HEME SYNTHESIS AND RED BLOOD CELL DYNAMICS IN NORMAL HUMANS AND IN SUBJECTS WITH POLYCYTHEMIA VERA, SICKLE-CELL ANEMIA, AND PERNICIOUS ANEMIA*

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Glycine is known to be specifically utilized for the biologic synthesis of the protoporphyrin of hemoglobin (1). The administration of N\textsuperscript{15}-labeled glycine results in the production of red blood cells containing labeled heme. After its formation, the heme as a constituent of hemoglobin remains in the cell until the cell disintegrates. Following the destruction of the red blood cell, little of the heme, if any, is reutilized for new hemoglobin synthesis. By following the isotope concentration in hemin isolated from the red blood cells the length of time that labeled hemoglobin remains in the blood, and thus the survival time of the red blood cells containing the labeled hemoglobin, can be determined. An analysis of such data reveals not only the age distribution at death of the red cell population but also the average life span of the red blood cells (2).

If \( n_t \) circulating red cells die at age \( t \), the average life span, \( T \), of the total circulating red cell population, \( N \), will be given by

\[
T = \frac{\sum n_t \cdot t}{N}
\]

(1)

In the normal subject it would appear that no significant number of circulating red cells die before they attain the age of 40 days. The death rate of the cells rises to a maximum value at approximately 120 days. This value of 120 days is close to the average life span, \( T \), of the circulating red blood cells. In the normal subject previously studied by this method the value for \( T \) was 127 days. This value corresponds well with figures obtained for the human and the dog by other reliable techniques (3–7).

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The average life span, $T$, is related to the rate at which red blood cells are delivered to the circulation. This relationship is shown in Equation 2,

$$T = \frac{M}{m}$$

in which $M$ is the total mass of circulating red cell hemoglobin and $m$ is the mass of red cell hemoglobin delivered to the circulation per day. This relationship holds only when $M$ is constant; i.e., the rate of synthesis of red cell hemoglobin is equal to the rate of its destruction.

This method of studying red cell dynamics has the unique advantage that it is possible to determine the rate of formation of hemoglobin and of red cells and their pattern of destruction in the same individual in whom the red cells are made and destroyed without altering the usual state of the organism, whether normal or abnormal. Since most disorders of red blood cells result from abnormalities in the synthesis of hemoglobin and red cells or from abnormal destruction of the cells, this method is particularly suited to the study of red blood cell dyscrasias. This report is concerned with studies performed in two normal subjects, male and female, and in subjects with pernicious anemia, sickle-cell anemia, and polycythemia vera.

**EXPERIMENTAL**

Labeled glycine was prepared from potassium phthalimide and chloroacetic ester (8). Glycine labeled with $^{15}$N was fed orally over a 48 hour period to each of the subjects. The glycine was fed hourly in equal doses except for triple doses at 12 midnight and 3 a.m., with no other doses between 12 midnight and 6 a.m. The normal male subject, the patient with pernicious anemia prior to treatment, and the patient with polycythemia vera each received 48 gm. of glycine containing 31.7 atom per cent excess $^{15}$N. The normal female subject received 41.2 gm. of glycine labeled with 30.8 atom per cent excess $^{15}$N. The patient with pernicious anemia 14 months after the start of liver therapy received 36.2 gm. of 31.7 per cent $^{15}$N glycine.

20 to 30 ml. of venous blood were drawn at frequent intervals during the course of the experiments and hemin was isolated by the usual procedure (9). In the case of the subject with polycythemia vera, 30 to 50 ml. samples were drawn.

Plasma volume was determined with the blue dye T-1824 and the single 10 minute point technique (10).

**Normal Subjects**—A 23 year-old white male medical student and a 24 year-old white female medical student served as subjects. Both had normal hematologic findings and had had no serious illnesses in the past.
There was no history of blood dyscrasia in their families. During the course of these experiments, both subjects remained in good health and there was no significant change in hematologic findings.

_Pernicious Anemia_—This subject was a 51 year-old Negro male with a 19 months history of gradually progressive weakness and weight loss of 25 pounds. Physical and laboratory examinations on admission to the Presbyterian Hospital in November, 1946, revealed a macrocytic anemia and gastric achlorhydria. X-ray examination of the gastrointestinal tract was negative. There was no jaundice or any evidence of combined degeneration of the spinal cord. Hemoglobin, 8.8 gm. per cent (Sahli); red blood cells, 2.0 million per c.mm.; white blood cells, 4800 per c.mm.; neutrophils 54 per cent (0-0-54), eosinophils 2 per cent, lymphocytes 38 per cent, monocytes 6 per cent; platelets 112,000; reticulocytes 0.9 per cent; hematocrit 31 per cent; marked macrocytosis of red blood cells, mean cell diameter 10.2 µ, mean cell volume 155 cu.µ, mean cell hemoglobin 46.5 µgm. (46.5 × 10^-12 gm.), mean cell hemoglobin concentration 27 per cent. A sealed wet smear was negative for sickling after 24 hours. Analysis of gastric juice 30 and 45 minutes after the subcutaneous injection of 1.0 mg. of histamine phosphate revealed no free acid and only 3 units of combined acid. Serum bilirubin 0.8 mg. per cent (indirect).

