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Research Article

PLASMA AND RED CELL IRON TURNOVER IN NORMAL SUBJECTS AND IN PATIENTS HAVING VARIOUS HEMATOPOIETIC DISORDERS [1]

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One of the obvious uses which may be made of a tracer in biological studies is the determination of rates. Zilversmitt and associates (1) and more recently London (2) have called attention to this use of a tracer. Only when the isotope can be added without effectively changing the amount or concentration of material in a steady state system can such an application be made. Not until recently has radio iron been available which had sufficiently high specific activity to tag plasma iron without changing its concentration. The amount of iron leaving and entering the plasma would be significant in determining an abnormal turnover rate in some other system of the body containing iron, for example, the red cells. The present theory of iron metabolism conceives of plasma as a pool into which iron is returned before being resynthesized into the complex organic substances, hemoglobin, myoglobin, cytochromes, peroxidases, and ferritin which are so important to body function. Since the approximate normal rate of turnover of red cell iron is known, and since the major portion of this element in the body resides in the red cells, it would be expected that abnormalities in this rate would be directly reflected in plasma iron turnover rates. This paper concerns the plasma iron and red cell iron turnover data on 75 human subjects who were given amounts of iron which did not alter the steady state systems. *It is shown that such turnover rates do, indeed, agree with the clinical and laboratory data concerning normal red cell life, abnormal rates of destruction and abnormal rates of formation of red cells.* It is believed that the data ascertained from the type of study described here are of value in the appraisal of hematologic abnormality. In some instances, information will be gained which is unique and which, together with the usual hematologic data, adds to the understanding and management of disorders of red cell formation and destruction.

Iron tagged with Iron-59 was injected intravenously into normal human subjects and patients having the following disorders: polycythemia vera, secondary polycythemia, chronic lymphatic leukemia, myelogenous leukemia, pernicious anemia, hemolytic anemia, and refractory anemia. The concentration of the Iron-59 in the plasma and the red cells was determined as a function of time. One or more total plasma iron analyses were made on each subject. Circulating red cell mass and plasma volumes were determined on each subject. In addition, clinical and laboratory studies were carried out.

METHODS

The radio iron was prepared from a deuteron bombarded cobalt target in the cyclotron or was obtained from the Atomic Energy Commission. That obtained from the A. E. C. was prepared by neutron bombardment of enriched Iron-58. The cobalt targets were dissolved in hydrochloric acid and extracted with isopropyl ether. The stock preparation was ferric chloride in dilute hydrochloric acid solution. The final preparation for injection was made up on the day of the study in the following manner: an appropriate aliquot of the stock solution was buffered to a pH of approximately 6 with sodium citrate, and this was autoclaved. Then 10–30 ml. of the subject's plasma were added and the specimen allowed to stand at room temperature for at least 10 minutes before intravenous injection. The amount of radioactivity injected was approximately 10^6 counts/min. (4 microcuries).

The iron injected varied from 4 to 80 micrograms. All blood samples were drawn in heparinized syringes and transferred to 15 ml. graduated centrifuge cones. At least five blood samples were taken during the first five hours after injection. The intervals differed in the various subjects. For example, if a fast plasma turnover

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was expected, most of the first day samples would be taken in the first hour; however, if the turnover was expected to be slow, sampling might be spread over an eight hour period. After the first day, nearly daily samples were taken for a period of two weeks in some cases. In other instances only one or two samples were taken at the end of two weeks. The packed cell volume was recorded from the graduated centrifuge cone after spinning for one-half hour at 3,000 rpm in an International centrifuge, size 2. Plasma aliquots were pipetted from all samples on the first day, and in some cases throughout the study, on which total iron and radio iron determinations were made. The remaining parts of the sample, containing all the red cells and an aliquot of plasma, were analyzed for radio iron. If the corresponding plasma sample contained a significant degree of radioactivity, the values for the cell samples were corrected. The amounts of iron per packed volume of red cells were taken as 1 mg./ml. unless the cells were particularly abnormal with respect to volume, shape, or hemoglobin content. In the latter case, the total iron value was calculated from a colorimetric hemoglobin determination.

Total plasma iron was determined by the method of Kitzes (3) and colleagues. If radio iron analyses were to be carried out on samples which had to be run for total plasma iron, carrier iron was added to the final colorimetric solution. Then this was subjected to the usual procedure for radio analysis, a modification of the method described by Peacock (4), which is as follows:

To each sample, in an Erlenmeyer flask, sufficient carrier iron was added in the form of ferric chloride to assure the presence of 10 mg. Fe. After the samples had been dried at 70° C., 3–10 ml. of concentrated nitric acid were added to each and this allowed to evaporate at a temperature of 90°–100° C. Then 1–3 ml. of concentrated sulfuric acid were added and the mixture brought to fuming. If any blackening occurred the mixture was cooled and 30% hydrogen peroxide or perchloric acid (3–10 gtts.) was added and the mixture again heated. The ashed samples were transferred to 100 ml. centrifuge cones by repeated washing with .1 N hydrochloric acid. The iron was precipitated with concentrated sodium hydroxide. Phenol red was used to indicate that a sufficient amount of hydroxide had been added. After 15 hours the samples were centrifuged, the supernatant decanted, and the precipitate dissolved in 1 ml. of concentrated hydrochloric acid. The precipitation and centrifugation were repeated and the ferric hydroxide was then dissolved in .1 ml. concentrated hydrochloric acid. The ferric chloride solution was transferred to electrodeposition cells with 35 ml. of plating solution (one part saturated solution of oxalic acid and five parts of saturated solution of ammonium oxalate). Electrodeposition was carried out at .8 ampere on 2.7 cm. copper planchets. After 20 minutes, 3 ml. of saturated oxalic acid solution were added and the electrodeposition continued for 40–60 minutes more, at which time the samples were spot tested with ammonium polysulfide. Usually the spot tests were negative at this time; if they were not, plating was continued.

