RADIOACTIVE IRON ABSORPTION IN CLINICAL CONDITIONS: NORMAL, PREGNANCY, ANEMIA, AND HEMOCHROMATOSIS*‡

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The experiments listed below suggest very strongly that when the *iron reserves are depleted* the human being will absorb iron in relative abundance—in fact 10 to 20 times normal. Although the iron stores of the body are mainly in liver, spleen, and bone marrow, these stores influence iron absorption in some obscure fashion. The iron content of the *mucosa of the gastro-intestinal tract* is very low (2) and there appears to be little if any iron storage here, but the cells of the mucosa do have the power to accept or refuse iron—a reaction totally different from that relating to most metals or salts. Low hemoglobin levels in the blood (anemia) are not enough to call out active iron absorption if the reserve stores are abundant (6). At the moment an adequate explanation of the observed facts is not at hand but continued study of the mucosa of the gastro-intestinal tract is obviously indicated.

For some good reason the body guards itself against too liberal an intake of iron, perhaps because it finds the elimination of iron difficult. There is some evidence that a considerable excess of iron will harm those cells in which the iron is stored—for example the liver and pancreas cells in hemochromatosis. The older clinical belief that iron was absorbed and the surplus removed by excretion through the mucosa of the large intestine must be put aside even though this belief has the prestige and academic tenure of many decades of acceptance by physician and physiologist. Some of this misconception was due to iron balance studies in which too much reliance should not be placed. Difficulties in iron analyses are considerable, particularly of feces, where phosphorus-iron compounds introduce serious errors and make the figures for iron recovery too low, thus giving an erroneous impression of positive iron balance.

Welch, Wakefield, and Adams (15) by means of an ileostomy in a single patient showed that the *excretion* of iron into the colon was negligible in

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amount. McCance and Widdowson (10, 11) by parenterally introduced iron showed that the human body could *eliminate* iron only in small amounts. Their general conclusions were in harmony with our belief that *absorption* controls iron balance and *reserve stores* in some way control absorption.

Radio iron is perfectly adapted to the study of the elusive iron element as it shuttles about within the body. Probably by no other means can we get a comprehensive understanding of the true internal iron metabolism. By use of radio iron in dogs we were able to bring convincing evidence (5) that the plethoric dog (with ample reserve stores) would absorb little or no radio iron, that the normal dog (hematocrit 50 per cent red cells) would absorb little radio iron, but the depleted anemic dog would absorb much (5 to 50 per cent) of the ingested radio iron which would promptly appear in new red cells in the circulation. Furthermore dogs, anemic or normal, can eliminate very little iron and that largely by way of the liver and bile (4).

Dogs are ideal experimental animals for the study of iron metabolism and we know a good deal about their capacity to form new hemoglobin under the stimulus of anemia. We believe their gastro-intestinal tract behaves toward iron much as does the human being. The experiments below support this claim. It is possible to do preliminary experiments in the dog with much better control than is possible with the human being but eventually we must always make similar, if incomplete, observations with human beings to exclude possible differences between the physiology of the dog and man. Furthermore, we can study diseased states in the human patient which have not been reproduced in the dog—for example, pernicious anemia and hemochromatosis.

As has been pointed out before (4), when *iron* is made *radioactive* by bombardment in the cyclotron, only an infinitesimal portion of the iron atoms is changed from the normal Fe^{58} isotope to the unstable and therefore radioactive isotope Fe^{59}. Every atom of iron that emits a beta ray detected by the Geiger counter has been during its whole previous history since bombardment in the cyclotron, an atom of iron differing from ordinary iron only by its small increase in atomic weight. Since there is abundant evidence to indicate that the animal organism cannot differentiate significantly between atoms with these small variations in mass, we can have every confidence that the path of these radioactive atoms that we can follow with the Geiger counter represents in every detail the pathway of the total amount of administered iron tagged by the radioactive iron isotope. Subsequently in this paper we refer to iron tagged in this manner as *radio iron*.