_Polycythemia Vera_—This subject was a 58 year-old white housewife with the characteristic physical and laboratory findings of polycythemia vera. Physical and laboratory findings revealed no significant pulmonary or cardiac disease. Laboratory findings included a marked increase in hemoglobin and red blood cell counts, and a mild leucocytosis. Hemoglobin 22.0 gm. per cent (Sahli); red blood cells, 7.2 million per c.mm., with normal differential count; platelets 240,000 per c.mm.; reticulocytes 2 per cent; hematocrit 75 per cent; plasma volume 2690 ml.; arterial hemoglobin oxygen saturation at rest 90 per cent; 1 minute after exercise 92 per cent.

_Sickle-Cell Anemia_—A 26 year-old Negro male with a history of frequent joint pains since childhood, the development of leg ulcers after minor trauma, frequent recurrent episodes of scleral icterus, and several crises with abdominal pain or generalized aching. The diagnosis of sickle-cell anemia was established on the basis of characteristic hematologic findings. On admission to the Presbyterian Hospital in July, 1947, for the purpose of this study, physical and laboratory findings revealed the following: hemoglobin 10.8 gm. per cent (Sahli); red blood cells 3.8 million

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1 P. H. 848800.
2 P. H. 687458.
3 P. H. 848837.
per c.mm.; white blood cells 2400 per c.mm.; neutrophils 58 per cent (0.1-57), lymphocytes 33 per cent, monocytes 8 per cent, eosinophils 1 per cent; platelets 282,000 per c.mm.; reticulocytes 10.2 per cent; hematocrit 31 per cent; mean cell volume 82 cu.μ, mean cell hemoglobin 28 μg/m., mean cell hemoglobin concentration 25 per cent. 100 per cent of the red blood cells were sickle-shaped after 24 hours in the sealed wet preparation.

**RESULTS AND DISCUSSION**

**Normal Subjects**—In Figs. 1 and 2 the N15 concentrations in hemin following the start of feeding labeled glycine to the two normal subjects are presented. These curves are similar to the one reported earlier (2). The isotope concentration in the hemin rises to a maximum value on about the 30th day; a decline along an S-shaped curve begins between approximately the 40th and 60th days. If, as previously (2), the function \( \phi(t) \) is defined as the probability that a red cell will survive in the circulation for a time greater than \( t \), then \( -\frac{d\phi(t)}{dt} \) represents the changing probability of survival of a cell population to age \( t \) and consequently...
mirrors the rate of destruction of these cells. In a stationary population \(-d\phi(t)/dt\) also represents the age distribution of the cells at death. In Figs. 1 and 2, Curves a represent the isotope concentration, \(C\), in the hemin throughout the course of the experiment, Curves b give the values of \(-dC/dt\), and Curves c the values of \(-C_0(d\phi(t)/dt)\). Curves c permit the evaluation of the average life span of the red cells, despite the fact that they have not all been made at one instant.

In the two normal subjects, Curves c attain their maximum value at 117 days (Fig. 1) and 120 days (Fig. 2). These values represent the time when the destruction of the labeled cells is most marked. Inasmuch as the curves are not symmetrical about their maximum ordinates, these values merely approximate the average life span of the red cells. It is possible, however, to determine the average red cell life span more precisely.
From the definition of \( \phi(t) \) it is clear that \(-N(d\phi(t)/dt)dt\) is the number of cells of age \( t \) to \( t + dt \) which die in the interval \( dt \). This is equal to \( n_t \). Substitution in Equation 1 yields

\[
T = \int_0^\infty t \frac{d\phi(t)}{dt} dt
\]

Graphical evaluation of this integral yields values for the average life span of the red cells of 120 and 109 days for the normal male and female subjects, respectively. These values correspond closely to the value of 127 days obtained by this method (2) and to values obtained by the modified Ashby (4, 5) technique in humans.