Red cell mass and plasma volume were determined by one or more of three methods. In a few subjects, iron tagged cells were used according to methods previously described by both Hahn (5) and Gibson (6). Red cell mass was measured in most subjects with P^{32} tagged cells (7). In most instances, the concentration of radio iron found by extrapolating the curve of plasma clearance of the injected radio iron to zero time, when used to calculate a plasma volume, gave a value which agreed well with the plasma volume calculated from the P^{32} red cell mass determination. In the event that the values were different, the latter was used in the turnover computations.

ANALYSIS OF DATA

Radio iron plasma concentration was plotted on semilogarithmic paper as a function of time (Figures 1a, 1b, 1c). In general, this plot was a straight line. It was expected that the concentration of radioactivity, a, would eventually reach some constant value, ka_0, where the value of k would be determined by the ratio of iron in plasma Fe_p to the amount of iron in the body, Fe_t. Usually no such horizontal component was observed. The ratio, $\frac{Fe_p}{Fe_t}$, is known to be of the order of 10^{-3}, and the value, a_0 (radioactivity/ml. of plasma), was approximately 10^3; thus it is obvious that it would be impossible to detect this component by sampling a few milliliters of plasma. Symbolically, the concentration of tracer as a function of time might be given as:

$$a_t = a_0e^{-Kt} + a_0k.$$

More precisely it might be expected that the function would be more complicated than above since there is no assurance that the feed-back from other systems could be considered as a single entity. Actually, as mentioned above, the value of a_0k was so small as to be neglected in the analysis. Likewise there was no evidence of any significant feed-back. Thus the equation becomes:

$$a_t = a_0e^{-Kt}.$$

The solution for K, the quantity of biological interest, is as follows:

$$K = \frac{-\ln \frac{a_t}{a_0}}{t}.$$

This constant represents the fraction of iron in

plasma, Fe_p, turned over per unit time. Thus K was considered as $\frac{r}{Fe_p}$, where r is given in mg./day. The K values for the various subjects are in column 5, Table I. The values for r for the various subjects constitute column 1, Table I; while column 2, Table I is mg./day/Kg. body weight. The evaluation of r is made as follows:

$$r = \frac{-\ln \frac{a_t}{a_0} (Fe)_p}{t}.$$

Experimentally Fe_p is determined by the product of V_p, plasma volume, and Fe_{cp}, the concentration of iron in plasma. The value for plasma iron concentration in some subjects was determined each time a plasma sample was analyzed for radioactivity; however, it became apparent that this was unnecessary since the value was nearly constant for the period of study. Thus in most instances, only one plasma iron determination was made. The methods for determining plasma volume have been discussed.

The third column of Table I contains the values, $r\frac{A_t}{A_0}$, where A_0 is the total amount of radioactivity injected and A_t is the amount of radioactivity in the circulating red cell mass when equilibrium of the radio iron in the red cells had been reached. This equilibrium state appears in many cases but not all. In the latter instance, the highest concentration achieved was the one used. The value, A_t, is the product of the circulating red cell mass and the radioactivity per unit of red cell mass. It is not thought that the value, $r\frac{A_t}{A_0}$, is a precise measure of red cell iron turnover, but rather that it is a good approximation. A discussion of its reliability and validity as an indicator of red cell iron turnover involves tracer theory that is beyond the scope of this paper.

The fourth column of Table I is the same value per Kg. of body weight.

The sixth column of Table I is:

$$\frac{r\frac{A_t}{A_0}}{\text{Fe cells}},$$

where r is in mg./day and Fe cells is the amount of iron in the circulating red cell mass.

TABLE I

Fe turnover data

Subject	Plasma iron turnover		Red cell iron turnover		Fraction of plasma iron removed/hour	Fraction of red cell iron renewed/day
	mg./day	mg./Kg./day	mg./day	mg./Kg./day		
Column	1.	2.	3.	4.	5.	6.
Normal subjects						
1. Wad.	30	.40	20	.26	.39	.0072
2. Perr.	28	.39	20	.28	.48	.0074
3. Scaz.	20	.31	14	.22	.42	.0076
4. Piet.	37	.32	31	.26	.38	.0101
5. Hut.	21	.31	15	.22	.37	.0103
Average	27	.35	20	.26	.41	.0085
Polycythemia vera Non treated cases* or treated cases with unsatisfactory remission						
6. *Yat.	41	.82	26	.50	.85	.0070
7. *Men.	245	3.70	165	2.40	3.10	.0370
8. *Mer.	65	.83	57	.72	.84	.0170
9. Mart.	111	1.40	74	.93	1.09	.0427
10. *Rem.$_1$	85	1.46	77	1.34	1.87	.0279
11. Rem.$_2$	246	4.19	209	3.58	2.97	.0695
12. *Rob.$_1$	104	1.62	—	—	3.15	—
13. Rob.$_2$	175	2.71	—	—	2.30	—
14. Rob.$_3$	148	2.28	70	1.79	3.15	.0202
15. Rob.$_4$	182	2.88	113	2.71	4.15	.0352
16. *San.	61	1.19	45	.87	1.65	.0213
17. *Vaut.	67	.72	57	.58	.93	.0118
18. *Mem.	88	1.24	47	.63	1.21	.0142
19. *Str.	107	1.55	—	—	2.77	—
20. *Opl.	68	.93	61	.84	1.04	.0350
21. *Prc.	44	.61	41	.57	1.26	.0120
22. Jen.	62	.84	—	—	1.73	—
Average	112	1.81	80	1.34	2.00	.0239
23. *Fin.	33	.51	21	.33	1.71	.0100
24. *Sch.	19	.36	15	.30	3.64	.0097
Polycythemia vera Treated: and having satisfactory remission						
25. Fin$_2$.	28	.41	—	—	.36	—
26. Kan.$_1$	41	.55	25	.30	.74	.0115
27. Kan.$_2$	29	.39	22	.27	.69	.0104
28. Sch.$_2$	16	.30	11	.20	.20	.0031
29. Has.	14	.16	11	.12	.14	.0030
30. Ltz.	30	.44	25	.39	.69	.0130
Average	26	.38	19	.26	.47	.0082
Patients referred with presumptive diagnosis of polycythemia vera						
31. Sett.	29	.33	25	.28	.73	.0084
32. Fred.	22	.28	20	.25	.70	.0073
33. And.	15	.15	9	.10	.38	.0034
Average	22	.28	18	.21	.60	.0064

