Recent Advances in the Knowledge of Total Red Cell Volume, Production and Destruction

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For evaluation of the erythropoietic state, the total volume of circulating red cells, the rate and site of production of red cells, and their life span must be known. 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 red cell life span or in the rate of production of red cells, or to both can then be determined from the total red cell volume, radioiron, and red cell life span studies.

Methods

Blood Volume and Total Red Cell 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, chromium-51, potassium-42, and thorium-234 can be incorporated into red cells in vitro, permitting the labeling of an individual's own red cells. Radioiron, on the other hand, must first be administered to a donor, whose labeled red cells are subsequently injected into the individual whose blood volume is to be determined. The radioiron method would be ideal for determining blood volume since, unlike P32, Cr51, K42, and ThB, once incorporated into the hemoglobin of the red cell, it remains there for the duration of the life span of the red cell. However, it has not found wide use in clinical work because of the necessity of maintaining a pool of donors from various blood groups and the need for cross-matching, blood typing, and the various serologic and clinical studies that are required before transfusion. Furthermore, the dose of radioiron to be administered to the donor is such 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 radioiron method has been of great value in the determination of blood volume.

For the P32, Cr51, and K42 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 individual and to "incubate" these cells with the isotope at 37 C. with constant mixing. With P32, approximately 30 per cent of the isotope will be incorporated in the red cells at the end of one hour. The red cells are then washed to remove the excess, unincorporated isotope. With K42 and Cr51, red cell uptakes of 80-90 per cent and better are obtained, and with corrections for amount of unincorporated isotope injected, washing of the red cells may be eliminated. The red cells are then resuspended in either saline or plasma, and the reconstituted blood administered to the patient. After adequate time for mixing (15 to 20 minutes) a sample is withdrawn. The total radioactivity administered is determined from an aliquot of the blood injected into the patient. Thus, knowing the total radioactivity administered and the radioactivity per milliliter of red blood cells after mixing, the total red cell volume can be calculated.

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total blood volume is determined from the total red cell volume and the hematocrit correcte for trapped plasma. This is a precise method for the determination of the total red cell volume and is independent of the venous hematocrit. The plasma volume can be calculated from the total red cell volume and the hematocrit. There has been objection to this method of calculation of the plasma volume, since several studies indicate that the venous hematocrit may not be the same as that in the smaller vessels and thus not be representative of the “total body” hematocrit. We do not believe that the “total body” hematocrit is significantly different from the venous hematocrit, and we feel that the plasma volume can be calculated with a high degree of precision from the total red cell volume and the venous hematocrit.

At present the isotope of choice for the determination of blood volume is Cr. It is easily measured in a well-type scintillation counter. The rate of elution of Cr from the red cell is relatively slow compared with that of P, and the physical half-life is long compared with K. While a number of studies have been made using P, many workers have now adopted the Cr method. The usefulness of K 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 ThB method has been explored by Hevesy and Nylin and should prove valuable. It has not been used in other laboratories although it does offer some promise for greater ease of labeling red cells than K, Cr, and P. The radiation dose involved in the use of ThB has been calculated and shown not to be a hazard. No radiation hazard is involved in the use of K, Cr, and P because of the rapid physical decay and quantities used. The results obtained in normals with these methods are presented in table 1.

**Red Cell Production**

At the present time there is only one method for the direct estimation of the rate of red cell production. This is done by measurement of the rate of disappearance of radioactive iron from the plasma and subsequent uptake of radioiron in the red cells. After intravenous injection approximately 90 per cent of the iron is removed from the plasma 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:

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\text{Plasma iron turnover in micrograms per day} = \frac{(0.693) \times (\mu g. \text{ Fe/mI plasma}) \times (\text{plasma volume in mL}) \times (24 \text{ hours})}{(\text{Plasma radioiron half-time in hours})}
\]
The plasma iron concentration is determined by the method of Kitzes et al.\textsuperscript{15} The plasma volume is determined from the blood volume by use of labeled cells, as described above. After the rate of removal of radioiron from the plasma is determined, a study is made of the uptake of radioiron in the red cells. At approximately 7 to 10 days, most of the radioiron that is going to appear in the red cells has been cleared from the plasma to the bone marrow, has been incorporated into hemoglobin, and the mature red cells containing radioiron are in the peripheral blood. Thus, the percentage of iron that passes through the plasma ultimately to reach the peripheral blood may be determined at approximately 7 to 10 days following the original injection of the isotope. Recently several workers have postulated more complex models of iron kinetics, indicating that in some instances this method of calculation may lead to an unduly large iron turnover for red cell formation.\textsuperscript{16–18}