It is clear that, in the normal human, the red blood cells are destroyed as a function of their age. Despite the rather wide range of red cell survival times (see Curves c, Figs. 1 and 2), the time span which encompasses the ages at death of half (the second and third quarters) of the cell population is relatively short. In the male subject this time span is 35 days (106 to 141 days), in the female 32 days (91 to 123 days).

The value of approximately 120 days corresponds to the production and destruction of approximately 0.83 per cent of the red cells per day. The absolute rate, in gm. per day, of production of circulating red cell hemoglobin can be calculated from Equation 2. The total mass of circulating red cell hemoglobin may be calculated from the values for the whole blood volume and the hemoglobin concentration in the blood. The determination of the whole blood volume may be performed by a variety of techniques, of which the use of T-1824 and the use of radioiron-tagged red cells have been most prominent. There is fairly general agreement (11) that the normal human adult male has a plasma volume of approximately 45 ml. per kilo of body weight, the female about 43 ml. per kilo of body weight. There is much less agreement concerning the total red cell volume. Gibson et al. (12) claim that the true red cell volume as determined by radioiron-tagged cells is about 15 per cent lower than that determined by T-1824. Values for the red cell volume which are consistent with most of the studies, in which T-1824 or cells tagged with radioiron or radiophosphorus have been used, are about 30 ml. per kilo of body weight for the normal man and about 25 ml. per kilo of body weight for the normal woman (11, 13). The hemoglobin concentration in the peripheral blood is, on the average, 16 and 14 gm. per 100 ml. of whole blood for the normal man and normal woman, respectively (14). Accordingly, there are about 12 and 9.5 gm. of circulating red cell hemoglobin per kilo of body weight in the normal male and female, respectively. With an average red cell life span of 120 and 109 days in our two normal subjects, one can calculate from Equation 2 that the rate of production
of circulating red cell hemoglobin in normal man is about 0.10 gm. of hemoglobin per kilo of body weight per day, and in the normal woman about 0.087 gm. of hemoglobin per kilo of body weight per day. With a mean corpuscular hemoglobin value of 29 µg/m. (2.9 × 10⁻¹¹ gm.) (14) these rates of hemoglobin production are equivalent to the production of 3.45 × 10⁹ red blood cells per kilo of body weight per day for normal man and 3.00 × 10⁹ red blood cells per kilo of body weight per day for the normal female. These values will serve as a basis for comparison of the rates of production of red cells and hemoglobin in the patients with polycythemia vera, sickle-cell anemia, and pernicious anemia.

Polycythemia Vera—Fig. 3 describes the isotope concentration in hemin, the hemoglobin concentration, and red blood cell counts, and indicates when phlebotomies were performed during the course of the experiment. The withdrawal of large amounts of blood produced a lowering of red blood cell and hemoglobin values. The shape of the N¹⁵ concentration curve in hemin, however, was not affected by the phlebotomies, because the blood withdrawn was a representative sample of the blood in circulation at the time, and the phlebotomies produced no significant change in the generative activity of red blood cells as indicated by reticulocyte counts, which never rose above 3 per cent.

The shape of the curve of N¹⁵ concentration in hemin is nearly identical with that of the normal curves. The red blood cells are destroyed, as in the normal, as a function of their age, and their average life span calculated in the manner previously described is 131 days. This value of $T$ is close to those values found in normal subjects and is probably within the normal limits. The time span which encompasses the ages at death of half (the second and third quarters) of the cell population is 34 days (116 to 150 days). As can be seen from Equation 2, a normal life span with an abnormally large circulating red cell hemoglobin mass, $M$, must be associated with an elevated rate of hemoglobin synthesis, $m$. The plasma volume for this subject at the start of the experiment was 2690 ml. With a venous hematocrit of 75 per cent, the red cell volume would be 7740 ml. But on applying a correction of about 15 per cent (12), the red cell volume is 6580 ml. The total blood volume, then, is 9270 ml. With a hemoglobin concentration of 22 gm. per 100 ml. of whole blood, the total circulating red cell hemoglobin is 2039 gm., or 29.5 gm. of hemoglobin per kilo of body weight (the subject weighed 69 kilos). Since the average life span of the red cells in this subject is 131 days, the rate of hemoglobin production is 0.225 gm. per kilo of body weight per day. With a mean corpuscular hemoglobin content of 3.0 × 10⁻¹¹ gm., the rate of red cell production is 7.50 × 10⁹ red cells per kilo of body weight per day. These rates of hemoglobin and red cell production are about
Fig. 3. $N^{15}$ concentration in hemin after feeding $N^{15}$-labeled glycine for 2 days. $C_0 = 0.56; \lambda = 0.17$ day$^{-1}$. 
2.5 times the rate in the normal female. It is to be noted that these values are based on the period during which the labeled red cells were produced and, therefore, are not significantly affected by the therapeutic phlebotomies which, for the most part, were carried out later. The blood withdrawn for hemin N16 analyses during the period when the great bulk of labeled cells was formed constitutes a small fraction of the total blood volume and does not affect the general conclusion.

