Ferrokinetics: a Biologic Model for Plasma Iron Exchange in Man

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Abstract A method is presented for calculating internal iron kinetics. An early reflux associated with extravascular exchange and a late reflux associated with erythropoiesis are described. A biologic model of iron exchange is proposed in which erythron iron turnover is divided into an effective portion (iron fixed in circulating red cells) and wastage iron of erythropoiesis (late reflux). Nonerythroid iron exchange also has a fixed portion (parenchymal uptake) and an early reflux (lymphatic circuit), both of which correlate in amount with the amount of plasma iron. Ferrokinetic measurements in normal subjects and in various pathologic states are presented to validate the model.

INTRODUCTION

Ferrokinetic measurements originally described by Huff et al. (1, 2) have provided the framework for much of our understanding of both the normal and disordered erythron. The plasma iron turnover (PIT), in which the daily transport of iron through the plasma is calculated from the plasma iron and the ^{59}Fe disappearance curve, has been extensively used as a quantitative measure of red cell production. However, the concept of a single exponential clearance of radioiron on which the PIT was formulated has been clearly disproved (3, 4). After the initial clearance of 80–90% of the injected tracer, the decline in radioactivity slows abruptly, and residual activity can be detected in the plasma over several days. This phenomenon has been ascribed to the return of part of the radioiron initially cleared from the plasma (5–7). There have been several attempts to formulate a reflux model by multicompartmental analysis and the digital computer (7–10). The importance of these models is compromised by the fact that no specific model has been validated in normal subjects or in disease states.

An alternate approach where no assumptions are required concerning the number or size of feedback compartments has been to analyze the radioiron disappearance curve in terms of probability theory (11). In the solution of that model in normal subjects, the distribution of sojourn times for returning particles was calculated numerically from an integro-differential equation by fitting the plasma iron disappearance curve with a linear combination of exponential functions. The present report deals with a method for estimating total percentage reflux based on the area underlying the disappearance curve; it has the advantage that no assumptions are required concerning the shape of the curve. Total reflux has been further divided into early and late components. These measurements have been performed in normal subjects and in selected patients with disorders in body iron stores and red cell production.

METHODS

Studies were performed in a total of 41 subjects admitted to the Clinical Research Center of the University Hospital. The control group was composed of six healthy adult males between the ages of 20 and 30 yr who were described in a previous report (11). The remaining 35 patients were selected to provide extremes in erythropoiesis and body iron stores. These patients were divided into seven categories as detailed later.

The protocol employed for ferrokinetic measurements has been previously outlined (11), and only the salient features will be noted here. Studies were performed in the morning with a dose of 0.3–0.5 μci/kg body weight of ^{59}Fe citrate (SA 11–15 mci/mg). The tracer was incubated for 30 min with sufficient heparinized plasma obtained on the same morning from a hepatitis-free donor to bind at least three times the quantity of radioiron employed. Immediately before injection and at frequent intervals over the next 14 days, blood was drawn into a heparinized syringe for measurement of the plasma iron level, microhematocrit, reticulocyte count, and ^{59}Fe activity in plasma and lysed

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TABLE I

Hematologic and Ferrokinetic Studies in Normal Subjects and in Patients with Disorders in Iron Metabolism and Red Cell Production