This now completes the data required for estimation of the rate of production of red cells. In normal males, it is found that approximately 0.26 milligram of iron is utilized for red cell production per kilogram of body weight per day (fig. 1). Much higher values are found in polycythemia, both primary and secondary. 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 the case of failure to produce red cells, such as in aplastic anemia, as low as one-fifth to one-third normal.\textsuperscript{13}

Finally, 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 of the distribution of radioiron in these tissues (fig. 2).\textsuperscript{19} This shows that there is a rapid clearance of iron by the bone marrow and a later release of the iron from the marrow to circulating adult red cells. 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 in radioiron content of the peripheral blood after 7 to 10 days. Several types of curves have been observed for in vivo counting over the spleen. In normal sub-

![Figure 1](chart.png)

**Fig. 1.** Milligrams of iron per kilogram per day for red cell production in normal subjects, in patients with polycythemia vera, and in patients with chronic leukemia.
FIG. 2. Uptake of Fe$^{59}$ by red cells, bone marrow (sacrum), liver, and spleen plotted against time in normals. Shaded area indicates range of normals.

jects there is a rapid rise in spleen count and fall (see fig. 2), and as compared to the activity observed over the marrow site the level is low. The second type is the secondary, or erythroclastic, spleen curve. In this instance, there is an early rise and fall followed by a secondary rise in counting rate over the spleen. The second rise occurs with the appearance of labeled cells in the peripheral blood. This type of curve has been interpreted as indicating random destruction of red cells. The third type is the primary, or erythrogenic. In this instance the counting rate over the spleen site rises rapidly to a higher than normal level and declines, the decline being associated with the rise in Fe$^{59}$ count in the peripheral blood. This curve is similar to that seen by the counting of the marrow in normal subjects and is associated with splenic production of red cells. The fourth type is that in which there is superimposed a second rise in the spleen activity on the curve of the third type. The combination may be interpreted as indicating both red cell production and red cell destruction in the spleen. A similar technic using Cr$^{51}$ labeled red cells has been used to demonstrate sequestration of red cells in the spleen.

**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 clinically only by the Ashby differential agglutination technic. To obtain satisfactory data with this method it is necessary to administer a large volume of blood (approximately 500 cc.) from a suitable donor. This method has usually involved 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. Many studies have also been carried out by transfusing cells from a donor having one type of anemia to a normal individual, and in this experiment the survival of abnormal cells in a
normal environment can be observed.\textsuperscript{31} The Ashby method, however, does not permit the determination of the life span of the red cells in their own environment.

With the demonstration by Shemin and Rittenberg\textsuperscript{24} that the nitrogen atoms of hemin are derived from glycine, it became possible to study the life span of the red cell in its own environment.\textsuperscript{25} This study demonstrated that the normal life span of the red cell is approximately 120 days. Valuable information on the red cell life span in sickle-cell anemia, pernicious anemia, and polycythemia vera was obtained.\textsuperscript{24} In our laboratory, alpha-\textsuperscript{C\textsubscript{14}} labeled glycine was introduced for such studies in man\textsuperscript{27} because it had been shown that the alpha-carbon of glycine is the source of 8 of the 34 carbon atoms of hemin.\textsuperscript{28} In addition, \textsuperscript{C\textsubscript{14}} is easier to measure and the sensitivity of the method for measurement of \textsuperscript{C\textsubscript{14}} is greater than that of \textsuperscript{N\textsubscript{15}}.

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 \textsuperscript{N\textsubscript{15}} or \textsuperscript{C\textsubscript{14}} methods, however, since 70 per cent to 90 per cent of the red cell iron liberated at the end of the life span of a red cell is reutilized in 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 \textsuperscript{Fe\textsuperscript{59}} hemoglobin activity as seen with \textsuperscript{C\textsubscript{14}} and \textsuperscript{N\textsubscript{15}}. This disadvantage can be overcome in experimental animals by loading the animal with nonradioactive iron after the red cells are labeled.\textsuperscript{29} Thus, in the method of Burwell and co-workers, when the red cell iron is released, it is released into a large pool of iron with a large reduction in the specific activity, so that as it is reutilized for red cell formation it is of a much lower specific activity than that originally present in the labeled cells. A sharp drop in the curve for the \textsuperscript{Fe\textsuperscript{59}} specific activity of hemoglobin is noted, from which the red cell life span can be determined.