Polycythemia vera, as exemplified by this patient, is characterized by an abnormally high rate of hematopoiesis and a normal red cell life span. These data throw light on the mechanism of the development of polycythemia vera. Theoretically, two factors, singly or in combination, might produce the marked increase in the total red cell mass which is characteristic of this disease. These are (1) an increased rate of hemoglobin and red blood cell synthesis and (2) prolonged life of the red blood cells. In the normal individual in the steady state, the rate of hemoglobin synthesis equals the rate of hemoglobin degradation, and the average life span of the erythrocyte is the reciprocal of the fraction of the total circulating red cell mass which is synthesized and degraded daily. Thus in the normal individual 0.83 per cent of the total red cell mass is synthesized and degraded daily, and the average life span of the erythrocytes is the reciprocal of 0.0083, or 120 days. The total red cell mass will increase whenever the rate of red cell synthesis is faster than the rate of degradation and will continue to increase until the degradative rate again equals the synthetic rate. A new steady state will then ensue. The finding of a normal red cell life span in this subject at a fully developed stage of the disease is conclusive proof for the existence of a functional hyperactivity of the blood-forming apparatus in the maintenance of the polycythemic state. It seems probable that the development of the polycythemia earlier in the disease is similarly characterized by an increase in hematopoietic activity with the maintenance of a normal erythrocyte life span. The existence of hematopoietic hyperactivity, at least in the maintenance of the polycythemic state, is consistent with the usual findings in polycythemia vera of hyperplasia of all bone marrow elements and evidence in the peripheral blood of increased bone marrow activity (polychromatophilia and basophilic stippling of erythrocytes, and leukocytosis with an increase in immature cells of the myeloid series). The fundamental cause of this functional hyperactivity, however, remains unknown.

A variety of etiologic theories has been proposed, but conclusive evidence in support of any of them is lacking. These theories have been reviewed by Harrop and Wintrobe (15). One of the theories is that of Minot and Buckman (16) who regard polycythemia vera as a form of
neoplasm. The persistent bone marrow hyperplasia involving all marrow elements, the development of leukemia in some cases of erythremia, and the development of erythremia in some cases of leukemia suggest that this is a neoplastic process. If polycythemia vera is indeed a benign neoplasm, then at least this neoplastic process is associated with an increase, above normal, of the synthetic activity of the hematopoietic system.

The maintenance of the polycythemic state in the presence of a normal erythrocyte life span requires that the amount of hemoglobin degraded, as well as the amount of hemoglobin synthesized, be increased. How-

![Graph](image-url)

**Fig. 4.** $N^{15}$ concentration in hemin after feeding $N^{15}$-labeled glycine for 2 days

ever, studies of the excretion of bile pigment in polycythemia vera, which are critically discussed by Watson (17), have revealed fecal urobilinogen values that are much lower than would be anticipated on the basis of the increased amounts of hemoglobin that are degraded. This discrepancy raises questions concerning the fate of the pigment that are pertinent to hemoglobin metabolism in general and warrant further investigation.

*Sickle-Cell Anemia*—The curve of $N^{15}$ concentration in hemin during the course of the experiment is shown in Fig. 4. During the period of the study, the patient suffered no “crises” and the red blood cell, hemoglobin, and hematocrit values remained essentially the same as at the start of the experiment.
The isotope concentration in the hemin rose rapidly to a peak on the 7th day following the start of the feeding of isotopic glycine and immediately began to fall. A direct plot of the isotope concentration in the hemin, $C$, after the 7th day in a semilogarithmic coordinate system (log $C$ versus time) gives a straight line through the major portion of the curve. Theory suggests that a small quantity, $\Delta$, should be subtracted from each value of the isotope concentration of the hemin, since this value is, at each instant, approaching not zero but a finite value representing the isotope concentration of the subject. $\Delta$ is not independent of time. However, it varies but slowly with time, approaching zero as a limit. Its value during the course of the last hundred days of this experiment must have been about 0.020 atom per cent excess. We have, therefore, plotted the logarithm of $(C - 0.020)$ against time (see Fig. 5). After the 30th day the data fit a straight line whose equation is

$$C = 1.02e^{-0.028t} + 0.020$$

(4)
If we assume that the bulk of the N\textsuperscript{15}-labeled red cells has been formed in a short time interval, then to a first approximation \( \phi \) is given by

\[
\phi = e^{-0.0238t}
\]  

