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Authors
Berlin, N.J.
Lawrence, J.H.

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ISOTOPES IN THE STUDIES OF TOTAL RED-CELL VOLUME, OF RATE AND SITE OF RED-CELL PRODUCTION AND DESTRUCTION, AND OF RED-CELL LIFE SPAN

N. I. Berlin and John H. Lawrence

Donner Laboratory
University of California
Berkeley, California

With the development of the cyclotron and nuclear reactors, it became possible to produce radioactive isotopes of elements associated with normal metabolic processes. As a result it has become possible to follow the metabolism of a given element or substance by the introduction of a labeled isotope into the body. Recent great improvements in the methods for measurement of radioactive isotopes, particularly the developments in scintillation counters, have made it possible to carry out tracer studies in laboratories or hospitals with relative ease. For most of these studies it is not necessary to have expensive or complex equipment.

In the evaluation of erythropoiesis, one must know the total volume of circulating red cells, the rate of production of red cells, and the life span of red cells. Knowing these three values, one can then determine whether an individual is normal, polycythemic, or anemic, and also whether an increase or decrease in the total circulating red-cell volume is due to a change in the life span of red cells or in the rate of production of red cells, or to both.

METHODS

Blood Volume

The availability of radioactive isotopes of iron, phosphorus, chromium, potassium, and thorium makes it possible to measure the total circulating red-cell volume with accuracy and ease. The isotopes phosphorus-32, [1] chromium-51, [2] potassium-42, [3] and thorium-C [4] may be incorporated into the red cell in vitro. This permits the labeling of an individual's own red cells. Iron, on the other hand, must first be administered to a donor, whose red cells are subsequently injected into the individual whose blood volume is to be determined. Although the radioiron method would be the ideal method for determining blood volume, it has not found wide usefulness in clinical work because of the necessity for maintaining a pool of donors of various blood groups and the need for cross-matching, blood typing, and the various serological studies that are required before transfusion. Furthermore,

the dose of radioiron to be administered to the donor is so large that the radiation to the bone marrow approaches tolerance values. In experimental animals, however--particularly in the rat, where inbreeding results in a uniform strain of animals in which cross-matching is no problem--the iron method has been of great value in the determination of blood volume.

For the P^{32}, Cr^{51}, and K^{42} methods in which red cells are labeled in vitro, it is necessary to remove only a small quantity of blood (10 to 15 cc) from the invididual and to "incubate" these cells with the isotope at 37°C with constant mixing. In general, more than 50% of the isotope will have been incorporated into the red cells at the end of one hour. The red cells are then washed to remove the excess, unincorporated isotope and are resuspended in either saline or plasma. The reconstituted whole blood containing the labeled red cells is then administered to the patient, and after adequate time for mixing a sample is withdrawn. An aliquot of the blood injected into the patient is kept and the total radioactivity administered is determined from it. Thus, knowing the total radioactivity administered and the radioactivity per milliliter of whole blood withdrawn from the patient, one may calculate the blood volume. The total red-cell volume is determined from the peripheral hematocrit corrected for trapped plasma. Actually, this method is precise. The plasma volume can be calculated from the total red-cell volume and the hematocrit. Although there has been some objection to this method (because several studies indicate that the hematocrit of the large-vessel blood may not be the same as that in the smaller vessels), the authors do not consider this to be a significant factor and believe that the plasma volume can be calculated with a high degree of precision from the total red-cell volume and the venous hematocrit.[5)]

Of P^{32}, Cr^{51}, and K^{42}, the isotope of choice for the determination of blood volume is Cr^{51}. It is easily measured in a well-type scintillation counter; the rate of elution of Cr^{51} from the red cell is relatively slow compared with that of P^{32}, and the physical half life is long compared with K^{42}. While many valuable studies have been made using P^{32}, most workers have now adopted the Cr^{51} method. The usefulness of K^{42} is limited by its short half life (12.4 hours), which means that the worker must have access to a facility for its production. The ThC method has been explored by Hevesy's and Nylin's groups, and should prove valuable. It has not been used extensively in other laboratories although it does offer some promise for greater ease of labeling red cells than K^{42}, Cr^{51}, and P^{32}. The radiation dose involved in the use of ThC remains to be established. The rapid physical decay of the other isotopes presents no radiation hazard to the individual receiving the isotope.

The results obtained with these methods show

for normal males:

average total blood volume	69.0 cc/kg of body weight,
average plasma volume	38.7 cc/kg,
average total red-cell volume	29.9 cc/kg;

for sixteen normal females studied in this laboratory:

average total blood volume	64.4 cc/kg of body weight,
average plasma volume	37.0 cc/kg,
average total red-cell volume	27.0 cc/kg.

(This was the first study of the blood volume of the normal female by isotopic methods.)

Subsequently, Wadsworth reported, for a series of eight normal women,

average total blood volume	66.5 cc/kg,
average plasma volume	43.1 cc/kg (by the Evans-Blue method),
average total red-cell volume	23.4 cc/kg (by the P^{32} method).

The value for the total red-cell volume in the males, 30 cc/kg, appears to be well confirmed.