Subject No.	Age	Sex	Hematocrit	Uncorrected reticulocyte count	Plasma iron	Transferrin saturation	Initial $t_{\frac{1}{2}}$	Plasma iron turn over*	Red cell incorporation of ^{59}Fe	Total area‡	End area§	Coefficient of variation‖	Total reflux
			%	%	μg/100 ml	%	min		%	%-hr	%	%	%
Normal subjects													
1		M	45	0.4	100	40	70	0.85	80	235	5.6	2.4	28
2		M	44	0.5	112	48	86	0.79	81	323	3.3	1.9	36
3		M	49	0.6	99	34	80	0.69	85	310	4.5	2.8	38
4		M	47	0.6	93	33	76	0.71	83	292	5.4	3.3	38
5		M	49	0.5	126	41	95	0.74	84	336	6.6	2.1	32
6		M	46	0.7	130	52	105	0.73	81	362	5.2	3.1	30
Mean			47	0.6	110	41	85	0.75	82	309	5.1	2.6	34
SEM			1	0.01	6	3	5	0.02	1	18	0.4		2
Iron overload													
7	32	F	40	3.8	203	84	133	1.00	79	442	0.5	4.7	28
8	46	M	41	2.8	225	99	94	1.52	76	331	2.4	1.2	32
9	75	F	44	1.4	185	93	111	1.01	85	360	1.3	2.2	26
10	37	M	44	3.0	192	85	105	1.13	76	371	3.8	1.8	32
11	49	F	42	2.0	162	81	105	0.96	67	338	4.0	1.9	25
12	38	M	41	4.6	233	85	105	1.40	73	351	1.7	3.6	28
13	44	F	31	4.8	177	83	95	1.34	92	325	2.5	1.0	30
Mean			40	3.2	197	87	107	1.19	78	360	2.1	2.3	29
SEM			2	0.5	10	2	5	0.08	3	15	0.6		1
Iron deficiency													
14	54	M	22	2.8	30	12	22	1.09	96	95	10.5	1.2	44
15	79	M	29	2.0	10	3	21	0.36	99	108	20.5	1.1	54
16	69	F	69	2.8	49	13	16	0.65¶	100	87	8.8	3.9	56
17	47	M	50	1.0	27	7	15	0.66¶	100	103	4.9	5.9	65
Mean			43	2.2	29	9	18	0.69	99	98	11.2	3.0	55
SEM			11	0.4	8	2	2	0.15	1	5	3.3		4
Erythroid hypoplasia													
18	60	M	26	0.0	201	79	334	0.46	0	1215	0.7	0.8	34
19	23	M	19	0.0	207	95	336	0.51	0	1249	1.2	0.5	35
20	75	M	32	0.0	158	57	185	0.61	8	729	2.7	1.0	39
21	52	M	31	0.7	123	70	275	0.32	3	1051	2.0	1.5	37
Mean			27	0.2	172	75	283	0.48	3	1061	1.6	0.9	36
SEM			3	0.2	20	8	35	0.06	2	118	0.4		1
Renal patients Group A													
22	27	M	19	1.8	286	80	178	1.33	34	649	3.9	1.5	34
23	30	M	34	1.0	282	89	150	1.31	29	557	1.7	4.6	35
24	41	F	20	4.0	275	93	245	0.93	24	1011	1.5	1.0	42
25	27	M	18	1.5	252	86	336	0.63	14	1425	1.3	1.7	43
26	44	F	25	0.9	196	71	220	0.69	15	972	1.3	1.1	46
27	44	F	26	1.6	261	89	202	1.00	23	820	1.3	6.5	41
Mean			23	1.8	259	86	221	0.98	23	906	1.4	2.7	40
SEM			2	0.5	14	2	27	0.12	3	127	0.7		2

TABLE I—(*Continued*)

Subject No.	Age	Sex	Hematocrit	Uncorrected reticulocyte count	Plasma iron	Transferrin saturation	Initial $t_{\frac{1}{2}}$	Plasma iron turnover*	Red cell incorporation of ^{59}Fe	Total area‡	End area§	Coefficient of variation	Total reflux
			%	%	μg/100 ml	%	min		%	%-hr	%	%	%
Group B													
28	48	M	31	1.6	76	48	105	0.52	71	408	2.3	3.9	38
29	35	F	18	2.1	105	45	132	0.67	76	489	8.3	2.0	35
30	29	M	30	3.3	73	21	70	0.77	96	294	6.3	0.4	43
31	34	M	35	5.6	104	34	60	1.19	84	221	7.8	4.3	35
32	74	M	31	1.3	107	17	64	1.21	74	254	3.9	1.7	39
33	31	M	30	3.2	60	45	38	1.15	81	160	5.4	1.1	43
Mean			29	2.9	88	35	78	0.92	80	304	5.7	2.2	39
SEM			2	0.6	8	5	14	0.12	4	50	0.9		1
34	17	F	31	15.8	50	19	16	2.23	60	83	27.0	0.2	53
35	38	F	36	5.5	126	54	29	2.94	68	120	18.2	1.6	42
36	15	F	30	7.0	102	40	22	2.32	49	120	18.7	2.5	55
37	10	F	28	21.5	195	77	34	4.33	59	176	19.0	4.0	54
Mean			31	12.5	118	48	25	3.20	59	125	20.7	2.1	51
SEM			2	3.8	30	12	4	0.44	4	19	2.1		3
38	72	M	31	4.6	175	88	32	3.96	24	210	28.7	3.8	63
39	79	F	28	3.9	201	83	36	4.20	16	396	22.0	3.0	78
40	85	M	17	1.6	240	89	94	2.17	21	478	6.1	2.7	53
41	57	M	32	2.3	159	87	50	2.29	31	300	8.7	1.3	60
Mean			27	3.1	194	87	53	3.15	23	346	16.4	2.7	62
SEM			4	0.6	18	32	14	0.54	3	58	5.4		5

* Expressed as mg iron/100 ml whole blood per day.

‡ Total area (%-hr) under plasma radioiron disappearance curve.

§ Area underlying plasma radioiron disappearance curve from day 14 to infinity expressed as a per cent of the total area.

‖ Standard deviation of total are derived from SD of individual net count rates and expressed as a per cent of total area.

