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Model of Reticuloendothelial Iron Metabolism in Humans: Abnormal Behavior in Idiopathic Hemochromatosis and in Inflammation

By Georges Fillet, Yves Beguin, and Laurent Baldelli

Iron transport in the reticuloendothelial (RE) system plays a central role in iron metabolism, but its regulation has not been characterized physiologically in vivo in humans. In particular, why serum iron is elevated and RE cells are much less iron-loaded than parenchymal cells in idiopathic hemochromatosis is not known. The processing of erythrocyte iron by the RE system was studied after intravenous (IV) injection of ^{59}Fe heat-damaged RBCs (HDRBCs) and ^{55}Fe transferrin in normal subjects and in patients with iron deficiency, idiopathic hemochromatosis, inflammation, marrow aplasia, or hyperplastic erythropoiesis. Early release of ^{59}Fe by the RE system was calculated from the plasma iron turnover and the ^{59}Fe plasma reappearance curve. Late release was calculated from the ratio of ^{59}Fe/^{55}Fe RBC utilization in 2 weeks. The partitioning of iron between the early (release from heme catabolism) and late (release from RE stores) phases depended on the size of RE iron stores, as illustrated by the inverse relationship observed between early release and plasma ferritin ($P < .001$). There was a strong correlation between early release and the rate of change of serum iron levels during the first three hours in normal subjects ($r = .85$, $P < .001$). Inflammation produced a blockade of the early release phase, whereas in idiopathic hemochromatosis early release was considerably increased as compared with subjects with similar iron stores. Based on these results, we describe a model of RE iron metabolism in humans. We conclude that the RE system appears to determine the diurnal fluctuations in serum iron levels through variations in the immediate output of heme iron. In idiopathic hemochromatosis, a defect of the RE cell in withholding iron freed from hemoglobin could be responsible for the high serum iron levels and low RE iron stores.

THE RETICULOENDOTHELIAL (RE) system plays a central role in iron metabolism, processing hemoglobin iron from senescent RBCs.[1-5] In normal conditions, the RE system provides most of the iron required for erythropoiesis,[6] and iron storage and release by the RE system are in equilibrium.

We previously reported a method using trace amounts of ^{59}Fe heat-damaged RBCs (HDRBCs) to investigate RE iron kinetics in dogs.[7] A model of iron transport by the RE system could be derived from that study. After an initial processing period required for heme catabolism, iron enters a labile pool from which it is either promptly returned to the plasma or incorporated into RE stores. Two distinct phases of radioiron release were observed: the early phase (immediate release from heme catabolism), which is completed within a few hours, and the late phase (release from RE stores), which develops over a period of days and weeks.

We characterized RE iron kinetics in humans, with a trace dose of a physiologic RE tag. We explored normal subjects as well as patients with various disorders of iron metabolism and/or erythropoiesis, including iron deficiency, inflammation, idiopathic hemochromatosis, marrow aplasia, and hemolysis. Iron transport in the RE system was quantified, and factors regulating RE iron release were identified. Investigation of patients with idiopathic hemochromatosis was of particular interest, because the reason why RE cells are relatively free of iron overload and serum iron is elevated in this condition is not known.[8]

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MATERIALS AND METHODS

Experimental subjects. Twelve normal male volunteers and 28 patients were studied (Table 1). Two patients had iron deficiency. Inflammation in nine patients was secondary to pneumonia (one), Dressler's syndrome after myocardial infarction (one), temporal arteritis (one), rheumatoid arthritis (five), or undetermined (one). The patients had a mean (±SD) WBC count of 8,100 ± 1,080/μl, erythrocyte sedimentation rate (ESR) of 62 ± 8 mm/hr, and fibrinogen of 6.7 ± 0.4 g/L. Eight patients had idiopathic hemochromatosis. Three had iron overload with tissue damage (subjects 24, 25, and 26), and two had no tissue damage (subjects 27 and 28). Subjects 24, 25, 27, and 28 were studied at diagnosis. Subject 26 was studied 1 year after he discontinued an incomplete phlebotomy program, subject 29 was studied while being regularly phlebotomized, and subjects 30 and 31 were studied 4 months after phlebotomy therapy was discontinued, when iron stores had returned to normal. Three patients with marrow aplasia were included, of whom two had aplastic anemia. The first (subject 32) had received ~40 L transfused blood in 3 years and developed acute myelogenous leukemia (AML) 4 months after the study. The other had received 2 L transfused blood (subject 33). The third patient had marrow aplasia after melphalan therapy for multiple myeloma but had never been transfused. Finally, six patients with hyperplastic erythropoiesis, due to hemolysis and/or ineffective erythropoiesis, were studied. Two had β-thalassemia minor, one had β-thalassemia intermedia, two had autoimmune hemolytic anemia, and one had megaloblastic anemia. Only one of them had been transfused (subject 39). The others had a mean plasma ferritin level of 213 ± 66 ng/mL.

Radioactive tags. ^{55}Fe-labeled transferrin was obtained as follows. Ten milliliters plasma were incubated for 30 minutes at 37°C with 0.5 to 0.8 μCi/kg body weight of $^{55}FeCl_3$ (specific activity 10 μCi/μg iron) previously mixed with 4% sodium citrate to ensure a molar ratio of citrate to iron of 50:1. The subject's own plasma was used except in patients with idiopathic hemochromatosis or marrow aplasia, for whom a normal donor plasma was used.