Recently, \textsuperscript{Cr\textsuperscript{51}} has been introduced as the labeling agent for the study of the life span of the red blood cells. With the demonstration that in normal subjects the rate of elution of \textsuperscript{Cr\textsuperscript{51}} from the labeled red cells in vivo is very slow (approximately 1\% per day), it has now become possible to obtain an estimate of the red cell life span by following the in vivo rate of disappearance of \textsuperscript{Cr\textsuperscript{51}}.\textsuperscript{*} These studies do not directly yield an absolute value for the red cell life span, but they may be used to compare red cell life span in a given state with that of normal.\textsuperscript{7, 10-12} It should be emphasized that this is a method for clinical determination of alterations in the life span of the red blood cell, but that the experimental values obtained are not the \textit{actual} values 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 the interpretation of the curves of the graphs obtained and for the calculation of the red cell life span.

In comparing the various methods for study of red cell life span, 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 normal individuals, the Ashby, \textsuperscript{C\textsubscript{14}}, and

\textsuperscript{*}The possibilities that the rate of elution may be altered in pathologic conditions and/or that chromium itself, while not injurious to normal red cells in the quantity used, may affect the survival of abnormal cells have not yet been adequately explored.
Fe\textsuperscript{59} methods give comparable results. In disease states we have seen one instance, however, in which the values by the Ashby technique indicated a longer red cell life than the C\textsuperscript{51} and Fe\textsuperscript{59} methods. In another case the Ashby technic showed a normal red cell life, but the C\textsuperscript{51} and Fe\textsuperscript{59} results showed a shortened red cell life. The conclusion that might be drawn from these two cases is that the individual’s own cells were qualitatively poor, while the destructive mechanisms were normal, and therefore the normal donor cells survived a normal or near normal life span.

**DISCUSSION**

With the development of these methods for determining total red cell volume, the rate and site of production of red cells, and the life span of the red cell, it has become possible to assess the erythropoietic state. Anemia or polycythemia can only arise from some change in the rate of production or in the “effective” life span of the red cell. This may even apply to bleeding states. Although the rate of production of red cells could conceivably be unaffected, the life span of the cells during bleeding would be shortened in the sense that some cells did not survive their normal life span. The resulting anemia could then be due to an over-all decrease in the red cell life span.

The methods described have been applied simultaneously in the study of various hematologic disorders, such as polycythemia, leukemia, aplastic anemia, hemolytic anemia, and hypersplenism. It is, of course, not implied that such studies are necessary or even helpful in the clinical management of most patients with disorders of erythropoiesis. Certainly the diagnosis of pernicious anemia, iron deficiency anemia, and the anemia associated with bleeding can be made by conventional procedures. It is in the diagnosis of the unusual anemia, the selection of patients for splenectomy, or in a clinical investigation program that these methods are of considerable value.

**Polycythemia**

There is a marked elevation of the total red cell volume in polycythemia vera (fig. 3).\textsuperscript{33} Figure 3 clearly indicates that the hematocrit cannot be used to predict the total red cell volume, although in general, as the hematocrit increases the total red cell volume increases. In 32 patients with a hematocrit above 55, the total red cell volume was elevated in all but two, ranging from 38.8 to 93.9 cc./Kg. of body weight. The plasma volume was low in 22 patients, in the lower range of normal in 8, and high normal in 2. Thus, in polycythemia vera in relapse with an elevated hematocrit, there is usually an increased total red cell volume and a low plasma volume, the latter being below the normal range in approximately two-thirds of the cases. In patients with hematocrits less than 55, the total red cell volume may be elevated and the plasma volume tends to be higher than in the group with hematocrits above 55.

In polycythemia vera it has been reported 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.\textsuperscript{*} The in vivo iron studies show that this increase cannot be explained entirely by the increased red cell mass, and some iron must be contributed by an increased production of red cells.