¶ When expressing the plasma iron turnover in relation to 100 ml whole blood, it is necessary to apply a correction when the total blood volume deviates significantly from normal. The plasma iron turnover values in these polycythemic subjects have therefore been corrected by the ratio of TBV_m:TEV_p where TBV_m = total blood volume derived from ^{59}Fe plasma volume and whole body hematocrit, and TBV_p = total blood volume predicted from body surface area (16).

whole blood. The number of samples in each study varied from 14 to 32 with a median of 18. Four of these were obtained during clearance of the initial 50% of injected activity and five more during the remainder of the first 24 hr. A final sample was obtained on day 14 of the study.

Radioactivity in 2 ml of plasma or whole blood was measured with a well-type scintillation detector. The volume of plasma counted was increased to 4 ml for samples drawn after the first 24 hr. A minimum of two 10,000 counts were obtained on each sample by an integral counting technique which gave a background of 200 cpm and an efficiency for ^{59}Fe of approximately 25% (12). The net plasma activities were expressed as a per cent of the extrapolated zero time value. The latter was obtained by plotting the activities of the first 6–8 plasma samples on semilogarithmic coordinates to determine the values which fell on the initial linear segment of the curve. A regression line was then fitted to the logarithm of these activities by the method of least squares and the zero time activity calculated as the antilogarithm of the y intercept.

The plasma iron turnover (PIT) (mg iron/100 ml whole blood per day) was calculated according to the following formula:

$$PIT = \frac{PI \times PCT_{wb}}{t_{\frac{1}{2}}}$$

where PI is the plasma iron (μg/100 ml plasma) of the time zero sample, PCT_{wb} is the whole-body plasmatocrit ($1 - Hct \times 0.0092$), and $t_{\frac{1}{2}}$ is the half-time of the initial clearance in minutes.

The red cell utilization of ^{59}Fe expressed as a per cent of the injected activity was calculated as follows (11):

$$\text{red cell utilization } (\%) = (WB_m/WB_0) \times 100 \times ([0.92 - HCT_{wb}]/PCT_{wb})$$

where WB_m and WB_0 are the net counting rates of whole

blood at the peak of the 14 day incorporation curve and at extrapolated zero time, respectively; and HCT_{wb} and PCT_{wb} are the whole body hematocrit and plasmatocrit values, respectively.

Plasma iron was determined by a modification of the method of Bothwell and Mallett (13) and iron-binding capacity by either the method of Ressler and Zak (14) or a modification of the technique of Morgan and Carter (15). Reticulocytes were based on counts of a minimum of 5000 red cells.

The method for calculating per cent reflux of plasma radioiron is based on two measurements: the initial ^{59}Fe clearance half-time and the area under the entire disappearance curve. The area subtended by the extension of the initial linear clearance represents that portion of iron fixed in tissues, whereas the total area represents both fixed and reflux turnover. Formal proof of the relationship between slope, area, and feedback is outlined in the Appendix. For practical purposes, separation of the iron turnover into fixed and feedback components may be expressed as follows:

$$\text{fixed tissue turnover (\%)} = \frac{100 \times t_{\frac{1}{2}}}{0.693 \times 60 \times (\text{area}/100)} = \frac{240 \times t_{\frac{1}{2}}}{\text{total area}}$$

$$\text{total reflux (\%)} = 100 - \text{fixed tissue turnover.}$$

Here, $t_{\frac{1}{2}}$ is the initial clearance half-time in minutes and area is the total area under the curve of ^{59}Fe activity (expressed as a per cent of the extrapolated zero time value) plotted against time in hours on ordinary rather than semilogarithmic graph paper.

While the total area under the radioiron disappearance curve can be determined by planimetric measurements, a more useful method used in the present study involves calculating the sum of the areas of trapezoids formed by connecting adjacent points with straight lines. Calculation of the area beyond the period of observation is based on the assumption that beyond day 4 of the study, the radioiron curve again becomes a single exponential function. Thus, if (0,100), (t_1,v_1), ... , (t_n,v_n) are the points on the disappearance curve with t's in hours and v's in per cent of the initial value, then the best estimate of the total area may be calculated from the following formula:

$$\tfrac{1}{2}[(t_1 - 0)(v_1 + 100) + (t_2 - t_1)(v_2 + v_1) + (t_3 - t_2)(v_3 + v_2) + \cdots + (t_n - t_{n-1})(v_n + v_{n-1})] + v_n(t_{\frac{1}{2}})/0.693$$

where $t_{\frac{1}{2}}$ is the half-time in hours of the line fitted to the tail of the curve on semilog paper and v_n is the value of that line at the final observation point t_n.

