THE ANEMIA OF INFECTION. XX. THE KINETICS OF IRON METABOLISM IN THE ANEMIA ASSOCIATED WITH CHRONIC INFECTION[1]

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(Submitted for publication July 7, 1955; accepted September 21, 1955)

Although the morphologic, biochemical and clinical characteristics of the anemia associated with chronic infection have been studied extensively (1), the pathogenesis of the anemia remains obscure. The profound alterations in iron and porphyrin metabolism which accompany this type of anemia have been interpreted as indicating a decreased rate of erythrocyte production as the result of a quantitative defect in the rate of conversion of protoporphyrin to hemoglobin (1, 2). The usual evidences of increased blood destruction, such as increased urobilinogen excretion and elevated serum bilirubin concentration, are lacking in the anemia of chronic infection (1).

Studies of the erythrocyte survival time have yielded conflicting results. Mollison (3), using the Ashby differential agglutination technique, found a normal disappearance rate for erythrocytes transfused into six patients with chronic sepsis and anemia. Brown (4), employing the same technique, observed a decreased life span of transfused erythrocytes in four of five cases. In pulmonary tuberculosis Hollingsworth and Hollingsworth (5) reported recently that the life span of autotransfused Cr^{51}-tagged erythrocytes is normal.

Since the anemia which is associated with rheumatic fever and rheumatoid arthritis is morphologically and biochemically similar to that associated with chronic sepsis (1), it seems appropriate to comment on several studies in these conditions. Reinhold (6), using the Ashby method, found the erythrocyte life span to be decreased in children with acute rheumatic fever. By the use of the Fe^{59} tracer technique and the Ashby method, Freireich, Ross, Bayles, Emerson, and Finch (7) found the erythrocyte life span to be decreased in rheumatoid arthritis. These investigators presented evidence suggesting that the shortened erythrocyte life span was due to an extracorpuscular hemolytic mechanism. Ebaugh, Peterson, and Bunim (8), employing the Ashby technique, the autotransfusion of Cr^{51}-tagged erythrocytes, and the Fe^{59} tracer technique, also found the erythrocyte life span to be shortened in rheumatoid arthritis.

Huff, Hennessy, Austin, Garcia, Roberts, and Lawrence (9) have developed a method for the determination of the turnover rate of iron through the plasma and red cells. By the use of these rates it is possible to understand better the alterations in the kinetics of iron metabolism which occur in various anemias (10–22). In addition, an estimation of the life span of the red cells can be obtained. The purpose of this report is to present the results of such studies on six patients with the anemia of chronic infection and, for comparison, on ten normal subjects. A preliminary report of this work has been published (23).

MATERIAL AND METHODS

The clinical and hematologic data are summarized in Table I. The normal subjects were healthy young males. All of the patients were males with no evidence of diseases other than those listed. During the period of study each subject was in a steady state with respect to erythrocyte production and destruction since no significant changes in the volume of packed red cells were noted. The reticulocyte values represent the means of at least 10 counts on each individual. All determinations were performed by the same technician. Although the individual mean values were all within normal limits for this laboratory, the difference between the mean value obtained on the normal subjects and that

[1] This investigation was supported in part by a contract (AT(11-1)-82), project No. 6, between the U. S. Atomic Energy Commission and in part by a research grant (C-2231) from the National Cancer Institute, National Institutes of Health, Public Health Service.

