THE LIFE SPAN OF THE HUMAN RED BLOOD CELL

By DAVID SHEMIN and D. RITTENBERG

(From the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York)

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Many different methods have been used to estimate the average life time of the human red blood cell. The different attempts have yielded estimates which range from 5 to 200 days. Estimates have been made by inducing polycythemia and noting the time required for the return to the normal red cell count; this yielded values of 18 to 30 days (1). Determinations of the amount of iron or pigment excreted have yielded estimates of 20 to 200 days (2).

Determinations of the survival time of transfused erythrocytes by means of differential agglutination (3, 4) yielded estimates ranging from 30 to 100 days. The use of agglutinogens M and N for tagging erythrocytes gave values of 80 to 120 days (5–7). More recently Callender, Powell, and Witts (8) using Ashby's differential agglutination method (3) in Rh-positive men (9) concluded from a mathematical analysis of their data that red blood cells live for approximately 120 days. Measurement of the time required for the disappearance of sulfhemoglobin from the blood of cyanosed workers (10) indicated the life span of the red blood cell to be 115 days. Hawkins and Whipple (11) by another technique obtained a value of 124 days for the dog erythrocyte.

Glycine has been shown to be a nitrogenous precursor of the protoporphyrin of hemoglobin in the rat (12). The feeding of glycine labeled with N^{15} to a man also results in the formation of heme containing a comparatively high concentration of N^{15} (13). After cessation of the feeding of the labeled glycine, the isotope concentration of the heme was followed over a long period of time by analysis of the hemin isolated at intervals. The values rose rapidly to a high level, remained practically constant for many weeks, and then fell quite sharply to a very low level. This finding indicates that the heme is neither involved in the dynamic metabolic state nor reutilized for hemoglobin formation. On these grounds, the curve of N^{15} concentration of the heme *versus* time can form the basis for a determination of the average life span of the human red blood cell. This was found to be about 127 days.

EXPERIMENTAL

Feeding Experiment—One of us (D. S.) ingested 66 gm. of glycine containing 32.4 atom per cent N^{15} excess (14). The glycine was divided into 60

doses and taken over a period of 3 days. At intervals blood was withdrawn and urine collected.

Preparation of Hemin and Protein Samples—Usually 20 cc. of venous blood were withdrawn, oxalated, and separated into red cell and plasma

TABLE I

N^{15} Concentration of Hemin

Time	Hemin N^{15} concentration	Time	Hemin N^{15} concentration
days	*atom per cent excess*	*days*	*atom per cent excess*
0	0.000	127	0.342
4	0.134	144	0.200
18	0.422	154	0.164
77	0.466	170	0.112
86	0.462	192	0.096
99	0.448	231	0.062