Experimental Methods

The radioactive isotope of iron (47 day half-life) used in these experiments was prepared in the cyclotron of the Radiation Laboratory of the University of California, using the methods described by Wilson and Kamen (16). Before feeding, it was usually further purified and freed from the last radioactive contamination by repeated ethyl ether extraction of the chloride adding neutral manganese and cobalt salts in excess as carriers. It was then made up as ferric ammonium citrate, in which form it was fed.

Radioactive measurements before January, 1941, were made, using the dipping counter technique described in an earlier paper (1, 5). Data obtained since that time were obtained by use of much more sensitive detection equipment. The other experimental techniques involved in blood studies, isolation of radio iron, electroplating, etc., have been described in earlier papers (3).

Direct determination of red cell circulating mass was not practicable in these cases. Therefore, the estimate of red cell mass was made as follows: By plasma dye dilution methods there is considered to be about 80 ml. of blood per kilo of body weight. However, it has been pointed out that the red cell volume as calculated from the plasma volume and venous hematocrit is about 25 per cent too high in dogs (7, 9) and this has been reported to be true also in human beings (14). Therefore, the red cell mass was estimated as follows:

$$\text{Body weight in kilos} \times 80 \times \text{venous hematocrit per cent} \times 0.75 = \text{cell mass}$$

We have expressly included in our tables values for per cent of the isotope fed per 100 ml. of red cells so in the event that other estimations of cell mass may prove more accurate, the total radio iron in circulation may be calculated on the latter basis. The circulating isotope in the tables is calculated by multiplying the concentration of isotope in red cells by the estimated mass of red cells.

The patients marked "*" in the following protocols received labelled iron at a time when our measuring equipment was 30 times less sensitive than that now used. Although the dosage activity was quite high in most of them, the amount of isotope appearing in the red cells was very near the limits of error of the methods as used at that time. Therefore, it is not possible to estimate accurately the amount of radio iron in the circulation. In each instance the figure given represents an *upper limit* which is reached by assuming a conservative count which could have been detected and calculating the amount which would have been present if this count had actually been found.

Clinical Histories[1]

Case 1. B. G. (Hospital No. 182641.) Male, age 57. *Peptic ulcer with hemorrhage.* The patient had ulcer symptoms for about 1 year. They increased in severity during the week before admission, and during the last 4 days of this week the patient noted black stools. There were increasing weakness and dizziness and vomiting of "coffee-ground" material. Admission blood studies: RBC 1,500,000; hemoglobin 4 gm. per cent. He was given small frequent transfusions and on the 4th hospital day 17 mg. of radio iron as ferric ammonium citrate was given. His hemoglobin on

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this day was 6.1 gm. per cent. Stool on the day of feeding showed 1+ guaiac, but subsequent examinations were negative for blood. He had been bleeding at least 10 days before the radio iron was given and probably longer. Neutral iron was begun 4 days after the radio iron was given. Blood samples were obtained at 5, 8, 11, 17, and 24 days following the feeding and activity measurements were made on the separated red cells. The 11 day cells showed 1.48 per cent of the fed iron per 100 ml. of red cells. An estimate of the patient's circulating red cell mass was 1005 ml. Thus, it was calculated that 15 per cent of the administered dose was present in the circulation at this time. This patient was sampled a number of times over the course of 45 days.

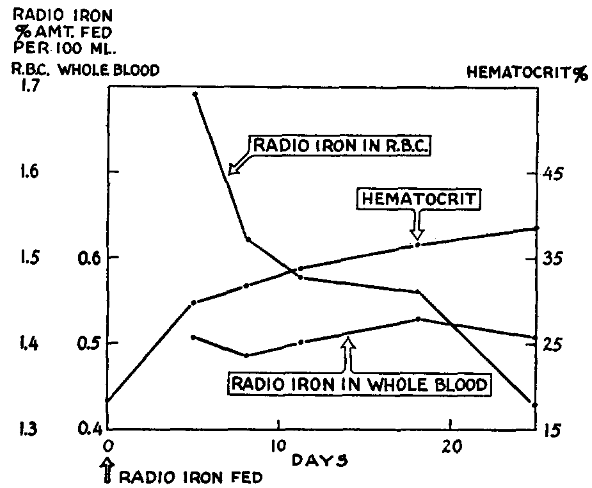

CHART A. Bleeding gastric ulcer. Case 1, Table 1, B. G.