The area underlying the terminal portion of the disappearance curve from day 14 to infinity was usually less than 5% of the total area, although higher values were encountered in certain cases with increased reflux. The assumption that the disappearance curve after 4 days is a single exponential was examined for the period between 4 and 14 days by comparing the area obtained from the sum of trapezoids with a calculated value based on an exponential decrease ([activity day 4 − activity day 14]/slope). In the 41 studies reported, the serial trapezoid area and the value derived from an exponential disappearance rate averaged 38.3 and 38.0%-hr, respectively, with a SD for the differences of ±4.2%-hr. Since the latter amounts to less than 1% of the area under the entire curve, the assumption of linearity of log clearance beyond day 4 seems justified. Values for the terminal area beyond day 14 have been expressed in the Results section as per cent of the total area.

The reliability of reflux measurements mainly depends on the accuracy with which the plasma ^{59}Fe disappearance curve can be constructed. The above formula for the area as a linear combination of the observed radioiron values $v_1,v_2,$ . . . . . $,v_n$ provides a means for assessing the reliability of radioactive measurements by computing the confidence interval for the total area from the SD of the individual net counts (12). These confidence limits, expressed in Table I as per cent of the total area (coefficient of variation), were less than 5%. Because of the pronounced diurnal variation in plasma iron of normal subjects, it is important to correct the individual plasma ^{59}Fe activities according to the serum iron value at the time they were obtained. This method has been described previously (17) and was employed for the normal subjects reported here. Because of the loss of normal circadian rhythm in clinical disorders in iron or red cell production, however, this type of correction did not offer an advantage in the studies of pathologic states and was not therefore employed.

To interpret the physiologic significance of total reflux measurements, the data were further analyzed in terms of the two compartment model for radioiron reflux described in a previous paper (11). The method employed in these studies consisted of searching several thousand possible combinations of rate and proportion of iron returned in an early and late reflux with a 7040/7094 IBM computer to determine which of these solutions gave the closest fit to the observed data. Further details of this mathematical approach are outlined in the Appendix. After multiplying the percentage reflux values by the PIT to convert to an absolute scale of mg iron/100 ml whole blood per day, the following additional calculations were performed:

Fixed Tissue Turnover (FTT)	= PIT − total reflux
Fixed Erythrocyte Turnover (FET)	= FTT × red cell utilization
Fixed Parenchymal Turnover (FPT)	= FTT − FET
Erythron Turnover	= FET + late reflux
Nonerythron Turnover	= FPT + early reflux

RESULTS

General ferrokinetic data and total feedback analysis

The results of hematologic, ferrokinetic, and reflux measurements are listed individually for each of the subjects in Table I.

Normal subjects. These subjects were between the ages of 20 and 40 yr, and had hematocrits greater than 42, morning plasma iron levels over 80 μg/100 ml, and a transferrin saturation greater than 25%. The usual calculations of PIT and utilization gave mean values of 0.75 mg of iron/100 ml whole blood per day and 82%, respectively. Total reflux averaged 34%.

Parenchymal iron overload. The criteria for admission of subjects into this group were a transferrin saturation above 80%, tissue parenchymal siderosis seen on biopsy of the liver, and a urinary iron excretion after

desferrioxamine in excess of 10 mg/24 hr (18). Of these seven subjects, two had idiopathic hemochromatosis, two had the additional history of alcoholism, two had portacaval shunts, and one had cutaneous porphyria. In none was the spleen palpable nor the impairment of hepatic function severe. The mean PIT of these subjects was 1.10 mg/100 ml whole blood per day and red cell utilization was 78%. The mean total reflux was 29%.

Iron deficiency. All subjects had red cell hypochromia and microcytosis on smear, a transferrin saturation below 16%, and absence of stainable iron on bone marrow aspirates. Chronic iron deficiency was the result of blood loss from benign gastrointestinal lesions in two subjects while in the remaining two, iron deficiency was the result of phlebotomy treatment for polycythemia vera. The mean PIT was 0.70 mg/100 ml whole blood per day and red cell utilization was 99%. Total reflux averaged 55% in this group.

Erythroid hypoplasia. Two of the four patients in this group had total absence of erythropoiesis resulting from benign thymoma (subject 18) and disseminated lymphosarcoma (subject 19). The greatly diminished erythropoiesis in the remaining two subjects was unexplained. The PIT in this group was 0.48 mg/100 ml whole blood per day and red cell utilization was 3% with an average total reflux of 36%.

Renal patients. Patients with renal disease were divided into two groups. Group A was comprised of six patients with diminished erythropoiesis as indicated by a high transferrin saturation, a diminished red cell utilization, and a need for repeated transfusions. The PIT of these patients was 0.98 mg/100 ml whole blood per day and red cell utilization was 23%. In contrast, the six subjects in group B did not require transfusions and had a normal transferrin saturation and red cell utilization. The PIT of these patients averaged 0.92 mg/100 ml whole blood per day and the red cell utilization, 80%. The per cent reflux in group A was 40, and in group B, 39.

