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Whole-Body Iron Loss in Normal Man, Measured with a Gamma Spectrometer

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The amount of daily loss of total-body iron in man has been difficult to determine, although many workers have measured radioactivity in the blood, stool, urine, sweat, and bile (1,2) after intravenous radioiron injection. Measurement of this parameter is important for the clinical evaluation of iron balance as well as in the basic study of iron metabolism in man. The best approach has been that of Finch (3), who injected radioiron and then measured the activity of Fe^{55} in the red cells over a period of several years, assuming that radioiron was uniformly mixed after one year. The whole-body counter seemed to us, and also to Price and co-workers (4) at Brookhaven, an excellent instrument for measuring total-body iron loss. Although this was seemingly a simple measurement, a number of problems complicated analysis of the data. We would like to present our results and interpretations which differ somewhat from those of Price and co-workers and include several factors of geometry and metabolism that they did not take into account.

MATERIALS AND METHODS

Twelve normal male human subjects, 19 to 43 years of age, were used in this study. A history of previous health was taken from each, and blood volume, hemoglobin, hematocrit, and differential blood counts were performed in order to recognize and exclude subjects with abnormalities of iron metabolism. A low-background whole-body counter, having a 9- by 4-in. crystal of NaI(Tl) with a 100-channel pulse-height analyzer, was used. The subjects were placed for counting on a special couch having a radius of curvature of 1 meter with the crystal at the center, i.e., the "1-meter-arc" geometry. The radioiron was administered intravenously as Fe^{59} citrate at a specific activity of 10 to 20 $\mu C/\mu g$. Ten subjects received a dose of 5 μC , injected without having been incubated with plasma. The radioactivity was more than body and room background until 240 days after the 5 μC injection. Two subjects received intravenous injection of 18 μC of radioiron that had been incubated with plasma. Their stool and urine samples were counted by placing them on top of the crystal.

RESULTS AND DISCUSSION

Immediately after injection, all the radioiron is in a subject's plasma, but after 24 hr the plasma-radioiron level is less than 3% and most of the

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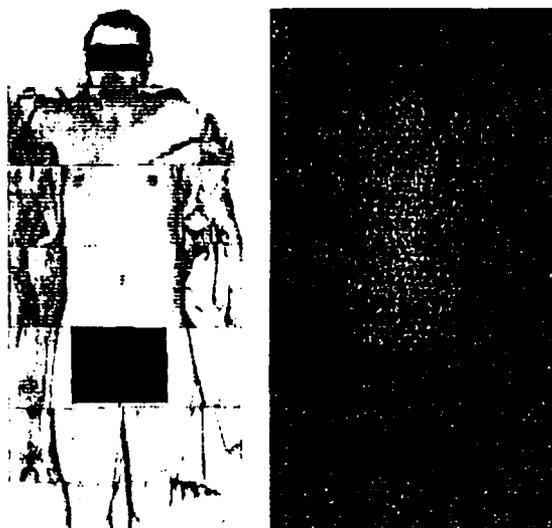


Figure 1. Subject scanned 30 hr after iv injection of 18 μ C Fe-59. The radioiron concentration in vertebral, pelvic, sacral, costal and caput humeri regions are shown. Storage retention in liver and slightly in spleen is also demonstrated. Blood-radioiron level at the time of the scan was 3% of the zero time activity.

JHL-3555

radioiron has been taken up by red-cell precursors in bone marrow, as known by autoradiography (5,6) and shown in Fig. 1. It is then released gradually into peripheral blood over a period of days. In our study the whole-body count of a subject immediately after injection was related to the counts on the succeeding days, as shown in Fig. 2. The whole-body activity decreased rapidly during the first several hours. By the time the radioiron in the marrow was greatest, approximately 1 day after injection, the whole-body count had decreased to 90% of the initial value; thereafter it rose slowly as newly labeled red cells were released to the circulating blood. This transient decrease of whole-body count can undoubtedly be related to the change of localization of radioiron. The count returned to an average of 97% after 10 days, and stayed at this level almost without change until 50 days.