Red-Cell Production

At present there is only one method for the direct determination of the rate of red-cell production, and that is the measurement of the rate of disappearance of radioactive iron from the plasma and subsequent uptake of radioiron in the red cells.[9] An analysis of the rate of disappearance of radioiron from the plasma indicates that approximately 80% to 90% of the iron is removed by a first-order process. That is, for each given time period a constant fraction of the radioiron remaining in the plasma is cleared, largely by the bone marrow and to some extent by the liver, spleen, and other tissues. From the rate of disappearance of radioiron from the plasma, the turnover of iron in the plasma can be calculated, as follows:

$$\frac{(0.693) \times (\mu g\ Fe/ml \text{ in plasma}) (\text{Plasma volume in ml}) \times (24 \text{ hours})}{\text{Half time in hours}}$$

This necessitates knowing the plasma iron concentration, which may be determined by several methods. We use the method of Kitzes et al.[10] The plasma volume is determined from the blood volume by use of labeled cells, as described above. After the determination of the rate of removal of radioiron from the plasma, a study is made of the uptake of iron in the red cells. At approximately 7 to 10 days, most of the iron that is going to appear in the red cells has been cleared from the plasma to the bone marrow and has been incorporated into hemoglobin, and the mature red cells containing the radioiron are in the peripheral blood. Thus, the percentage of iron that passes through the plasma, ultimately to reach the peripheral blood as red cells, may be determined at approximately 7 to 10 days following the original injection of the isotope.

This now completes the data required for determination of the rate of production of red cells. In general it is found that approximately 0.26 milligram of iron is utilized for red-cell production per kilogram per day in a normal male. Much higher values are found in polycythemia. In leukemia these values are usually normal or increased to two to three times normal, whereas in certain anemias the values may be normal, increased, or (in case of failure to produce red cells) as low as one-fifth to one-third normal.[9]

The use of scintillation counters placed over the spleen, liver, and sacrum--the latter representing bone marrow--makes it possible to determine the time course in distribution of the radioiron in these tissues.[11] (Fig. 1). This shows that there is a rapid clearance of iron from the bone marrow and a later release of the iron from the marrow to the peripheral red cells.

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Some iron reaches the liver and the spleen and thereafter is slowly removed from these tissues. It is this slow release from the spleen and liver that accounts for the slight rise of radioiron in the peripheral blood after 7 to 10 days.

Red-Cell Life Span

Prior to the introduction of isotopes into the study of red-cell physiology, it was possible to measure the life span of the red cell by the Ashby Differential Agglutination technique.[12] To obtain valuable data by use of the Ashby technique, one must administer a large volume of blood (approximately 500 cc) from a suitable donor. This method has usually been applied to the transfusion of normal red cells to an individual with a disease state, and yields information regarding only the fate of normal cells in an abnormal environment. Some studies have been carried out by transfusing cells from a donor having sickle-cell anemia to a normal individual, and in this experiment the survival of sickle cells in a normal environment could be observed. The Ashby method, however, does not permit the determination of the life span of the red cell in its own environment.

In a comparison of the various methods for study of red-cell life, the Ashby method might be criticized because normal cells are placed in a pathologic environment or pathologic red cells in a normal environment, and red cells are never studied in their normal environment. In most normal individuals, the three methods give comparable results. We have seen one instance, however, in which the values by the Ashby technique indicated a longer red-cell life than those techniques using the C^{14} and Fe^{59} methods. There was another case in which the Ashby technique showed a normal red-cell life, but the C^{14} and Fe^{59} results showed a shortened red-cell life. The conclusion might be drawn from these findings that the individual's own cells were qualitatively poor, while the destructive mechanisms were normal, and therefore the normal donor cells survived their normal life span.

With the demonstration by Shemin and Rittenberg[13] that the nitrogen atom of glycine is specifically used for the four nitrogen atoms of hemin and the preparation of N^{15}-labeled glycine, it became possible to study the life span of the red cell in its own environment. This study demonstrated that the normal life span of the red cell was approximately 120 days, and valuable information on the red-cell life in sickle-cell anemia and in pernicious anemia was also obtained. In our laboratory C^{14}-labeled glycine was introduced for such studies[14] because it has been shown that the alpha-carbon atom is the source of 8 of the 34 carbon atoms of glycine[15], and N^{15} is much more difficult to measure.

Some indication of red-cell life span may also be obtained by following the activity of radioiron in the peripheral blood. This method is less satisfactory than the C^{14} method, however, since 70% to 90% of red-cell iron liberated at the end of the life span of a red cell is reutilized for the formation of new red cells. As the red cells reach the ends of their life spans, the radioiron is released and reutilized, and there is no sharp drop in the curve of Fe^{59} hemoglobin activity. This disadvantage can be overcome in experimental animals by loading the animal with iron after the red cells are labeled.[16] Thus, when the red-cell iron is released, it is released into a large pool of iron, and the specific activity becomes considerably lower, so that when it is reutilized in red-cell formation it is of a much lower specific activity than that originally present in the first labeled cells. A sharp

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drop in the curve for the Fe^{59} hemoglobin specific activity is noted, and the red-cell life can be determined.

Recently, Cr^{51} has been introduced as the labeling agent for study of the life span of the red blood cells.

Although the demonstration of the rate of elution of Cr^{51} from the labeled red cells in vivo is very slow, it has now become possible to obtain a measure of the red-cell life span by following the rate of disappearance of Cr^{51} following the labeling of the cells; these studies do not give an absolute value for the red-cell life span, but they may be used to compare a red-cell life span in a given state with that of a normal.[17-19] It should be emphasized that this is a valuable method for clinical determination of alterations in the life span of the red blood cell, but that the values obtained--even with the present methods of analysis--are not comparable with the actual value of red-cell life span, and must be compared with the normal as determined with this method. Various methods of analysis have been proposed for interpretation of the curves of the graphs obtained. At the present time, however, we feel that these have not been completely substantiated.

DISCUSSION

With the development of methods for determining total red-cell volume, the rate of production of red cells, and the life span of the red cell, it became possible to assay the erythropoietic state. Anemia or polycythemia can only arise from some change in the rate of production or in the life span of the red cell. This may even apply to bleeding states. Although the rate of production of red cells would conceivably be unaffected, the life span of the cells would be shortened in the bleeding state in the sense that cells did not survive their normal life span, and thus the resulting anemia would be due to a loss of red cells rather than to a failure to produce.