*As mentioned earlier, there has recently been some question of the theoretical basis on which the calculation was made.\textsuperscript{14, 17} Calculations by Sharney et al.\textsuperscript{18} indicate that this value may be high.*
crease is due to hyperactivity of the bone marrow. In most patients with polycythemia vera the spleen is not a significant site of red cell formation, although occasional instances of splenic erythropoiesis are observed. Studies of the life span of the red cell with N\textsuperscript{15} indicated a normal life span.\textsuperscript{26} Data from the C\textsuperscript{14} studies, however, can 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.\textsuperscript{34} This hypothesis fits well with the iron data, which show that iron utilization or turnover is usually greater than the volume of red cells of normal life span would require and that there must be a considerable increase in the amount of iron released from red cells per day. 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.

The blood volume changes in polycythemia secondary to congenital heart disease, acquired pulmonary disease, or residence at high altitude are the same as those in polycythemia vera, and for this reason primary and secondary polycythemia cannot be distinguished on the basis of such studies.

In individuals with polycythemia secondary to these conditions, it is found that there is an increase in the rate of utilization of radioactive iron for the production of red cells.\textsuperscript{34} However, unlike the situation in polycythemia vera, the increase is directly proportional to the increase in total red cell volume. Likewise, studies of individuals having a polycythemia secondary to residence at high altitude show a normal red cell life span.\textsuperscript{36} 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.\textsuperscript{36} During the period of acclimatization to altitude the red cell life span is not altered.\textsuperscript{39} 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. The increased urobilinogen excretion found by Merino shortly after descent to sea level has been interpreted as indicating that premature destruction of red cells is part of the mechanism of the readjustment of the polycythemic level of total red cell volume to normal. Studies of the life span of the red cell by the C⁴⁵ method recently completed indicate that following descent from high altitude there is no shortening of red cell life span, but it is not possible definitely to eliminate premature destruction of some fraction of the circulating red cells.

Relative polycythemia or the polycythemia of stress is a condition in which the total red cell volume is normal and the plasma volume is pathologically low, resulting in a falsely high hematocrit. Iron turnover studies indicate a normal rate of formation of red cells as expressed in the rate of utilization of iron for red cell formation. Thus, the diagnosis rests upon a blood volume determination.

Chronic Leukemia

In chronic leukemia, studies with P³² labeled red cells show again that the hematocrit is of little value for the prediction of total red cell volume (fig. 4). This is particularly true of patients with chronic myelogenous leukemia with splenomegaly. Most of the patients with chronic myelogenous leukemia studied had total red cell volumes within the normal range, but their hematocrits were less than normal, presumably owing to large plasma volumes. In chronic lymphatic leukemia there did appear to be a less consistent relationship between splenomegaly and large plasma volumes, and in addition there was a greater incidence of true anemia as shown by the low total red cell volumes. It should be mentioned, however, that the measurement of plasma volume in some of these patients with

**Fig. 4.** The total red cell volume in chronic leukemia plotted against the hematocrit. The ellipse represents area for normal values.
marked splenomegaly is open to question since recent studies indicate that the spleen may act as a reservoir for a cell-rich, plasma-poor blood.41

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 in production of red cells. This has not been borne out by radioiron studies of patients with chronic myelogenous and chronic lymphatic leukemia. Patients with chronic leukemia have been shown to produce a normal, and in many instances a greater than normal, number of red cells per day.13

In all patients with chronic myelogenous leukemia studied by the in vivo iron method, there has been evidence of production of red cells in the spleen and possibly some destruction in the spleen.23 Extramedullary erythropoiesis does not occur in most patients with chronic lymphatic leukemia.

C14 red cell life span studies in chronic myelogenous leukemia show that the red cell life span is shortened but is finite (as compared with the pattern of random destruction seen in a hemolytic process).42 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 chronic 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.42 Shortening of the red cell life span associated with random destruction of red cells presumably is extracorporeal in origin, e.g., hypersplenism and antigen-antibody reactions, while a finite but shortened red cell life span can be due either to an intrinsic defect of the red cell or possibly to some extracorporeal factor. It is not possible at present to determine which type of mechanism is operative, but it is thought that it is an intrinsic defect in the red cell which leads to a shortened but finite life span.

Aplastic Anemia

Studies of a patient with aplastic anemia (F.W.) showed that the rate of production of red cells as indicated by the radioiron studies was considerably decreased. Figure 5 shows that the rate of clearance of iron from the plasma is slow and that the uptake of iron in the peripheral blood is approximately one-fourth that of normal. The in vivo studies show that the amount of radioactivity in the liver is considerably greater than in the normal and that the marrow radioiron content is less than that of the normal. Figure 6 shows the red cell life span to be approximately 90 days, which is a slightly shortened but finite red cell life span. Thus, in this patient, it was demonstrated 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. With regard to life span, the red cells that were produced were apparently almost normal qualitatively. This patient represents the true aplastic anemia, where there is failure of production of cells in a marrow which is truly hypoplastic or aplastic as studied by bone marrow biopsy.