In Chart A are plotted the concentrations of radio iron per 100 ml. of the red cells of case 1, Table 1, the concentrations per 100 ml. of whole blood and the red blood cell hematocrit readings for the corresponding values. One notes that over the whole experimental period the concentration of radio iron per 100 ml. whole blood remains constant. During this time the hematocrit had risen from about 19 per cent to 37 per cent. At the same time there had been a progressive decrease in the red cell isotope concentration. If one assumes that the total circulating blood volume of this patient remained constant over this period, an assumption probably valid in the light of other recently published data (3), this result would indicate that at least all of the absorbed radio iron which was going to be utilized to form hemoglobin had been so used as early as the 5th day after feeding. Since there was a need for new hemoglobin and in view of the fact that hematopoiesis was progressing rapidly at this time, it would be a fair inference that the figure of 15 per cent *utilization* also represented the major portion of the radio iron *absorbed* from the gastro-intestinal tract.

Case 2. V. P. (Hospital No. 80540.) Male, age 27. *Duodenal ulcer with hemorrhage.* A third admission to this hospital with severe hemorrhage from the duodenal ulcer. Other admissions were 8 and 3 years previously. Following each he remained on iron and diet therapy for short periods. Short follow-ups revealed a hemoglobin remaining around 12 gm. per cent. The third hemorrhage began after long continued dietary indiscretions. Symptoms of bleeding apparently began 6 days before admission. The red cell count on admission was 1,600,000, and the hemoglobin 6 gm. per cent. He was treated with bed rest and progressive Sippy diet, and the hemorrhage stopped after one hospital day. Two small transfusions were given. On the 14th hospital day, when his red count was 3,800,000 and his hemoglobin 10.2 gm. per cent, he was given a single feeding of 20 mg. of radio iron as ferric ammonium citrate. 7 days later the red cells contained 1.85 per cent of the administered dose per 100 ml. This corresponded to an estimated 20.2 per cent of the fed dose in circulation. 46 days after feeding, the hematocrit had risen to 47 per cent and the red cell isotope amounted to 1.42 per cent of the amount fed. The amount estimated in circulation was essentially the same (20.6 per cent) due to the increased mass of red cells (3).

Case 3. A. D.* (Hospital No. 127050.) Female, age 22; para 2, gravida 4. *Incomplete abortion.* Last menstrual period 2 months previously. Slight bleeding began 2 weeks before and *profuse bleeding* with passage of clots 4 days before admission. Admission blood studies: red blood cells 2,280,000; hemoglobin 5 gm. per cent. On the 2nd hospital day she was given 200 cc. of whole blood. Because of repeated chills and fever, the abortion was not completed until the 17th hospital day. 10 days before curettage and 3 weeks after the bleeding began, she was given by mouth 423 mg. of radio iron as ferric ammonium citrate. At this time the red count was 3,000,000 and hemoglobin 6.8 gm. per cent. 4 days later a blood sample revealed that the red cell radio iron was 0.2 per cent of the dose fed per 100 ml. of red cells, or an estimated 1.3 per cent of the dose in circulation.

Cases 1, 2, and 3 (Table 1) may be considered together and show an iron absorption many times normal. With *severe anemia* due to bleeding from a duodenal *ulcer* (case 2) there had been previous episodes of bleeding and probably much of the *iron reserve* had been exhausted. For this amount of ingested iron (18 to 20 mg. Fe) we may say that an expected normal absorption would be 0.5 to 1.5 per cent (case 14). The absorption in these two cases is 15 and 21 per cent respectively—more than 10 times the expected absorption of a normal individual. The intestinal mucosa accepts this iron readily and the hemopoietic mechanism builds it very rapidly into hemoglobin within new red cells.