TABLE I—*Continued*

Subject	Plasma iron turnover		Red cell iron turnover		Fraction of plasma iron removed/hour	Fraction of red cell iron renewed/day
	mg./day	mg./Kg./day	mg./day	mg./Kg./day		
Column	1.	2.	3.	4.	5.	6.
Polycythemia secondary (34, 35, 37: Congenital heart disease)						
34. Ferr.	27	.82	19	.56	.59	.0074
35. Syk.$_1$	26	.36	18	.25	1.31	.0086
36. Syk.$_2$	60	.82	42	.57	1.98	.0159
37. John.	59	.88	46	.70	.35	.0101
38. Hrnd.	38	.70	26	.48	.57	.0140
39. Roch.	35	.50	24	.33	.56	.0107
Average	41	.68	29	.48	.89	.0111
Chronic myelogenous leukemia (Case 45: Acute myelogenous leukemia)						
40. Hof.	94	1.46	63	.98	.73	.0342
41. Hod.	75	1.13	27	.40	.87	.0120
42. Fer.	29	.40	15	.25	.35	.0128
43. Can.	53	.94	22	.39	.54	.0150
44. Pet.	21	.35	54	.10	.40	.0033
45. Rya.	49	.95	17	.33	.36	.0089
46. Will.	36	.62	30	.49	.38	.0180
Average	51	.84	33	.42	.62	.0149
Chronic lymphatic leukemia						
47. Mill.	36	.52	34	.49	.41	.0150
48. Bach.	37	.42	31	.35	.54	.0134
49. Berd.	50	.80	40	.64	.53	.0304
50. Cow.	13	.17	11	.15	.27	.0101
51. Edw.	30	.39	26	.33	.40	.0119
52. Hag.	39	.66	33	.68	.45	.0190
53. Hen.	39	.44	37	.43	.32	.0171
54. Hop.	19	.30	19	.30	.30	.0098
55. Hug.	35	.49	31	.44	.53	.0220
56. Jam.$_1$	46	.49	28	.30	.44	.0182
57. Jam.$_2$	44	.47	12	.13	.20	.0087
58. Mus.$_1$	31	.40	12	.16	.24	.0094
59. Mus.$_2$	26	.33	8	.02	.26	.0013
60. Morg.	27	.38	23	.26	.39	.0113
61. Pek.	73	.76	73	.74	.61	.0270
62. Shp.	59	.85	43	.63	.69	.0300
63. Stlz.	30	.53	27	.44	.44	.0226
64. Trn.	45	.64	—	—	.44	—
65. Wlls.	32	.43	27	.36	.56	.0150
Average	37	.50	30	.38	.42	.0162
Pernicious anemia—untreated						
66. Brt.	78	1.89	11	.26	.94	.0340
67. Hrs.$_1$	137	2.70	27	.50	1.65	.0650
Pernicious anemia—treated						
68. Hrs.$_2$	50	.84	35	.60	.74	.0138

TABLE I—*Continued*

Subject	Plasma iron turnover		Red cell iron turnover		Fraction of plasma iron removed/hour	Fraction of red cell iron renewed/day
	mg./day	mg./Kg./day	mg./day	mg./Kg./day		
Column	1.	2.	3.	4.	5.	6.
Refractory anemia						
69. Park.	127	1.74	4	.05	.63	.0040
70. Bck.	35	.42	11	.13	.22	.0132
71. Med.	13	.26	1	.01	.10	.0001
72. Sch.	220	3.89	17	.22	.73	.0110
73. Fich.	43	.52	36	.34	.20	.0340
74. Bent.	158	2.14	112	1.51	1.66	.1270

Summary of Symbols

A_0 quantity of radioactivity injected.

A_t quantity of radioactivity present at any time, t, after injection.

a_0 concentration radioactivity/ml. plasma (extrapolated value to t = 0).

a_t concentration of radioactivity/ml. plasma at any time, t, after injection.

Fe_{pc} plasma iron concentration (micrograms/ml.).

Fe_p total circulating plasma iron.

Fe_{cells} total circulating red cell iron.

r turnover rate of plasma iron (in Table I, mg./day).

V_p plasma volume.

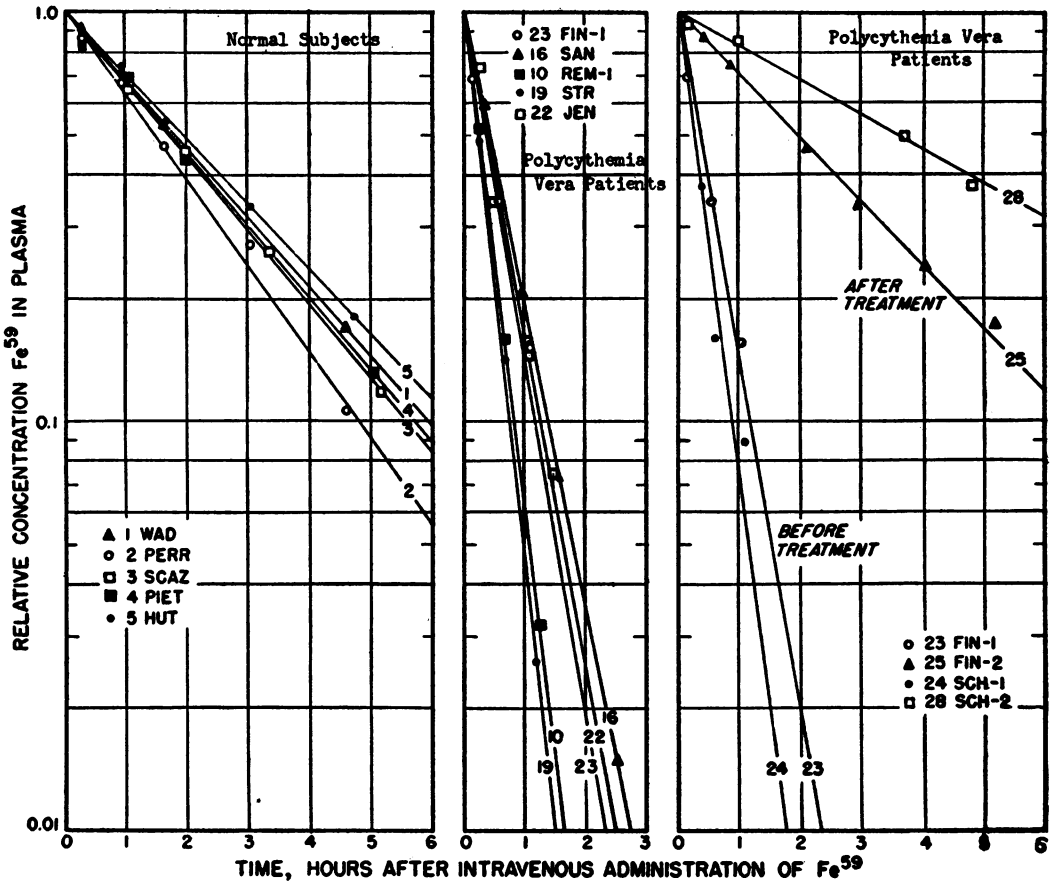
Fe_t total iron in the body.