Hemolytic anemia. This group was composed of four patients with hereditary spherocytosis studied before splenectomy. In addition to splenomegaly, elevated reticulocyte counts were present in all subjects which promptly returned to normal levels after subsequent splenectomy. The PIT averaged 3.2 mg/100 ml whole blood per day of which 51% represented reflux. The mean red cell utilization was 59%; this value was undoubtedly an underestimate since red cell sequestration in the spleen was not measured.

Ineffective erythropoiesis. The four subjects in this group had refractory anemia characterized by a hyperplastic erythroid marrow with a near normal reticulocyte count. Plasma transferrin levels were in excess of 80% saturation but parenchymal iron overload as defined by urinary iron excretion in excess of 10 mg/24 hr after desferrioxamine was present in only one subject. The mean PIT was 3.15 and red cell utilization was 23%. Reflux represented 63% of the iron turnover.

Early and late reflux

The results of resolution of feedback into early and late components are summarized in Table II. A clear separation of the two components was obtained in each instance with an early portion returning within a few hours and the late component returning over several days.

The reentry $t_{\frac{1}{2}}$ for the early component was similar in all subjects for the first seven groups with means ranging from 7.0 to 10.3 hr. The proportion of total iron cleared which was returned in this component was lowest in subjects with hemolytic anemia (4.7%) and highest in patients with erythroid hypoplasia with or without associated renal failure (31.8 and 29.3%, respectively).

The reentry $t_{\frac{1}{2}}$ of the late component was more variable. The mean in normal subjects was 7.7 days. A

TABLE II
Analysis of Reflux

	Total reflux	Early reflux		Late reflux	
	%	$t_{\frac{1}{2}}$, *hr*	%	$t_{\frac{1}{2}}$, *days*	%
Normal subjects	33.6 ±1.6*	10.3 ±1.5	7.9 ±1.4	7.7 ±1.3	25.7 ±1.1
Iron overload	28.6 ±1.0	10.3 ±2.0	9.5 ±1.2	4.7 ±0.6	19.0 ±1.2
Iron deficiency	54.9 ±4.3	8.5 ±2.5	15.3 ±4.5	7.2 ±1.8	39.6 ±1.1
Erythroid hypoplasia	36.3 ±1.1	8.0 ±1.2	31.8 ±1.6	5.9 ±1.3	4.5 ±0.6
Renal Patients					
Group A	40.1 ±1.9	7.7 ±1.7	29.3 ±3.0	6.2 ±1.2	10.8 ±1.7
Group B	38.8 ±1.4	7.0 ±0.4	12.6 ±1.3	6.5 ±0.7	26.3 ±0.5
Hemolytic anemia	51.1 ±3.1	10.0 ±4.9	4.8 +1.3	15.3 ±2.4	46.3 ±2.3

* Standard error of the mean.

slightly earlier return was seen in parenchymal iron overload (4.7 days) and erythroid hypoplasia (4.8 days), while a much slower return (15.3 days) occurred in patients with hemolytic anemia. In normal subjects, 26% of the total iron cleared was returned in this late component with extremes in the remaining groups varying from 4.5% in erythroid hypoplasia to 39.6 and 46.3% in iron deficiency and hemolytic anemia, respectively.

DISCUSSION

An understanding of internal iron exchange in man depends not only on a kinetic definition of exchange, but on an anatomical localization and a functional explanation of this exchange. The observations made here arbitrarily divide iron exchange into reflux and fixed components. The patient groups studied permit conclusions as to the biologic nature of these components.

The area calculation described in the present report provides a relatively simple way to determine the proportion of plasma iron flow which is recircuited through the plasma compartment before reaching its ultimate intracellular location. The *total reflux* amounted to roughly ⅓ of total plasma iron turnover in normal subjects and in four of the seven clinical disorders studied. Significant increases in reflux to more than ½ of the total clearance occurred in hemolytic anemia (51%), iron deficiency anemia (55%), and patients with ineffective erythropoiesis (62%). These differences become more meaningful from a clinical standpoint when expressed in absolute terms and when they are further divided into early and late components.

As shown in Table III, the absolute amount of iron returned as *early reflux* was similar in all subjects of the first seven groups with a mean of 0.14 mg iron/100 ml whole blood per day and a SD of ±0.08. The level did not appear to be affected by differences in erythropoietic activity (compare groups 4 and 7) and only slightly by tissue iron load (groups 2, 4, and 5). A significant correlation was observed with the level of plasma iron supplied to the tissues calculated as the product of the plasma iron and plasmatocrit ($r = 0.711$, $P < 0.001$). Previous studies have shown that a fraction of transferrin-bound iron leaves the circulation and returns without iron exchange, presumably through lymphatics (19). Both the reentry time of 7–10 hr and the amount of iron involved in this early exchange are consistent with this lymphatic shunt.