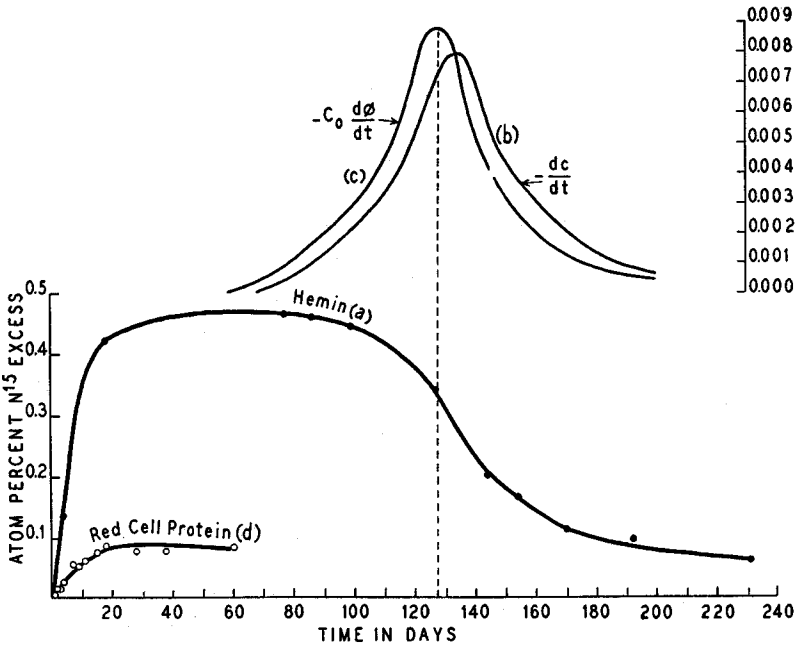

FIG. 1. N^{15} concentration in hemin after feeding N^{15}-labeled glycine for 3 days

fractions by centrifugation. On the 4th and 18th days of the experiment 300 cc. quantities of blood were withdrawn. These larger samples were taken to permit isolation of individual amino acids from the blood proteins. The results obtained on the amino acids and proteins will be reported in a later publication.

The hemin was isolated in a manner previously described (15). The red blood cell proteins were obtained by treating hemolyzed red cells with trichloroacetic acid.

Urinary Urea Nitrogen—The urinary urea nitrogen was obtained as ammonia by treating urine, from which ammonia had been adsorbed with permutit, with urease.

Isotope Concentrations—The isotope concentrations found in the hemin at various periods of the experiment are given in Table I. For comparison there are plotted in Fig. 1 the isotope concentrations found in the hemin (Curve *a*) and red cell proteins (Curve *d*).

DISCUSSION

If during the administration of an isotopic compound a nitrogenous constituent of a cell is continuously being degraded and resynthesized, though morphologically the cell remains intact, the N^{15} concentration will increase to a maximum value, and then decline. The average life span of the cell cannot be thus determined but only the half life time of the constituent of the cell, which can be estimated from the rate of decrease of the N^{15} concentration of the particular component (16, 17). This problem has been mathematically treated by Zilversmit, Entenman, and Fishler (18).

The shape of the curve in which the isotope concentration of the hemin is plotted against time is different from that found previously for any cellular constituent. Instead of rising during the period in which the labeled material is fed and then decaying along an exponential curve, the curve for hemin rises rapidly for about 25 days after the cessation of feeding the labeled glycine, remains practically flat for the next 70 days, and then falls along an S-shaped curve. Such a curve cannot be the result of a single process involving molecules which are synthesized and degraded by a random procedure, a process which would result in a curve similar in shape to that we found for the liver proteins of the rat (17). The curve for hemin cannot be described by a simple exponential function; it appears that the probability that a hemoglobin molecule may be degraded is a function of its age. Since the concept of "aging" is not applicable to a molecule, another explanation must be sought.

If a constituent of a cell is not involved in the flux of synthesis and degradation, the presence of N^{15} in the constituent of this cell must be the result of synthesis of the component and its incorporation during the formation of the cell. The original molecules of this constituent will then remain with the cell until the cell disintegrates or dies. In such a case the N^{15} concentration of the component will, if the cells are not indiscriminately destroyed, reach a maximum value and remain constant for a length of time depending on the life span of the cell. On the destruction of the cell the N^{15} concen-

