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IRON ABSORPTION. MEASUREMENT OF INGESTED IRON^{59} BY A HUMAN WHOLE-BODY LIQUID SCINTILLATION COUNTER

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The availability of radioactive isotopes of iron led to the development of reliable methods of studying iron kinetics (1), but the absorption of iron by the gastrointestinal tract was still determined indirectly by the amount of unabsorbed iron excreted in the stools or by quantification of isotopic iron incorporated into hemoglobin (2–7). The availability of a whole-body counter in a clinical facility has made possible the direct assay of iron^{59} retention in human subjects and has eliminated sample collecting and preparation. The purpose of this paper is to describe this method and demonstrate some of the findings.

METHODS AND MATERIALS

Thirty-five volunteers, 25 normal and 10 iron-deficient adults, were the subjects of the study. The iron-deficient subjects met one or more of the following criteria: *1*) virtual absence of iron in bone marrow smears stained by the Prussian-blue reaction (8); *2*) serum iron less than 70 μg per 100 ml with total iron-binding capacity in excess of 350 μg (9, 10); *3*) recent loss of blood without replacement by iron or transfusion. Five subjects (Tables IV and V, numbers 1, 3, 4, 7 and 9) had been purposely phlebotomized to induce iron deficiency. They were in various stages of recovery following the loss of 4 or 5 units (2.0 to 2.5 L) of blood in a period of 1 month. In Subjects 4 and 9 the peripheral parameters had become normal at a time when one could be sure from the history, amount of blood loss and the lack of iron in the marrow, that the body iron stores had not been replenished.

The normal iron-replete subjects met none of these criteria. Serum iron was in excess of 80 μg per 100 ml; there was no evidence of bleeding or history of recent blood loss. Red cell indices were established in all subjects. None of the subjects was known to have neoplastic, infectious or inflammatory disease.

Iron absorption studies were performed in each subject with radioactive iron^{59} and a whole-body liquid scintillation counter. The instrument was a 4π liquid scintillation counter simliar in design to the Los Alamos counter (11). The major differences were an increase in diameter of the sample well to 20 inches and the substitution of thirty 5-inch photocathode tubes. This instrument was operated as a two-channel analyzer, specifically to detect body cesium^{137} and potassium^{40} with maximal efficiency. The two ranges of γ-energy discrimination were wide (0.3 to 0.8 and 0.8 to 2.0 Mev) and useful for detecting a large number of γ-emitting nuclides with good efficiency.

The standards used were a 2.5 L water-filled plastic bottle to which iron^{59} was added in amounts equal to half the dose given the subject. A 250 ml plastic bottle similarly prepared was used as a standard for measurement of radioactivity in feces.

Prior to administration of the radionuclide, the volunteers were assayed in the body counter. They lay supine on a canvas sling within the sample chamber (Figure 1).

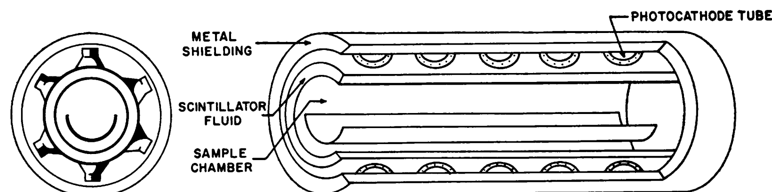

Fig. 1. Human whole-body liquid scintillation counter. The subject is placed in the internal cylinder which is 6 feet long and 20 inches in diameter. The scintillator solution surrounds the patient except at the ends of the tank. Thirty 5-inch photomultiplier tubes in the outer wall of the tank detect the scintillations; the signals generated by the tubes are collected and conducted to a two-channel analyzer where they are quantified by appropriate scaling devices.