The *late reflux* usually represents a larger fraction of the plasma iron turnover and varies considerably in different clinical disorders. That the amount of body iron has no significant effect on the late reflux component is shown by comparing the mean reflux value of 0.23 mg/100 ml whole blood per day in iron deficiency with the similar value of 0.19 mg in parenchymal iron overload. Erythropoiesis, on the other hand, has a profound effect. Late reflux is virtually absent in erythroid aplasia (0.02 mg), whereas in hemolytic anemia, it is greatly increased both in amount (1.49 mg) and in the time of return ($t_{\frac{1}{2}}$ of 15.3 days). These findings identify the late reflux with wastage iron of erythropoiesis, i.e., iron taken up by the marrow but released without permanent incorporation into circulating red cells.

Having defined these two reflux pathways, it is possible to construct a general model of iron exchange (Fig. 1). When ^{59}Fe activity disappears from plasma, there is a reciprocal buildup over the erythroid marrow (7, 17). Analysis of aspirated marrow indicates that

TABLE III

Iron Turnover Compartments

	Fixed			Reflux			Total erythron
	Total	RBC	Parenchymal	Total	Early	Late	
	mg/100 ml whole blood per day						
Normal subjects	0.50 ±0.03*	0.41 ±0.02	0.09 ±0.01	0.25 ±0.01	0.06 ±0.01	0.19 ±0.01	0.60 ±0.02
Iron overload	0.85 ±0.05	0.66 ±0.05	0.18 ±0.03	0.34 ±0.03	0.12 ±0.02	0.23 ±0.02	0.89 ±0.07
Iron deficiency	0.32 ±0.10	0.32 ±0.09	0.01 ±0.01	0.37 ±0.06	0.10 ±0.03	0.27 ±0.06	0.59 ±0.15
Erythroid hypoplasia	0.31 ±0.04	0.01 ±0.01	0.30 ±0.03	0.18 ±0.02	0.16 ±0.03	0.02 ±0.002	0.03 ±0.01
Renal patients							
Group A	0.60 ±0.09	0.15 ±0.04	0.45 ±0.05	0.38 ±0.03	0.27 ±0.02	0.11 ±0.02	0.26 ±0.06
Group B	0.56 ±0.08	0.45 ±0.06	0.11 ±0.02	0.36 ±0.05	0.11 ±0.02	0.24 ±0.04	0.69 ±0.10
Hemolytic anemia	1.56 ±0.20	1.43‡ ±0.12	0.13‡ ±0.07	1.65 ±0.27	0.15 ±0.05	1.49 ±0.25	2.92‡ ±0.32
Ineffective erythropoiesis	1.07 ±0.11			2.08 ±0.32			2.67‡ ±0.49

* Standard error of the mean.

‡ Values calculated indirectly by estimating the nonerythron iron turnover from plasma iron and plasmatocrit as illustrated in Fig. 3.

within minutes, 80–90% of the tagged iron is incorporated into heme (20). Most of this iron appears subsequently in circulating red cells. The remainder, after a delay of some days, reenters the circulation. The source of this late reflux, as discussed above, is iron associated with erythropoiesis but not fixed in the circulating red cell mass. It has been previously suggested that malformed red cells, cytoplasmic hemoglobin attached to the extruded red cell nucleus, and sideroblast iron may all contribute to this reflux (21–23). This red cell iron is processed by the reticuloendothelial tissue. Thus the movement of radioiron through the erythron and reticuloendothelial cells comprises a single intracellular iron pathway.

Fixed tissue turnover in the context of the present study means the amount of iron cleared from the plasma and not returned during the subsequent 2 wk. It consists of iron incorporated into red cells as hemoglobin and iron taken up by other tissues. That portion of injected ^{59}Fe which appears in the circulating red cells within 2 wk (i.e. the red cell utilization) may be used to calculate *fixed erythrocyte turnover* provided the life-span of the tagged circulating red cells exceeds the 2 wk interval and that all iron in the erythron cycle is located in the circulating red cell mass at 2 wk. Fixed erythrocyte turnover should then indicate the rate of turnover of circulating cells (effective erythropoiesis),

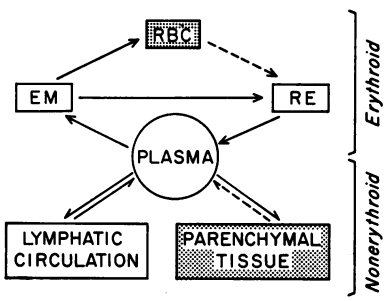

FIGURE 1 A biologic model for plasma iron turnover. The arrows leading to and from the plasma compartment depict pathways of iron turnover expressed in mg/100 ml whole blood per day. Calculation of these flows involves the initial division of the plasma iron turnover (PIT) into iron which remains localized in tissue (denoted by shaded areas) and iron which will be returned to the plasma within the 2 wk period of observation. Total reflux consists of a relatively short sojourn in the lymphatic circulation and a longer sojourn via the reticuloendothelial cell from nonviable erythrocyte precursors. The intracellularly fixed tissue turnover is partitioned on the basis of the red cell incorporation of radioiron into an erythrocyte and parenchymal fraction. No evidence was seen in the first six study groups for a return of iron from these sites (represented by interrupted lines) during the 2 wk period of observation. The total erythron iron turnover, which includes both effective and ineffective erythropoiesis, is represented by the sum of the fixed erythrocyte and late reflux values.