(5)

The average time the red cells spend in the circulation, \( T \), is given by Equation 3. Differentiating Equation 5 and substituting in Equation 3 give

\[
T = \int_{0}^{\infty} -0.0238 e^{-0.0238t} dt
\]  

(6)

On integration,

\[
T = -\frac{e^{-0.0238t}}{0.0238} (1 + 0.0238t)
\]  

(7)

Therefore

\[
T = \frac{1}{0.0238} = 42 \text{ days}
\]

Since \( \phi(t) \) is by definition the fraction of a stationary population of cells which are born at a particular moment and will survive to age \( t \), \( -(d\phi(t)/dt) \) is the fraction of the population which will die in the interval \( t \) to \( t + dt \) and \( -(1/\phi)(d\phi(t)/dt) \) or \( -(d \ln \phi(t)/dt) \) is the death rate of the population of age \( t \). In this case, when \( \phi(t) = e^{-0.0238t} \), the death rate is independent of age for \( -(d \ln \phi(t)/dt) = 0.0238 \). This means that heme is removed from the circulation at a rate which is independent of the age of the heme at the time of its degradation. Such a curve reflects the random occurrence of a single event or of a complex of events which leads to the death of the cell once the initial event has occurred.

This curve could result from (1) a random destruction of the red blood cells and a consequent loss of labeled heme from the circulating blood; or (2) a random degradation and synthesis of heme in circulating red blood cells which are morphologically intact; or (3) random synthesis and degradation of heme in red blood cells which are themselves undergoing random destruction. In the light of our findings in normal subjects which demonstrate that hemoglobin in circulating red blood cells is not in the dynamic state, the second, and, consequently, the third of these possibilities would appear most unlikely. Recent studies (18), however, make it difficult to rule out completely the possibility that some synthesis of heme in the circulating red cells of patients with sickle-cell anemia may occur.

When the whole blood of patients with sickle-cell anemia was incubated with N\textsuperscript{15}-labeled glycine, the hemin isolated from the red cells was found to contain significant concentrations of N\textsuperscript{15} (18). This indicated
that heme was synthesized from glycine in vitro. Similar significant concentrations were not obtained with the blood of normal subjects or of patients with sickle-cell trait. The synthesis of heme in vitro in the blood of patients with sickle-cell anemia occurs at the rate of 0.1 to 0.2 per cent of the red cell heme in 24 hours. If all the hemoglobin in the circulating red blood cells of sickle-cell anemia subjects were synthesized in the peripheral blood at the same rate as in the in vitro experiments, the average survival time of the labeled hemoglobin in the circulation would be of the order of 500 to 1000 days. Actually the average survival time, as determined in our subject, is about 40 days.

The major part of the disappearance of heme described in Fig. 4 must be due to a random destruction of cells in sickle-cell anemia. Studies with the Ashby technique (19) are in accord with this view. Any random synthesis of heme that may occur in the peripheral blood of these patients can play only a minor part in the hemoglobin turnover in this disease.

Since the red cells of sickle-cell anemia are destroyed in an indiscriminate fashion rather than as a function of their age, their survival is better designated in terms of their half life time $t_4$. The half life time of these cells in the circulation is given by the expression

$$t_4 = T \times \ln 2$$

(8)

$t_4$ is therefore equal to 29 days.

It is to be noted that the curve representing Equation 5 consistently lies below the experimental points from the 6th to the 22nd day. A priori the contrary would be expected, since during this period some labeled heme is being delivered to the circulation and would tend to result in low values of $(d \ln C/dt)$ rather than high ones. When the logarithms of the deviations of the observed data (Curve b) from the extrapolation of the major portion (Curve a) of the data are plotted against time, Curve c, Fig. 5, is obtained. $t_4$ for this curve is approximately 7 days. This suggests the possibility that there may exist a small fraction of the total red cell population which has a $t_4$ of approximately 7 days. This value of $t_4$ is a maximum value, since, as we have indicated above, the accession to the circulation of newly formed red blood cells containing N\textsuperscript{15} would tend to lower the rate of fall of the N\textsuperscript{15} concentration of the total circulating heme during this period. Further studies in patients with sickle-cell anemia and analysis of N\textsuperscript{15} concentrations in the bile pigment excreted during the course of the experiment may throw light on this problem.

With measurements of the total red cell mass and the mean survival time of the red cells, it is possible to calculate the rate of red blood cell and hemoglobin production in this patient. For the mean survival time
we shall employ the value of 42 days, which is representative of the great majority of the red cells in circulation.

