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Plasma radioiron kinetics in man: Explanation for the effect of plasma iron concentration

(transferrin/erythrocytes)

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ABSTRACT **The plasma iron turnover was measured in 19 normal subjects. A correlation was found between plasma iron concentration and plasma iron turnover. In addition to the turnover of ^{55}Fe at normal plasma iron concentration (predominantly monoferric transferrin), a second turnover in which the labeled plasma was saturated with iron (to produce predominantly diferric transferrin) was studied with ^{59}Fe. It was demonstrated that diferric transferrin had a greater rate of iron turnover but that the distribution between erythroid and nonerythroid tissues was unchanged. It was concluded that plasma iron turnover is dependent on the monoferric/diferric transferrin ratio in the plasma but that the internal distribution of iron is unaffected.**

The literature contains reports of plasma radioiron kinetics in normal subjects and in patients with hematologic disorders, based for the most part on the determination of the turnover of plasma radioiron and the amount of radioiron appearing in the circulating erythrocyte mass at 2 weeks (1). This simple formulation of iron turnover has been useful in evaluating erythropoiesis but understates the complexity of internal iron exchange. Detailed analyses of the plasma iron disappearance curve over a 2-week period have also been carried out and represent a more elegant approach to the problem (2, 3). The considerable variation in turnover among normal subjects, even by these elaborate techniques, however, has suggested that other variables are present. This study demonstrates that changes in the plasma iron concentration are responsible for much of the variation in plasma iron turnover observed in normal subjects and suggests that this variation is due to a difference in clearance rate between monoferric and diferric transferrin.

MATERIALS AND METHODS

The 19 subjects, 8 men and 11 women, had a mean age of 28 years (range, 21–39 years). Informed consent was given by all. Their mean (±SD) hemoglobin concentration was 13.8 ± 1 g/100 ml and all were nonanemic by World Health Organization criteria (4). Other mean (±SD) values were: plasma iron, 106 ± 28 μg/100 ml; transferrin saturation, 34 ± 7%; and ferritin, 51.3 μg/liter (range, 23–113 μg/liter). Iron deficiency was excluded in all subjects by a transferrin saturation of more than 16% and a ferritin concentration of more than 12 μg/liter. In this study, freshly drawn plasma was divided into two aliquots to which were added tracer doses of ^{55}Fe and ^{59}Fe, respectively. To the second aliquot, nonradioactive iron was then added to near saturation. These two plasmas were injected intravenously in rapid sequence. Blood samples were drawn from the opposite arm every 5 min for 20 min and then every 20 min until 2 hr after the injection to determine the plasma radioiron disappearance rate. An additional blood sample was obtained at 14 days.

For labeling the plasma, radioiron in the form of $^{59}FeCl_3$ and $^{55}FeCl_3$, in 0.2 M HCl (specific activities, 7–20 and 15–30 mCi/mg, respectively) was used. Citrate was added to the radioiron in a molar ratio of 20:1, and 0.6 ml of the diluted isotope solution was added to 6 ml of the plasma and mixed on a turntable for 15 min. Five microcuries of plasma-bound ^{55}Fe in 2 ml of plasma and 3 μCi of ^{59}Fe in 2 ml of plasma were injected intravenously into each subject. In an attempt to nearly saturate the transferrin of the second plasma sample tagged with ^{59}Fe, an amount of iron calculated to represent 90% of the unsaturated binding capacity of plasma in the form of a freshly prepared solution of ferrous ammonium sulfate, pH 5.4, was added immediately after the radioiron and before *in vitro* incubation.