The methods described earlier have been applied simultaneously in the study of various hematopoietic disorders, such as polycythemia, leukemia, aplastic anemia, hemolytic anemia, and hypersplenism.

Polycythemia

In polycythemia vera it has been clearly demonstrated that there is a considerable increase--in some patients as much as 10 times normal--in the quantity of iron incorporated into hemoglobin per day. The in vivo iron studies show that this increase is due to hyperactivity of the bone marrow. In most patients with polycythemia vera the liver and spleen are not significant sites of red-cell formation, although occasional instances of splenic erythropoiesis are observed. Studies with N^{15} of the life span of the red cell indicated a normal life span.[20] Data from the C^{14} studies,[21] however, can best be explained by postulating the presence of two populations of red cells, one having a normal life span and the other a shortened red-cell life. This hypothesis fits well with the iron data, which show that there must be a considerable increase in the amount of iron released from red cells per day, and this can be explained only by supposing that either all the cells have a shortened life span or that some of the cells have a short cell life.

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In individuals with polycythemia secondary to either congenital heart disease, acquired pulmonary disease, or residence at high altitude, it is found that there is an increase in the rate of utilization of radioactive iron for the production of red cells.[22] However, the increase is directly proportional to the increase in total red-cell volume. Studies of individuals having a polycythemia secondary to residence at high altitude show a normal red-cell life span.[23] When sea-level dwellers are taken to high altitude there is an immediate and rapid increase in the rate of utilization of iron for red-cell formation.[22] However, their red cells have been found to have a normal life span during the period of acclimatization.[24] Therefore, the mechanism for the development of polycythemia at altitude is entirely confined to an increase in the rate of production of red cells and does not involve an alteration of the life span of the red cell. When individuals with polycythemia secondary to residence at high altitude are taken to sea level, there is a remarkable decrease in the rate of production of red cells.[22] The increased urobilinogen excretion shortly after arriving at sea level might be evidence that there is premature destruction of red cells under these conditions. Studies of the life span of the red cell by the C^{14} method are now in progress to determine whether there is actually a shortening of cell life following descent from high altitude.

Leukemia

Until recently the anemia associated with leukemia has been attributed to a crowding out of the erythropoietic elements of the bone marrow by leukemic tissue, and it has been assumed that the anemia resulted from a failure to produce red cells. This has not been borne out by studies of chronic myelogenous and lymphatic leukemia with radioiron and C^{14}-labeled glycine. Patients with chronic leukemia have been shown to produce a normal, and in many instances a greater than normal, number of red cells a day.

In all patients with chronic myelogenous leukemia studied by in vivo iron methods, there has been evidence of production of red cells in the spleen and possibly some destruction in the spleen.[25] This does not occur in most patients with chronic lymphatic leukemia.

C^{14} studies in myelogenous leukemia show that the red-cell life span is shortened but is finite (as compared to the pattern of random destruction as seen in a hemolytic process).[26] This was true of all patients studied. In chronic lymphatic leukemia, however, the life span is either normal or shortened, but if it is shortened, the pattern is one of random destruction of red cells. In myelogenous leukemia there is a relatively uniform red-cell life span of about 75 to 90 days. In lymphatic leukemia, when shortening of red-cell life was observed, the average life span of the cells was approximately 20 to 30 days.[23]

Aplastic Anemia

Studies of one patient with aplastic anemia (F. W.) showed that the rate of production of red cells as indicated by the rate of disappearance of radioiron from the plasma was considerably decreased. The in vivo iron studies showed a normal pattern (Fig. 2). The red-cell life span was found to be 100 days, which is a low normal, or at most a slightly shortened life span

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(Fig. 3). Thus, in this individual we were able to demonstrate that the anemia, or decreased total red-cell volume, could be attributed to a failure of the bone marrow to produce an adequate number of red cells. The red cells that were produced were apparently almost normal qualitatively.

Hemolytic Anemia

The following case history illustrates one diagnostic problem studied by these methods.

A 23-year-old male (Patient C.O.) was referred for diagnostic studies in August 1953. He had experienced several episodes of jaundice, and three years previously a diagnosis of hemolytic anemia of unknown etiology had been made. His initial red-cell count was 2.55 million, with 9.1 grams of hemoglobin. The reticulocyte count was 5.4%. A bone-marrow biopsy showed hyperplasia of the hematopoietic tissue, with replacement of the adipose tissue. There appeared to be a considerable increase in the number of cells of the erythroid series. The Coombs test was negative. Serum bilirubin was elevated, and the icteric index was 26 units. Total red-cell volume was 20.9 cc/kg of body weight; plasma volume, 48.9 cc/kg.

Radioiron studies on this patient, who was anemic by red-cell volume standards, showed a rapid turnover of radioiron and an increase in the rate of production of red cells (Fig. 4). In vivo iron studies showed a rapid rise in the counting rate over the spleen, which was interpreted as indicating an early destruction of red cells. Also, it should be noted that only 30% of the iron could be detected in the red cells at any one time (as compared with 70% to 90% of the injected activity in normals). This would occur if there were a fast cycling of the iron through red cells and back into the bone marrow and spleen.

A splenectomy was performed, and a normal in vivo iron pattern was observed (Fig. 5), with 80% to 90% of the injected activity noted in red cells at 7 to 10 days. The repeat studies now showed the plasma iron turnover to be decreased to approximately half its former rate, and the rate of incorporation of iron into red cells was also decreased, although it remained faster than normal. In vivo iron studies showed no secondary spleen curve as before, and the marrow curve was of more normal pattern. Thus, in this individual we have been able to demonstrate a remarkable change in the turnover of radioiron following a splenectomy.