The plasma volume as determined by T-1824 was reported to be 5000 ml. With a venous hematocrit of 31 per cent, the red cell volume would be 2160 ml. The 15 per cent correction yields a red cell volume of 1836 ml. With a total blood volume of 6836 ml. and a hemoglobin concentration in the blood of 10.8 gm. per 100 ml. of whole blood, the total circulating red cell hemoglobin is 738 gm., or 11.71 gm. of hemoglobin per kilo of body weight (weight of subject, 63 kilos). Since the mean survival time of the red cells is 42 days, the rate of hemoglobin production is 0.279 gm. of hemoglobin per kilo of body weight per day. With a mean corpuscular hemoglobin value of $2.8 \times 10^{-11}$ gm., the rate of red cell production is $9.96 \times 10^9$ red blood cells per kilo of body weight per day. These rates of hemoglobin and red cell production are about 2.8 times the rate in normal man. Since the hemoglobin and red blood cell counts remained at the same level during the course of the study, it is safe to assume that the patient was also destroying red blood cells at a rate 2.8 times the normal. If there is a fraction of the total red cell population which has a half life time of about 7 days, then this rate of production and destruction, 2.8 times the normal, is minimal. The very rapid formation of erythrocytes is consistent with the markedly hyperplastic bone marrow and the reticulocytosis in the peripheral blood characteristic of this disease. Similarly, a rapid rate of destruction is consistent with the hyperbilirubinemia and increased fecal urobilinogen excretion.

These findings indicate that there is no deficiency in the ability of the hematopoietic organs to make adequate numbers of red blood cells and adequate amounts of hemoglobin. It is clear, however, that the red blood cells are defective in their capacity to survive for a normal erythrocyte life span. That the defect is intrinsic to the cell and not ascribable to any factor in the plasma was shown by Huck (20) and more recently by the use of the Ashby technique (19, 21, 22). The nature of the defect in the cell is still unknown. It would appear to be a defect in the structure of the red cell membrane. This defect may be associated with the sickling process but cannot be ascribed to this phenomenon alone, inasmuch as the erythrocytes of individuals with sickle-cell trait are not abnormally susceptible to destruction (22).

Pernicious Anemia—At the start of the experiment, this patient had never received any form of therapy for pernicious anemia. The isotope concentrations in hemin, and the hemoglobin, red cell, and reticulocyte values are shown in Fig. 6. The isotope concentration in the hemin rose rapidly and was approaching its maximum value on the 15th day. It was considered unwise to withhold treatment longer, and liver extract in
large doses was administered intramuscularly. The dosage of liver extract is shown in Fig. 6. The reticulocyte count rose to a peak of 12.8 per cent on the 7th day after the start of therapy, and there was a satisfactory rise in hemoglobin and red cell values. After the start of liver therapy, a fall in the isotope concentration in the hemin occurred. This fall was expected, because the influx of large numbers of new cells formed when the isotope concentration in the glycine had fallen to a low value should result in the dilution of the average isotope concentration in the hemin of circulating red blood cells. The curve continues to decline, however, when the hemoglobin and red blood cell values have approached normal levels, and a mere dilution effect should be minimal. To differentiate between the effect of the dilution and the actual destruction of the labeled red cells, we have calculated total heme N\textsuperscript{15} in circulation during the course of the experiment. Gibson (23) has shown that the
hemoglobin concentration in the peripheral blood is a good index of the total hemoglobin in circulation before and after the start of liver therapy in pernicious anemia. By multiplying the hemoglobin concentrations in the peripheral blood by the isotope concentrations in the hemin during the course of the experiment, a curve representing the changes in the total amount of N\textsuperscript{15} in the heme of circulating red blood cells is obtained (Fig. 7).

As in Fig. 6, the curve rises rapidly and is approaching its maximum value on the 15th day. With the administration of liver extract there is a further rise in the total heme N\textsuperscript{15}. This additional rise is due to the influx of many new cells. Although the isotope concentration in the heme of these newly formed cells is relatively low, the large number of these cells newly added to the circulation represents a considerable increment in the total heme N\textsuperscript{15}. Within 2 weeks after the start of liver therapy the peak of the total heme N\textsuperscript{15} curve is reached. If the cells of untreated pernicious anemia enjoyed a normal life span, the curve would have maintained a plateau until about the 40th to 60th day and would then have begun to decline. The curve declines, however, in linear fashion almost immediately after reaching its peak. The linear decline indicates that many but not all of the cells are being destroyed in indiscriminate fashion. If all the cells were destroyed in an indiscriminate manner, the decline of the curve would have been exponential in character. The linear decline suggests that the cell population is mixed, with many of the cells destroyed indiscriminately and others as a function of their age.