^{59}Fe-Labeled nonviable HDRBCs were prepared in the following manner. Ten milliliters normal plasma were incubated with $^{59}FeCl_3$ as described above and injected intravenously (IV) into a donor subject to obtain a circulating activity of ~50,000 cpm/mL whole

Table 1. Ferrokinetic and RE Kinetic Parameters in Normal Subjects and Patients

Subject No.	Plasma Ferritin (ng/mL)	SeFe (μg/dL)	^{59}Fe t½ (min)	PIT (μg/dL/day)	Early Release %	Early Release t½ (min)	Late Release %	Late Release t½ (days)	Late Release ^{59}Fe RCU (%)	^{59}Fe RCU (%)
Normal subjects										
1	45	89	65	0.83	68	34	27	6	81	75
2	56	70	48	0.82	89	38	18	3	88	89
3	204	120	100	0.68	43	23	51	6	73	63
4	68	148	94	0.98	71	32	22	3	82	84
5	60	152	100	0.93	53	29	41	7	86	77
6	110	150	124	0.69	58	37	32	5	86	80
7	72	122	102	0.72	53	35	42	7	86	76
8	90	126	101	0.75	68	40	32	5	82	76
9	97	138	85	0.89	73	28	—	—	—	—
10	128	45	51	0.50	58	25	41	8	83	71
11	108	100	68	0.91	46	35	46	7	89	79
12	29	120	95	0.77	87	37	18	6	87	84
Mean	89	115	86	0.79	64	33	34	6	84	78
SEM	13	10	7	0.03	4	2	3	1	1	2
Iron deficiency										
13	15	27	22	0.83	100	32	0	—	96	97
14	14	22	26	0.59	103	30	0	—	90	90
Mean	15	25	24	0.71	102	31	0	—	93	94
SEM	1	3	2	0.12	1	1	0	—	3	4
Inflammation										
15	199	38	39	0.59	35	40	74	21	93	47
16	135	41	48	0.52	35	42	65	9	95	72
17	231	20	30	0.46	28	40	76	18	88	47
18	655	55	53	0.71	28	43	72	24	88	42
19	92	25	14	1.10	59	28	37	5	81	72
20	77	30	26	0.71	69	40	37	4	87	87
21	165	40	29	0.92	63	38	37	5	100	96
22	425	30	23	0.94	42	60	—	—	—	—
23	314	23	21	0.79	35	43	65	11	80	52
Mean	254	34	31	0.75	44	42	58	12	89	64
SEM	62	4	4	0.07	5	3	6	3	2	7
Hemochromatosis										
24	2,889	149	123	0.83	52	40	43	∞	70	40
25	1,970	216	132	1.01	60	36	37	∞	63	49
26	624	262	180	0.90	60	40	41	22	72	53
27	533	270	185	0.88	51	36	44	20	66	59
28	183	298	158	1.17	55	38	48	29	81	52
29	2,238	177	71	1.60	59	32	40	15	72	56
30	28	152	123	0.74	90	30	8	≤ 0.5	85	85
31	117	205	117	1.08	94	28	0	0	86	86
Mean	1,073	216	136	1.03	65	35	33	—	74	60
SEM	395	19	13	0.10	6	2	6	—	3	6
Marrow aplasia										
32	4,836	230	240	0.57	22	—	—	—	0	0
33	867	240	264	0.62	25	—	—	—	0	0
34	246	250	281	0.67	52	—	—	—	0	0
Mean	1,983	240	262	0.62	33	—	—	—	0	0
SEM	1,438	6	12	0.03	10	—	—	—	0	0
Hyperplastic erythropoiesis										
35	71	149	80	1.20	64	36	30	5	73	62
36	167	102	62	1.10	71	44	32	11	80	72
37	152	195	109	1.30	50	40	47	8	48	34
38	215	70	21	2.70	49	30	48	4	82	57
39	1,640	123	43	2.10	30	32	—	—	—	—
40	462	247	58	3.20	53	38	—	—	—	—
Mean	451	148	62	1.93	53	37	39	7	71	56
SEM	244	26	12	0.36	6	2	5	2	8	8

blood. Two donors were selected according to the following criteria: (a) vegetative state and life expectancy not exceeding a few months; (b) normal marrow function as assessed by PB cell counts; (c) blood compatible with the largest number of potential recipients (ideally O Rh−); (d) normal liver function tests and negative serology for hepatitis and syphilis; (e) absence of antecedent or current hepatitis or neoplasia. The HIV status of the donors was not tested because the study was performed long before the first case of AIDS was reported. Both donors were admitted for cerebral thrombosis. They died 3.5 and 4 months after admission, respectively. The injection of ^{59}Fe produced no effect on their hematologic parameters. Compatibility between the donor and recipient was checked by cross-match test. At least ten days after the ^{59}Fe injection, donor blood was drawn on heparin and plasma was removed by centrifugation. The RBCs of 1 mL whole blood were washed three times in 4 mL saline, resuspended in 4 mL ACD formula A (8 g citric acid, 22 g sodium citrate, 22.3 g dextrose per liter), and heated to 46°C for nine minutes. After cooling on ice, the RBCs were washed three times with saline at room temperature, resuspended in saline, and injected within 30 minutes.