Hypersplenism

Recently we have had an opportunity to study a number of individuals with a syndrome that we call the short-red-cell-life syndrome. All these patients had splenomegaly, and their bone marrows were histologically abnormal, generally showing extensive areas of myelofibrosis with little if any hematopoietic tissue remaining. These patients show a rapid disappearance of iron from the plasma, and all had adequate or greater than adequate iron turnover for red-cell formation. The in vivo iron patterns showed an early, marked rise in the counting rate over the spleen, with maintenance of this counting rate over the spleen, indicating early destruction of red cells. The C^{14} red-cell life span studies showed random destruction of the red

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cells, with a red-cell life to 20 to 30 days. In one patient (M. B.) a normal red-cell life span was observed following administration of adrenal cortical hormones (Fig. 6). In another patient (A. F., Figs. 7-9), the red-cell life span returned to normal following a splenectomy.

CONCLUSION

In conclusion, then, it may be stated that these three methods--namely, blood-volume determination, iron studies, and the measurement of the life span of the red cell--permit an adequate description of the pathogenesis of anemia or polycythemia. They have been of great value in selecting patients with unusual anemias that may or may not be related to hypersplenism when splenectomy is under consideration.

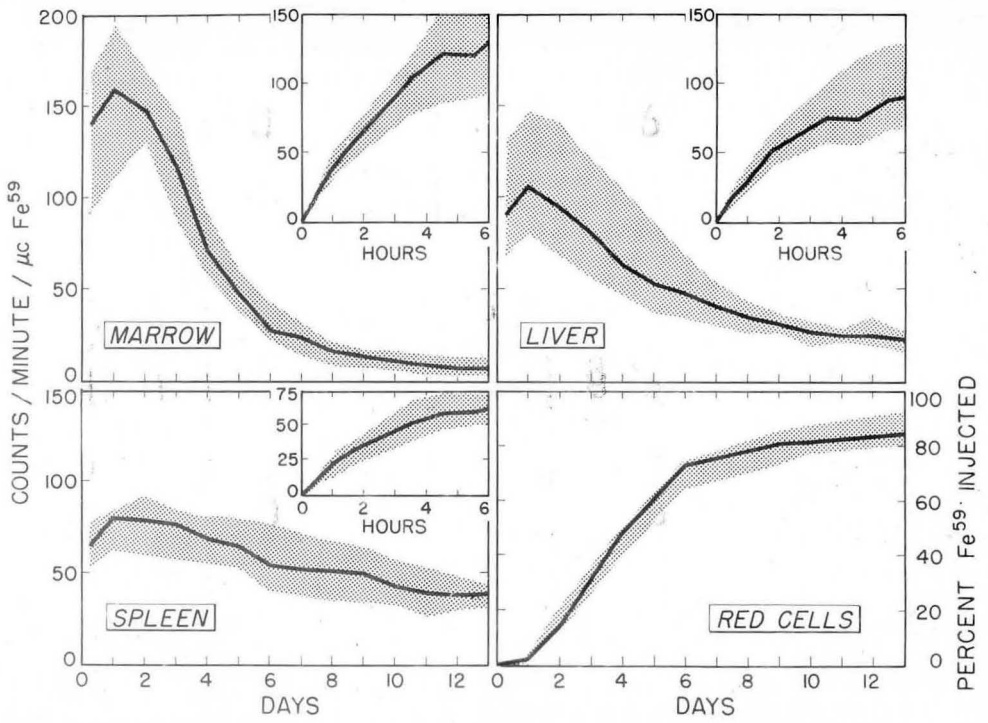

Fig. 1 Uptake of Fe^{59} by red cells, bone marrow, liver, and spleen plotted against time in normals. Shaded area indicates range.

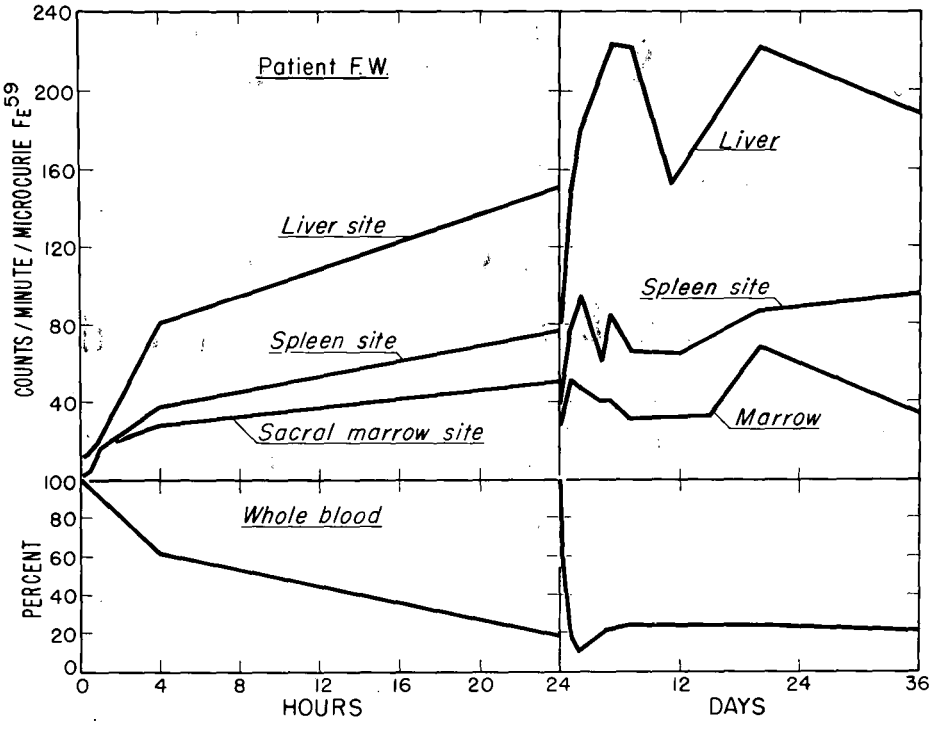

Fig. 2 Uptake of Fe^{59} in red cells and by bone marrow, liver, and spleen with time in Patient F. W.

