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Whole-body Iron Loss In Normal Man Measured with a Gamma Spectrometer[1]

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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 was that of Finch (3), who injected radioiron and then measured the activity of iron-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 way to measure 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 which 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 to recognize and exclude subjects with abnormalities of iron metabolism. A low-background whole-body counter, having a 9 × 4-inch 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

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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 plasma, but after 24 hours plasma radioiron level is less than 3 per cent and most of the radioiron been taken up by red cell precursors in bone marrow, as known by autoradiography (5,6) and shown in Figure 1. It is then released gradually into peripheral blood over a period of days. In our study the whole-body count of a subject im-

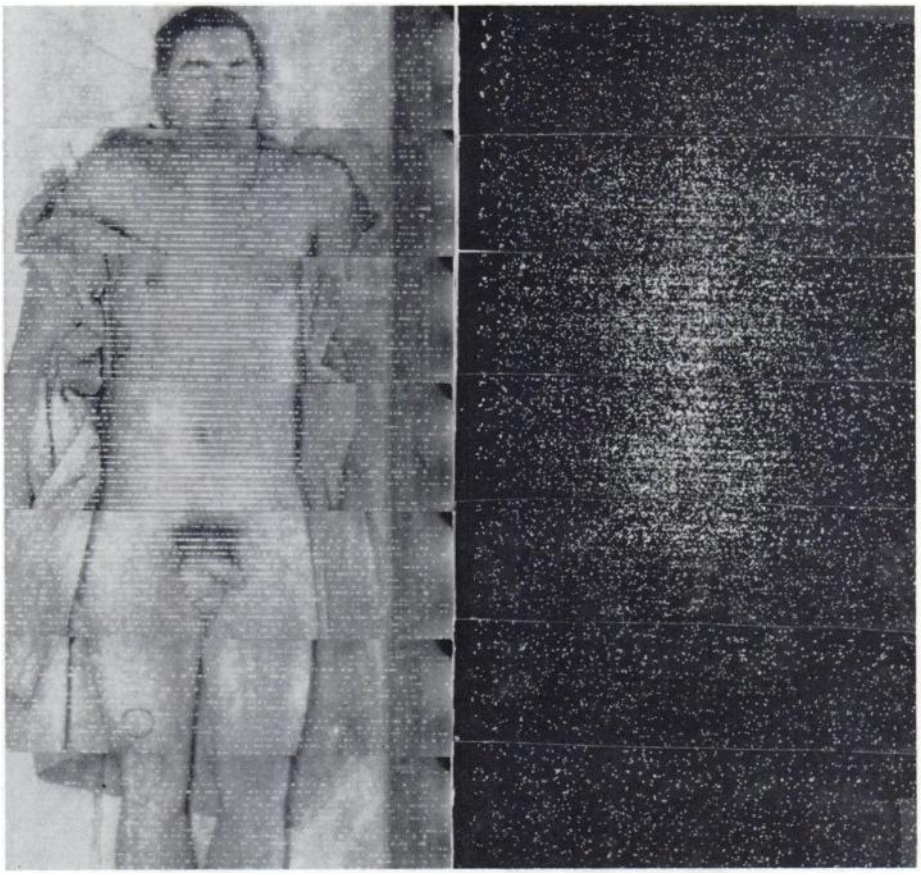

Fig. 1. Subject T.L. scanned 30 hours after iv injection of 18 μC Fe^{59}. The radioiron concentration in vertebral, pelvic, sacral, costal, and caput humeri regions are shown. Storage retention in liver and slightly in spleen are also demonstrated. Blood radioioron level at the time of the scan was 3 per cent of the zero-time activity.

mediately after injection was related to the counts on the succeeding days, as shown in Figure 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 per cent 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 per cent after 10 days, and stayed at this level almost without change until 50 days.

Loss of radioiron during the first 10 days cannot be so large as to account for this 3 per cent decrease; activity found in stool and urine during this time amounted to less than 0.5 per cent. 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 (8). As seen above, this movement increases the whole-body count. The daily stools showed constant radioactivity during this time.