Case 3, an incomplete *abortion* with bleeding and *anemia* 2 to 3 weeks before iron feeding, shows considerable iron absorption. The dose of radio iron was large (423 mg. Fe) because the radio activity was low. In standard dogs with this dose we expect 5 to 7 per cent absorption and utilization and we could hardly expect more than this in a human. In other words, this human absorption (1.3 per cent) is 20 to 25 per cent of maximal, or twice or three times an expected normal. It is probable that this bleeding had not decreased the reserve stores of iron as completely as in cases 1 and 2. Moreover, the interval between feeding and sampling was only 4 days, which may not have allowed sufficient time for the total radio iron utilization and appearance as new hemoglobin (7).

Case 4. J. H. (Hospital No. 163765.) Male, age 11 months. *Impetigo* contagiosa. This boy had had a series of infections of the skin which, because of poor environmental conditions, were difficult to control. Impetigo on this admission had been present about 2 months. Blood studies showed a red count of 4,700,000 and a hemoglobin of 11 gm. per cent. There was no fever and little systemic reaction. It was thought that there might be a nutritional basis for the anemia, although not necessarily due to iron deficiency. 3 days after feeding 8 mg. of radio iron as ferric ammonium citrate, 0.75 per cent of the administered iron was present per 100 ml. of red cells. 5 and 7 days after feeding, the amounts present were 1.21 per cent and 0.98 per cent per 100 ml. in the cells. The amount present in the circulation as determined by concentration in 7 day cells was 2.2 per cent of the amount fed.

Case 5. H. K.* (Hospital No. 172905.) Male, age 21. Chronic *pyelonephritis* with severe secondary anemia. This patient had been sick for several months. On admission the red count was 1,600,000 and the hemoglobin 8.5 gm. per cent. The urine contained albumin and white cells with no red cells. The N.P.N. was 180 mg. per cent. Iron and a long trial of intramuscular liver had had little effect on the anemia. A single dose of 42 mg. of radio iron was given as ferric ammonium citrate. 7 days later there was no detectable activity in the red cells. Because of the counter used and the low activity of the sample given, it is possible that the maximum absorption in this case was 3.7 per cent. However, it is highly probable that the absorption was less than 1 per cent.

Case 6. L. S.* (Hospital No. 167067.) Female, age 44. *Hypochromic anemia* with gastric anacidity. The history dates back about 10 years. During this period she had been treated elsewhere for anemia by various oral liver products with little effect. Symptoms recently increased. She noted difficulty in swallowing and a tendency toward spoon-shaped nails. Admission blood studies: red count 4,200,000; hemoglobin 7.5 gm. per cent. No free gastric HCl after histamine. She was given a single dose of 204 mg. of radio iron and 6 days later activity measurements of the red cells showed a very small count which would represent a maximum of about 2.0 per cent of the amount fed in circulation. On a régime of ferrous sulfate and HCl, her symptoms gradually decreased over a period of 2 months. During this time her hemoglobin rose to 13.7 gm. per cent with a slight increase in the count.

Cases 4, 5, and 6 (Table 1) are considered together—anemias in which there should be some absorption but a relatively insignificant response is recorded. Case 4, *impetigo*, and case 5, *pyelonephritis*, show a utilization perhaps not in excess of normal. It should be noted that the degree of anemia in case 4, *impetigo*, is only slight or close to a low normal hemoglobin. It has been shown in standard anemic dogs that an *infection* will not prevent absorption but will delay the utilization of the hemoglobin building material (13). So it is possible that some iron was absorbed but not utilized in the 7 day period between feeding and sampling for the radio iron.

Case 6 is an interesting type of *hypochromic anemia* which had been under treatment at various times. Lack of gastric HCl may have been a factor. She did absorb somewhat more than normal (2 per cent) of a 204 mg. dose of radio iron. The maximum anemic absorption would be 5 to 10 per cent and the normal absorption less than 1 per cent. Therapy of ferrous sulfate and HCl did bring her blood back to normal within 2 months.