Loss of radioiron during the first 10 days is not large enough to account for this 3% decrease because activity found in stool and urine during this time amounted to less than 0.5%. Most of the radioiron is fixed in the red-cell mass; thus the whole-body count is influenced by the death of the labeled red cells. The whole-body curve is almost flat after 10 days (Figs. 2 and 3), but this does not mean that no radioiron is lost from the body. It can be explained by loss of radioiron compensated by the movement of radioiron from miscible tissue to red-cell mass, i.e., further utilization (7). As seen above, this movement increases the whole-body count. The daily stools showed constant radioactivity during this time. The initial radioiron injection can be considered as equivalent to simultaneous death of one generation of labeled red

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WHOLE-BODY IRON LOSS IN NORMAL MAN

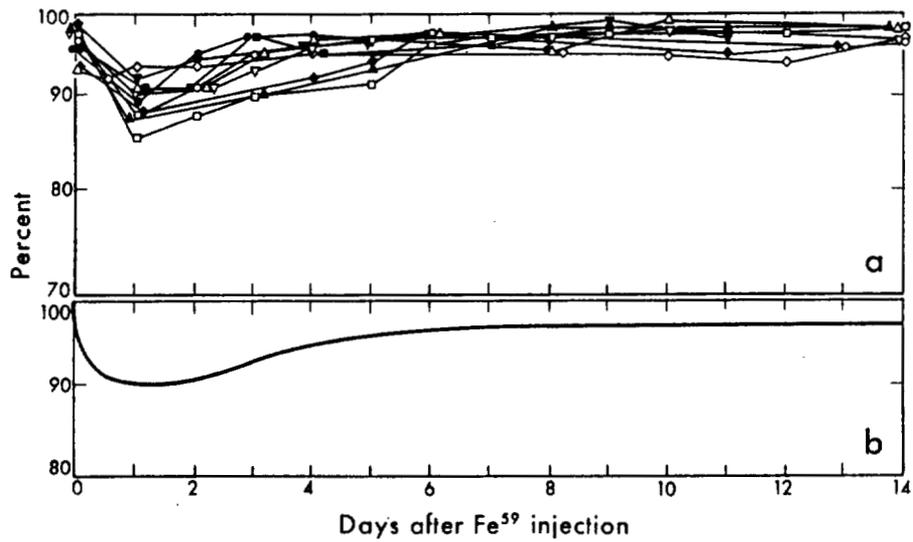


Figure 2. Change of whole-body activity after intravenous injection of radioiron. 2a shows experimental data. Curve of 2b represents average of 2a. MUB-2062

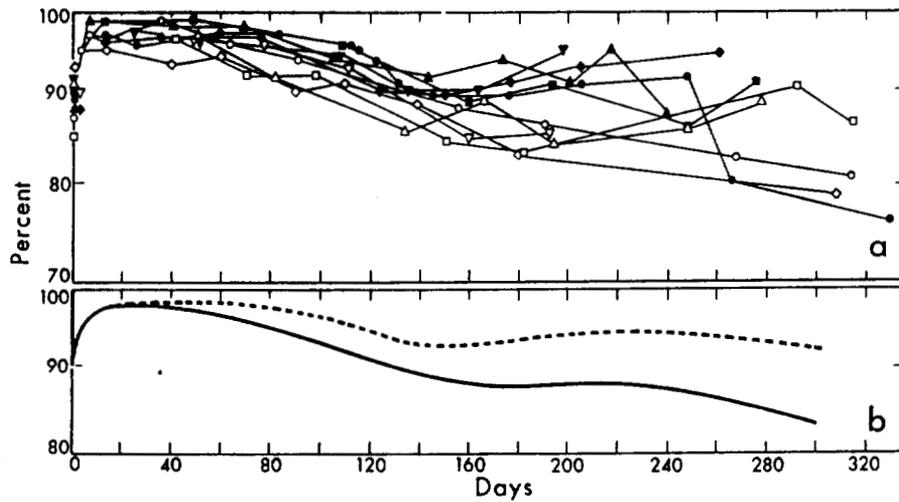


Figure 3. Whole-body activity of ten normal subjects. 3a shows experimental data. In 3b the solid line represents the average of 3a. The broken line is the constructed no-loss curve. MU-31545

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cells, causing the sudden appearance of radioiron in the circulating serum iron, after passing through the reticuloendothelial cells. This same situation can be expected to occur again approximately 120 days later, except that then the death of the red cells takes place over a wider time distribution. Therefore, the daily whole-body counts would again change as in Fig. 2, except that the spread in time of death of red cells would tend to make the dip wider and shallower.