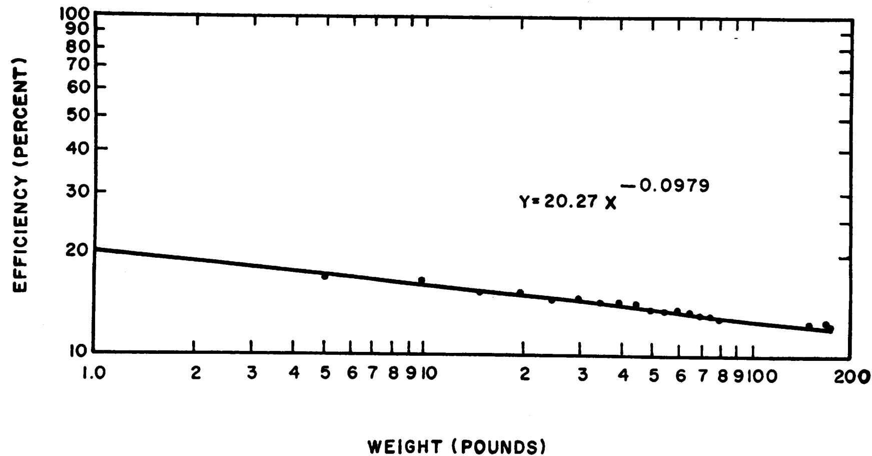

FIG. 2. THE EFFICIENCY OF THE WHOLE-BODY COUNTER FOR MEASURING IRON^{59} RADIOACTIVITY DECREASES AS THE SAMPLE WEIGHT INCREASES. The calibration up to 80 pounds was performed with bottle phantoms. The equation was derived from calibration by means of the phantoms as well as subjects weighing up to 78 kg.

Total counting time was 200 seconds. The resultant count rate represented the background quantities of radioactivity present in each subject. This baseline count, due to potassium^{40}, cesium^{137} and any previously administered radioisotope, was subtracted from all subsequent determinations.

The test dose of iron consisted of 1 μc of iron^{59} as citrate in 50 ml of water in which there was a carrier dose of 1 mg of elemental iron as ferrous sulfate. The dose was drunk in the morning after an overnight fast. No food was taken for an additional 90 minutes. The subjects were then counted in the body counter.

For two weeks after ingestion of the iron, 9 of the subjects made complete collections of feces and 3 made complete collections of urine. These were counted in the whole-body counter.

In the beginning of the study, 4 subjects were counted at intervals of 20 to 30 minutes until they had attained a maximal count rate. This occurred from 3 to 4 hours after ingestion of the iron^{59}. Subsequently, the remaining subjects were assayed 4 hours after the test dose. The explanation for this phenomenon is that, immediately after oral administration, the dose of radioisotope is comparable with a point source surrounded by a large mass of tissue which acts as shielding. After several hours the radioisotope is more widely distributed in the gastrointestinal tract and body. It is then a diffuse source with relatively less shielding and produces a higher count rate. Failure to consider this phenomenon may result in errors of 5 to 10 per cent in calculating the initial retention values. In the third to fifth hour period, count rates vary by 2 per cent or less.

After each count of the subject the standard was also counted. The counts of the subjects and standard were corrected for resolving time loss (12). The ratio of the subject's count to that of the reference standard, 4 hours after administration of the radionuclide, was used as the 100 per cent reference value. Thereafter any comparison of a similar fraction to the reference value represented the fraction of radionuclide retained by the body at that time.

Count rate of patient/count rate of standard $= R_0$; R_0 represents 100% retention. Count rate of patient/count rate of standard $= R_x$ (any day x); $R_x/R_0 \times 100 =$ % retention on day x.

Similarly when fecal excretion was followed, fecal samples were assayed with the appropriate standards. Fecal count rate/standard count rate $\times$ 100 = % excreted; 100 − % excreted = % retained.