RESULTS AND DISCUSSION

Cases 1–5. Normal Male Subjects:

All normal subjects were males over the age of 40 except for one who was 32. This age group was chosen because it most nearly matched the average age of the patients. The average plasma iron turnover in the five subjects was .35 mg./Kg. body wt./day, with a range of .31–.39 or 27 mg./day, range 21–37. The average value for the red cell iron turnover was .26 mg./Kg. body wt./day or 20 mg./day with ranges of .22–.28 and 15–31 respectively. The K values ranged from .37–.48 with an average of .41 (hours^{-1}). (See Table I and Figure 1a). The average value for the fraction of red cell iron renewed/day was .0085. There is little previous experimental data or discussion indicating what quantity of iron

FIG. 1a. FIG. 1b. FIG. 1c.



$$\text{Relative Concentration} = \frac{\text{Concentration at any t}}{\text{Concentration at t} = 0}$$

might be expected to enter or leave the plasma per unit time. As pointed out by Hahn, no adequate studies of the true distribution of iron in the human body have been made (8). Estimates of 60–75% of the body iron being present in red cells are probably not greatly in error, and erythrocyte longevity studies indicate that .85% of the red cells are removed from the circulation per day (9). Other studies have shown that the iron in red cells does not exchange exclusive of cell destruction (10) except in the case of reticulocytes (11). Values for circulating red cell mass determined by cell isotope dilution techniques in normal human males are approximately 30 ml./Kg. body wt. (6, 12, 13). There is about 1 mg. of iron/ml. of packed red cells. It might be expected then that .26 mg./Kg. body wt./day would enter and leave the plasma from the destruction and for the preparation of red cells. It would then appear that, at least in normal subjects, the method used here in estimating the red cell iron turnover is valid. (Table I, columns 6 and 4, normal subjects). This is about 75% of the total plasma iron turnover of .35 mg./Kg. body wt./day and indicates that the total remaining iron in the body has almost the same turnover rate as the red cells, although it does not preclude the possibility that some particular chemical entity containing a small amount of iron may metabolize at an altogether different rate. Iron turnover in the cytochromes has been shown to occur but the rate has not been determined (14). So far as is known there are no data on the rate at which iron enters and leaves the liver in human subjects. If its rate is proportional to the fraction of the total body iron present there, then it might be expected to be one-

tenth of the total plasma turnover or about 2.7 mg./day. Feeding experiments in this laboratory with normal foodstuffs labeled with radio iron and fed to normal subjects indicate that probably less than 1% of the total plasma iron turnover is concerned with absorption. These results are, in general, in agreement with the hypothesized system of iron metabolism of Moore, Doan, and Arrowsmith (15), except it would seem that Fe should be returning from cytochrome to the plasma. Since their description, more has been learned about the mechanism of carriage of iron in the plasma. Vannotti has demonstrated by various chemical methods the presence of what he believes are at least four types of iron circulating in the plasma (16). By his method of determination the total plasma iron is from two to three times the amount gotten by methods like that of Kitzes and co-workers (3). It is suggested by Vannotti that the "biologically active" plasma iron is the part corresponding to the fractions which he has called A and B. The method for plasma iron used in the present experiments includes only the iron fractions referred to as A and B by Vannotti. In some of the cases here reported, recovery experiments for tracer in the residue from samples which had been subjected to the usual analysis for plasma iron were carried out. In such an instance the colorimetric solution as finally developed and read and the precipitated residue were analyzed separately for radioactivity. No significant amount of radio iron was ever found in the residue. This indicates that the turnover of the tracer, as manifest in these experiments, is the same as the iron in the plasma classed by Vannotti as A and B. With the isolation of the plasma globulin which carries iron (17), following the work of Schade (18, 19) and Laurell and Holmberg (20, 21), additional correlative work was carried out by Rath and Finch (22), and Cartwright and Wintrobe (23), who confirmed the fact that clinically this protein was normally only about one-third saturated with iron. The method for plasma iron used by these investigators corresponds to the one used in these experiments. It is apparent that this protein iron complex furnishes the iron which corresponds to the Vannotti parts A and B of the plasma iron.

It appears that at least the iron from such a protein-iron compound is susceptible of removal

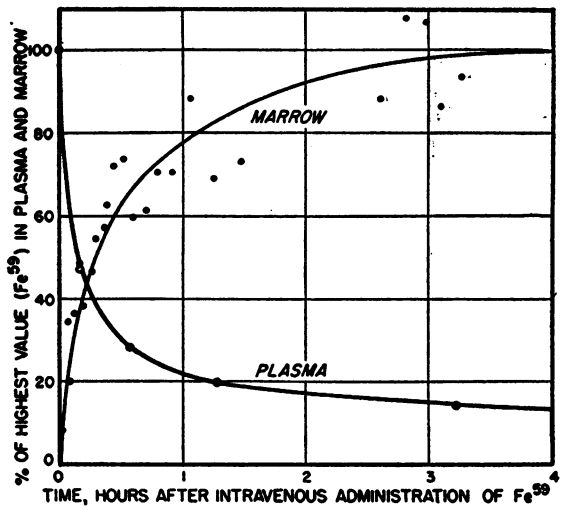


FIG. 2.