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. This condition is
Fig. 5.—The in vivo liver and bone marrow time curves and the rate of disappearance of radioiron from the plasma and uptake in red cells for patient F.W., aplastic anemia.

Fig. 6.—The specific activity of the hemoglobin (Fe$^{59}$ and C$^{14}$) as a function of time for patient F.W., indicating that the red cell life span is approximately 90 days. This figure also demonstrates that Fe$^{59}$ can be used for the determination of the red cell life span in this instance probably because the reutilization of iron is only 20 per cent instead of 80 per cent.

found associated either with chronic lymphatic leukemia or in individuals whose bone marrows show extensive areas of myelofibrosis or hypoplasia. In the past the latter group were classified as "aplastic anemia." All the patients had splenomegaly. It is probable, although we have not studied it, that the anemia of other splenomegalic disorders, such as Hodgkin's disease, has a similar pathogenesis involving premature random destruction of red cells. The patients showed 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 turnover patterns showed an early, marked rise in the counting rate over the spleen, indicating
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Fig. 7.—Pre-splenectomy radioiron studies in patient A.F., with hypersplenism, showing the high, maintained level of activity over the spleen.

Fig. 8.—Post-splenectomy radioiron studies in patient A.F., showing that the blood and bone marrow values returned toward normal following splenectomy.

extramedullary production of red cells in the spleen, with maintenance of the counting rate over the spleen, interpreted as indicating early destruction of red cells. Figures 7 and 8 show pre- and post-splenectomy blood and in vivo studies in one of these patients with lymphatic leukemia. Before splenectomy there was a rapid clearance of radioiron from the plasma which returned toward normal following splenectomy. Prior to splenectomy there was an early, rapid rise in activity over the spleen site, with maintenance at a high level of activity for the period of observation. This has been interpreted as indicating premature destruction of red cells in the spleen. There is also a somewhat lower marrow radioiron
The specific activity of the hemoglobin as a function of time for patient A.F., indicating that before splenectomy the red cells were randomly destroyed with a life span of approximately 30 days. Following splenectomy the red cell life span was normal.

uptake following splenectomy. Figure 9 shows a comparison of the red cell life span studies before and after splenectomy and indicates that before splenectomy the red cell life span was approximately 30 days with a pattern of random destruction and that after splenectomy the red cell life span was normal. In another patient, conversion of the short red cell life span with random destruction to a normal life span was effected by adrenal cortical hormones (fig. 10). This is the first instance in which it has been demonstrated that adrenal cortical hormones can effect a conversion toward a normal red cell life span from a shortened red cell life span with random destruction.

Hemolytic Anemia

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

A 23 year old male (Patient C.O.) was referred for diagnostic studies. 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 per cent. 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 in the bone marrow. The Coombs test was negative. Serum bilirubin was elevated. Total red cell volume was 20.9 cc. Kg. of body weight; plasma volume was 48.9 cc. Kg.

Radioiron studies on this patient showed a rapid turnover of radioiron and an increase in the rate of production of red cells (fig. 11). In vivo iron studies showed a rapid rise in the counting rate over the spleen, which was interpreted
Fig. 10.—The specific activity of the hemoglobin as a function of time for patient M.B., with Felty's syndrome, before and after treatment with adrenal cortical hormones. The life span of the red cells after treatment approaches normal.

as indicating an early destruction of red cells. Also, it should be noted that only 30 per cent of the iron could be detected in the red cells at any one time (as compared with 80 to 95 per cent 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, owing to premature red cell destruction.

Following splenectomy a normal in vivo iron pattern was observed (fig. 12), with 80 to 90 per cent of the injected activity noted in red cells at 7 to 10 days. The repeat studies showed that although the rate of utilization of iron for red cell formation was decreased, it remained greater than normal. Thus, in this individual there was a conversion toward normal in the turnover of radioiron in plasma and tissues following splenectomy.

The Erythropoietic Stimulus

With the development of the methods for measurement of total red cell volume, the rate of production of red cells, and the life span of the red cell, it became possible to study in experimental animals and man certain aspects of the mechanisms whereby the total red cell volume is regulated.