The average utilization of radioiron was 90%; this means that about 10% was retained in tissues. This redistribution appears to account for most of the decrease of the whole-body count from 100% immediately after injection and the return to 97% 10 days after. This same effect should be observed again at the time of death of labeled red cells. Although the capacity for utilization should be the same in the same subject, storage partition would be expected to result in appearance of less radioiron in the second generation of labeled red cells. This kind of stepwise decrease ought to be reflected in the whole-body count and to occur repeatedly until the radioiron in the body is uniformly mixed. It may take about a year (3) for virtually complete mixing.

Figure 3a shows individual whole-body retention curves over 300 days, and the solid line of Fig. 3b shows an average curve obtained from Fig. 3a. Although the average curve is derived from widely varying individual curves, it does suggest the stepwise decrease expected. Because of the effect of changes in localization of radioiron on the counting rate, interpretation of loss curves obtained with whole-body counters by ourselves and others (4) has not been as straightforward as had been hoped. Nevertheless, we feel that by utilizing the known localization effects and known hematological processes, the data can be treated in such a way that useful information may be derived from them. Although a number of assumptions are required in what follows, none of them are unreasonable, and the results for average daily loss of iron are in good agreement with those obtained by the rather different method of Finch (3).

A decrease of whole-body activity after 50 days does not necessarily mean a higher rate of loss of radioiron from the body. This decrease reflects death of the labeled population of red cells, in turn causing movement of radioiron to miscible tissue iron. This radioiron is then incorporated into new red cells, but some fraction remains in tissues. A small loss of radioiron from the body may also occur at this time, as is discussed later. After the observed decrease, the curve was nearly flat again from 160 to 250 days. The combined effects of the death of the first generation of labeled red cells and of reutilization by the second generation produce a decrease of whole-body count. The effect of the redistribution after the initial injection was a 3% decrease in whole-body count, and the utilization of the first labeled red-cell generation was 90%. The initial radioiron injection is comparable to red-cell death, exclusive of the route through the RE cells. However, the high reutilization

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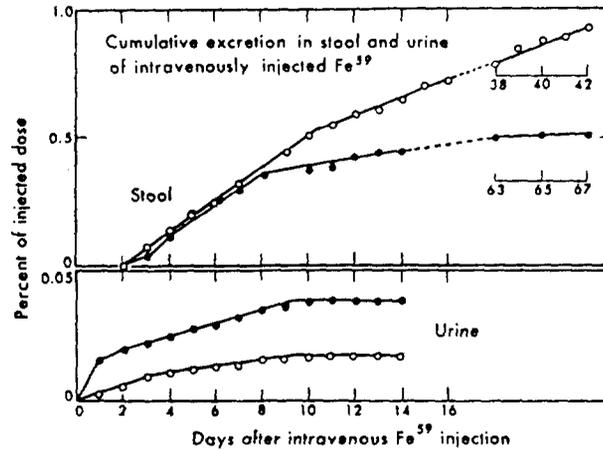


Figure 4. Cumulative excretion in stool and urine of intravenously injected Fe^{59} . Subject ■■■ open circles; subject ■■■ closed circles. MU-30674

(7,8) suggests that the effect on the whole-body count of such radioiron retention in RE cells is negligible. Therefore the effect of red-cell death should be 2.7% (90% of 3%).

If, on the whole-body activity curve (solid curve in Fig. 3b), we draw a line from the first peak to the second peak, it shows a slope of 6.3% per 120 days. (The 120-day period was chosen as a normal red-cell life span. The effect of storage retention occurs once per red-cell life span, and this occurred between the peaks.) Therefore we can obtain the loss in 120 days by subtracting 2.7% (the effect of storage retention) from 6.3% (loss plus effect of storage retention). Thus $6.3\% - 2.7\% = 3.6\%$; 3.6% per 120 days = 0.030% per day. It should be noted that even without the above allowances for redistribution effects, simply by taking the average loss over the 300 days period, a value of 0.05% loss per day can be obtained. However, this is an unnecessarily rough approximation for obtaining a loss figure, inasmuch as redistribution and reutilization are known to occur, and their effects on whole-body counting cannot be neglected. Thus we prefer to conclude that 0.030% per day is the most reasonable loss figure.