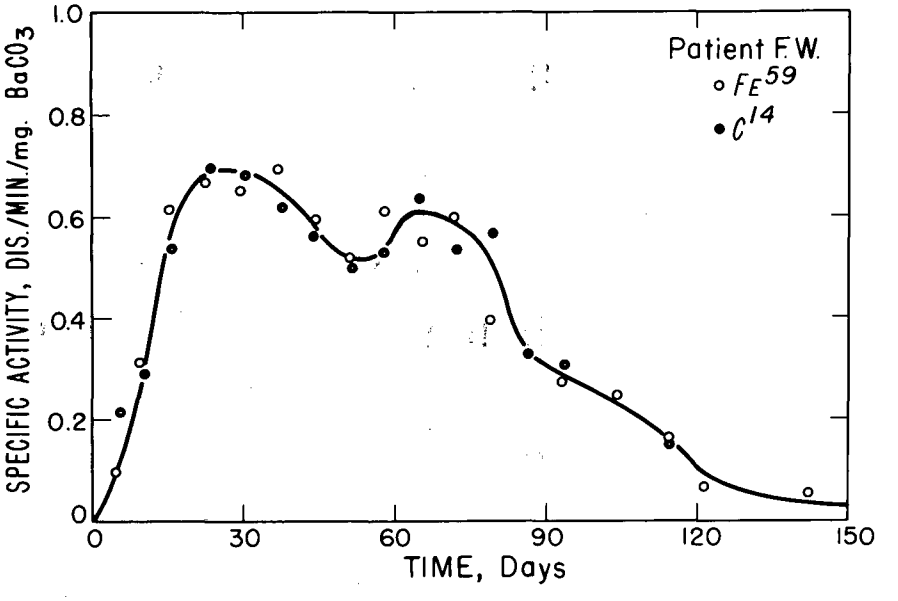

Fig. 3 The life span of the red cell in Patient F. W., as measured with Fe^{59} and C^{14}.

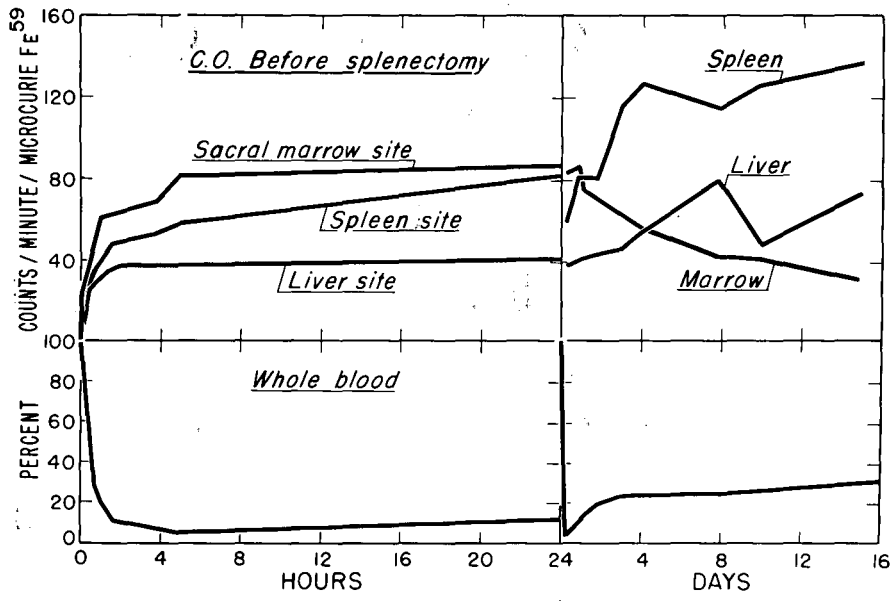

Fig. 4 Uptake of Fe^{59} in red cells and by bone marrow, liver, and spleen in Patient C.O. before splenectomy.

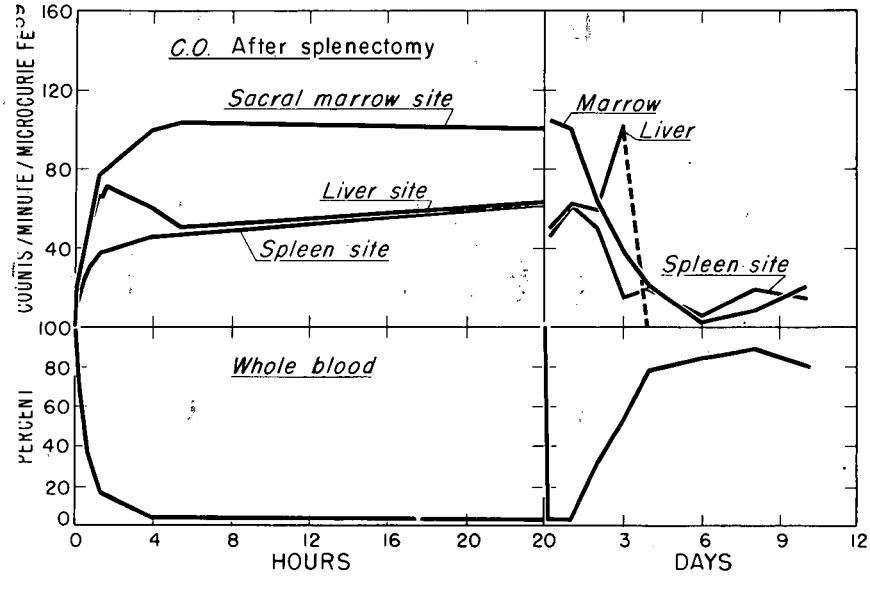

Fig. 5 Uptake of Fe^{59} in red cells and by bone marrow, liver, and spleen in Patient C.O. after splenectomy.