The accumulation of radio iron (detected by external counter) in the bone marrow of a patient with polycythemia vera; and the loss of radio iron from plasma (*in vitro* analysis).

by the bone marrow without further reaction, since the time concentration relationship of the radio iron in the marrow when compared to the plasma is for all practical purposes inversely identical. Figure 2 demonstrates an *in vivo* study of a polycythemic patient (No. 12). This subject was given enough radio iron so that an external counter placed over the proximal end of the femur and pointed toward the distal end gave the simple growth curve of Figure 2. Simultaneously

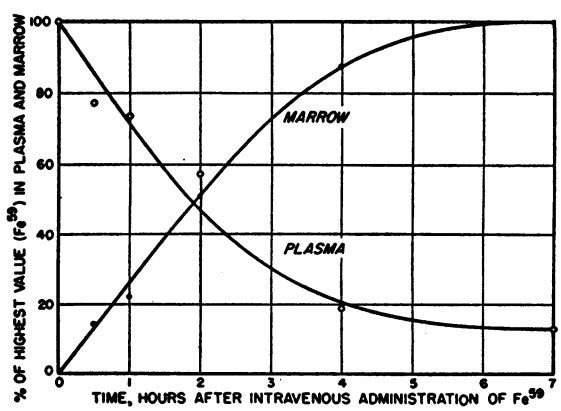


FIG. 3.

The accumulation of radio iron in the bone marrow of rabbits and the loss of the radio iron from plasma; the animals were killed serially and the analysis performed *in vitro*.

the plasma concentration of radioactivity was determined and is also plotted on the graph. *If there were an interposed metabolic reaction it would be expected that the rise in concentration in the marrow would be influenced so that it would not be a simple exponential growth curve,* ($f(t) = 1-e^{-Kt}$). This same phenomenon has been observed in serial studies on the concentration of radioactivity in the bone marrow of animals following a single intravenous injection of iron-59. In the case of the animals, the marrow was actually removed. Figure 3 shows the time concentration relationship of radio iron in the plasma and bone marrow of rabbits killed serially following single intravenous injections of radio iron.

Polycythemia vera (non-treated or treated with radio-isotopes and having an unsatisfactory remission). Studies 6–24:

Subjects having an asterisk before the name in Table I were not treated prior to the experiment. Those having no asterisk in this group are cases which were treated by marrow inhibition at some time prior to the experiment (24). Such subjects were in clinical relapse at the time of the study. Cases 23 and 24 are excluded from the group average because it was thought that their plasma iron level was so low that turnover rates were limited by circulatory rates to the various removing tissues and were not purely a function of the maximum removal rate of the areas utilizing iron.

The average value of plasma iron turnover in 17 studies on 13 cases was 1.81 mg./Kg. body wt./day. This is approximately five times the normal value of .35. Figure 1b illustrates the concentration of radio iron in plasma of polycythemic subjects as a function of time after intravenous injection. Comparison with Figure 1a shows the rapidity of iron turnover in the plasma of polycythemic subjects. Some of the K values were extremely high, for example, study 15. This subject had extraordinary splenomegaly, so much so that she felt it necessary to wear a maternity garment to appear presentable. After the first three determinations of plasma iron turnover it was thought possible that the subject's marrow was not responsible for the very rapid rate but that the spleen might be playing a significant role in iron removal. The *in vivo* study presented in Figure 2 is from this patient. Although this evidence of the accumulation of tracer iron in marrow is not quantitative, the fact that the usual amount of tracer appeared in the red cells in the subsequent days is highly indicative of an extremely avid utilization of iron by the marrow for red cells. A counter which was placed over the spleen during the study showed no accumulation of radioactivity.

Nearly all the high values for plasma iron turnover were associated with values for red cell iron turnover which were high. This was to be expected, since the subjects had a greater than normal red cell mass; however, the red cell iron turnover values were often inordinately high. That is, if the longevity of the red cells was normal, the values for red cell iron turnover would be expected to be in accordance with the red cell mass provided the patient's red cell mass was in a steady state. The usual increase in the cell mass in these subjects is by a factor of 1.5–3.0 of normal; yet some of them showed red cell iron turnover values as great as 10 times normal. This may indicate that the longevity of red cells in polycythemia vera is significantly shortened, perhaps by virtue of malformation or simply by increase in the number of collisions. Since the latter increases as the square of the concentration, it could play a significant role. However, a single study of a case of polycythemia vera red cell life by means of glycine tagged red cells showed a normal longevity (25).

No analyses were done in these experiments to show that the iron in the cells was present in the form of hemoglobin. Thus it is possible that this rapid turnover rate does not involve cell longevity but only the cellular function of hemoglobin synthesis or a peculiar exchange reaction. Miller and associates (26) studied stool urobilinogen excretion in six cases of polycythemia vera and found the values low; however, as was pointed out, the low excretion can be explained without the implication of decreased cell destruction. That stool urobilinogen excretion in excess of the value expected from the circulating red cell mass does occur in subjects having polycythemia was demonstrated by Merino recently in two cases of soroche. These subjects had red cell

masses of approximately three times normal, no greater than some of the cases of polycythemia vera included in this study (27).

Polycythemia vera (treated with radio-isotopes and having a satisfactory remission at the time of study). Studies 25–30:

The range of plasma iron turnover in these subjects is somewhat greater than in the normals: 14–41 mg./day. Likewise the red cell iron turnover shows a more marked variation than the normal. The important point is the absence of any high turnover values in the group, either of plasma or cells. It is to be noted that in studies 28 and 29 (Table I, column 6) the fraction of the red cell iron which is being renewed per day is quite low. This indicates that if this condition continues to prevail, the red cell mass will gradually diminish. Two of the subjects studied before treatment were studied again after treatment when they were in remission: Nos. 23 and 24, 25 and 28 respectively. Figure 1c demonstrates the change in K values for these patients following treatment.