Experimental procedures. Experimental subjects were fasted overnight. ^{55}Fe Transferrin and ^{59}Fe HDRBCs were injected IV simultaneously, between 10:30 and 11:30 AM, on day 0. Blood samples (5 mL) were drawn from the opposite arm through an inline catheter at 5, 10, 15, 30, 45, 60, 90, 120, 140, 160, 180, 210, 240, 270, 300, 360, and 420 minutes after injection. ^{59}Fe Activity was also monitored by external counting over the spleen, liver, and sacrum by scintillation detectors. Radioactivity was measured for 60 minutes after injection and for ten minutes at 240 and 420 minutes on day 0. Blood samples (5 mL) and external counting were also obtained on eight to ten occasions in a 2-week period.

Ferrokinetics. The plasma iron turnover (PIT) was calculated according to the standard formula[6]:

$$\text{PIT (mg/dL WB/day)} = \frac{\text{SeFe}(\mu\text{g/dL}) \times (100 - \text{Hct} \times 0.9)/100}{t_{1/2}}, \quad (1)$$

where WB is whole blood, $t_{1/2}$ is the clearance half-time of transferrin ^{55}Fe, and 0.9 is the correction for trapped plasma in the Hct (hematocrit) (0.98) and to convert venous Hct to whole body Hct (0.92).

Plasma activity was extrapolated to 0 time, and RBC utilization (RCU) at day N was calculated by the formula:

$$\text{RCU (\%)} = \frac{\text{cpm/mL WB at day N}}{\text{cpm/mL plasma at 0 time}} \times \frac{100}{(100 - \text{Hct} \times 0.9)}, \quad (2)$$

RE iron kinetics. The calculation of early RE release of radioiron was based on the rate of clearance of ^{55}Fe transferrin and the reappearance curve of transferrin ^{59}Fe derived from HDRBCs (Fig 1A). If X represents the amount of ^{59}Fe which reappeared in the plasma at any time (percentage of dose injected), then:

$$X(t) = \int_0^t R(t - z)G(z)\,dz, \quad (3)$$

where R is the ^{55}Fe transferrin disappearance curve (percentage of dose injected), G is the rate of entry into the plasma of ^{59}Fe released by the RE system (percentage of dose injected per minute), and z is the total time from injection to appearance of ^{59}Fe on transferrin; $z = y + x$, where y is the time from injection to uptake by the RE system and x is the transit time within the RE. The convolution equation [eq. 3] was solved by a computer to obtain G from the experimental curves R and X. Because ^{59}Fe in HDRBCs is not immediately taken up by the RE system and disappears from circulation according to an exponential curve of slope r, then:

$$F(t) = G(t) - G'(t)/r, \quad (4)$$

where F represents the rate of entry into the plasma of ^{59}Fe released by the RE system (percentage of dose injected per minute) if ^{59}Fe uptake by the RE system is immediate. G' is the derivative of G. Integration of $F(t)$ with respect to time gives a hypothetical plasma ^{59}Fe radioactivity time curve $P(t)$ which represents the pattern of accumulation of ^{59}Fe in the plasma that would occur in the absence of uptake by tissues. In all studies, $F(t)$ had declined to virtually 0 by 420 minutes, resulting in a plateau of $P(t)$. The value of $P(420)$ was therefore considered equal to $P(\infty)$ and taken as the proportion of

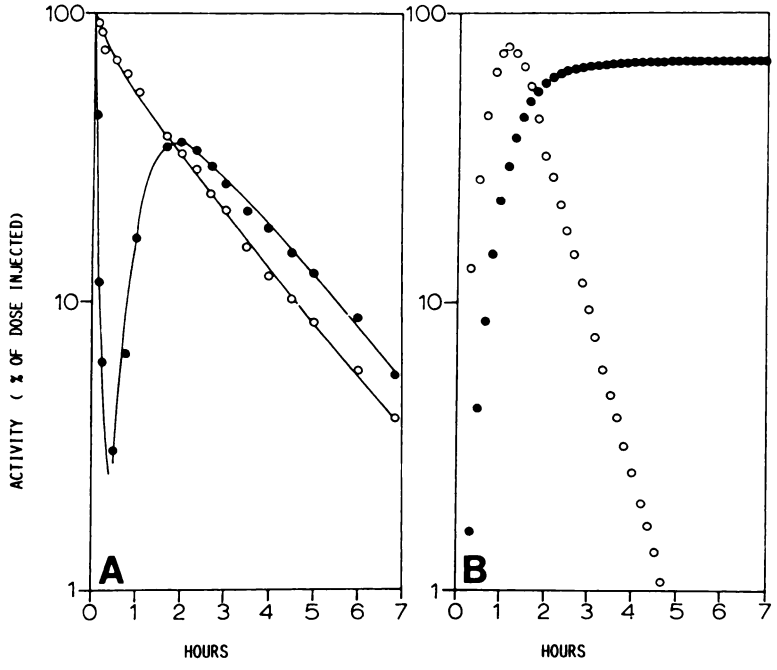

Fig 1. RE iron kinetics in a normal subject. (A) Transferrin ^{55}Fe plasma disappearance curve (○), and ^{59}Fe plasma disappearance as HDRBCs and reappearance as transferrin (●). (B) Rate of entry $G(t)$ into the plasma of ^{59}Fe released by the RE system expressed as percent of dose per minute multiplied by 100 (○), and hypothetical plasma ^{59}Fe radioactivity time curve $P(t)$ that would occur in the absence of uptake by tissues. (●).