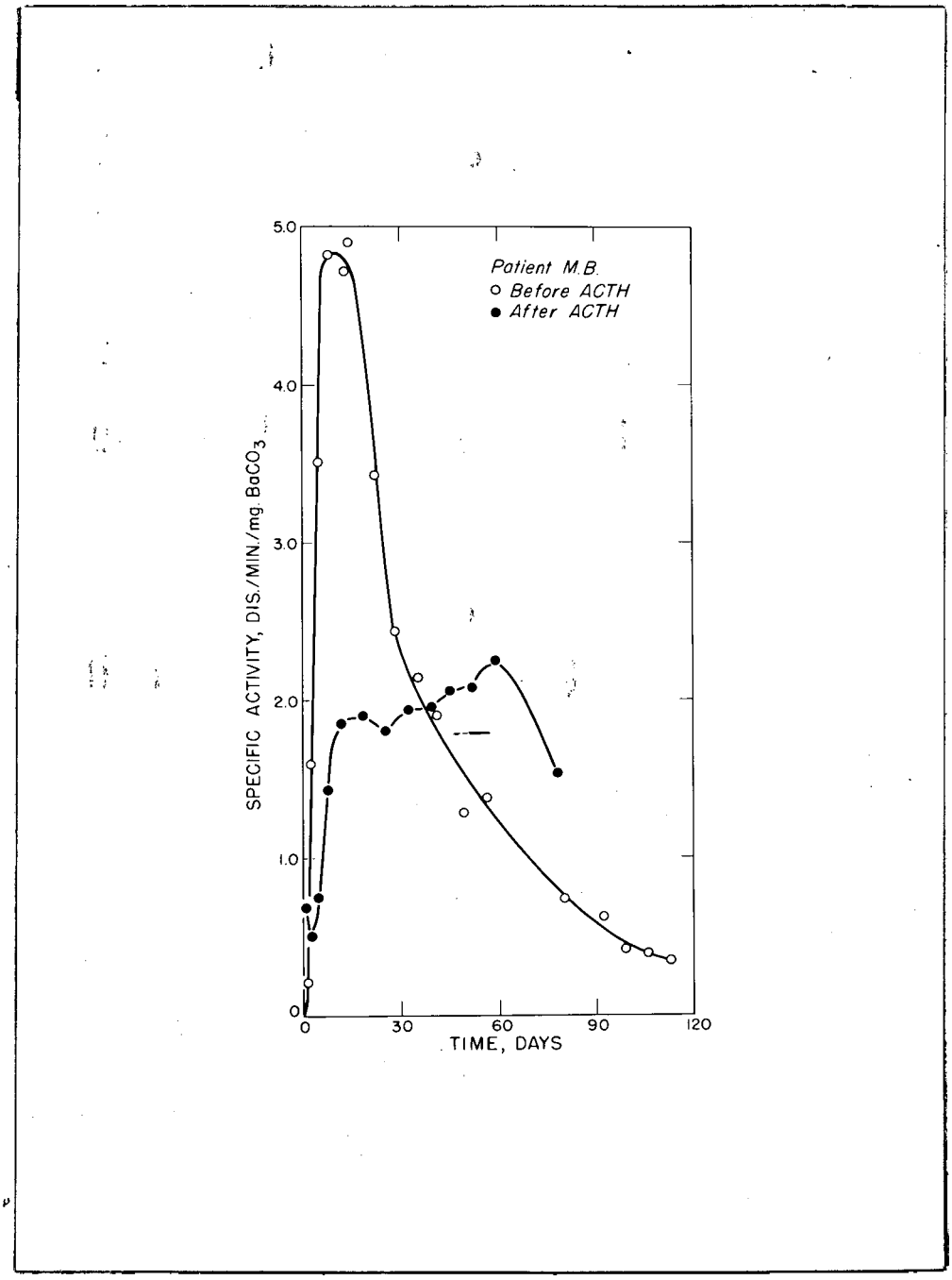

Fig. 6 The life span of the red cell in Patient M. B. before (open circles) and after (black dots) treatment with ACTH.