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TABLE I

Clinical and laboratory findings on normal subjects and patients

Patient	Age (*years*)	Diagnosis	Duration of infection (*weeks*)	V.P.R.C.* *ml./100 ml.*	Body temperature (average daily maximum)	White count *cells/cu. mm.*	R.B.C. vol. *ml./Kg.*	MCHC % †	Plasma iron *μg./100 ml.*	Reticulocytes %	Serum bilirubin *mg./100 ml.*	Hemolytic index
Normal subjects		Mean		48			36.3	34	125	0.8		
		Range		46–51			30.7–43.8	32–35	94–150	0.4–1.3		10–24
A. M.	64	Silicotuberculosis with secondary aspergillosis	48	36	99.6	7,500	34.9	32	34	1.1		18
F. B.	65	Tuberculosis (osseous and pulmonary)	18	33	100.5	8,200	27.0	32	30	1.7		3
W. S.	46	Chronic lymphangitis and cellulitis, right leg	14	43	99.0	9,800	32.8	33	57	1.5	0.8	
W. F.	60	Unresolved pneumonia	3	35	100.0	13,500	36.0	33	30	1.6	0.4	11
P. S.	64	Severe tuberculosis (pulmonary)	14	36	100.0	14,400	26.9	32	39	1.1	0.8	
R. D.	33	Tuberculous pneumonia	10	39	102.0	16,900	25.7	34	38	0.9	0.4	15
Mean				37			30.6	33	38	1.3		

* Volume of packed red cells.
† Mean corpuscular hemoglobin concentration.

obtained on the patients with infection is significant ($t = 7.35$, $P < .01$).

Plasma iron determinations were performed by the method of Hamilton, Gubler, Cartwright, and Wintrobe (24). Fecal urobilinogen was determined by the method of Schwartz, Sborov, and Watson (25). The hemolytic indices were calculated by the method of Miller, Singer, and Dameshek (26). The volume of packed red cells, hemoglobin concentration, and reticulocyte per cent were determined daily.

The radioactive iron was supplied as ferric (Fe^{59}) chloride on allocation from the U. S. Atomic Energy Commission. Ferric chloride, containing 3 to 6 μg. of iron and 5 to 20 μc of radioactivity, was buffered to pH 6.0 with 4 per cent sodium citrate and incubated for 30 minutes at room temperature with 12 ml. of the subject's own plasma. Ten ml. of this material were injected intravenously at 9:00 A.M. with the subject in the post-prandial state. Plasma samples were obtained at 15 to 30-minute intervals following the injection until the activity in 1 ml. of plasma had decreased to twice the background count. The samples were counted for 4,096 counts in a well-type, thallium-activated sodium iodide crystal, scintillation counter. A standard was prepared by diluting 1 ml. of the remaining plasma-Fe^{59} mixture to 500 ml. with water. One ml. of this material was pipetted and retained as a reference standard for subsequent counts.

The plasma iron turnover rate in ml. per Kg. of body weight per day was determined by the method of Huff and his co-workers (9), by the use of the following formula:

$$\frac{.693 \times \text{Plasma iron (mg./ml.)}}{T\,1/2\text{ (hours)} \times \text{body weight (Kg.)}} \times \text{Plasma volume (ml.)} \times 24\text{ (hours)} = \text{Plasma iron turnover rate}$$

The number 0.693 is the natural log of 2.

The plasma iron value taken for the calculations was the mean of the values of all samples from time zero to T ½.

The T ½ is the time at which the activity in the plasma had decreased to half of the initial concentration. For this determination the counts for the individual plasma samples were plotted against time on semi-logarithmic paper. A line was fitted to the points by eye and extrapolated to time zero.

The plasma volume was determined on the last day of the study with 5 μc of I^{131} human serum albumin (27). A few ml. of normal human plasma were added to the standard in order to eliminate attraction of the protein to the walls of the glassware (28).

Samples of blood were obtained daily at 9:00 A.M. until the activity in the red cells failed to increase for three successive days. For counting, 1 ml. of blood was hemolyzed with saponin; whole blood, rather than washed red cells, was counted because no significant radioactivity was present in the plasma after the day of injection. In order to correct for radioactive decay, the standard was counted daily.

The total blood volume was determined by appropriate calculations from the plasma volume and the volume of packed red cells. The total amount of radioactivity present in the red cells was determined by multiplying the counts in 1 ml. of blood by the blood volume. The frac-

tion of injected Fe^{59} incorporated into the red cells was determined by dividing the total amount of radioactivity found in the red cells by the activity injected (counts per minute per ml. of standard × 500 (dilution of standard) × 10 (ml. of plasma injected)).