tration should then, unless the component is reutilized, abruptly decline. In such a system, the average life time of the cell, as will be shown below, can be estimated from the curve obtained by plotting the N^{15} concentration of the component against time. During the period in which the labeled compound was fed in this experiment, heme containing isotopic nitrogen was synthesized and incorporated into newly formed erythrocytes. These cells as they are discharged into the circulation raise the isotope concentration of the heme of the total red blood cells. Up to about the 25th day the rise takes place rapidly. After this period, and probably even earlier, the isotope concentration of the newly synthesized heme is lower than that of the average N^{15} concentration of the heme in circulation. The addition of this heme of relatively low isotope concentration to the circulating heme would lower the average isotope concentration if the destruction of heme were a random process. However, the molecules to be destroyed are those in the red blood cells which had been synthesized before feeding the labeled glycine. As these contain no excess N^{15}, the isotope concentration of the heme in the red cells rises, even though the newly added material has a lower isotope concentration than the average circulating heme. Eventually the time arrives at which the red cells, synthesized during the period in which labeled glycine was fed and which contain heme with the highest isotope concentrations, begin to be destroyed. As the bulk of these red cells is replaced by others containing heme with very low isotope concentration the N^{15} concentration in the total heme drops abruptly. This decrease of N^{15} concentration begins at about the 80th day. This phenomenon can occur only if the porphyrin moiety of hemoglobin is not reutilized for new hemoglobin formation. Were the heme used again, the isotope concentration of the isolated hemin would decrease very slowly instead of showing an abrupt drop. In this respect the protoporphyrin of the hemoglobin differs from the iron, which is reutilized after the destruction of the red cell (19, 20).

These considerations indicate that the heme of the non-nucleated red blood cells, unlike the constituents of nucleated cells, is not continuously formed and degraded within the cell. The red cells are not indiscriminately destroyed, but their rate of destruction is a function of the age of the cell.

A mathematical treatment of the data gives a figure of the average life time of the red cell of 127 days, which is the same time as that separating the mid-points of the rising and declining portions of the curve.

An equation relating the isotope concentration $C(t)$ of the hemin as a function of time is derived below.

Let

$f(\theta)$ = the N^{15} concentration in the hemin synthesized at the time θ. $f(\theta)$ is also the isotope concentration in the hemin precursor at time θ

a = rate of formation of hemin nitrogen in atoms per day
N = total hemin nitrogen (atoms) in the blood
$G(t)$ = atoms of N^{15} in circulating hemin nitrogen at time t
$\phi(t)$ = probability that a red cell will have a life span greater than t

Then

$$G(t) = \int_0^t af(\theta)\phi(t - \theta)d\theta \tag{1}$$

Since

$$100\frac{G(t)}{N} = C(t) \tag{2}$$

$$C(t) = 100\frac{a}{N}\int_0^t f(\theta)\phi(t - \theta)d\theta \tag{3}$$

Let

$$\frac{N}{a} = \bar{T}$$

Then

$$C(t) = \frac{100}{\bar{T}}\int_0^t f(\theta)\phi(t - \theta)d\theta \tag{4}$$

There does not exist a general solution of this integral equation.[1] An approximate solution may be obtained as follows: Assume $f(\theta)$ is zero every place except at $t = 0$ and that $\int f(\theta)d\theta$ is finite. Physically this assumption concerning $f(\theta)$ is equivalent to the sudden introduction into the circulation at zero time of newly formed red cells whose hemin is labeled with N^{15}. Equation 4 becomes

$$C(t) = \frac{100}{\bar{T}}\phi(t) \tag{5}$$

and

$$\frac{dC}{dt} = \frac{100}{\bar{T}}\frac{d\phi}{dt} \tag{6}$$

From the definition of ϕ it is apparent that $-d\phi/dt$ is proportional to the rate of destruction of red cells. Therefore under these conditions the rate of destruction will also be proportional to $-dC/dt$.

We have graphically evaluated $-dC/dt$ for the period $t > 80$ days. These values are plotted in Fig. 1, Curve *b*. This curve shows that cells formed during the period in which high concentration isotope was being incorporated into red cells, *i.e.* the 3 day feeding period, begin to die at

[1] A treatment of the Volterra equation is given by Margenau and Murphy (21).