We have found these studies of value in the handling of such patients because of the great changes which can occur in red cell production before there is clinical or routine laboratory evidence of an increased red cell mass. Moreover, there is a tendency because of the slow effect of radiophosphorus to over-treat. If therapy appears to be unsatisfactory but it is thought that further effects may be seen later, then a radio iron study of this type can be extraordinarily helpful. In study 29, precisely this question arose. The usual clinical and laboratory data were not decisive. Reference to Table I shows that radiophosphorus therapy at this time would have been inadvisable. Further observation of this patient over several months sustained this contention; for there was no increase, but rather a slight decrease, in red cell mass.

Patients referred with presumptive diagnosis of polycythemia vera. Studies 31–33:

Routinely polycythemia vera is suspected when there is a high hematocrit, hemoglobin, or red cell count. In most clinics, the diagnosis is considered made if in addition to this finding a red cell mass determination gives a value in excess of normal, and all evidence of secondary polycythemia has been ruled out. In this laboratory this situation has been observed on occasion, when the plasma iron turnover and red cell iron turnover values have been normal. It is believed that transitory rises in red cell mass may occur "normally." This might occur because a production rate of 10% above normal had prevailed for a year or so for some unknown reason and then had become normal. A hypothetical situation might be as follows: a patient may have been having chronic hemorrhage—enough to cause a higher production of red cells—then the hemorrhage stops with a consequent transient rise in red cell mass. We believe that the definition of the disease, polycythemia vera, should include: "an abnormally high production rate of red cells occurring in the absence of any known cause." Further, it should be realized that the production rate of erythrocytes is only one of the factors which controls the quantity of circulating red cell mass. An equally important part is played by the rate of destruction, which is not only a function of the environment of the cell but of the nature of the cell itself. As long as a polycythemia vera subject continues to show evidence of a high rate of production of red cells, he should retain that diagnosis even though he may have become anemic. The most immediate manifestation of abnormal production rates can be attained by determining the quantity of a precursor which is used in the synthesis of the product, red cells. We believe that the studies described here, in which iron is the precursor quantitated, offer such an approach. It is evident that isotope therapy should be directed at an excessive production rate of erythrocytes and not at an excessive red cell mass. The latter is easily reduced by venesection.

Cases 31–33 were referred with a presumptive diagnosis of polycythemia vera; yet the iron turnover data indicated no excessive utilization of iron (Table I).

Secondary Polycythemia. Studies 34–39:

As in the studies of polycythemia vera, the plasma iron turnover and the red cell turnover are above normal. In these cases, however, the mean value of the plasma iron turnover, .68 mg./

Kg. body wt./day, is only approximately twice normal, and the variations of the individual values are not markedly great, in contrast to the cases of polycythemia vera where there may be a conspicuous difference. The red cell iron turnover values are also less variable than in the polycythemia vera subjects and are certainly not so high. It appears that in such a subject the turnover of iron is regulated so that it quite closely approximates a nearly normal turnover rate for circulating red cell mass. Note column 6, Table I, where the mean value of fraction of red cell iron renewed per day for the five studies was .0111. It is hoped that studies of this nature may be used diagnostically in the separation of secondary polycythemia and polycythemia vera where the differential diagnosis might be difficult.

The data on polycythemia bring into contrast the apparently complete lack of regulation of iron turnover and possible lack of control of cell production in polycythemia vera, with that of secondary polycythemia, and emphasize the similarity of polycythemia vera to neoplastic disorders. It is also an argument against the type of pathogenesis for polycythemia vera suggested by Reznikoff (28), namely, bone marrow anoxia subsequent to arteriosclerosis.

Myelogenous Leukemia (all chronic except No. 45). . Studies 40–46:

These subjects were studied without selection for any particular reason except the clinical diagnosis of leukemia. They were in varying states of remission and relapse, although all of them were ambulatory. It should be noted that only one of the group had a normal plasma iron turnover value (Study 44), and that the group average, .84 mg./Kg. body wt./day, is higher than even the secondary polycythemias. Some of the cases, for example Study 40, had an almost normal uptake of radio iron in red cells so that the value for red cell iron turnover in such a case was particularly high. In spite of this the patient was slightly anemic, had been so for several months, and is now, one year later, developing a more severe anemia. The tendency here for iron turnover to be accelerated is apparent and although it is not as excessive as in polycythemia vera, the similarity is striking. Again it is not certain that the excessive rate indicates that cell destruction is going on at a greater than normal pace or that a peculiar cellular iron exchange is occurring. In addition, there is the possibility that abnormal white cell production in such subjects is concerned *per se* with excessive iron turnover. No cell separations were made; thus it is possible that radio iron was associated with white cells. Urobilinogen excretion studies were not carried out simultaneously. Nevertheless, these data suggest the possibility that in leukemia, red cell production may be adequate but abnormal.

Chronic Lymphatic Leukemia. Studies 47–65:

These cases were also unselected and are similar to the myelogenous leukemia subjects insofar as iron turnover is concerned. In general the acceleration of iron turnover is not as great as in the latter. Studies 56 and 57, at two different times on the same subject, are of interest from the standpoint of prognosis. The first study showed a quite high turnover value in plasma and a significant cell utilization so that the fraction of red cell iron renewed per day was high: .0220. The study was repeated several months later and the plasma iron turnover remained practically the same; however, the amount of tracer appearing in red cells was low. Shortly after the last test was performed, the patient became severely anemic and died.

Although corroborative studies for cell longevity are not available, it appears that in both polycythemia vera and the leukemias, the red cells have a lack of staying power and that as the clinical condition becomes worse, the ability of the red cells to sustain themselves becomes negligible. One of the polycythemia cases, Study 9, falls into this category. This subject was studied at an earlier time. The data for the earlier study does not appear in the table; but at that time the patient had an excessive turnover and a rather high red cell mass. Two years elapsed and the test was repeated. At this time the patient was slightly anemic; the turnover rate continued to be high. It is rather certain that the anemia did not appear in relation to isotope therapy; for bleeding had constituted the major form of therapy. The rate of venesection had not been increased.