The red cell iron turnover rate in mg. per Kg. per day was determined by the following formula (9):

Plasma iron turnover rate × maximum fraction of Fe^{59} incorporated into red cells = Red cell iron turnover rate, where:

The maximum fraction of Fe^{59} incorporated into red cells is the mean of the values obtained on the three consecutive days in which the proportion of radioactivity in the erythrocytes was no longer increasing.

The total amount of red cell iron, expressed in mg., was calculated by the use of the following formula:

$$\frac{\text{Blood volume (ml.)} \times \text{Hemoglobin (gm./100 ml.)} \times 3.4}{100} = \text{Red cell iron}$$

The fraction of red cell iron renewed each day was determined by the following formula (9):

$$\frac{\text{Red cell iron turnover rate} \times \text{Body weight in Kg.}}{\text{Red cell iron}} = \text{Fraction of red cell iron renewed per day}$$

The reciprocal of the fraction of red cell iron renewed each day is the red cell life span.

Body-surface counting was performed by the use of a mobile scintillation counter (10). The skin was marked with ink at appropriate points over the liver, spleen, sacrum, and rib cage. Since the liver and spleen were not palpable in any of the subjects, the sites for counting these organs were determined by percussion, and the counter was directed at the organ through an intercostal space. In order to eliminate the influence of activity emanating from costal bone marrow, an intercostal space in the right axilla was counted also and this value was subtracted from that obtained at the same time over the liver and spleen, respectively.

The counts obtained at each site were corrected for activity due to blood circulating through the area in the following manner (13): Immediately after injection of the labelled plasma, serial counts were obtained at each site. By plotting these counts on semi-logarithmic paper and extrapolating the line to time zero, the activity present at a particular site when 100 per cent of the injected activity was in the blood was determined. At the time of each *in vivo* count, the counts obtained were corrected for activity due to blood by the use of the following formula (10):

$$CPM^s - CPM^c \times \frac{Bl^{act}}{100} = CPM^c$$

where:

CPM^s is the gross counts per minute obtained at any counting site;

CPM^c is the activity present in the counting site when 100 per cent of the injected activity was in the blood;

Bl^{act} is the per cent of injected activity present in the blood (plasma and/or red cells) at the time of the particular counting;

CPM^c is the corrected counts per minute.

RESULTS

Turnover of Fe^{59} through plasma

The injected Fe^{59} disappeared from the plasma at a single exponential rate in all 6 patients with the anemia of infection and in 8 of the 10 normal subjects. Two of the normal subjects exhibited a slightly more rapid phase of isotope clearance during the first hour. However, during the next four hours the activity in the plasma diminished at a single exponential rate. The second rate was considered to represent the true rate of Fe^{59} disappearance in these 2 cases (21).

The curves of disappearance of Fe^{59} from the plasma of the normal subjects and of the patients

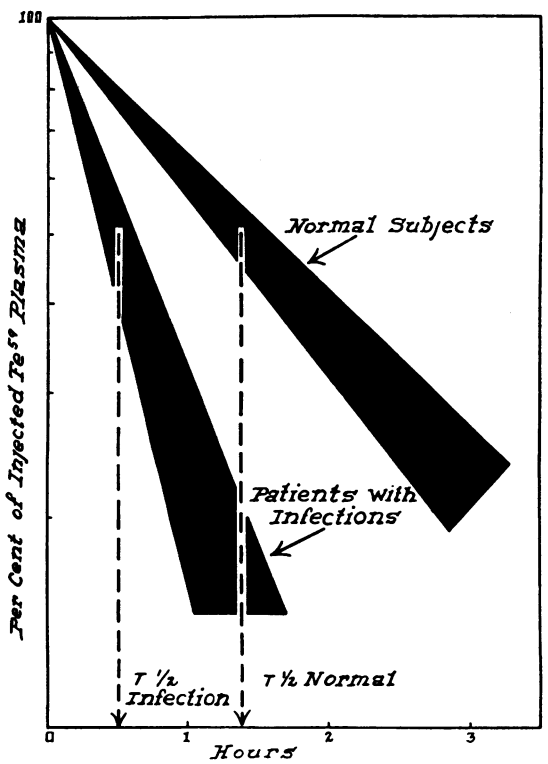

FIG. 1. DISAPPEARANCE OF Fe^{59} FROM PLASMA OF PATIENTS WITH ANEMIA OF INFECTION COMPARED TO DISAPPEARANCE FROM PLASMA OF NORMAL SUBJECTS (RANGE OF VALUES)