iron released in the early phase. After 180 minutes, $F(t)$ could be described by an exponential curve, the t½ of which was used to characterize the speed of early release (Fig 1B). The mean transit time (TT) of the early phase was defined as:

$$TT \text{ (minutes)} = \frac{\int_0^\infty tF(t)\,dt}{\int_0^\infty F(t)\,dt}. \quad (5)$$

The lag period was calculated by subtracting the mean release time (release t½ × 1.44) from the TT. The late RE release was calculated from the cumulative ^{59}Fe and ^{55}Fe RCU over a 2-week period (Fig 2A). The ratio of ^{59}Fe/^{55}Fe RCU at time t represents the total fraction of ^{59}Fe released by the RE system (early release + late release at time t). When the complement of this ratio was plotted against time on a semilogarithmic paper (Fig 2B), late release appeared to be a single exponential curve. The proportion of the injected dose released in the late phase was obtained by extrapolating the curve back to the half-reappearance time of ^{55}Fe in the RBC mass. The t½ of the exponential, determined by least squares, represents the speed of late release. Formal proof of this method has been discussed previously.[7]

Miscellaneous. Serum iron and total iron binding capacity (TIBC) were measured by standard procedures.[9,10] Serum iron was measured at zero, one, two, three, five, and seven hours after injection, and the rate of change (μg/dl/h) during the first three hours was determined from the slope of the regression line fitted through serum iron values at zero, one, two, and three hours. Plasma ferritin was measured by a radioimmunoassay (RIA).[11] Hemoglobin (Hb), WBC, and platelet counts were measured in a Coulter S Counter (Coulter Electronics Inc, Hialeah, FL). Hct was measured by the micromethod.

Blood samples were processed by a modification of the method of Eakins and Brown,[12] and radioactivity was measured in a liquid scintillation counter with appropriate correction for cross-counting. Background activity and standards were measured in triplicate. Statistical significance of the differences observed between normal subjects and groups of patients was assessed by Student's t-test or by Wilcoxon rank sum test when the number of patients was very small.

Consent. The University of Liège Human Subjects and Ethic Committee was not formed at the time of the study. Therefore, a special committee was established, including the heads of the departments of internal medicine and neurology and the senior attending physician on the neurology ward. The committee approved of the experimental protocol and monitored selection of the donor subjects. Informed consent was obtained from all experimental subjects and from the families of the donor subjects.

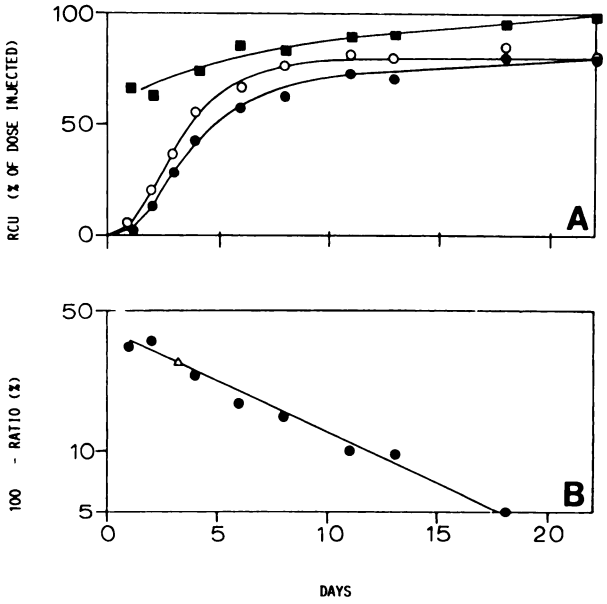

Fig 2. Late release in a normal subject. (A) RBC utilization of transferrin ^{55}Fe (○) and HDRBCs ^{59}Fe (●), and ratio of ^{59}Fe/^{55}Fe (percentage) in the RBC mass (■) in 3 weeks. (B) Late RE release. The complement of the ^{59}Fe/^{55}Fe ratio in the RBC mass was plotted against time (●). The t½ of the curve represents the speed of late release. The proportion of the injected ^{59}Fe released in the late phase was obtained by extrapolating the curve back to the half-reappearance time of ^{55}Fe in the RBC mass (△).

RESULTS

The disappearance of HDRBCs from the circulation of the recipient subject occurred as a single exponential with a t½ of 4.2 ± 0.3 minutes (mean ± SEM). Intravascular hemolysis, as monitored by the activity present in the plasma 15 minutes after injection, amounted to 1.1% ± 0.1%.