TABLE 1

Radioactive Iron Uptake by Red Cells in Various Clinical Conditions

Case	Diagnosis	Weight	Estimated red cell mass	Hematocrit at feeding	Hematocrit at sampling	Interval between feeding and sampling	Dose iron	Per cent amount fed per 100 ml. red cells	Per cent amount fed circulating estimated
		kg.	*ml.*	*per cent*	*per cent*	*days*	*mg.*	*per cent*	*per cent*
1. B. G.	Gastric ulcer—hemorrhage	49.4	1005	18.4	33.8	11	18	1.48	15
2. V. P.	Duodenal ulcer—hemorrhage	51.8	1455	29.4	46.7	7	20	1.37	20.2
3. A. D.*	Abortion, hemorrhage	50.0	630	22.1	20.8	4	423	0.20	1.3
4. J. H.	Impetigo	9.8	220	35.3	37.9	7	8	0.98	2.2
5. H. K.*	Chronic pyelonephritis	52.6	600	18.4	19.0	7	42		0–3
6. L. S.*	Hypochromic anemia	64.6	1150	32.0	29.4	6	204	0.17	2.0
7. A. H.	Pernicious anemia	60.0	1090	23.4	30.3	10	20	0.12	1.3
8. S. M.*	Leukemia	8.9	130	24.5	—	6	125	0.47	0.6
9. J. V.*	Familial icterus	11.8	215	—	30.5	7	93	0.13	0.3
10. A. Z.*	Mediterranean anemia	14.3	46	6.6	5.3	4	15		0–1.5
11. C. Y.*	" "	13.0	175	23.2	22.7	5	64	0.26	0.5
12. H. V.	Hemachromatosis	61.2	1610	45.6	43.8	6	53	0.04	0.7
12. "	"	61.2	1610	41.0	43.4	7	14		0.8
13. S. V.	Polycythemia	59.0	1750	—	49.6	6	204	0.03	0.5
14. A. K.	Normal	81.0	2260	48.5	46.6	7	20	0.08	1.8

Case 7. A. H. (Hospital No. 184793.) Male, age 64. Untreated *pernicious anemia*. Symptoms had been gradually increasing over a period of 4 months. Recently evidence of spinal cord damage had appeared. On admission, the patient was almost moribund. Blood studies: red blood cells 1,300,000; hemoglobin 5.9 gm. per cent; white blood cells 2,450. Bone marrow smear was typical of Addisonian type of macrocytic anemia. Two doses of intramuscular liver had been given when he received 20 mg. of radio iron as ferric ammonium citrate. At the time of feeding the red cell hematocrit was 23.4 per cent. A considerable hematopoietic effect was induced by continued intramuscular liver and 18 days later the hematocrit was 38 per cent. 10 days after feeding the isotope, the red cell radio iron was 0.115 per cent of

the administered dose per 100 ml. of red cells corresponding to an estimated 1.3 per cent of the dose in circulation. No therapeutic iron was given during the course of these observations. He was discharged on the 26th hospital day with normal blood studies and evidence of considerable improvement in the cord changes.

Case 7 (Table 1) is of particular interest. A typical case of *pernicious anemia* rapidly producing new hemoglobin due to the liver therapy would presumably absorb iron rapidly. The iron absorption (1.3 per cent) is not more than that of a normal control (1.8 per cent) given the same dose of radio iron. Obviously the iron needed for new red cell and hemoglobin production (case 7) comes from iron *reserve stores* which are well known to be much above normal in untreated pernicious anemia. We believe that the presence of these reserve stores of iron in some fashion inhibits iron absorption which takes place so readily in simple anemia due to blood loss when the reserve stores are depleted.