T ½ is the time at which the concentration of Fe^{59} in the plasma had decreased to half of its initial value.

TABLE II

Plasma iron turnover data on normal subjects and patients

Patient		Half time of plasma Fe^{59} disappearance (*Hours*)	Plasma iron pool (*mg.*)	Plasma iron turnover rate *mg./Kg./day*	No. of times/day plasma iron pool turned over
Normal subjects	Mean	1.39	3.5	0.56	11.9
	Range	1.05–1.73	2.36–4.63	0.46–0.75	9.6–15.9
A. M.		0.57	0.9	0.65	29.3
F. B.		0.42	0.8	0.65	39.6
W. S.		0.62	1.9	0.65	26.8
W. F.		0.47	1.0	0.58	35.3
P. S.		0.62	1.1	0.54	27.0
R. D.		0.38	0.9	0.59	36.7
Mean		0.51	1.1	0.61	32.5

TABLE III

Red cell iron turnover data on normal subjects and patients

Patient		Mean maximal Fe^{59} uptake in red cells	Red cell iron turnover rate *mg./Kg./day*	Red cell iron renewed daily %	Red cell life span (*days*)
Normal subjects	Mean	95	0.52	1.28	79
	Range	83–100	0.43–0.72	0.97–1.45	69–103
A. M.		96	0.62	1.60	62
F. B.		98	0.64	2.15	47
W. S.		100	0.65	1.78	56
W. F.		92	0.53	1.52	66
P. S.		95	0.51	1.61	62
R. D.		87	0.51	1.75	57
Mean		95	0.58	1.73	58

with chronic infections are shown in Figure 1. The average time at which half of the activity initially present had disappeared (T ½) in the patients with infection was 0.51 hours as compared to 1.39 hours in the normal subjects (Table II).

The size of the plasma iron pool of the patients with infection is compared with that of the normal subjects in Table II. It can be seen that the total amount of iron in the plasma of the patients was about one-third that of the normal subjects. Despite the marked reduction of the plasma iron pool in the patients, the amount of iron turned over per day (plasma iron turnover rate) did not differ significantly from that of the control subjects (Table II). The reason for the similarity between the plasma iron turnover rates of the patients and the control subjects is apparent when one considers how much more rapidly the radioiron tracer was moved (Table II) in the patients as compared to the normal subjects. This relationship is shown more clearly by the calculation of the number of times each day the plasma iron pool is turned over. The plasma iron pool of the patients was turned over an average of 32.5 times a day as compared to 11.9 times a day in the normal subjects (Table II).

Utilization of Fe^{59} by erythrocytes

The uptake of Fe^{59} by the erythrocytes of the patients with chronic infection is compared to that of the normal subjects in Figure 2. The average proportion of injected Fe^{59} in the erythrocytes of the patients was higher on the 1st, 2nd, and 3rd days after injection than in the normal subjects. Maximal incorporation was attained in the normal subjects between the seventh and the tenth day, and in the patients between the fifth and eighth day after the injection. At the time of the maximal incorporation of Fe^{59} into the red cells, the patients had incorporated an average of 95 per