The dotted line in Fig. 3b was constructed by adding the loss rate, i.e., 0.030% per day, to the average whole-body activity curve (solid line) in Fig. 3a. This dotted curve then represents the expected whole-body activity curve if no loss of radioiron occurred. If we subtract 5.7% (total of 3.0% and 2.7%) from 100% (at zero day), this level represents the decrease of whole-body activity by the effect of storage retention and intersects the constructed curve at 120 days. This serves as confirmation that the 120-day red-cell life span chosen for the calculation of loss and the 2.7% figure for the storage retention

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were adequate. The radioiron loss in stool and urine was analyzed as follows. Total excreted activity (cumulative as of each day) was plotted as a function of days after injection. There were two components in the cumulative Fe^{59} excretion curve; the first prevailed until from 8 to 10 days and the second component prevailed thereafter. Samples taken much later in subject [redacted] and somewhat earlier in subject [redacted] showed losses at the same respective rates, as indicated in Fig. 4a. Radioiron excretion was larger during the first 10 days than during the period of the second component. Subject [redacted] excreted 0.66% and T.L. 0.45% of the total radioiron in the stool within 14 days. The first slope, ending within 10 days, coincides with the period of exfoliation of the mucous epithelia as described in the review by Leblond and Walker (9). By autoradiography, Saito (10) found that the radioiron injected intraperitoneally in the rat appeared in the serum and was incorporated in the gastrointestinal epithelia, which forms nonhemoglobin iron: hemosiderin and ferritin. Therefore, it can be concluded that the first slope represents the loss of non-hemoglobin radioiron occurring by exfoliation of mucous epithelia following intravenous radioiron injection.

A small quantity of radioiron may be lost with bile (11); however, it is difficult to conceive of continuous loss of radioiron through bile when the serum-radioiron level is very low. Leblond found no sign of renewal of liver cells, pancreas cells, etc. (9) by which radioiron could be lost. It is therefore concluded, on the basis of this circumstantial evidence and in the absence of evidence to the contrary, that the loss of radioiron represented by the second slope means the loss of blood in the intestinal tract. Such a route of loss in normal subjects was suggested by studies by Ebaugh and Beeken (12) and by Harris and Belcher (13). However, they used the benzidine test, which is perhaps too sensitive to detect blood only; and in radiochromium studies the label is known to leak from red cells. The results presented here would imply daily intestinal bleeding at around 2.0 ml (1.0 mg) in subject [redacted] and 0.6 ml (0.3 mg) in subject [redacted]. According to the above interpretation, the peeling analysis of the radioiron-loss curves in the stool yields a ratio of hemoglobin iron to nonhemoglobin iron of 10:1 in [redacted], and 3:1 in [redacted], per red-cell life span of 123 days and 120 days respectively, which were obtained from the peripheral red-cell activity and whole-body activity curves (8).

The loss of radioiron in the urine is shown in Fig. 4b. This has to represent iron loss by exfoliation of the urogenital epithelia, since it occurred in the same 10-day period as loss from the intestine, and hemoglobin is not ordinarily lost via the urine. The total amount lost within 10 days in urine was 0.018% for [redacted], and 0.039% for [redacted]. The fractional amount excreted into the urine in the form of nonhemoglobin iron was 0.035 for [redacted] of the amount excreted in the stool during 10 days after injection, and 0.083 for [redacted]. The radioactivity in the urine after 10 days was undetectable in a 24-hr count

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Table 1. Loss rate per RBC life span

Subject	RBC life span (days)	Loss in stool & urine (%)	Loss in sampled blood (%)	Total loss in stool, urine, and sampled blood (%)	Loss as shown by whole-body count (%)
████	123	3.8	2.0	5.8	5.5
████	120	1.6	1.0	2.6	2.9

with urine at the center on top of the 9- by 4-in. crystal. One subject, not included in these data, showed a larger amount of urine radioactivity than did the subjects studied. Analysis of the gamma energy proved that this was caused by Co⁶⁰, apparently a contaminant of the injected Fe⁵⁹ (Abbott Laboratory, Oak Ridge, Tennessee). The whole-body activity curves of each subject showed a loss of radioactivity very close to that found in the stool and urine, as shown in Table 1. Therefore, it is concluded that the iron loss took place mainly via the stool.