The whole-body liquid scintillation counter was a 4π counting system in which low levels of γ-emitting isotopes can be detected. The statistical precision and stability of this type of instrument has been previously reported (11). The γ-energy of iron^{59} can be detected in the channel that measures potassium^{40}. Detailed calibration of the whole-body counter for iron^{59} led to determination of absolute efficiencies for standards and subjects of various sizes, ranging in weight from 1 to 78 kg. Radioactivity in a large mass was less efficiently de-

TABLE I

Comparison of two methods of determining iron^{59} activity

Subject	Absolute calibration	Single standard method
	Percentage retained in body	
A	21.1	21.0
B	4.1	4.1
C	2.2	2.0
	Iron^{59} in a unit of blood (500 ml)	
A	20.1 mμc	19.2 mμc
B	3.7 mμc	3.6 mμc

TABLE II

Normal volunteers

Patient	Sex	Hb	MCV	MCH	MCHC	Serum iron	Total iron-binding capacity	Fe^{59} retained based on	
								Body count	Fecal assay
		g/100 ml				*μg/100 ml*	*μg/100 ml*	%	%
1	M	16.1	104	33	32	83	353	10.0	
2	M	15.3	98	32	32	105	327	10.0	
3	M	16.1	92	32	35	160	329	10.0	
4	M	13.6	94	29	31	87	317	9.0	
5	M	15.5	100	31	34	133	335	8.8	
6	M	15.7	91	31	34	82	339	8.6	
7	M	14.5	95	29	31	81	303	6.8	
8	M	14.6	86	28	32	83	240	6.5	11.0
9	M	15.3	92	29	31	181	218	5.4	0.0
10	M	15.1	97	30	34	108	319	5.4	
11	F	14.5	92	30	33	130	390	4.0	0.0
12	M	15.1	97	33	34	110	240	3.0	
13	M	15.5	99	30	31	103	327	2.5	
14	M	14.6	99	31	32	135	240	2.4	0.0
15	M	14.0	90	28	31	82	286	1.7	
16	M	12.1	97	28	30	132	294	1.5	6.2
17	M	14.5	91	30	33	92	292	1.5	
18	M	14.2	96	30	31	91	315	0.8	
19	M	15.7	86	29	33	100	286	0.5	
20	M	14.2	89	29	32	96	243	0.5; 3.5*	
21	M	15.4	95	31	32	99	258	0.5	
22	M	15.5	100	31	34	133	335	0.4	
23	M	12.8	93	30	32	93	242	0.2	6.0
24	M	16.2	91	30	33	105	367	10.3	
25	M	14.5	84	28	33	136	350	7.1	

* Done twice, 1 year apart.

tected because of self-absorption. A log-log plot of the data provided a linear relationship between detector efficiency and sample weight, and a least squares analysis of the data yielded a power function (Figure 2). The efficiency of the subjects was about 12 per cent.

In the clinical studies absolute calibrations were not necessary. It was sufficient to compare the activity of subject or sample with a reference standard. The use of the standard in an appropriate geometry obviated corrections for physical decay of the radioactive isotope and for flutuations in counter efficiency. The retention of iron[59] and amount of circulating iron[59] removed by phlebotomy were calculated by using the power function and the simple ratios, and the results were compared (Table I).

RESULTS

Normal, iron-replete adult humans absorbed 10 per cent or less of a 1 μc dose of iron[59] to which 1 mg of elemental iron was added (Table II). Iron-deficient subjects absorbed 29 to 71 per cent

TABLE III

Iron-deficient subjects

Patient	Sex	Hb	MCV	MCH	MCHC	Serum iron	Total iron-binding capacity	Bone marrow iron	Fe^{59} retained based on	
									Body count	Fecal assay
		g/100 ml				*μg/100 ml*	*μg/100 ml*		%	%
1	M	13.8	88	27	31	51	551		71	
2	F	9.6	76	21	28	0	464	Dec.	67	
3	M	10.6	97	31	32	15	426		66	
4	M	15.7	91	31	33	121	285	Dec.	64	58
5	M	13.4	80	24	29	58	379	Dec.	55	
6	M	14.2	72	23	27	43	380		45	
7	M	10.3	73	21	29	24	504		40	37
8	M	13.5	91	29	32	94	264	Dec.	31	20
9	M	14.0	91	28	31	107	255	Dec.	30	
10	M	15.5	105	34	33	85	362		29	