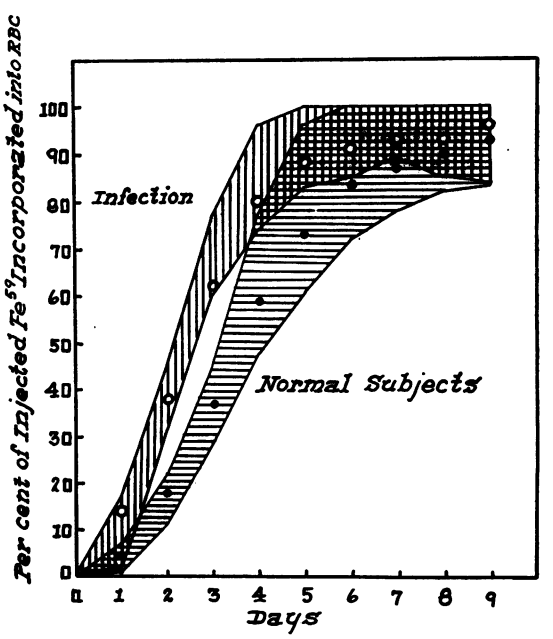

FIG. 2. UPTAKE OF Fe^{59} INTO ERYTHROCYTES OF PATIENTS WITH ANEMIA OF INFECTION COMPARED WITH NORMAL SUBJECTS

The vertical lines represent the range of values in the patients with infections. The horizontal lines represent the range of values in the normal subjects. The circles and dots represent the mean values in the patients and normal subjects, respectively.

cent of the isotope. This was the same as the value obtained in the normal subjects (Table III).

The mean red cell iron turnover rate in the patients was 0.58 mg. per Kg. per day as compared to 0.52 mg. per Kg. per day in the control subjects (Table III). The average per cent of red cell iron renewed each day in the patients with infection was 1.73 as compared with 1.28 in the normal subjects (Table III), and the average red cell life span was 58 days as compared to 79 days in the normal subjects.

Body-surface counts

Graphs of the body-surface counts in two normal subjects (L. L. and J. J.) and in two patients with infection (R. D. and A. M.) are shown in Figure 3. The pattern did not differ significantly between the two groups. The activity at the bone marrow site (sacrum) increased rapidly and peak activity was attained between 4 and 6 hours after the time of injection. Thereafter the activity decreased at a rate that was roughly the inverse of the curve of uptake of Fe^{59} into the red cells (Figure 2). A small proportion of activity entered the spleen and liver in one of the normal subjects (J. J.) and one of the patients with infection (R. D.). In the other two subjects no significant activity was detected in either the liver or the spleen.

DISCUSSION

The data presented indicate that the amount of iron turned over through red cells each day by the patients with infections was approximately the same as that turned over by normal subjects. Expressed in another way, the patients with infection made and destroyed each day the same amount of hemoglobin as did the normal subjects. Since the patients were anemic and the total red cell iron was reduced, the fraction of red cells renewed each day $\left(\frac{\text{red cell iron turnover rate}}{\text{total red cell iron}}\right)$ was increased and, hence, the erythrocyte life span was shortened.

At first glance, it would seem that the anemia is explained by the shortened life span of the erythrocytes. However, the reduction in red cell longevity is slight when compared to that observed in hemolytic anemia (29). In our patients with infections a 50 per cent increase in erythropoiesis would have been sufficient to prevent the occurrence of anemia. When consideration is given to the observation that the normal bone marrow is capable of increasing erythrocyte production 6 to 8-fold (29), it is apparent that there is marked inhibition of the bone marrow in patients with infection. Furthermore, although anemia would not develop in the absence of a decrease in red cell life span, the inability of the bone