if all circulating cells have a finite life-span. The figure of 0.41 mg/100 ml whole blood per day obtained in normal subjects is in close agreement with the value of 0.37 mg based on the known red cell life-span of 120 days.[1]

The remaining fixed tissue turnover will be referred to as *fixed parenchymal turnover* and is accounted for by uptake into various nonerythroid tissues and by loss of a small amount from the body. In normal man, losses amount to only about 3% of the total plasma iron turnover, and even maximum iron losses in iron overload (blood loss excluded) probably do not exceed 2 mg/day or approximately 0.04 mg/100 ml whole blood per day (24).

It is not possible to identify areas of parenchymal uptake beyond citing the liver as the most important organ (25). A more precise estimate of parenchymal iron uptake may be obtained in patients with no functional erythron; localization in reticuloendothelial tissue can be excluded in these patients since this tissue cannot receive iron directly from transferrin (17). Fixed parenchymal turnover in the four subjects with aplastic anemia averaged 0.3 mg/100 ml whole blood per day while an average of 0.45 mg was found in patients with renal failure and iron overload. The higher plasma iron values in the latter two groups suggested that parenchymal uptake might be related to the level of plasma iron supplied. Indeed, a close relationship was observed in subjects of the first six groups between fixed parenchymal turnover and the concentration of transferrin iron/100 ml whole blood ($r = 0.895$, $P < 0.001$).

These interpretations of tissue uptake and plasma reflux make it possible to characterize the erythron component of iron turnover in a clinical setting. Data obtained from subjects in groups 1–6 appear consistent with the assumption that, although there is some early recircuiting, all of the ^{59}Fe in the erythron has been fixed in circulating red cells by 2 wk (Fig. 2 [top]). This assumption is not valid in hemolytic anemia where circulating red cells are being destroyed during the 2 wk study, and some of the erythron iron at 14 days remains in the expanded erythroid marrow, the reticuloendothelial tissues, and in red cells sequestered in the splenic pulp (Fig. 2 [middle]). Ineffective erythropoiesis also fails to meet the criteria of the proposed model, since an appreciable amount of radioiron cycles continuously through the erythroid marrow-reticuloendothelial circuit, and by 2 wk only a portion of this iron has located in the circulating red cell mass (Fig. 2 [bottom]). This recircuiting of iron with ineffective erythropoiesis also produces a more complex curve of plasma radioiron

[1] This figure is derived from the iron content of whole blood (50 mg/100 ml) corrected for the mean body hematocrit of 0.9 and divided by the red cell life-span of 120 days.

disappearance which presents further difficulty in resolving reflux into early and late components.

The principal use of ferrokinetics is in the evaluation of erythropoiesis. The convenient parameters for clinical use are the measurement of the initial disappearance rate of radioiron from which the plasma iron turnover may be determined and the percentage utilization at 14 days. Present observations indicate that nonerythroid turnover, including both early reflux and fixed parenchymal uptake, correlates with plasma iron supply (Fig. 3) and may be derived from the formula:

$$\text{Nonerythron Turnover} = \text{PI } (\mu g/100 \text{ ml}) \times \text{plasmatocrit} \times 0.0035.$$

If nonerythron turnover is subtracted from PIT, *erythron turnover* is obtained. This calculation is considered valid in all patients regardless of the nature of red cell production or breakdown. The magnitude of this turnover in relation to the circulating red cell mass and the red cell utilization obtained in comparison with that expected from the nonerythron turnover and the PIT, characterize the efficiency of erythropoiesis.