Ferrokinetic and RE iron kinetic measurements in normal subjects are shown in Table 1. After a lag period of 40 ± two minutes, 64% ± 4% (range 43% to 89%) of the activity injected as HDRBCs was released into the plasma with a t½ of 33 ± 2 minutes. The mean transit time within the RE cell was 86 ± 2 minutes. As shown in Fig 1 by the cumulative ^{59}Fe release, most of this early release takes place in the first two hours. A plateau is reached after five to seven hours, indicating that early release is virtually terminated by that time. Analysis of the RCU curve over a 2-week period demonstrated a further (late phase) release of ^{59}Fe by the RE cell with a t½ of 6 ± 1 days, involving 34% ± 3% of the activity injected. The sum of early and late releases, calculated independently, accounted for 97% (range 90% to 107%) of the radioactivity injected as HDRBCs. As determined by the ratio of ^{59}Fe/^{55}Fe RCU, 92.9% of the ^{59}Fe had been released after the second week, and >99% had been released after the third week. External counting demonstrated a rapid uptake of ^{59}Fe HDRBCs by the spleen and liver, followed by a quick release of >50% of the activity (Fig 3). Thereafter, radioactivity over the two organs remained unchanged because increasing circulating RBC activity compensated for the late ^{59}Fe release by the RE system. Although there was no correlation of the percentage of early release with the initial serum iron level, a highly significant association was found with the rate of change of serum iron level during the first three hours ($r = .85$, $p < .001$) (Fig 4). There was also a significant negative correlation between the percentage of early release and plasma ferritin ($r = -.70$, $P < .01$) (Fig 5).

RE radioiron release was also studied in a variety of disorders of iron metabolism (Table 1). Two patients with iron deficiency had 102% ± 1% of the radioiron injected as HDRBCs released in the early phase ($P < .05$ by Wilcoxon rank sum test), with a t½ similar to that of the normal subjects. The ^{59}Fe and ^{55}Fe RCU curves were identical, indicating no iron release in the late phase.

Eight patients with inflammation were studied. As compared with normal subjects, they had significantly lower

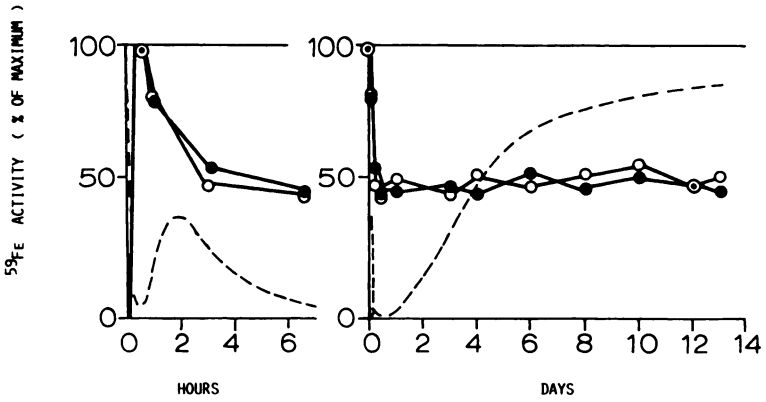

Fig 3. External counting over the spleen (O) and liver (●), in percentage of maximum uptake, using scintillation detectors. Whole blood radioactivity (---) is expressed as percentage of dose injected.

serum iron levels and higher plasma ferritin levels. Early release was reduced to 44% ± 5% ($P < .001$), and late release increased to 58% ± 6% ($P < .005$). Although the mean transit time and lag period were not changed significantly, the t½ of early release (42 ± 3 minutes, $P < .05$) and the t½ of late release (12 ± 3 days, $P < .05$) were increased. The relationship between the percentage of early release and plasma ferritin ($R = -.71$, $P < .05$) for these patients is also shown in Fig 5. No correlation was found between the percentage of early release and ESR, fibrinogen, or α_2-globulin.

The pattern of radioiron release by the RE cell was also analyzed in eight patients with idiopathic hemochromatosis. The proportions of early release (51% to 60%) and late release (37% to 48%) were rather constant in the six patients studied before or during treatment. Thus, despite transferrin saturation and increased iron stores, the early release phase was within the normal range. The t½ of late release was increased to relatively high values in the three patients with moderate iron overload, to very high values in the two patients with important iron overload, and to only 15 days in the patient heavily overloaded but being phlebotomized. In the three patients with tissue damage, external counting disclosed an accumulation of ^{59}Fe over the liver, whereas the clearance of iron from the spleen was normal. This indicated early redistribution of iron processed by the spleen into the hepatic parenchyma. The iron stores of two patients studied after treatment had returned to normal levels; 90% and 94% of the ^{59}Fe activity was released in the early phase, and 8% and 0% was released in the late phase, respectively.

Three patients with marrow aplasia had a saturated circulating transferrin. The proportion of radioiron released in the early phase was 22%, 25%, and 52%, respectively ($P < .02$ by Wilcoxon rank sum test). Late release could not be determined because of the absence of significant RCU.

Six patients presented hyperplastic erythropoiesis and thus increased amounts of iron entering the RE system secondarily to hemolysis and/or ineffective erythropoiesis.

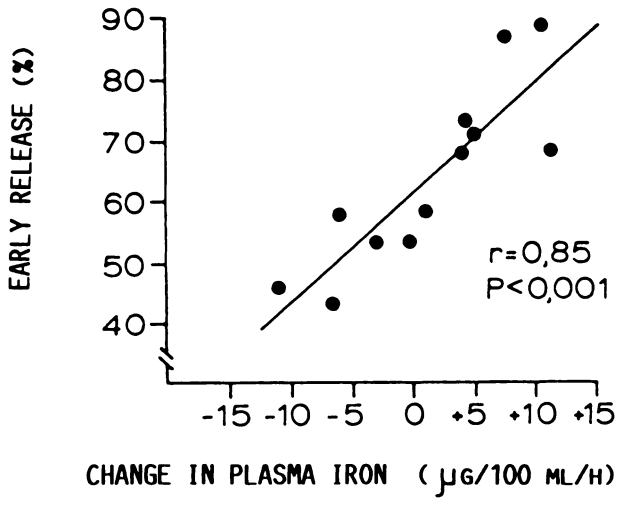

Fig 4. Correlation in normal subjects between early release and rate of change of serum iron level during the first three hours.