The plasma iron disappearance rate was determined from plasma activity in samples drawn on the day of the radioiron injection; the slope was calculated by the least squares method. The initial plasma radioiron concentration was determined by extrapolating this curve to the vertical axis at 0 time. Blood volume (BV) was determined by dividing the cpm injected by the extrapolated 0 time value for each isotope according to the following formula (1):

$$\text{BV (ml)} = \frac{\text{cpm injected}}{\text{whole blood activity extrapolated to 0 time}} \times \frac{1 - \left(\frac{\text{hct}}{100} \times 0.98\right)}{1 - \left(\frac{\text{hct}}{100} \times 0.90\right)}. \quad [1]$$

Plasma iron turnover (PIT) was calculated from the plasma iron concentration (PI), the hematocrit value (hct), and the determined $t_{1/2}$ plasma radioiron clearance rate according to the following formula:

$$\text{PIT/100 ml whole blood per day} = \frac{\text{PI (}\mu\text{g/100 ml)} \times (100 - 0.9\ \text{hct})}{t_{1/2}\ \text{(min)} \times 100}. \quad [2]$$

The value for PI in this formula was the average of approximately six plasma iron values from samples drawn between 0 time and the half-time of radioiron disappearance.

Erythocyte utilization (RBCU) of each isotope was calculated from the extrapolated 0-time plasma activity when all activity was in the plasma and the peak activity of whole blood 12–14

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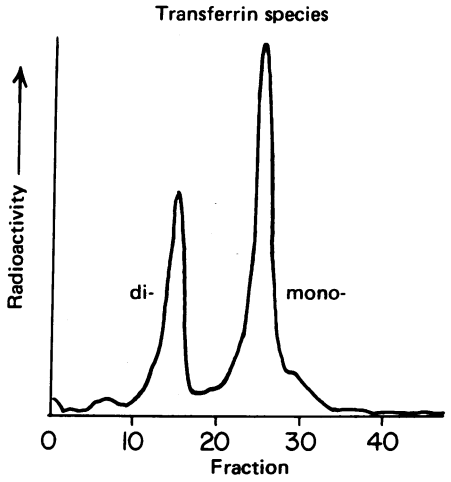

FIG. 1. Profile of normal plasma trace-labeled with ^{59}Fe. The plasma iron and iron binding capacity of this sample were 100 and 318 μg/100 ml of plasma, respectively, with 31% saturation of transferrin. Each fraction in the displayed profile consisted of a gel slide 2 mm thick. The proportions of monoferric and diferric radioiron are estimated to be 67.7 and 33.3%, respectively.

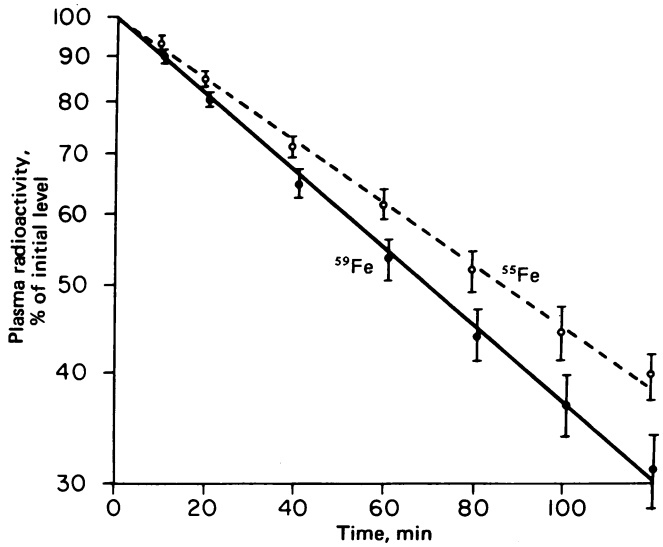

FIG. 2. Mean (±SEM) clearance rates of predominantly monoferric (- - -○- - -) and predominantly diferric (—●—) transferrin in 19 subjects. Both rates appear to be exponential over a 2-hr period.

days later when virtually all activity going to the erythrocytes was located there. Because the hematocrit value of venous blood differs from the mean body hematocrit value, corrections were made according to the following formula (1):

$$\text{RBCU (\%)} = \frac{\text{wba on day 14}}{\text{wba extrapolated 0 time}} \times 100 \times \left[\frac{0.92 - 0.9\left(\frac{\text{hct at day 14}}{100}\right)}{1 - 0.9\left(\frac{\text{hct at 0 time}}{100}\right)}\right] \quad [3]$$

in which wba = whole blood activity.