Case 8. S. M.* (Hospital No. 168514.) Female, age 7 months. *Leukemia*, probably monocytic (autopsy). This Italian baby had several admissions because of *severe anemia*. Splenomegaly and hepatomegaly were marked. The diagnosis of Mediterranean anemia (Cooley's type) was considered. The leucocyte count was low-normal with a high percentage of lymphocytes. During one admission she was given 125 mg. of radio iron in the form of ferric ammonium citrate. Red cells taken 2 hours after feeding showed no radio iron. Cells taken 6 days after administration showed 0.47 per cent of the amount fed per 100 ml. of red cells, or about 0.6 per cent of the amount fed in circulation. No more blood samples were obtained. Her red count at the time of feeding was 3,140,000 and hemoglobin 8.2 gm. per cent. She was admitted again 4 months later in poor condition with a very severe anemia and bronchopneumonia. At *autopsy* specimens of the *liver*, *spleen*, and heart were obtained. The 15 gm. liver sample contained an amount of radio activity which would represent about 0.83 per cent of the administered dose in the whole 530 gm. liver. A 26 gm. aliquot of the 1100 gm. spleen indicated a radio iron content in this organ of 0.56 per cent of the administered dose. The heart, by similar analysis, contained about 0.09 per cent. Thus, 4 months after feeding radio iron 1.5 per cent of the dose given was present in these unperfused organs.

Case 8 (Table 1) supplies us with data on autopsy material in *leukemia*. The patient was given radio iron and 6 days later showed 0.6 per cent in the circulation, but at *autopsy* 4 months later the liver and spleen contained 1.5 per cent of the dose fed 4 months previously. Obviously 1 per cent of the dose of radio iron (or more) was absorbed but not incorporated in new red cells within 6 days in spite of the anemia. The severe anemia of leukemia is probably due to choking of the red marrow with abnormal white cells thus impairing red cell production—a blockade of the hematopoietic process. This case with severe anemia did absorb about four times normal (or more) but did not utilize the radio iron to make hemoglobin, perhaps due to an abnormal marrow.

Case 9. J. V.* (Hospital No. 170116.) Male, age 2 years. Familial *hemolytic icterus*. This patient was admitted with symptoms of a severe anemia without jaundice. Blood studies revealed small cells with increased fragility and tendency toward spherocytosis. Similar changes were found in maternal blood. 93 mg. of labelled iron was fed as ferric ammonium citrate in milk. His red count at this time was 2,000,000 and hemoglobin 4.6 gm. per cent. 7 days later the activity in the red cells was extremely low, but the amount of isotope in circulation could be placed at a maximum of 0.3 per cent of the amount fed. Following transfusions and general supportive measures, splenectomy was performed with complete relief of symptoms.

Case 10. A. Z.* (Hospital No. 121099.) Female, age 4 years. *Erythroblastic anemia*. This Italian girl had had the diagnosis made at 14 months of age. A brother had died of the same disease. During 19 hospital admissions there was a history of repeated intercurrent infections and she had been transfused many times. Red cell count was 0,830,000 and hemoglobin 2 gm. per cent at the time a single dose of radio iron of 15 mg. was given as ferric citrate. 4 days later the red cell activity was very near the background count, but it was estimated that less than 1.7 per cent of the material fed was in circulation.

Case 11. C. Y.* (Hospital No. 128287.) Female, age 3½ years. *Erythroblastic anemia*. At the time of feeding the red count was 2,800,000 and the hemoglobin 7.9 gm. per cent. 532 nucleated red cells per 100 white cells were counted. She was given 64 mg. of radio iron in a single dose as ferric citrate. 5 days later the red cell activity was extremely low, and the maximum amount of the isotope estimated to be in circulation was about 0.45 per cent of the dose fed.

Cases 9, 10, 11, and 12 (Table 1). *Familial icterus*, *Mediterranean anemia* (Cooley), and *hemochromatosis* have several important abnormalities in common and some striking differences. All three conditions show *maximal* figures for *iron storage*, 10 to 20 or more times normal in the liver for example. Iron absorption in all three conditions is *minimal* in spite of severe anemia in the children with Mediterranean anemia and familial icterus. There was no anemia in hemochromatosis but bleeding was instituted to deplete the reserve iron stores if possible. Anemia due to blood loss was produced but there was still no significant iron absorption. Again it appears that the presence of *abundant iron stores* in the body in some way inhibits iron absorption. Obviously at some time, probably *early* in these chronic disease conditions, iron was absorbed in abundance.