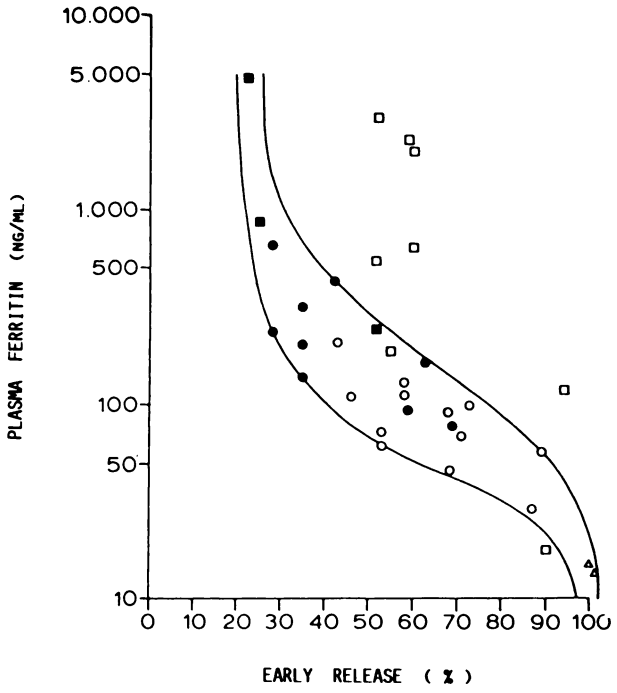

Fig 5. Correlation between percentage early release and plasma ferritin in normal conditions (O), iron deficiency (△), inflammation (●), marrow aplasia (■), and idiopathic hemochromatosis (□). The area between the two curves includes all but idiopathic hemochromatosis subjects.

Early release accounted for 53% ± 6% and late release for 39% ± 5% of the activity injected as HDRBC. The t½ of the early (37 ± 2 minutes) and late (7 ± 2 days) phases did not differ from the values observed in normal subjects. The only discrepancy with normal subjects was noted in the patient heavily transfused in whom the proportion of early release was only 30%.

DISCUSSION

The present study is the only one to characterize iron processing and release by the RE system in humans, using a physiologic RE tag. The mean iron dose of 6 μg/kg body weight is equivalent to the amount entering the RE system in 30 minutes under normal conditions. When a much higher dose of HDRBCs (500 μg Fe/kg) is injected, serum iron levels are raised up to 300 μg/dL, thus redistributing part of the radioiron to the hepatocyte.[13] However, ^{59}Fe RCU in three subjects who received only 10 μg Fe/kg was similar to our findings.[13] On the other hand, colloidal iron is catabolized more slowly than RBC iron, and iron in some big particles may not be available at all.[14]

In contrast to methods based on a preestablished multicompartmental model,[15] the present method is independent of any assumption, except that HDRBCs are cleared by the RE system and that all radioiron released is returned to the plasma. A lag period of ~40 minutes, presumably required for phagocytosis of HDRBCs and heme catabolism, was observed in normal subjects as well as in patients. In normal conditions, two thirds of the radioiron freed from Hb were promptly returned to the plasma with a t½ of 33 minutes, and one third was incorporated into RE stores and released at a much slower rate (t½ = six days). Two distinct phases of radioiron release were also identified by external counting (Fig 3). This biphasic rate of release has also been recognized in macrophage cultures.[16-18] The difference in rate of mobilization of RE storage iron and newly catabolized heme iron is consistent with the observation that normal individuals phlebotomized to an Hct of 25% to 30% will deliver a maximum of 40 to 60 mg storage iron per day to the marrow, whereas iron supply from hemolyzed RBCs is much more efficient.[19]

Most[16,20,21] but not all[22] in vitro studies with macrophages have failed to show any effect of excess apotransferrin or of transferrin saturation on RE iron release. Injection of a large dose of apotransferrin in animals did not modify iron donation by tissues.[23,24] Saturating plasma transferrin in dogs[7] or in humans[13] did not change iron release by the RE cell. Similarly, we and other investigators[15] observed no correlation between the initial plasma iron level and the early phase of RE radioiron release.

However, in normal subjects, there was a strong correlation between the percentage of early release and the rate of change of serum iron in the three hours after injection of HDRBCs (Fig 4). A mean rate of change of 10 μg/100 mL corresponded to 18.1% early release. The total flux of iron through the RE system represents 80% of the PIT (ie, 44.5 μg/100 mL/h). Therefore, 80% of the variation of serum iron can be accounted for by changes in the percentage of early release (44.5 × 18.1/100 = 8 μg/100 mL/h). This indicates that the RE activity is responsible for most of the diurnal variations in serum iron levels.[7,25]

It is important to make the distinction between radioiron release as we define it and the absolute iron output from the RE cell. Thus, because radioiron is diluted in a higher iron input into the RE system, the normal percentage of early radioiron release in patients with hemolysis or ineffective erythropoiesis means that the absolute amount of iron released by the RE system is enhanced in such cases.