Inasmuch as the disappearance of radioiron was found to be affected by the serum iron concentration or the percentage saturation of transferrin, it seemed desirable to examine the distribution of radioiron between monoferric and diferric transferrin molecules. Accordingly, samples of normal plasma were labeled in a fashion identical to those used in *in vivo* injections and subjected to isoelectric focusing (5). In these studies, 2 ml of the ^{55}Fe- or ^{59}Fe-tagged plasma was applied to gel chromatography column (0.8 × 29 cm, Sephadex G-25 coarse) equilibrated with 1 mM Tris·HCl buffer (pH 7.6) and elution was performed with the same buffer at 2.5 ml/min. Fractions of 5 ml were collected. The radioiron-containing fractions emerging at the void volume of the column were concentrated to 0.5 ml by plasma filtration (Amicon 8 MC filtration unit, PM 10 filter) and subjected to isoelectric focusing on polyacrylamide gel columns (1.4 × 18 cm). The 75% polyacrylamide gel was prepared as described by Karlsson *et al.* (5). The gel contained 2% Ampholine (pH range, 5–8). The isoelectric focusing was carried out in an electrophoresis cell (Bio-Rad model 155)

Table 1. Blood radioiron kinetics in 19 normal subjects

Subject	Sex; age, yrs	Initial hct, %	PI, μg/100 ml	Sat, %	^{55}Fe sat, %	^{59}Fe sat, %	Blood volume, liters ^{55}Fe	Blood volume, liters ^{59}Fe	Disappearance time ($t_{1/2}$), min ^{55}Fe	Disappearance time ($t_{1/2}$), min ^{59}Fe	RBCU, % ^{55}Fe	RBCU, % ^{59}Fe	PI turnover*
1	M; 29	44	81	31	44	91	5.86	5.93	95	81	83	86	0.515
2	M; 28	43	108	40	50	77	5.82	6.29	106	87	82	82	0.625
3	M; 39	44	107	34	39	85	5.03	5.30	65	53	85	82	0.987
4	M; 39	47	91	30	38	100	4.97	4.87	85	66	85	82	0.618
5	M; 23	44	68	28	26	100	4.27	4.80	73	52	86	81	0.564
6	M; 23	43	41	12	25	91	6.17	6.15	55	40	84	80	0.458
7	M; 25	42	182	52	65	82	4.94	5.58	119	111	80	83	0.954
8	M; 26	40	90	32	46	99	6.73	6.61	76	59	78	77	0.753
9	F; 27	38	80	34	37	85	4.07	3.96	107	74	94	88	0.492
10	F; 31	43	90	26	31	84	4.66	4.44	63	46	91	83	0.878
11	F; 26	40	139	39	43	76	3.77	3.70	145	125	85	82	0.614
12	F; 28	40	186	53	59	89	3.39	3.46	110	98	85	83	1.088
13	F; 36	38	46	18	26	98	3.37	3.37	45	39	84	82	0.668
14	F; 22	46	44	18	30	100	4.55	4.66	63	42	87	83	0.409
15	F; 21	45	159	37	59	100	4.41	4.56	102	83	73	73	0.927
16	F; 21	40	194	52	40	100	3.93	3.89	145	107	86	85	0.850
17	F; 22	44	197	43	49	82	4.45	4.44	124	99	85	84	0.960
18	F; 22	41	127	44	49	87	3.80	3.90	96	75	82	84	0.835
19	F; 35	40	131	47	51	95	4.69	4.91	85	65	88	87	0.988
Mean		42	114	35	42	91	4.68	4.78	93	74	84	82	0.746
SD		±2	±51	±12	±12	±8	±2.14	±2.24	±7	±6	±6	±1	±0.210

Abbreviations: hct, hematocrit value; PI, plasma iron; Sat, saturation; RBCU, erythrocyte utilization (see Eq. 3).
* Clearance of ^{55}Fe was used in this calculation. Units are mg/100 ml per day.

for 20 hr at 1000 V and 10°. Thereafter, the gel was cut into slices of 2 mm thickness. The radioiron content in these slices was measured in a gamma spectrometer (Packard model 5330).