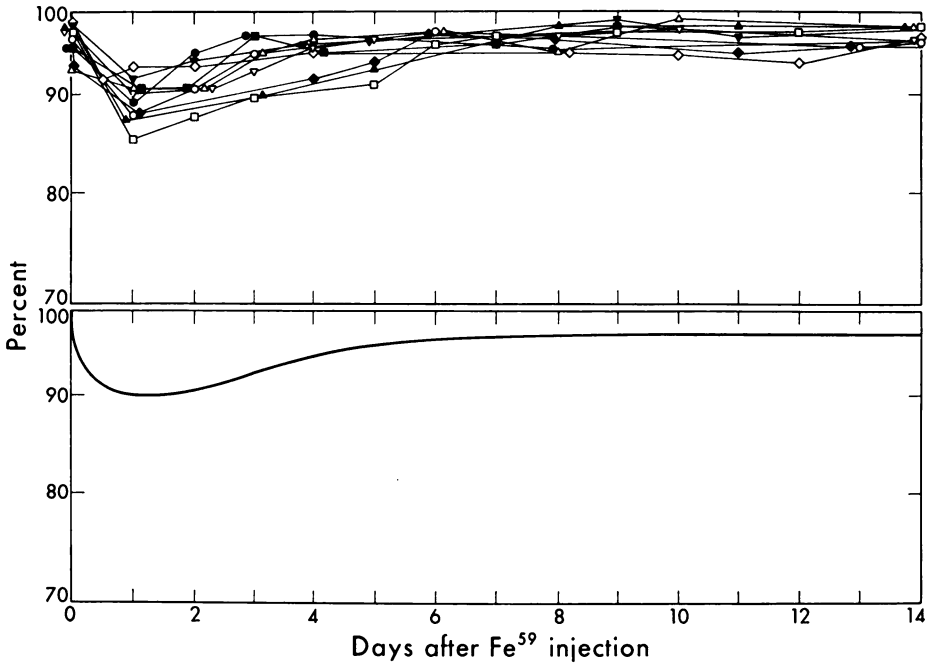

Fig. 2. Change of whole-body activity after intravenous injection of radioiron. (a) Experimental data. (b) Curve representing average of (a).

The initial radioiron injection can be considered as equivalent to simultaneous death of one generation of labeled red 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 Figure 2a, 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 per cent; this means that about 10 per cent was kept in tissues. This redistribution appears to account for most of the decrease of the whole-body count from 100 per cent immediately after injection and the return to 97 per cent 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 should result in appearance of less radioiron in the second generation of labeled red cells. This kind of stepwise decrease should be reflected in the whole-body count. It would be expected 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 Figure 3b shows an average curve obtained from Figure 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 ob-

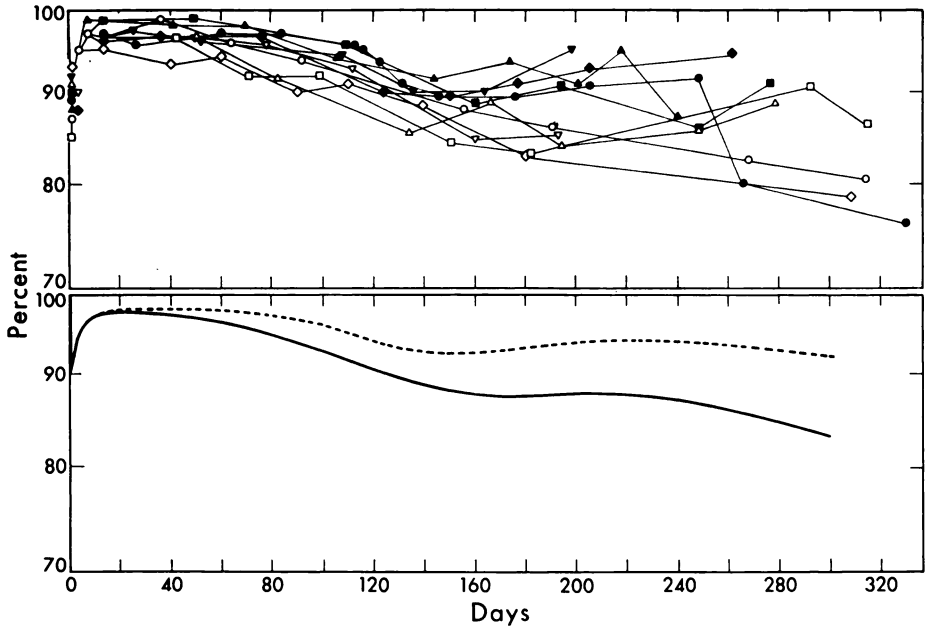

Fig. 3. Whole-body activity of ten normal subjects. (a) Experimental data. (b) Solid line: average of (a); broken line: constructed no-loss curve.