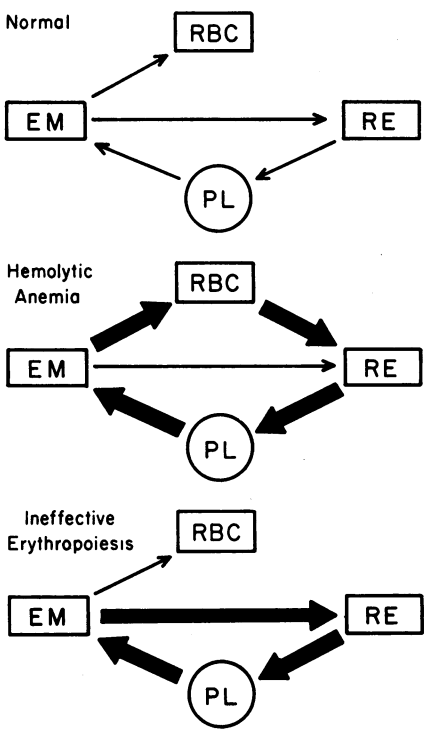

FIGURE 2 Iron kinetics in qualitative disorders of erythropoiesis. Top part portrays iron kinetics in groups 1–5 where all erythron iron becomes localized in the circulating red mass by day 14. In hemolytic anemia (middle) radioiron is revolving continuously through the erythron-reticuloendothelial pathway. The lower red cell utilization in hemolytic anemia thus reflects not only uptake by parenchymal tissues, but in addition, a more general distribution of radioiron in other components of the erythron circuit. In ineffective erythropoiesis (bottom), the major portion of radioiron is revolving through an erythroid marrow-reticuloendothelial short circuit. Only a small portion of the iron in this circuit has localized in the circulating red cell mass by 2 wk.

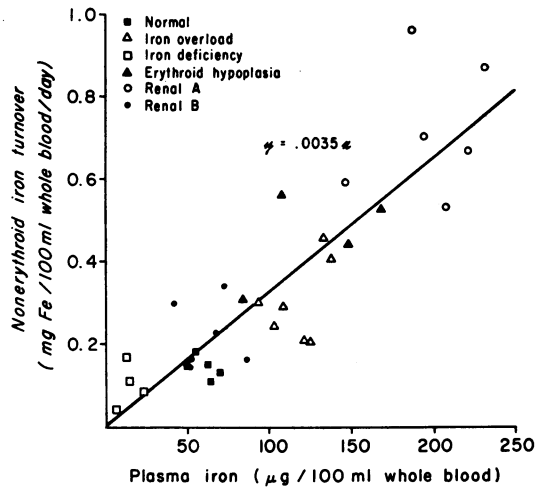

FIGURE 3 Relationship of the nonerythron iron turnover to plasma iron supply. The former has been calculated as the sum of early reflux and fixed parenchymal turnover. The plasma iron has been multiplied by the plasmatocrit to express transferrin iron concentration/100 ml whole blood. The highly significant correlation coefficient obtained for this relationship ($r = 0.873$, $P < 0.001$) indicates that nonerythron iron turnover can be predicted with reasonable accuracy from the plasma iron and hematocrit. The value of 0.0035 is the slope of a least squares regression line fitted through the origin (26).

APPENDIX

The basic equation relating the amount of tracer, f(t) in a compartment and its feedback density function g(x) is:

$$f'(t) = -hf(t) + hp\int_0^t f(t-x)g(x)dx \qquad (1)$$

where $-h$ is the initial slope of f(t), $h = -f'(0)$, p is the probability a departing particle will return, and g(x) is the sojourn density function of returning particles (11, 27). If A is the area under the disappearance curve f(t), scaled so that $f(0) = 1$, we integrate both sides of equation 1 from 0 to infinity to get $-1 = -hA + hpA$.

(The area under the convolution of two functions is the product of their areas, and g, being a density function, has area 1.) Thus

$$p = 1 - \frac{1}{hA},$$

and substituting $h = 1/(2.4\ t_{\frac{1}{2}})$, where $t_{\frac{1}{2}}$ is in minutes, gives

$$p = 1 - \frac{2.4\ (t_{\frac{1}{2}} \text{ in minutes})}{\text{area under disappearance curve}}.$$

When f(t) is a linear combination of 3 exponentials,

$$f(t) = p_1e^{-a_1t} + p_2e^{-a_2t} + p_3e^{-a_3t}, \qquad (2)$$

then g(x) will be a linear combination of 2 exponentials:

$$g(x) = c_1e^{-b_1x} + c_2e^{-b_2x}. \quad (3)$$

If h, p, and g(x) in the form 3 are given, then f(t) will have the form (2), where a_1,a_2,a_3 are roots of the cubic equation

$$x^3 - (b_1 + b_2 + h)x^2 + [b_1b_2 + h(b_1 + b_2 - pc_1 - pc_2)]x - h(b_1b_2 - pc_1b_2 - pc_2b_1) = 0$$

and

$$p_1 = \frac{a_1h + b_1b_2 - a_1(a_2 + a_3)}{(a_1 - a_2)(a_1 - a_3)}$$

$$p_2 = \frac{a_2h + b_1b_2 - a_2(a_1 + a_3)}{(a_2 - a_1)(a_2 - a_3)}$$

$$p_3 = \frac{a_3h + b_1b_2 - a_3(a_1 + a_2)}{(a_3 - a_1)(a_3 - a_2)}.$$

Thus by using the calculated initial slope h and the calculated probability of feedback p, one may use the above relations to fit an f(t) of the form 2 to experimental data by searching through a representative set of feedback densities of the form 3. Note that only three parameters need be assigned to determine g(x), since it is a density function and must have unit area, i.e., $c_1/b_1 + c_2/b_2 = 1$.

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