Case 12. H. V. (Hospital No. 138938.) Male, age 49. *Hemochromatosis*. History dates back about 5 years with first symptom being increasing pigmentation of the skin. This was followed by the onset of diabetes mellitus and finally by distention of the abdomen due to fluid. On the first admission, the diagnosis was confirmed by skin biopsy which showed hemosiderin granules and iron-staining pigment about the sweat glands. Infra-red photography revealed extensive collateral circulation in the abdominal wall. There were pronounced muscular weakness and evidence of myocardial damage. Loss of hair, impotence, decrease in testicular size, and a low basal metabolic rate (−43 per cent) indicated involvement of other endocrine glands. The diabetes was well controlled by diet and 60 units of protamine insulin per day. On a subsequent admission a single dose of *60 mg. of radio iron* was given by mouth as ferric ammonium citrate. Samples were taken at intervals for 3 hours after feeding to determine whether any absorption had occurred as indicated by presence of the isotope in the plasma. Further sampling was done 1, 2, 5, and 6 days following administration, and the amount of radio iron expressed as per cent of the dose fed found in the red cells was 0.012 at the highest reading.

The blood picture in hemochromatosis is usually near normal, as it was in this case; and it might be argued that such a patient might absorb iron and not utilize it for hemoglobin formation, since there was no demand for increased hematopoiesis. Therefore, the patient was subjected to a phlebotomy amounting to 500 ml. to provide a stimulus for red cell formation. 2 days after bleeding the isotope concentration in the red cells was 0.017 per cent of the amount fed. 10 weeks later there was 0.04 per cent of the amount fed per 100 ml. of red cells. It was thought proper to determine whether intermittent heavy bleeding might be effective in marshalling deposited iron from the tissues for hemoglobin formation. If the hemosiderin were reversibly deposited, it might be expected that eventually the excess iron could be removed and possibly the fibrotic changes in the liver and endocrine glands arrested. If radio iron had been absorbed and deposited along with the other iron in the tissues, it might be possible to follow the removal of this iron. However, after each of several bleedings the red cell count in the patient dropped progressively, and even though the hematocrit and hemoglobin content of the blood remained near normal, it was felt advisable to suspend attempts at therapy at this time. Following the second bleeding, the patient was given 14 mg. of radioactive iron, and even though his red count was about 3,800,000 subsequent sampling showed he had absorbed only 0.8 per cent of this small dose. Subsequent bleedings and samplings showed no rise in the red cell isotope concentration.

Case 13. S. V.* (Hospital No. 167389.) Male, age 42. Chronic bronchitis and bronchiolitis with secondary *polycythemia.* This patient had numerous phlebotomies with symptomatic relief, the last one several months before the iron was given. The red count was 6,500,000 and the hemoglobin 15 gm. per cent at the time he was fed a single dose of 204 mg. of radioactive iron and 6 days later the red cell activity was practically 0. The maximum estimated amount of the iron in circulation was 0.5 per cent of that fed. Two phlebotomies were performed following the feeding of the iron, and the red cell activity did not change.

Case 14. A. K. (Hospital No. 157144.) Male, age 25. Normal. This medical student was fed radio iron as a normal control. Labelled iron was given in very low doses of 4 mg. per day for 5 days since this was felt to favor maximum absorption. 7 days after the last feeding there was 0.08 per cent of the administered radio iron per 100 ml. of red cells. This corresponded to an estimated 1.8 per cent of the dose in circulation. Since the blood picture was normal and there was presumably no stimulus for hematopoiesis over and above the replacement demands, 500 ml. of blood was removed in order to provide the stimulus. 7 days later the red cells showed 0.09 per cent of the administered dose per 100 ml. corresponding to an estimated 1.8 per cent of the dose in circulation.

Case 13 (Table 1). *Polycythemia*. It might be suspected that the over production of red cells in this disease might be due to an abnormally high iron absorption. Here is definite evidence again that surplus reserves (even if in the circulation) inhibit iron absorption. This case shows only traces of radio iron in the circulating red cells, 6 days after feeding.