The average survival time of the cells of this mixed population (cells formed prior to and after the start of liver therapy) can be estimated from the declining portion of the curve. Approximately three-fourths of the labeled heme was formed prior to the start of liver therapy. It is reasonable to assume that the survival of the cells containing this portion
of the labeled heme is not influenced by liver therapy administered after formation and release of these cells into the circulation. The decline in the total hemin $N^{15}$ (Fig. 7) must therefore reflect the disappearance of these labeled cells from the circulation. The time required for the total heme $N^{16}$ to decline from any given value on the declining portion of the curve to one-half that value is about 90 days. This is the half life time for the disappearance of the labeled heme and consequently of the cells containing the labeled heme. As the declining curve is for the most part linear, the half life time and average survival time are equal.

Inasmuch as therapy supervened during the course of the experiment, this value represents the average survival time of the mixed red cell population; i.e., cells formed prior to and after the start of liver therapy. An approximate value for the average survival time of the cells formed before the start of treatment can be obtained.

Fig. 7 represents the sum of the hemin $N^{15}$ of the cells formed before and after the start of liver therapy. It is reasonable to assume that the average survival time of the cells formed after the start of liver therapy is at least as long as the survival time of the cells formed prior to liver therapy. If the survival times of these two groups of cells are equal, the untreated cells will have an average survival time of approximately 90 days; i.e., the same as the survival time of the mixed population. This value represents an upper limiting value for the average survival time of the untreated cells. To establish a lower limiting value, let us assume that the cells formed after the start of treatment have an infinite life span. Inasmuch as those cells contain heme $N^{16}$ representing approximately one-fifth of the total heme $N^{15}$, were they to have an infinite life span they would account for a lengthening of the mean survival time of the mixed population of about 20 per cent. The survival time of the cells formed prior to treatment would then be approximately 72 days; i.e., about 20 per cent less than that of the mixed population. This represents the lower limiting value. In the fully treated subject with pernicious anemia the average life span of the cells is normal (see below). Following the start of treatment, however, there is most likely an interval prior to the formation of thoroughly normal cells during which cells of varying abnormality are produced. Accordingly, a value closer to the upper limiting value than to the lower will closely approximate the real value for the mean survival of cells of the untreated state. For purposes of further calculation we may choose a value of 85 days. In two cases of untreated pernicious anemia studied by means of the Ashby technique, Loutit (24) found that the red cells survived for 20 and 60 days. Such variations, however, are to be expected, for the severity of the disease and the corresponding abnormalities of the red cells vary widely among patients.
The rate at which newly formed red blood cells and hemoglobin were released into the circulation prior to liver therapy can be calculated by Equation 2. Before the start of treatment with liver extract, the hemoglobin concentration in the peripheral blood was 8.8 gm. per 100 ml. of whole blood, 55 per cent of the average value for the normal adult male. Inasmuch as the hemoglobin concentration in the peripheral blood provides an index of total circulating red cell hemoglobin, one may estimate that this patient had approximately 6.6 gm. of hemoglobin per kilo of body weight (55 per cent of 12.0 gm. of hemoglobin per kilo). The rate of hemoglobin production, based on a mean red cell survival time of 85 days, is 0.0786 gm. of hemoglobin per kilo of body weight per day. This value is 78 per cent of the normal rate. With a mean corpuscular hemoglobin content of 4.65 X 10^{-11} gm., the rate at which red cells are released into the circulation is 1.67 X 10^9 cells per kilo of body weight per day. This is only 48 per cent of the normal rate.

The findings of a diminished production of red blood cells capable of reaching the peripheral blood and of a diminished average survival of the red cells in circulation are consistent with the view that the red blood cell of untreated pernicious anemia is intrinsically defective. The absence of an abnormal hemolytic factor in the plasma is suggested by early studies with the Ashby technique, and is demonstrated by recent studies with the improved technique in which normal cells transfused to recipients with pernicious anemia enjoyed normal survival.

The production of circulating red cell hemoglobin is somewhat diminished. It remains to be determined, however, whether this represents a diminished capacity to synthesize normal hemoglobin or whether it is merely a reflection of the fate of the abnormal red blood cell. But even a normal rate of circulating red cell hemoglobin production and destruction falls far short of providing an adequate explanation for the very large amounts of bile pigment produced in this disease. This discrepancy is explained by the finding in this patient that a very large portion of the stercobilin in the feces apparently was derived from a source other than the hemoglobin of circulating red blood cells. These studies, to be reported later, suggest that this portion of bile pigment is derived from one or more of the following sources: (1) hemoglobin of red blood cells which are destroyed shortly after reaching the peripheral blood or which never reach it and are destroyed in the bone marrow; or (2), porphyrins which are not utilized for hemoglobin production; or (3), direct synthesis of bile pigment via a pathway which does not involve degradation of a porphyrin ring.