Pernicious Anemia (without therapy). Studies 66–67:

Pernicious Anemia (with therapy). Study 68:

The plasma iron turnover values in the two untreated cases were as great as those of some of the most severe polycythemics: 1.89 and 2.70 mg./Kg. body wt./day. The red cell uptake of radio iron was low; but the red cell iron turnover was excessively high in relation to the circulating red cell mass, the fraction of red cell iron renewed per day being .0340 and .0650. In pernicious anemia there is good corroborative evidence for erythropoietic hyperplasia, the formation of abnormal erythrocytes, lack of staying power of the red cells (25, 29), and excessive urobilinogen excretion (26).

The treated pernicious anemia patient showed a definite tendency to return to normal; however, there remained a tendency for accelerated iron turnover. The subject was studied about three months after the institution of therapy. Morphologic cellular studies and other clinical studies showed him to be in a satisfactory remission.

Refractory Anemia. Studies 69–74:

These cases fall into two categories, in relation to plasma iron turnover. Cases 69, 72 and 74 show excessively high values which correspond to polycythemia vera; whereas cases 70, 71, and 73 are nearly normal or below normal. (Table I, column 2.) Of the first group, the last two had outright indications of excessive cell destruction such as reticulocytosis, splenomegaly and hyperplastic marrow; but the first showed no reticulocytes and a marrow which avoided classification in the usual sense. The characteristic finding of the marrow was numerous young cells which appeared to be of the red cell series. Both marrow aspiration and biopsy were unsuccessful in case 70. The marrow of case 71 was described as hypoplastic while that of case 73 was similar to that of case 69. The red cell iron turnover differed markedly among the members of the group also. Note case 74, 1.51 mg./Kg. body wt./day, and compare to cases 69 and 70, which are .05 and .13 mg./Kg. body wt./day. In cases where extremely rapid destruction of marrow cellular output occurs the method of computation of red cell iron turnover previously described breaks down and a more complicated analysis must be used. Some of these cases, for example case 69, where the marrow was questionably hypoplastic and at the same time presented a great plasma iron turnover, probably fall into this class. The accuracy of the computation of red cell iron turnover in case 73 is questionable because there appeared to be no smooth function of accumulation of radio iron in the circulating red cells; instead the function was very irregular and paralleled those cases of hemolytic anemia studied by Dubach, Moore and Minnich (30). Calculations of the fraction of red cell iron turnover in these cases are also not valid when it is impossible to determine the amount of circulating red cells which have been transfused.

In some of the refractory anemia cases the loss of radio iron from the plasma was not a simple exponential but was complicated by a rapid early loss which accounted for approximately 60% of the tracer. The K value of the rapid component was about four hours^{-1} while the K value of the slow component (on which the calculations were made when such a situation arose) was from one-tenth to two-tenths hours^{-1} $\frac{.693}{T\frac{1}{2}\ (\text{hrs.})}$. It was thought that since these patients had high plasma iron values, there might be insufficient protein for binding or that the protein was abnormal, and the fast component merely represented diffusion into the extracellular fluid. Case 71 was repeated incubating the radio iron, not with the subject's plasma, but with a normal individual's plasma; however, the result was the same. Simultaneous *in vivo* marrow studies would be very helpful in such cases.

SUMMARY

The plasma iron turnover rate has been determined and red cell iron turnover rate approximated in 75 subjects. Five of the subjects were normal males in which the average turnover rate of plasma iron and red cell iron were respectively .35 mg./Kg. body wt./day and .26 mg./Kg. body wt./day. These values are discussed in terms of what might be expected from known rates of red cell destruction and known quantities of iron in the body and in cells. The data indicate that if iron from all systems enters the plasma at one time or another, then the rate constant of iron other

than that in red cells is approximately equal to the rate constant of iron in the red cells. This does not preclude the possibility that small quantities of iron in some system may have rate constants which are altogether different.

Nineteen of the subjects studied were patients with polycythemia vera who had either not been treated or had been treated and were in relapse. The turnover rate for these patients was invariably high and the average value, 1.81 mg./Kg. body wt./day, was approximately five times the normal value. The red cell iron turnover value was also high. It appeared that the rate of renewal of red cell iron was out of proportion to the amount of circulating red cell mass and that either a faster rate of destruction of red cells or a peculiar exchange of iron occurred.

Six studies were made on secondary polycythemia subjects. Again the plasma and red cell iron turnover values were above normal, but in this instance they approached very closely the expected values for the increased red cell mass.

The effect of radio-isotope therapy on iron metabolism in polycythemia vera subjects is demonstrated in two cases which were studied before and after therapy. The iron turnover data are presented in six cases of polycythemia vera which are in satisfactory clinical remission following therapy. There is a marked difference in plasma iron turnover values following therapy. They may be restored to normal or may even fall below normal. The red cell iron turnover values may also become lower than normal following therapy.

The use of iron turnover data in the management and diagnosis of polycythemia vera is discussed.

Plasma iron turnover was found to be consistently high in seven cases of myelogenous leukemia but not as great as in polycythemia vera. Likewise the red cell iron turnover was increased in some instances, indicating again the possibility of rapid red cell destruction.

In 19 cases of lymphatic leukemia the plasma iron turnover was often above normal. In general this increase was mild when compared to the cases of polycythemia vera.

In two cases of pernicious anemia in relapse, values for plasma iron turnover were as high as some of the most severe polycythemics. Likewise the red cell iron turnover was far above the amount needed to replace the iron in the circulating red cell mass at a rate corresponding to a cell life of 125 days.

CONCLUSIONS

1. Plasma iron turnover in normal men is approximately one and one-half times the amount required for the renewal of red cell iron at a rate of .85% per day.
2. Plasma iron turnover is increased in the polycythemias, in the leukemias, in pernicious anemia, and in hemolytic anemia.
3. Radio-isotope therapy reduces the excessive plasma iron turnover rates in polycythemia vera.
4. In some cases of refractory anemia the plasma iron turnover rate is reduced; in others it is increased.
5. Iron turnover data are useful in the management and diagnosis of polycythemia vera.
6. The excessive plasma iron turnover rate of a pernicious anemia patient in relapse returned to near normal with therapy.
7. The turnover rate of plasma iron is a much more sensitive indicator of abnormalities of iron metabolism than a plasma iron determination alone.