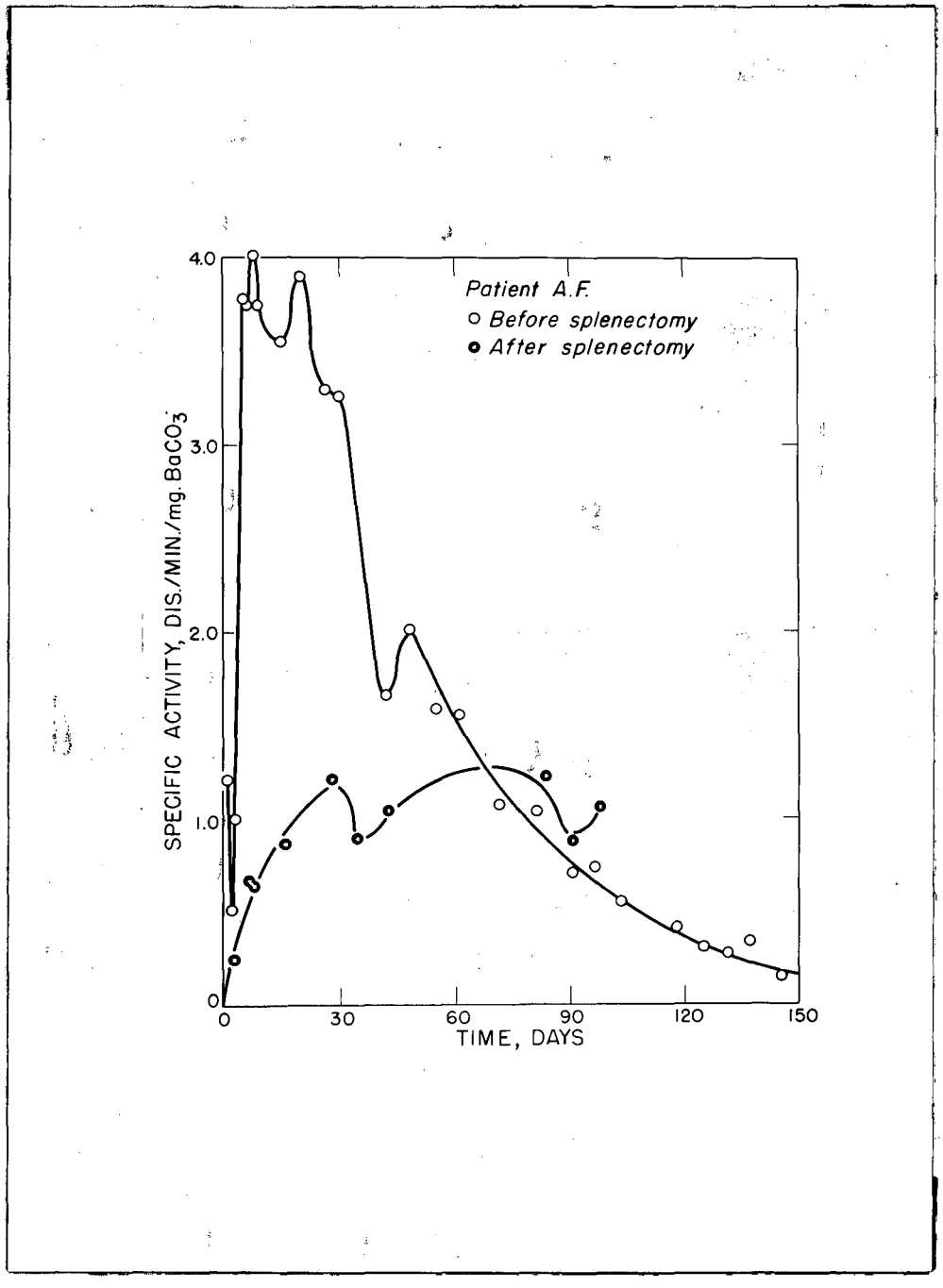

Fig. 7 The life span of the red cell in Patient A. F. before (open circles) and after (black dots) splenectomy.