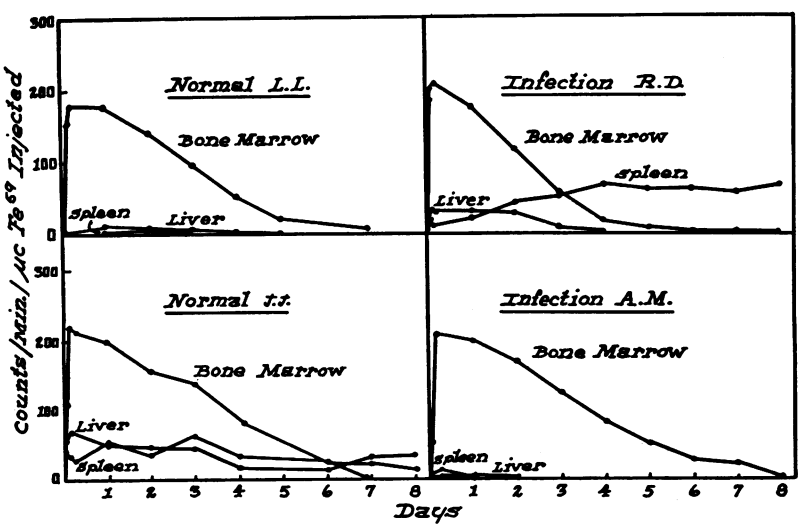

FIG. 3. BODY-SURFACE COUNTS OBTAINED OVER LIVER, SPLEEN, AND SACRAL BONE MARROW OF TWO PATIENTS WITH ANEMIA OF INFECTION (R. D. AND A. M.) AND TWO NORMAL SUBJECTS (L. L. AND J. J.)

marrow to increase erythroycte production is a defect of greater magnitude than the decrease in red cell longevity.

It should be pointed out that all six of the patients in this study had chronic infections and a mild degree of anemia which was neither improving nor becoming more severe. Therefore, these observations pertain only to patients of this type and it cannot be inferred that all patients with infection will have a similar ferrokinetic pattern.

The patients in this study incorporated an average of 95 per cent of the injected activity into the erythrocytes. Furthermore, the rate of incorporation was more rapid than in the normal subjects. These observations are in contrast to those of earlier investigators (30, 31) including studies in our own laboratory (32). In these investigations it was found that both the rate and the amount of radioiron incorporated into the red cells were decreased in patients with chronic sepsis. Furthermore, the extent of this decrease was dependent on the severity of the infection. A reasonable explanation to account for this difference is that the earlier investigators administered considerably larger quantities of carrier iron. For example, Dubach, Moore, and Minnich (30) administered 2.5 to 22 mg. of iron; Finch Gibson, Peacock, and Fluharty (31) gave 0.1 to 0.5 mg. and Greenberg, Ashenbrucker, Lauritsen, and Wintrobe (32), 3 to 4 mg. The amount of carrier iron used in the present study was between 3 and 6 μg. Since the plasma iron pool in patients with infection is about 1 mg. (Table II), the amounts of iron injected in the earlier studies appreciably increased this pool. As a consequence, it is probable that a portion of the administered isotope was shunted into stores together with the plasma iron with which it had mixed.

In order to test this hypothesis, 3 mg. of iron containing 10 μc of Fe^{59} were administered intravenously to a patient with the anemia of infection (33). The rate of uptake of Fe^{59} by the erythrocytes was found to be retarded and a maximum of only 60 per cent of the injected activity was found in the red cells after 12 days. Unfortunately it was not possible to restudy this patient with a tracer dose of radioiron.

By the use of a variety of direct methods for determining red cell life span, it has been shown that the normal human erythrocyte persists in the circulation for 110 to 120 days (34). The red cell life span as calculated from the ferrokinetic data was 79 ± 11 days in our normal subjects. This is in close agreement with the values for life span calculated from ferrokinetic data reported by some workers (20, 21), although it is somewhat shorter than the mean life span calculated from the data reported by Huff and his associates (9). There are several possible explanations for these low values for erythrocyte life span in normal subjects. First, the blood volume determinations may be erroneously high. The calculation of total blood volume on the basis of plasma volume determinations has been shown to give values which are too high, especially when the subjects are not anemic (35). On the other hand, when the total blood volume is calculated by an erythrocyte-tagging method, the value is too low (35). The true blood volume is probably obtained by the summation of the plasma volume and the red cell mass, determined simultaneously by appropriate specific methods. However, the validity of this assumption does not lend itself readily to experimental confirmation. Because of the simplicity and reproducibility of the determination of plasma volume with I^{131} human serum albumin, this method was employed with a full realization of its limitations. Furthermore, no correction was made for the difference between body and venous hematocrit because it has been our experience that this ratio is not constant from one individual to another. No correction was made for trapped plasma in the hematocrit since the correction curve had not been determined for the conditions of this laboratory at the time these studies were carried out. Had these corrections been used, however, the calculated blood volumes would have been lower and as a consequence the percentage of administered Fe^{59} incorporated into the erythrocytes would have been less and the erythrocyte life span would have been longer. A second possible explanation is that the plasma iron turnover rate is subject to a diurnal cycle (36, 37). It is possible that if the studies had been initiated at a different time of day the turnover rates would have been lower and the calculated erythrocyte life span would have been longer. However, all of the studies were initiated at the same time of day. Another possibility is that a portion of the iron which is ultimately incor-