tained 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 can 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. It includes death of the labeled population of red cells, 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 integral effect of the death of the first generation of labeled red cells and reutilization by the second generation produces a decrease of whole-body count. The effect of the redistribution after the initial injection was a 3 per cent decrease in whole-body count, and the utilization of the first labeled red cell generation was 90 per cent. The initial radioiron injec-

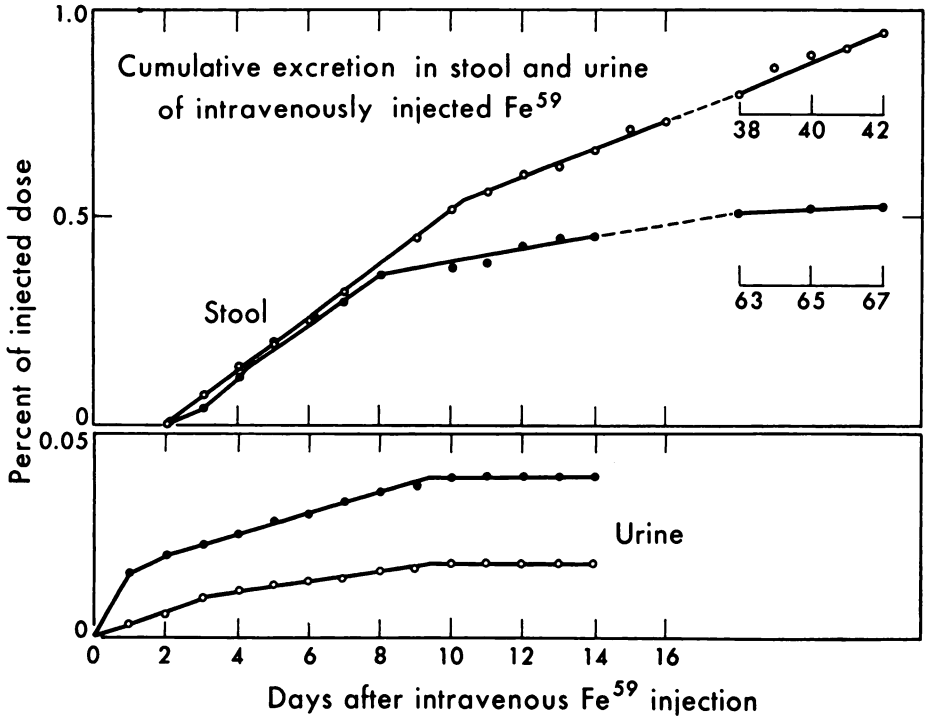

Fig. 4. Cumulative excretion in stool and urine of intravenously injected Fe^{59}.
○ Subject J. Z.
● Subject T. L.

tion is comparable to red cell death though excluding the route through the RE cells. However, the high retutilization (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 percent (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 cent 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 per cent (the effect of storage retention) from 6.3 per cent (loss plus effect of storage retention). Thus 6.3 per cent − 2.7 per cent = 3.6 per cent; 3.6 per cent per 120 days = 0.030 per cent per day. It should be noted that even without the above allowances for redistribution effects, simply by taking the average loss over the 300 day period, a value of 0.05 per cent loss per day can be obtained. However, this is an unnecessarily rough approximation for obtaining a loss figure, since 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 cent per day is the most reasonable loss figure.

The dotted line in Figure 3b was constructed by adding the loss rate, *i.e.*, 0.030 per cent per day, to the average whole-body activity curve (solid line) in Figure 3a. This dotted curve then represents the expected whole-body activity curve if no loss of radioiron occurred. If we subtract 5.7 per cent (total of 3.0% and 2.7%) from 100 per cent (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 per cent figure for the storage retention 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 8 to 10 days and the second component prevailed thereafter. Samples taken much later in subject J.Z., and slightly less later in subject T.L., showed losses at the same respective rates, as indicated in Figure 4a. Radioiron excretion was larger during the first 10 days than during the period of the second component. Subject J. Z. excreted 0.66 per cent and T.L. 0.45 per cent 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.