Hypertransfusion[23] or aplasia reduces, whereas venesection,[23] exchange transfusion with reticulocytes,[24] or erythroid hyperplasia enhances iron release by the RE cell. Patients with marrow aplasia still showed significant early release despite transferrin saturation and suppression of erythropoiesis. This 22% early release could represent a limit for the ability of the RE system to retain iron from digested RBCs.

Although marrow requirements largely influence the total amount of iron leaving the RE system, the importance of RE iron stores appears to influence the partitioning of radioiron between the early and late release phases. When iron stores are absent, as in iron deficiency, iron ouput is totally contributed entirely by recently digested RBCs.[13,14,26,27] When RE iron stores increase, RE radioiron release decreases,[23] and this was particularly true for early release (Fig 5). This was also observed in rats in which the percentage of early release (ER) and the rate of late release were inversely related to the amount of ferritin iron in the spleen (G. Fillet, unpublished observations, July 1977).

The following model of RE iron metabolism in humans can thus be proposed. Iron output from the RE system is responsible for diurnal variations of serum iron levels. However, the total amount of iron leaving the RE system remains relatively constant over time under basal conditions. When body (marrow) requirements are enhanced, more iron is donated, particularly through an increase in the immediate output of heme iron. When body (marrow) requirements are decreased, iron supply is diminished. Plasma iron levels decrease when marrow requirements cannot be matched by iron mobilization and increase when marrow activity is not sufficient to use the minimum amount of iron the RE cell is not able to retain. When the amount of iron entering the RE system is increased by transfusions and erythropoiesis is not stimulated to a similar extent, the RE cell will accumulate storage iron.

However, abnormalities in regulation of RE activity can appear in certain diseases. RE iron output is reduced by inflammation.[13,23,26,28-31] The negative correlation of the percentage of early release with plasma ferritin (Fig 5) and the absence of correlation with the biologic markers of inflammation apparently indicate that early release is decreased because RE stores are increased and not because of the intensity of inflammation itself. However, hyposideremia occurs within hours of inflammation,[31,32] when the iron content of RE cells cannot yet be increased.[32] Ferritin production is enhanced in inflammatory RE cells,[29,33] and this precedes the decrease in serum iron levels. Thus, increased ferritin synthesis (as a nonspecific acute-phase reactant?) may be a mechanism accounting for the reduction in iron output from the RE cell.[29]

Idiopathic hemochromatosis is characterized by high serum iron levels even before significant iron overload has developed.[8] The high transferrin saturation is responsible for a progressive iron loading of the hepatocytes.[34] IH patients with limited iron stores released >90% of HDRBC radioiron in the early phase, whereas those with heavy stores still had a normal percentage of early release. At each ferritin level, early release was much higher in idiopathic hemochromatosis patients than in the other patients with similar plasma ferritin levels (Fig 5). However, the rate of late release appeared to be related to the size of iron stores. These results suggest that the RE cell in idiopathic hemochromatosis could have a defect in withholding iron freed from Hb that would explain the high serum iron levels observed early in the course of the disease and the relatively low RE iron stores. Because iron input into the RE cell is mostly contributed by heme iron from senescent RBCs, any reduction in transferrin iron uptake would probably not be of sufficient magnitude to render RE cells iron deficient. The abnormal behavior of the RE cell should be paralleled to that of the mucosal cell, which also releases excessive amounts of iron from absorption into the portal circulation.[8] An inverse relationship between plasma ferritin and iron absorption is observed in normal subjects as well as in patients with idiopathic hemochromatosis, but absorption in idiopathic hemochromatosis is higher in relation to iron stores at any level.[35] The basic mechanism responsible for this abnormal RE activity is not known. The rate of ferritin synthesis in monocytes isolated from idiopathic hemochromatosis patients has been either normal[17] or decreased (although not significantly).[36] However, monocytes process HDRBCs and release iron with much less efficiency than macrophages[18] and these studies could therefore deal with cells not normally involved in iron metabolism. The behavior of the RE cell in idiopathic hemochromatosis warrants further investigation.

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REFERENCES

1. Lynch SR, Lipschitz DA, Bothwell TH, Charlton RW: Iron and the reticuloendothelial system, in Jacobs A, Worwood M (eds): Iron in biochemistry and Medicine I. London, Academic, 1974, p 563

2. Hershko C: Storage iron regulation, in Brown EB (ed): Progress in Hematology. Orlando, FL, Grune & Stratton, 1977, p 105

3. Deiss A: Iron metabolism in reticuloendothelial cells. Semin Hematol 20:81, 1983

4. Haurani FI, Ballas SK: Iron metabolism, in Reichard SM, Filkins JP (eds): The Reticuloendothelial System. London, Plenum, 1984, p 353

5. Finch CA, Huebers HA, Cazzola M, Bergamaschi G, Bellotti V: Storage iron, in Albertini A (ed): Ferritins and Isoferritins as Biochemical Markers. Amsterdam, Elsevier, 1985, p 3