After treatment with concentrated liver extract for 1 year, the patient was studied again to determine the effects of the treatment on hemoglobin
metabolism and red blood cell dynamics. Despite the treatment with concentrated liver extract in a dosage which averaged slightly more than 15 units per week over the 12 months period, a very slight increase in mean cell size persisted: hemoglobin 15.0 gm. per 100 ml.; red blood cells, 4.7 million per c.mm.; hematocrit 46 per cent; mean corpuscular volume 98 cu.μ; mean corpuscular hemoglobin 33 μg.; mean corpuscular hemoglobin concentration 33 per cent. To determine whether increased dosage and a cruder extract would reduce the mean cell size, 30 units of concentrated liver extract per week and 60 ml. of Valentine's oral liver extract daily were administered. After 10 weeks of this schedule, the hematologic picture remained essentially unchanged. Examination of the bone marrow revealed no abnormality. To repeat the study, 36.2 gm. of glycine labeled with 31.7 atom per cent excess N15 were administered over a 48 hour period. The results of this study are plotted in Fig. 8.

The shape of the curve of isotope concentration in the hemin and the death rate curve, (dφ(t)/dt), are normal. The red blood cells are destroyed as a function of their age and not in indiscriminate fashion. Half of the red cells (the second and third quarters) die within a 33 day period, 116 to 149 days. The average life span is 129 days, a value very close to the values in the two normal male subjects.

Fig. 8. N15 concentration in hemin after feeding N15-labeled glycine for 2 days. C0 = 0.37; λ = 0.11 day⁻¹.
Fig. 9. $N^{15}$ concentration in hemin after feeding $N^{15}$-labeled glycine for 2 days

*Hemoglobin Synthesis and Release of Erythrocytes into Circulating Blood*—The concentrations of $N^{15}$ in the hemin during the early part of the experi
ments in the normal subjects and in the subjects with pernicious anemia (prior to treatment), sickle-cell anemia, and polycythemia vera are shown in Fig. 9. It is noteworthy that red blood cells containing labeled hemo-
globin appear in the peripheral blood within several hours after the start of feeding labeled glycine. These findings indicate a rapid utilization and conversion of dietary glycine to protoporphyrin. In addition, they sug-
gest that the human red cell is released into the circulation at a time which approximates the completion of hemoglobin deposition in the cell. If the red cell is mature and not reticulated, hemoglobin deposition is prob-
cably completed shortly before the cell enters the circulation. The absence of significant synthesis in vitro of heme by normal human blood supports this view (18). If, however, the red cell entering the circulation is reticu-
lated, the process of hemoglobinization may not yet be completed and further hemoglobin synthesis may occur. This hypothesis is supported by the finding that normal mammalian (rabbit) reticulocytes can synthesize heme in vitro. The absence of significant heme synthesis in vitro in blood from some patients with elevated reticulocyte counts (18) suggests that reticulocytes, although morphologically similar, may differ in their func-
tional capacity to synthesize heme.

SUMMARY

1. The average life span of the circulating red blood cell in a normal human adult male has been found to be 120 days, in a normal human adult female 109 days.

2. A subject with polycythemia vera was shown to have a normal red cell life span of 131 days and a normal pattern of red cell destruction, but a rate of red cell and hemoglobin production about 2½ times the normal. The mechanism of the development of polycythemia vera is discussed in the light of these findings.

3. In sickle-cell anemia the red blood cells were shown to be destroyed indiscriminately rather than as a function of their age. Their mean survival time in the subject studied was 42 days, their half life time 29 days. The rates of hemoglobin and red cell formation and destruction were about 2.8 times the rates in normal man.

4. Study of a subject with untreated pernicious anemia disclosed an abnormal pattern of red cell destruction and a mean survival time of approximately 85 days. The rate of production of circulating red cell hemoglobin was found to be about four-fifths the normal, the rate of production of circulating red cells about half the normal rate. Treatment with liver extract resulted in restoration of the pattern of red cell destruction to normal and in a normal red cell life span of 120 days.

5. The utilization of dietary glycine for the synthesis of the protoporphyrin of hemoglobin is very rapid, and cells containing labeled hemoglobin are released into the circulation within several hours after the start of feeding labeled glycine. The time relationships between hemoglobin deposition in the red cell and release of the red cell into the circulation are discussed.

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