Therefore, the first slope represents the loss of nonhemoglobin 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, pan-

creas cells, etc. (9) by which radioiron could be lost. Therefore it is 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 radiochromium leaks from red cells. The results presented here would imply daily intestinal bleeding at around 2.0 ml (1.0 mg) in subject J.Z. and 0.6 ml (0.3 mg) in subject T.L. 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 J.Z. and 3:1 in T.L. per red cell life span of 123 days and 120 days respectively, which were obtained from their peripheral red cell activity and whole-body activity curves (8).

The loss of radioiron in the urine is shown in Fig. 4b. This must represent iron loss by exfoliation of the urogenital epithelia, since it occurred for the same 10-day period as loss from the intestine, and hemoglobin is not lost via the urine.

The total amount lost within 10 days in urine was 0.018 per cent in J.Z. and 0.039 per cent in T.L. The fractional amount excreted into the urine in the form of nonhemoglobin iron was 0.035 for J.Z. of the amount excreted in the stool during 10 days after injection, and 0.083 in T.L. The radioactivity in the urine after 10 days was undetectable in a 24-hour count with urine at the center on top of the 9 × 4-inch crystal. One subject not included in these data showed a larger amount of urine radioactivity than these subjects, and analysis of the gamma energy proved that it was cobalt-60, apparently as a contaminant of the injected Fe^{59} (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 I. Therefore, it is concluded that the iron loss is mostly into stool.

There may be a slight difference in the radioiron loss rate between the time of the first labeled red cell generation, and after mixing of radioiron with body iron is complete. This would occur because loss of radioiron from the red cell mass will decrease, and loss from nonhemoglobin iron will increase, as the 10 to

TABLE I

LOSS RATE PER RBC LIFE SPAN

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

TABLE II

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*)
C. D.	43	81	173	5.37	46.0	15.0	68	422	490	13.9	2129	2571	4700	2355
R. G.	40	87	180	5.72	50.0	16.5	114	374	488	23.4	2487	2586	5070	2794
K. K.	27	51	158	5.18	46.0	15.3	110	289	397	27.7	1350	2317	3667	1874
R. M.	26	70	177	5.35	47.0	15.0	75	389	464	16.4	2109	2516	4619	2314
M. M.	35	62	160	4.95	48.5	16.0	136	334	470	28.9	1935	2550	4485	2397
H. P.	35	79	171	5.01	45.5	14.8	85	345	430	19.8	2251	2413	4664	2306
T. Y.	19	54	171	5.03	48.0	15.2	150	363	513	29.2	1773	2475	4248	2157
H. W.	32	68	180	5.02	47.5	15.3	101	205	366	27.6	1836	2559	4395	2246
B. T.	31	77	191	5.43	47.5	15.8	107	206	313	34.2	1927	2814	4941	2607
C. R.	29	74	170	5.19	45.0	15.2	144	155	299	48.2	2053	3050	5103	2591
Average	32	70	173	5.23	47.1	15.4	109	308	417	26.9	1985	2585	4589	2364
J. Z.	37	77	173	5.06	46.5	15.6	62	411	475	13.1	2238	2644	4882	2543
T. L.	24	74	180	5.05	45.0	14.6	138	352	490	28.2	2142	3118	5260	2565

20 per cent 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 Figure 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 2364 mg, as shown in Table II (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 2964 mg. The loss of radioiron at the rate of 0.030 per cent 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 used for the loss study; 9 per cent absorption for 15 mg daily iron intake makes 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 was 0.61 mg per day, which is smaller than ours. This may be due to differences in the subjects; the average age of his was 70 years and 32 years for ours, and total miscible iron was 2685 mg for his and total miscible iron 2964 mg for ours. There may, of course, be differences due to differences in method employed. However, the daily percentage loss rate found here, 0.030 per cent is in reasonable agreement with the 0.023 per cent 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 20-100 days, they believe that a normal range of 0.103-0.182 per cent 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

1. 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 mostly in the stool, mainly as blood loss and partly as the loss by exfoliation of mucous epithelia. A small quantity of radioiron loss in the urine due to the exfoliation of urogenital epithelia was suggested.

2. 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.

3. The whole-body counter can detect a very minute amount of iron loss within a short period of time. This is the simplest and most accurate method for measuring the loss of body iron.

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