6. Cook JD, Marsaglia G, Eschbach JW, Funk DD, Finch CA: Ferrokinetics: A biologic model for plasma iron exchange in man. J Clin Invest 49:197, 1970

7. Fillet G, Cook JD, Finch CA: Storage Iron Kinetics VII. A biologic model for reticuloendothelial iron transport. J Clin Invest 53:1527, 1974

8. Bothwell TH, Charlton RW, Cook JD, Finch CA: Iron Metabolism in Man. Oxford, Blackwell Scientific, 1979

9. International Committee for Standardization in Haematology: Recommendations for measurement of serum iron in human blood. Br J Haematol 38:291, 1978

10. International Committee for Standardization in Haematology: The measurement of total and unsaturated iron-binding capacity in serum. Br J Haematol 38:281, 1978

11. Lipschitz DA, Cook JD, Finch CA: A clinical evaluation of serum ferritin as an index of iron stores. N Engl J Med 290:1213, 1974

12. Eakins JD, Brown DA: An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. Int J Appl Rad Isotopes 17:391, 1966

13. Noyes WD, Bothwell TH, Finch CA: The role of the reticuloendothelial cell in iron metabolism. Br J Haematol 6:43, 1960

14. Henderson PA, Hillman RS: Characteristics of iron dextran utilization in man. Blood 34:357, 1969

15. Stefanelli M, Bentley DP, Cavill I, Roeser HP: Quantitation of reticuloendothelial iron kinetics in humans. Am J Physiol 247:R842, 1984

16. Brock JH, Esparza I, Logie AC: The nature of iron released by resident and stimulated mouse peritoneal macrophages. Biochim Biophys Acta 797:105, 1984

17. Bassett ML, Halliday JW, Powell LW: Ferritin synthesis in peripheral blood monocytes in idiopathic hemochromatosis. J Lab Clin Med 100:137, 1982

18. Custer G, Balcerzak S, Rinehart J: Human macrophage hemoglobin-iron metabolism in vitro. Am J Hematol 13;23, 1982

19. Hillman RS, Henderson PA: Control of marrow production by the level of iron supply. J Clin Invest 48:454, 1969

20. Esparza I, Brock JH: Release of iron by resident and stimulated mouse peritoneal macrophages following ingestion and degradation of transferrin-antitransferrin immune complexes. Br J Haematol 49:603, 1981

21. Saito K, Nishisato T, Grasso JA, Aisen P: Interaction of transferrin with iron-loaded rat peritoneal macrophages. Br J Haematol 62:275, 1986

22. Fedorko ME: Loss of iron from mouse peritoneal macrophages in vitro after uptake of ^{55}FE ferritin and ^{55}FE ferritin rabbit antiferritin complexes. J Cell Biol 62:802, 1974

23. Lipschitz DA, Simon MO, Lynch SR, Dugara J, Bothwell TH, Charlton RW: Some factors affecting the release of iron from reticuloendothelial cells. Br J Haematol 21:289, 1971

24. Finch CA, Huebers H, Eng M, Miller L: Effect of transfused reticulocytes on iron exchange. Blood 59:364, 1982

25. Uchida T, Akitsuki T, Kimura H, Tanaka T, Matsuda S, Kariyone S: Relationship among plasma iron, plasma iron turnover, and reticuloendothelial iron release. Blood 61:799, 1983

26. Beamish MR, Davies AG, Eakins JD, Jacobs A, Trevett D: The measurement of reticuloendothelial iron release using iron-dextran. Br J Haematol 21:617, 1971

27. Bentley DP, Cavill I, Ricketts C, Peake S: A method for the investigation of reticuloendothelial iron kinetics in man. Br J Haematol 43:619, 1979

28. Kanakakorn K, Cavill I, Jacobs A: The metabolism of intravenously administered iron-dextran. Br J Haematol 25:637, 1973

29. Konijn AM, Hershko C: Ferritin synthesis in inflammation I. Pathogenesis of impaired iron release. Br J Haematol 37:7, 1977

30. Quastel MR, Ross JF: The effect of acute inflammation on the utilization and distribution of transferrin-bound and erythrocyte radioiron. Blood 28:738, 1966

31. Hershko C, Cook JD, Finch CA: Storage iron kinetics. VI. The effect of inflammation on iron exchange in the rat. Br J Haematol 28:67, 1974

32. Roeser HP: Iron metabolism in inflammation and malignant disease, in Jacobs A, Worwood M (eds): Iron in Biochemistry and Medicine II. London, Academic Press, 1980, p 605

33. Birgegard G, Caro J: Increased ferritin synthesis and iron uptake in inflammatory mouse macrophages. Scand J Haematol 33:43, 1984

34. Fawwaz RA, Winchell HS, Pollycove M, Sargent T: Hepatic iron deposition in humans. I. First-pass hepatic deposition of intestinally absorbed iron in patients with low plasma latent iron-binding capacity. Blood 30:417, 1967

35. Walters GO, Jacobs A, Worwood M, Trevett D, Thomson W: Iron absorption in normal subjects and patients with idiopathic hemochromatosis: Relationship with serum ferritin concentration. Gut 16:188, 1975

36. Jacobs A, Summers MR: Iron uptake and ferritin synthesis by peripheral blood leucocytes in patients with primary idiopathic haemochromatosis. Br J Haematol 49:649, 1981