TABLE IV

Normal volunteers, per cent retention Fe^{59}

Days	Patient number																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20a	20b	21	22	23	24	25
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	55	100						24	95	80	93		60	46	6.4	69	89	100			67	100		74	71	
2		78			89	84		22	18	26	53		8.0	5.3	4.8	1.6		100		72	67		1.4	13		26
3		48									54			3.6	4.3	1.6		16				3.0		0.4		24
4	25	35		36								35			3.6	1.6	5.0	3.5			25			0.4	12	
5	25		16							14			4.0				4.0		13					0.3	10.8	
6	23					25	19	8.2		12.4	12.2	15.4			2.7	1.5	4.0			2.1				0.4	10.6	10
7	24	19			12.7			7.7	5.6	10.9	8.1		4.0	2.7	2.5	1.5	4.0	1.9				0.5		0.3	10.3	
8	23	14				24		7.5			8.2		3.0	2.6	2.4	1.5	3.5					0.6		0.3		7
9		14						7.0	5.6	10.7	4.7		3.0	2.4	2.1	1.5				1.6	5.3	0.5		0.3		7.1
10		13					8.9											1.3	1.5							
11																		1.1			4.7	0.5				
12																	2.4									
13										6.8	4.0		2.6		1.7									0.2		
14	12								5.4		4.0			2.4			1.8									
15								6.9		5.9								0.8			4.3					
16								7.0		5.4	4.0															
17																			0.5							
18	10											3.0					1.5									
19			10		8.8		7.6																			
20											4.0		2.5													
21			10	9.0		8.6	6.8	6.5												0.5	3.5					
23		10																					0.4			
24												3.0														
26						8.6														0.5						

(Table III). The procedure provided a clear-cut distinction between normal and iron-deficient subjects.

The gradual decay of radioactivity after ingestion of the test dose was due to loss of unabsorbed iron in the feces. After loss in the feces stopped, the retention value remained constant, indicating no excretion of the absorbed iron (Tables IV and V). However, with chronic loss of blood, there was a corresponding loss of radioactivity.

The accuracy with which blood loss can be quantitated is shown in Figure 3. The curve is that of a chronic blood donor (number 8, Tables III and V). When iron^{59} retention had reached equilibrium, 500 ml of whole blood was removed on three occasions at weekly intervals. The iron^{59} retention values decreased by 17, 12 and 12 per cent, respectively. In another patient of this series (Table V, number 2) there was a continuing decrease in retention after equilibrium should have occurred. This was regarded as evidence of bleeding. From Days 31 to 35, the subject's retention decreased from 60 to 52 per cent or 13 per cent. She weighed 62 kg, and her blood volume was estimated to be about 4,000 ml. She was believed to have lost, therefore, approximately 500 ml of blood over a 4 day interval. The radioactivity in a 24 hour fecal sample was assayed and corresponded to 100 ml of blood. At a subsequent laparotomy an oozing hemangioma of the lower jejunum was removed.

Nine subjects collected all stools for 2 weeks following oral administration of iron^{59}. Retention of iron computed by this method is shown in Tables II and III. Small variations in fecal sample size produced significant changes in counting efficiency. Thus, measurement of gastrointestinal absorption by collection of feces and counting the total collection was unreliable. The method could be improved by using standardized aliquots,

TABLE V

Iron-deficient subjects, per cent retention Fe^{59}

Days	Patient number									
	1	2	3	4	5	6	7	8	9	10
0	100	100	100	100	100	100	100	100	100	100
1	76	97	87	96	97	60	50	42		
2	72		72	85	61	52	45	35		
3		68		68		48	45	34		
4	71		67				40			85
5					64		40		30	44
6				70	61		40			
7				64			40	30	29	32
8		68		64		47	41	31	30	
9		67		65		45	40	31		
10						45				
11										
12					58					
13				62			39			
14		67				46		30		
15				64						
16						45	38			
17		64				45				28
18										
19					55					
20				64		45				
21								25*		29
22										
23				64						
24		61								
25										
26										29
27										
28		60						25/22†		
35		52						21/18.5†		
40	66		66							

* Phlebotomies.
† Count before/count after.

