. F., Jr., and Harris, J. W. Studies of the role of the spleen in the destruction of erythrocytes. *Trans. Ass. Amer. Phycns* 1955, 68, 131.
18. Cruz, W. O., Hahn, P. F., Bale, W. F., and Balfour, W. M. The effect of age on the susceptibility of the erythrocyte to hypotonic salt solutions. Radioactive iron as a means of tagging the red blood cell. *Amer. J. med. Sci.* 1941, 202, 157.
19. Stewart, W. B., Stewart, J. M., Izzo, M. J., and Young, L. E. Age as affecting the osmotic and mechanical fragility of dog erythrocytes tagged with radioactive iron. *J. exp. Med.* 1950, 91, 147.
20. Simon, E. R., and Topper, Y. J. Fractionation of human erythrocytes on the basis of their age. *Nature (Lond.)* 1957, 180, 1211.
21. Marks, P. A., and Johnson, A. B. Relationship between the age of human erythrocytes and their osmotic resistance: A basis for separating young and old erythrocytes. *J. clin. Invest.* 1958, 37, 1542.
22. Motulsky, A. G., Cassard, F., Giblett, E. R., Broun, G. O., Jr., and Finch, C. A. Anemia and the spleen. *New Engl. J. Med.* 1958, 259, 1215.