The fundamental stimuli to red cell production are discussed in a comprehen-
sive review by Grant and Root. They consider that there are only three ways in which the normal total red cell volume can be increased. These are: (1) reduction in oxygen tension, resulting in a secondary polycythemia, such as is seen in experimental animals subjected to low oxygen tension, in high altitude dwellers and in patients with certain types of congenital heart disease and chronic fibrosis of the lung; (2) administration of cobalt; and (3) the presence of a humoral factor. Recent work in the Institute of Experimental Biology and this laboratory suggests that the pituitary gland may elaborate an erythropoietic factor which plays a role in the control of red cell production.

Studies in this laboratory have shown that in polycythemia secondary to a
reduced oxygen tension, whether this be a reduced oxygen tension in the atmosphere or an anoxia resulting from pulmonary or heart disease, the rate of red cell formation is increased, but the red cell life span is unchanged, and there is a true increase in total circulating red cell volume. It has also been shown that a true polycythemia can be produced in the rat by the continued administration of cobalt in adequate doses, that the resulting polycythemia is due to an increased rate of red cell production, and that cobalt does not influence the life span of the red cell. These two erythropoietic stimuli—reduction in oxygen tension and administration of cobalt—are probably not the usual mechanisms for control of total red cell volume. It is possible that in man and in the experimental animal a hormone or hormones elaborated by the pituitary and/or other tissues are necessary for the regulation of erythropoiesis. Evidence for this is based upon studies of the changes in blood volume following hypophysectomy, and the finding of a humoral factor present in the blood of anemic animals. The relation of the pituitary to erythropoiesis has been discussed by several workers, but recent studies by the Institute of Experimental Biology and this laboratory have indicated that this pituitary erythropoietic effect is not mediated through any of the other endocrine glands. A sheep pituitary extract has been prepared, which repairs the anemia of the newborn rat; repairs the anemia of hypophysectomy in the presence or absence of pituitary target organs; produces a polycythemia in normal and hypophysectomized rats; and apparently is not identical with ACTH acting through the adrenals. Now in press is an article by Van Dyke, Contopoulos, et al. which adds further evidence to the probability that there is a pituitary erythropoietic factor separate from ACTH. Contopoulos and Lawrence have also recently demonstrated an erythropoietic factor present in the plasma of patients with polycythemia vera. This latter agent may or may not be pituitary in origin.

Summary

Since anemia or polycythemia can result only from a change in rate of formation of red cells or a change in the life span of the red cells, a determination of the total red cell volume, F'e studies to estimate the rate and site of production of red cells, and C-labeled glycine or F'e studies to determine the life span of red cells permit a description of the pathogenesis of any alterations in the erythropoietic state. This has been of great value in clinical hematology from the standpoint of understanding the basic nature of the various diseases and, in particular, the selection of patients with unusual anemias possibly related to hypersplenism who might benefit from splenectomy. Splenic erythropoiesis can be easily differentiated from splenic red cell destruction.

Since two of these methods—the determination of the blood volume and the F'e studies—are now possible in general community hospitals and since modifications of the F'e method permit the determination of the life span of the red cell, adequate evaluation of the erythropoietic state is now possible in general hospitals.

Summary in Interlingua

Proque anemia o polycythemia resulta necessarimente de alterate valores del production o del duration vital del erythrocytos, le determination del volumine
erythrocytic total, studies to Fe\textsuperscript{59} in re le nivello e le sito del generation de erythrocytos, e studios a glycine marked per C\textsuperscript{14} in re le duration vital del erythrocytos permitte un description del geneese de onne alteration pathologic in le stato erythropoietic. Iste facto se ha provate de grande valor ab le puncto de vista del hematology clinic, proque ilo avanta nostre compression del natura fundamental de varie morbos e guida le selection de patientes con anemias unusual que es possibilemente relationate a hypersplenismo e assi deberea beneficiar de splenectomia. Erythropoiese splenic es facilmente differentiabile ab destruction splenic de erythrocytos.

Proque duo del methodos mentionate—le determination del volume de sanguine e le studios a Fe\textsuperscript{59}—non exceede le ressources del moderne hospital general e proque modificationes del methodo a Fe\textsuperscript{59} es usabile in le determination del duration vital del erythrocytos, le adequate evallation del stato erythropoietic es nunc possibile in hospitales general.

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Recent Advances in the Knowledge of Total Red Cell Volume, Production and Destruction

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