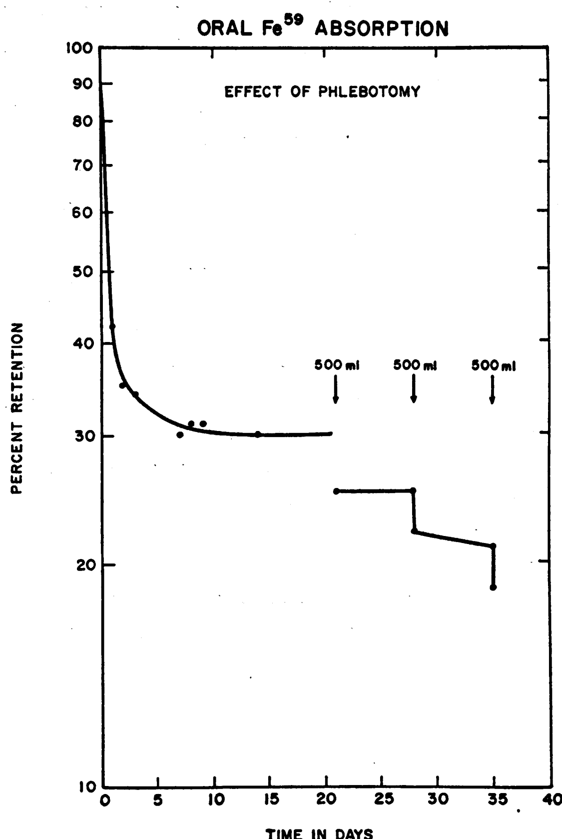

FIG. 3. INCREASED ABSORPTION OF IRON^{59} WAS DEMONSTRATED IN THIS CHRONIC BLOOD DONOR. He was shown to have depleted marrow stores of iron. Phlebotomies of 500 ml each, 21, 28, and 35 days after administration of an oral dose of radioactive iron^{59} showed an abrupt loss of labeled iron from the body.

but larger doses of iron^{59} would be required. Cumulative urine collections in three subjects contained less than 1 per cent of the administered dose. This confirmed in humans the previous observations in animals that significant quantities of radioactive iron are not excreted in the urine (13, 14).

DISCUSSION

A liquid scintillation counter large enough to receive a human permits accurate determination of the total body content of most γ-emitting nuclides. Determination of the percentage of retention by the body and rate of disposal in excreta may be obtained. The gastrointestinal absorption of iron^{59} was studied by this method. The small quantities of radioactive iron needed for study reduce the radiation hazard and permit the performance of repeated studies when indicated. Many methods of studying iron absorption have been used in the past. The availability of radioisotopes of iron led to the development of several methods more reliable than chemical balance techniques (14). Measuring the amount of orally administered radioactive iron that is incorporated into hemoglobin is an undependable method of studying iron absorption because of the variability of incorporation of iron into erythrocytes (3–5). Measurement of the unabsorbed fraction of radioactive iron in the stools has the technical problem of ensuring complete collection of specimens (2). Further, when the proportion absorbed is small, technical errors of measurement become magnified (4). The most reliable method employs two isotopes (6, 7). The method assumes that equal amounts of oral and intravenously administered iron are incorporated into hemoglobin. The procedure is technically difficult and requires the administration of an isotope with a long half-life.

In contrast, the present method directly measures iron absorption by the body and permits accurate determination of the amount of retention and the rate of loss. Collection of excreta is not necessary. The method permits an estimation of blood loss over a long period of time.

SUMMARY

Iron absorption was measured in normal and iron-depleted subjects by using radioactive iron^{59} and a whole-body liquid scintillation counter.

Normal iron-replete adult humans absorbed 10 per cent or less of the orally administered test dose. Iron-deficient subjects absorbed 29 to 71 per cent of the dose. The method requires a small dose of radioisotope, permitting repeated studies. It provides an index of the rate of iron loss, including loss by bleeding.

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