Plasma iron was determined according to the recommendations of the International Standardization Committee (6); total iron binding capacity was measured by the magnesium carbonate method as described by Cook (7). Hemoglobin was determined by the cyanmethoglobin method and hematocrit value, by the micro method. Ferritin was measured by the method of Miles *et al.* (8); blood and tissue samples were processed by wet ashing and prepared for scintillation counting as described by Eakins and Brown (9). Samples were corrected to an accuracy of ±2% and cross counting of ^{59}Fe in ^{55}Fe was 5%.

RESULTS

Isoelectric focusing of the radioiron-tagged plasma revealed two radioiron coding bands (pH 5.6 and 6.0), the former being the diferric and the latter the monoferric transferrin species. A typical example of tracer ^{59}Fe-labeled plasma after the isoelectric focusing is shown in Fig. 1. In these studies, in which the transferrin saturation was 23, 31, and 49% (calculated percentages: monoferric, 77, 69, and 51; diferric 23, 31, and 49), the values found by isoelectric focusing for monoferric were 75, 68, and 70 and for diferric 25, 33, and 30. In plasma samples to which sufficient ferrous ammonium sulfate (freshly dissolved in saline, pH 5.4) had been added to saturate transferrin to 90%, isoelectric focusing revealed that only about 60% of the radioiron was bound to the diferric transferrin species, indicating that the ferrous ammonium sulfate as added was not a very efficient transferrin iron donor.

The blood radioiron kinetics on 19 normal subjects injected with labeled plasma at a mean transferrin saturation of 42 ± 10% are shown in Table 1. Individual plasma iron values varied from 41 to 197 μg/100 ml, providing an opportunity to examine the effect of differences in plasma iron concentration on internal ferrokinetics. There was a correlation between plasma iron concentration and plasma iron turnover; regression equation, PIT = 0.327 + 0.0026 PI; $r = 0.764$. No correlation was found between either plasma iron concentration or plasma iron turnover and the amount of radioiron appearing in the circulating erythrocyte mass at 2 weeks.

In the same subjects, ^{59}Fe in near-saturated plasma had been simultaneously injected and differed from the ^{55}Fe clearance study in that sufficient iron had been added to the tagged plasma to saturate its transferrin to 91 ± 8% (Table 1). The clearance of this second isotope was found to be more rapid, with a mean $t_{1/2}$ of 74 min compared to 93 min for ^{55}Fe (Fig. 2). Blood volume calculations based on the dilution of the injected radioiron showed an average increase of 2.7% for the saturated plasma tagged with ^{59}Fe over the ^{55}Fe-labeled plasma (SD of differences, ±4.53%). This difference in the dilutional pool was of the same magnitude as the 2.3% difference in erythrocyte utilization of the two isotopes (Table 1).

DISCUSSION

Transferrin is known to be the carrier protein whereby iron is distributed to body tissues, but the manner in which it functions has been in question (10). The suggestion that the two iron-binding sites behave differently in *in vivo* exchange (11) is no longer tenable (12–14). On the other hand, certain animal studies suggest that plasma iron turnover is increased at high levels of plasma iron and transferrin saturation (15–17). In the present study in man, the *in vivo* behavior of predominantly diferric transferrin has been compared to that of predominantly monoferric transferrin. The diferric molecule was shown to deliver iron at a greater rate. There is, therefore, a heterogeneity in the turnover of iron within the circulating blood plasma, depending on the relative amounts of each molecular species present. Because the loading of iron is random, the monoferric/diferric ratio depends on the total number of binding sites available for iron loading. This indicates the importance of the level of circulating transferrin and explains previous clinical observations in iron-deficiency anemia indicating that transferrin saturation was a better measure of iron supply than was plasma iron concentration *per se* (18).