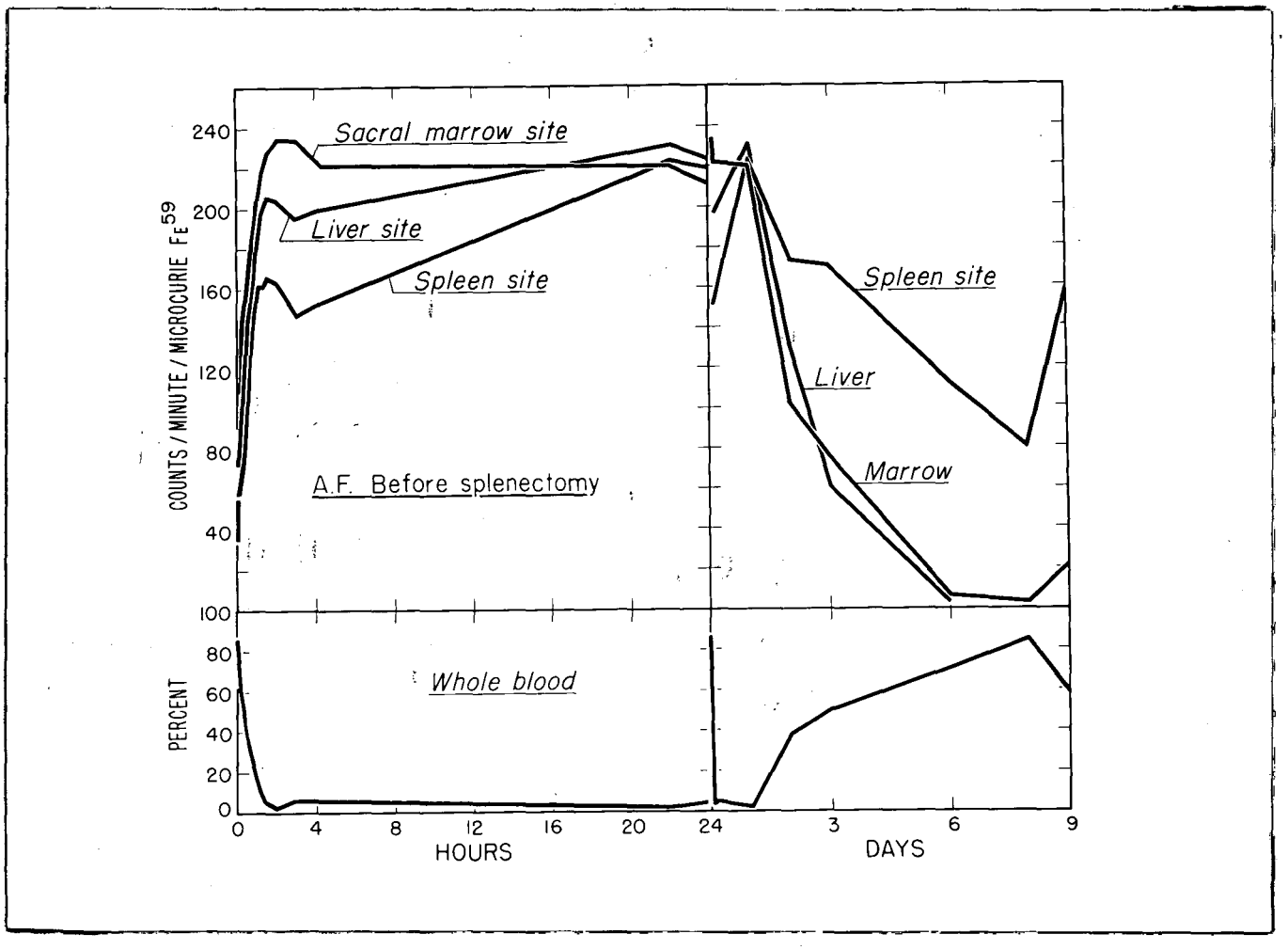

Fig. 8 Uptake of Fe^{59} in red cells and by bone marrow, liver, and spleen in Patient A. F. before splenectomy.

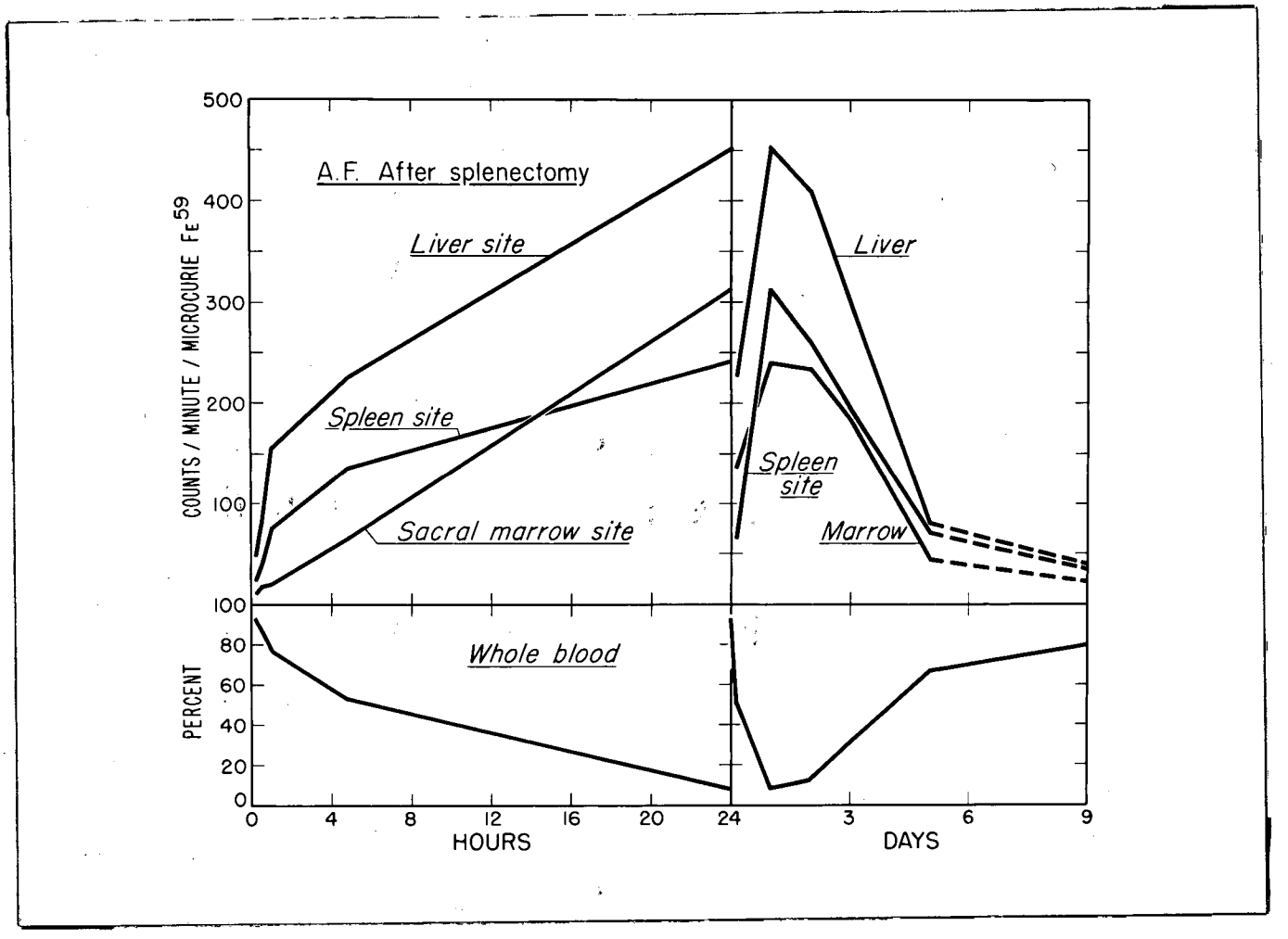

Fig. 9 Uptake of Fe^{59} in red cells and by bone marrow, liver, and spleen in Patient A. F. after splenectomy.

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