about the 70th day. Therefore, from $t = 0$ to $t = 70$, $\phi(t)$ must be constant at the value 1. $-d\phi/dt$ attains its maximum value at $t = 133$ days. As a first approximation this may be taken to be the average life span of the red cells, $\overline{T}$. During the initial period, certainly up to $t = 30$, $\phi(t)$ is constant at unity; *i.e.*, all cells survive for more than 30 days. During this period when $\phi(t - \theta)$ is equal to 1, equation 4 can be simplified to

$$C(t) = \frac{100}{\overline{T}} \int_0^t f(\theta) d\theta \tag{7}$$

Differentiating (Equation 7) with respect to t, we get

$$\frac{\overline{T}}{100} \frac{dC}{dt} = f(t) \tag{8}$$

If we take 133 days to be the value of $\overline{T}$, we can from the slope of the initial portion of the curve calculate $f(\theta)$, the concentration of the nitrogenous precursor of hemin. We have not attempted this since our experimental values in this region ($t = 0$ to $t = 30$) are too scanty to define the curve precisely. Further, this value of $\overline{T}$ is certainly too large, since the labeled red cells have been formed over a period of time and not at an instant. To correct for this factor the initial portion of the curve must be considered.

Returning to the general equation (No. 4), let

$$x = t - \theta$$

$$dx = -d\theta$$

Then

$$C(t) = -\frac{100}{\overline{T}} \int_t^0 f(t - x)\phi(x) dx = \frac{100}{\overline{T}} \int_0^t f(t - x)\phi(x) dx \tag{9}$$

As mentioned before, for an arbitrary function $f(t - x)$ Equation 9 cannot be generally solved. The initial part of Curve *a*, Fig. 1, can, however, be fitted with sufficient accuracy up to the 30th day by an equation of the form

$$C(t) = C_0(1 - e^{-\lambda t}) \tag{10}$$

with $C_0 = 0.48$ and $\lambda = 0.11$.

Then

$$\frac{dC}{dt} = C_0 \lambda e^{-\lambda t} \tag{11}$$

It follows from Equations 8 and 11 that

$$f(t) = \frac{\bar{T}}{100} C_0 \lambda e^{-\lambda t} \tag{12}$$

Substituting this into Equation 9 yields

$$C(t) = \int_0^t C_0 \lambda e^{-\lambda(t-x)} \phi(x) dx \tag{13}$$

$$C(t) = C_0 \lambda e^{-\lambda t} \int_0^t e^{\lambda x} \phi(x) dx \tag{14}$$

Differentiating with respect to t yields

$$\frac{dC}{dt} = -\lambda \cdot C(t) + C_0 \lambda \cdot \phi(t) \tag{15}$$

$$C_0 \cdot \phi(t) = \frac{1}{\lambda} \frac{dC}{dt} + C(t) \tag{16}$$

$$C_0 \frac{d\phi}{dt} = \frac{1}{\lambda} \frac{d^2C}{dt^2} + \frac{dC}{dt} \tag{17}$$

Equation 17 shows that the death rate of the cells is not proportional to $-dC/dt$ as we initially assumed, but that another factor $(1/\lambda)(d^2C/dt^2)$ must be added to correct for the fact that the labeled red cells were not generated in an instant but have been produced over an extended interval of time, though with a rapidly declining isotope concentration.

By a graphical procedure we have evaluated d^2C/dt^2 and have used Equation 17 to compute values of $-C_0(d\phi/dt)$ for values of the argument between $t = 80$ to $t = 220$; the result is shown in Fig. 1, Curve *c*. In confirmation of our earlier assumption $d\phi/dt$ is found to be zero, *i.e.* $\phi(t) = 1$, from $t = 0$ to $t = 70$. The maximum value of $-C_0(d\phi/dt)$ occurs at $t = 127$ days. Since the death curve $-(d\phi/dt)$ *versus* time is seen to be symmetrical about the ordinate $t = 127$, the average life span of the red cell must also be 127 days.

If half the red cells have a life span between $\bar{T} - \Delta$ and $\bar{T} + \Delta$, then

$$\frac{\int_{\bar{T}-\Delta}^{\bar{T}+\Delta} \left(-\frac{d\phi}{dt}\right) dt}{\int_0^{\infty} \left(-\frac{d\phi}{dt}\right) dt} = \frac{1}{2}$$

By graphical integration of the curve $-C_0(d\phi/dt)$ *versus* time, Δ is found to be 14 days. This means that half the red cells survive for them 113 to 141 days, while the other half die before and after this period.