In a previous analysis of the plasma iron disappearance curve in normal and pathologic states, it was suggested that the amount of iron taken up by nonerythroid tissues increased as the plasma iron concentration increased (19). In the present study of 19 normal subjects, the same general relationship was observed, but it could now be explained by the increasing amounts of diferric transferrin present. Furthermore, because the amount of radioiron appearing in the erythrocyte mass was the same for both monoferric and diferric transferrin, all tissue receptors appear to behave similarly. It thus appeared that, within the limits imposed by the density of tissue receptors and blood flow, the delivery of iron throughout the body, including erythroid and nonerythroid tissues, is modulated according to the relative amounts of monoferric and diferric iron present in the serum.

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1. Finch, C. A., Deubelbeiss, K., Cook, J. D., Eschbach, J. W., Harker, L. A., Funk, D. D., Marsaglia, G., Hillman, R. S., Slichter, S., Adamson, J. W., Ganzoni, A. & Giblett, E. R. (1970) *Medicine (Baltimore)* **49**, 17–53.
2. Hosain, F., Marsaglia, G. & Finch, C. A. (1967) *J. Clin. Invest.* **46**, 1–9.
3. Cavill, I. & Ricketts, C. (1974) in *Iron in Biochemistry and Medicine*, eds. Jacobs, A. & Worwood, M. (Academic, London), pp. 614–647.
4. World Health Organization, (1968) "Report of a WHO Scientific Group" World Health Organization Technical Report Series No. 405 (World Health Organization, Geneva, Switzerland), pp. 3–37.
5. Karlsson, C., Davies, H., Ohman, J. & Andersson, U. B. (1973) *Application Note 75* (LKB-Produkter AB, Bromma, Sweden), pp. 1–14.
6. International Committed for Standardization in Hematology/Expert Panel on Iron (1971) *Br. J. Haematol.* **20**, 451–453.
7. Cook, J. D. (1970) *J. Lab. Clin. Med.* **76**, 497–506.
8. Miles, L. E. M., Lipschitz, D. A., Beiber, C. P. & Cook, J. D. (1974) *Anal. Biochem.* **61**, 209–224.
9. Eakins, J. D. & Brown, E. A. (1966) *Int. J. Appl. Radiat. Isot.* **17**, 391–397.
10. Aisen, P. & Brown, E. B. (1977) *Semin. Hematol.* **14**, 31–53.
11. Fletcher, J. & Huehns, E. R. (1967) *Nature* **215**, 584–586.
12. Harris, D. C. (1977) in *Proteins of Iron Metabolism*, eds. Brown, E. B., Aisen, P., Fielding, J. & Crichton, R. R. (Grune and Stratton, New York), pp. 197–204.
13. Pootrakul, P., Christensen, A., Josephson, B. & Finch, C. A. (1977) *Blood* **49**, 957–966.
14. Huebers, H., Huebers, E., Csiba, E. & Finch, C. A. (1977) *Blood* **50** (Suppl), 93.
15. Brown, E. B., Okada, S., Awai, M. & Chipman, B. (1975) *J. Lab. Clin. Med.* **86**, 576–585.
16. Fletcher, J. (1971) *Clin. Sci.* **41**, 395–402.
17. Hahn, D., Baviera, B. & Ganzoni, A. M. (1975) *Acta Haematol.* **53**, 285–291.
18. Bainton, D. F. & Finch, C. A. (1964) *Am. J. Med.* **37**, 62–70.
19. Cook, J. D., Marsaglia, G., Eschbach, J. W., Funk, D. D. & Finch, C. A. (1970) *J. Clin. Invest.* **49**, 197–205.