porated into hemoglobin does not enter the bone marrow directly from the plasma but may be cycled first through other channels. If such were the case the calculated values for the turnover rates would be falsely high and the red cell life span would be falsely low. Certainly additional unknown errors may be present in the ferrokinetic studies and for the present the values for life span must be considered as relative and not absolute values. However, it is likely that the observed differences between the two groups are real since similar methods were used and similar errors were involved.

The pattern of movement of Fe^{59}, as determined by body-surface counting, was not different in the patients with the anemia of infection as compared with that in the normal subjects. The appearance of almost all of the radioactivity in the bone marrow between 4 and 6 hours after injection is in keeping with the high proportion of the administered radioactivity which was ultimately incorporated into hemoglobin. It is interesting that the patterns observed in both the normal subjects and the patients in this study are in marked contrast to those observed by Huff and his co-workers (10). These investigators noted considerable activity in the liver and spleen during the first five days of the study. However, although it was necessary for them to direct their counter at the liver and spleen through intercostal spaces, these workers did not make a correction for activity originating in the bone marrow of the ribs. When our data are plotted without the correction for activity originating in rib marrow, the patterns are similar to those described by Huff, and his co-workers. In view of the considerable quantity of active marrow which is present in the ribs, it seemed appropriate to correct the counts obtained over the liver and spleen sites. Furthermore, since in our subjects an average of 95 per cent of the administered activity was incorporated into hemoglobin, it would be surprising if a significant proportion of the radioactivity entered organs other than the bone marrow.

SUMMARY

1. Plasma and red cell iron turnover studies were carried out on 6 patients with the anemia of infection and on 10 normal subjects. Body-surface counts were made with a mobile scintillation counter over the liver, spleen, and sacral bone marrow sites in two patients and in two control subjects.

2. The average half time of plasma Fe^{59} disappearance in the patients was 0.51 hours as compared with 1.39 hours in the control subjects. The average plasma iron turnover rate and red cell iron turnover rate in the patients were 0.61 mg. per Kg. per day and 0.58 mg. per Kg. per day, respectively, as compared with 0.56 mg. per Kg. per day and 0.52 mg. per Kg. per day in the normal subjects. The incorporation of Fe^{59} into the red cells of the patients was initially more rapid than in the control subjects. The ultimate percentage of injected Fe^{59} incorporated into erythrocytes was the same in both groups. The average "apparent" erythrocyte life span as calculated from the above data, was significantly shorter in the patients than in the control subjects. The body-surface counting patterns of uptake and delivery of Fe^{59} by the liver, spleen, and bone marrow in the patients with infection did not differ significantly from those in the normal subjects. Very little activity entered the liver or spleen.

3. These data suggest that the erythrocyte life span in patients with chronic infection is shortened. However, the bone marrow is unable to compensate for this modest shortening of the erythrocyte life span. When consideration is given to the fact that a normal marrow is capable of increasing its production 6 to 8-fold, it is apparent that there is marked inhibition of erythropoiesis in such patients.

ACKNOWLEDGMENTS

We are indebted to Misses Doris Kurth and Mitsue Yanagita for technical assistance.

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