Variations in the value of C_0 and λ have but small effect upon the value of $\overline{T}$. The value for the average life span of the red cells, which is presumably much the same for all normal male human adults, corresponds to the production (and destruction) of 0.79 per cent of red cells per day. The value of $\overline{T}$ only relates to the period the red cell is in the circulatory system. The period between the introduction of heme into the red cell and its discharge into the circulation cannot exceed a few days. On the 4th day after the beginning of the experiments the red cells in the blood contained an appreciable concentration of labeled heme. It is likely that the time between the period in which the heme is formed and the moment it appears in the circulation cannot exceed 1 to 2 days.

By the isotope technique here described, in contrast to the other techniques, the life span of the red cells can be determined in the individual in which they are produced and destroyed under physiological conditions.

With this knowledge of the life span of the red blood cell it is possible to confirm the fact that glycine is the precursor of the protoporphyrin of hemoglobin in the human as well as in the rat. 10 days from the start of the experiment the N^{15} concentration of the protoporphyrin was 0.34 atom per cent N^{15} excess. Since the average life time of the red blood cell is about 127 days, approximately one-thirteenth of the cells are, at this time, newly formed and contain isotopically labeled heme. The newly formed red cells must contain heme having an average N^{15} concentration 13 times as high as the total heme or 4.4 atom per cent N^{15} excess (*i.e.* 13×0.34). The nitrogenous source of the heme in this 10 day period, therefore, must have had an average N^{15} concentration of 4.4 atom per cent N^{15} excess. It is clear from quantitative considerations that glycine, the isotopic amino acid fed, is the only compound that could have had as high an average N^{15} concentration for the first 10 days. It is known that ammonia and glutamic acid have the next highest N^{15} concentrations. Ammonia can easily be eliminated. The N^{15} concentration of excretory urea, which is derived from ammonia, was determined through this period, and found to be far below the required 4.4 per cent. The isotope concentration in the urinary urea rose rapidly to a maximum concentration of 3.3 atom per cent excess on the 3rd day of the experiment and then rapidly fell to 0.37 atom per cent excess on the 7th day of the experiment and had fallen to 0.188 atom per cent excess by the 10th day. The average isotope concentration in the urinary urea was less than 1.2 atom per cent excess during this period. Glutamic acid can also be eliminated. The N^{15} concentration of glutamic acid is always found to be approximately equal to that found for the whole protein, for glutamic acid nitrogen rapidly equilibrates with most of the nitrogen of the protein (17). Even the plasma proteins, which are among those with the highest N^{15} concentration after the feeding

of an isotopic compound, had an average N^{15} concentration far below 4.0 atom per cent N^{15} excess. The maximum isotope concentration attained by the plama proteins was only 0.39 atom per cent excess.

The finding that the isotope concentration in the red cell proteins, which consists mainly of globin, are low (Fig. 1, Curve *d*) is consistent with the view that the proteins, like the heme, of the erythrocytes differ from the proteins of other organs in not being involved in the dynamic state. The red cell, when formed, is supplied with its store of hemoglobin, which remains intact during its lifetime. From this it may be inferred that only about 0.79 per cent of the globin is synthesized per day.[2]

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SUMMARY

1. A study of the isotope concentrations found in the heme of the human red blood cell, after the feeding of glycine labeled with N^{15}, indicates that the erythrocyte is not subjected to indiscriminate destruction but has a life span. This was found to be about 127 days.

2. Evidence has been presented which shows that the protoporphyrin of hemoglobin is not reutilized for hemoglobin synthesis.

3. Glycine is the nitrogenous precursor of the protoporphyrin of hemoglobin in man.

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[2] In an experiment in which labeled histidine was fed to rats it was found that about 1 per cent of the globin was synthesized per day (private communication from Dr. C. Tesar).

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