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BIBLIOGRAPHY

1. Zilversmitt, D. B., Entenman, C., and Fishler, M. C., On the calculation of "turnover time" and "turnover rate" from experiments involving the use of labeling agents. J. Gen. Physiol., 1943, **26**, 325.
2. London, I. M., The use of stable isotopes in biological and medical research. J. Clin. Invest. 1949, **28**, 1255.
3. Kitzes, G., Elvehjem, C. A., and Schuette, H. A., The determination of blood plasma iron. J. Biol. Chem., 1944, **155**, 653.
4. Peacock, W. C., Evans, R. D., Irvine, J. W., Jr., Good, W. M., Kip, A. F., Weiss, S., and Gibson,

J. G., II, The use of two radioactive isotopes of iron in tracer studies of erythrocytes. J. Clin. Invest., 1946, **25**, 605.

5. Hahn, P. F., Balfour, W. M., Ross, J. F., and Bale, W. M., Red cell volume, total and circulating, as determined by radio iron. Science, 1941, **93**, 87.
6. Gibson, J. G., II, Peacock, W. C., Seligman, A. M., and Sack, T., Circulating red cell volume measured simultaneously by the radioactive dye and iron method. J. Clin. Invest., 1946, **25**, 838.
7. Berlin, N. I., Lawrence, J. H., and Gartland, J., The blood volume in polycythemia as determined by P–32 labeled red blood cells. To be published.
8. Hahn, P. F., The use of radioactive isotopes in the study of iron and hemoglobin metabolism and the physiology of the erythrocyte, *in:* Advances in Biological and Medical Physics. (Lawrence, J. H., and Hamilton, Joseph G., Editors.) Academic Press, Inc., New York, 1948, p. 288.
9. Shemin, D., and Rittenberg, D., The utilization of glycine for the synthesis of a porphyrin. J. Biol. Chem., 1945, **159**, 567.
10. Hahn, P. F., Bale, W. F., Hettig, R. A., and Whipple, G. H., Radio-iron in plasma does not exchange with hemoglobin iron in red cells. Science, 1940, **92**, 131.
11. Walsh, R. J., Thomas, E. D., Chow, S. K., Fluharty, R. G., and Finch, C. A. Iron metabolism. Heme synthesis *in vitro* by immature erythrocytes. Science, 1949, **110**, 396.
12. Mayerson, H. S., Lyons, C., Parson, W., Nieset, R. T., and Trautman, W. V., Jr., Comparison of results of measurement of red blood cell volume by direct and indirect techniques. Am. J. Physiol., 1948, **155**, 232.
13. Reeve, E. B., and Veall, N., A simplified method for the determination of circulating red cell volume with radioactive phosphorus. J. Physiol., 1949, **108**, 12.
14. Beinert, H., and Maier-Leibnitz, H., Cytochrome C labeled with radioactive iron. Science, 1948, **108**, 634.
15. Moore, C. V., Doan, C. A., and Arrowsmith, W. R., Studies in iron transportation and metabolism. II. The mechanism of iron transportation: its significance in iron utilization in anemic states of varied etiology. J. Clin. Invest., 1937, **16**, 627.
16. Vannotti, A., and Delachaux, A., The circulating iron, *in:* Iron metabolism and its Clinical Significance, p. 45. Grune and Stratton, New York, 1949.
17. Surgenor, D. M., Koechlin, B. A., and Strong, L. C., Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXXVII. The metal-combining globulin of human plasma. J. Clin. Invest., 1949, **28**, 73.
18. Schade, A. L., and Caroline, L., Raw hen egg white and the role of iron in growth inhibition of Shigella dysenteriae, Staphylococcus aureus, Escherichia coli and Saccharomyces cerevisiae. Science, 1944, **100**, 14.
19. Schade, A. L., and Caroline, L., An iron binding component in human blood plasma. Science, 1946, **104**, 340.
20. Holmberg, C. G., and Laurell, C. B., Studies on the capacity of serum to bind iron. Acta physiol. Scandinav., 1945, **10**, 307.
21. Laurell, C. B., Studies on the transportation and metabolism of iron in the body, with special reference to the iron binding component in human plasma. Acta physiol. Scandinav., 1947, **14**, Supp. 46.
22. Rath, C. E., and Finch, C. A., Chemical, clinical and immunological studies on the products of human plasma fractionation. XXXVIII. Serum iron transport. Measurement of iron-binding capacity of serum in man. J. Clin. Invest., 1949, **28**, 79.
23. Cartwright, G. E., and Wintrobe, M. M., Chemical, clinical and immunological studies on the products of human plasma fractionation. XXXIX. The anemia of infection. Studies on the iron-binding capacity of serum. J. Clin. Invest., 1949, **28**, 86.
24. Lawrence, J. H., The control of polycythemia by marrow inhibition. J. A. M. A., 1949, **141**, 13.
25. London, I. M., Shemin, D., West, R., and Rittenberg, D., Heme synthesis and red blood cell dynamics in normal humans and in subjects with polycythemia vera, sickle cell anemia and pernicious anemia. J. Biol. Chem., 1949, **179**, 463.
26. Miller, E. B., Singer, K., and Dameshek, W., Use of daily fecal output of urobilinogen and hemolytic index in measurement of hemolysis. Arch. Int. Med., 1942, **70**, 722.
27. Merino, C. F., Studies on blood formation and destruction in the polycythemia of high altitudes. Blood, 1950, **5**, 1.
28. Reznikoff, P., Foot, N. C., and Bethea, J. M., Etiologic and pathologic factors in polycythemia vera. Am. J. M. Sc., 1935, **189**, 753.
29. Singer, K., King, J. C., and Robin, S., The life span of the megalocyte and the hemolytic syndrome of pernicious anemia. J. Lab. & Clin. Med., 1948, **33**, 1068.
30. Dubach, R., Moore, C. V., and Minnich, V., Utilization of intravenously injected radioactive iron for hemoglobin synthesis and an evaluation of the radioactive iron method for studying iron absorption. J. Lab. & Clin. Med., 1946, **31**, 1201.