There may be a slight difference in the radioiron-loss rate between the life span of the first labeled red-cell generation and the time when mixing of radioiron with body iron is complete. This difference would occur because loss of radioiron from the red-cell mass decreases, and loss from nonhemoglobin iron will therefore increase, as the 10 to 20% of radioiron moves from hemoglobin to stores in the course of mixing. However, the total change in loss rate after our experimental period would be very small, since the whole-body activity curve of Fig. 3b suggests that the mixing of radioiron was almost complete after the death of the first labeled red-cell population. Moreover, the decreased loss from hemoglobin radioiron would be mostly compensated by the increased loss from nonhemoglobin radioiron.

The average hemoglobin iron, calculated from each subject's blood volume and hemoglobin concentration, was 2,364 mg, as shown in Table 2 (0.334% of hemoglobin as iron by weight), and total miscible tissue iron was taken as 600 mg (3). Therefore, miscible total-body iron was taken as 2,964 mg. The loss of radioiron at the rate of 0.030% per day from this total miscible iron gives an average total-body iron-loss figure of 0.89 mg per day. This is smaller than the absorption figure obtained from the same subjects when used for the loss study; 9% absorption for 15 mg of daily iron intake amounts to 1.35 mg per day (14). However, when iron is absorbed from food, the absorption is less than from the elemental iron (15-18).

The loss figure obtained by Finch (3) was 0.61 mg per day, a figure smaller than ours. This may be due to differences in the subjects: the average

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Table 2. Summary of experimental data.
Miscellaneous data of 10 subjects (5 µC group) and 2 subjects (18 µC group)

Name	Age	Wt (kg)	Height (cm)	RBC (million)	Ht (%)	Hb (g)	SI (µg/dl)	UIBC (µg/dl)	TIBC (µg/dl)	Sat. (%)	RCV (cc)	PV (cc)	BV (cc)	Hb iron (mg)
●●		81	173	5.37	46.0	15.0	68	422	490	13.9	2129	2571	4700	2355
●●		87	180	5.72	50.0	16.5	114	374	488	23.4	2487	2586	5070	2794
●●		51	158	5.18	46.0	15.3	110	289	397	27.7	1350	2317	3667	1874
●●		70	177	5.35	47.0	15.0	75	389	464	16.4	2109	2516	4619	2314
●●		62	160	4.95	48.5	16.0	136	334	470	28.9	1935	2550	4485	2397
●●		79	171	5.01	45.5	14.8	85	345	430	19.8	2251	2413	4664	2306
●●		54	171	5.03	48.0	15.2	150	363	513	29.2	1773	2475	4248	2157
●●		68	180	5.02	47.5	15.3	101	205	366	27.6	1836	2559	4395	2246
●●		77	191	5.43	47.5	15.8	107	206	313	34.2	1927	2814	4941	2607
●●		74	170	5.19	45.0	15.2	144	155	299	48.2	2053	3050	5103	2591
Aver-														
age	32	70	173	5.23	47.1	15.4	109	308	417	26.9	1985	2585	4589	2364
●●		77	173	5.06	46.5	15.6	62	411	475	13.1	2238	2644	4882	2543
●●		74	180	5.05	45.0	14.6	138	352	490	28.2	2142	3118	5260	2565

age was 70 years for his and 32 years for ours; and total miscible iron was 2,685 mg for his and 2,964 mg for ours. There may, of course, be variations because of differences in method employed. However, the daily percentage loss rate found here, 0.030%, is in reasonable agreement with the 0.023% found by Finch in a study lasting 4.5 years. Price and co-workers (4), also using a whole-body counter, studied loss in patients with hematological disorders. On the basis of only 3 normal subjects studied over periods of from 20 to 100 days, they believe that a normal range of 0.103 to 0.182% loss per day is indicated. This is much higher than our data or those of Finch and cannot be reconciled with daily iron absorption values.

SUMMARY

The average whole-body iron loss of twelve normal subjects was analyzed and explained by the change of distribution and a small amount of radioiron loss. The normal radioiron loss occurred mainly in the stool, mostly as blood loss and partly as the loss by exfoliation of mucous epithelia. A small quantity of radioiron loss in the urine from the exfoliation of urogenital epithelia was suggested. The average normal iron loss rate was 0.030% per day or 0.89 mg per day. This normal iron-loss figure is in reasonable agreement with the daily amount of iron absorption. Because the whole-body counter is capable of detecting a very minute amount of iron loss within a short period of time, studies with the counter are the simplest and most accurate approach for measuring the loss of body iron.

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