Case 14 (Table 1). A *normal control* was given very small amounts (4 mg. Fe) of active samples of radio iron over 5 consecutive days to get a *maximal absorption*. In anemia due to blood loss with repeated similar small feedings one might expect 50 to 60 per cent utilization of iron. The maximum after 7 days in the normal case was 1.8 per cent. Subsequent bleeding to bring out of storage any unused absorbed radio iron showed a maximum figure of 1.8 per cent radio iron in the circulating red cells. Compared with cases 1 and 2 (Table 1) this represents not less than a 10 to 1 differential in favor of the human case of ulcer and anemia due to blood loss. Actually the differential was greater because the ulcer patients received larger single doses of iron which are not utilized so effectively as are the small doses.

Clinical Histories—Pregnancy

Case 21. M. C. (Hospital No. 143507.) Age 37; para 2, gravida 5. Pregnancy with hypertension and paroxysmal auricular tachycardia. Gestation 2 months; therapeutic abortion. Patient complained of vertigo, scotomata, headache, and paroxysmal dyspnea. Heart was enlarged, with presystolic and systolic murmurs. Blood pressure was 220/115. At the time of iron feeding, red cells were 4,200,000; hemoglobin 14.7 gm. per cent; hematocrit 43.3. She was given 16 mg. of radio iron and delivered by hysterotomy 1 week later. Subsequent sampling showed an estimated 2.7 per cent of the amount fed in circulation.

Case 22. P. F. (Hospital No. 165477.) Age 27; para 2. Pregnancy with rheumatic heart disease, Class II-A. Gestation 3 months; therapeutic abortion. Pregnancy was subjectively uneventful except for dyspnea on exertion. Examination disclosed mitral and aortic stenosis and insufficiency. Blood pressure 140/42. Urine showed occasional red and white cells. At the time of iron feeding, blood studies showed red count of 4,110,000; hemoglobin 14.1 gm. per cent; hematocrit 37.5 per cent. She was given 53 mg. of radio iron and pregnancy was interrupted 7 days later. Subsequent sampling revealed an estimated 4.9 per cent of the amount fed in circulation.

Case 23. J. F. (Hospital No. 101086.) Age 24; para 3, gravida 4. Pregnancy, abnormal, multiple, with chronic nephritis and bilateral hydronephrosis. Gestation 4 months; therapeutic abortion. In her two previous pregnancies the patient developed nephritic toxemia with chronic nephritis. The first pregnancy was complicated by pre-eclampsia. During the present pregnancy, she developed headaches, nausea, vomiting, ankle edema, and albuminuria. Blood pressure was 125/90. Retrograde x-ray studies showed bilateral hydronephrosis. At the time of feeding, the red count was 4,430,000; hemoglobin 14 gm. per cent; hematocrit 43.7 per cent. She was given 122 mg. of radio iron and the pregnancy was interrupted 2 days later. Sampling revealed 4.2 per cent of the amount fed appearing in the circulation.

Case 24. E. L. (Hospital No. 171540.) Age 25 years; para 2, gravida 3. Pregnancy with nephritic toxemia, without convulsions. Gestation 4½ months; therapeutic abortion. 3 years previously she had had a pregnancy complicated by albuminuria. During this pregnancy she had headaches, ankle edema, blood pressure 185/120, and albuminuria amounting to 0.5 gm. per liter. At the time of feeding, blood studies showed 4,000,000 red cells, hemoglobin 14.1 gm. per cent, and hematocrit 42 per cent. She was given 62 mg. of radio iron. 2 days following, hysterotomy was performed. Sampling revealed 3.2 per cent of the amount fed in circulation.

Case 25. V. G. (Hospital No. 164558.) Age 41 years; para 7. Pregnancy with nephritic toxemia, hypertensive cardio-vascular disease, mild diabetes mellitus. Gestation 5 months; therapeutic abortion. The patient was relatively asymptomatic. There were no signs of decompensation. Blood pressure was 188/102. The urine showed 2+ albumin. The glomerular filtration was one-third normal. She was given 16 mg. of radio iron and 2 days later delivered by hysterotomy. 3.2 per cent of the dose fed was the amount estimated to be present in the total circulation.

Case 26. J. T. (Hospital No. 84390.) Age 42 years; para 10, gravida 11. Pregnancy, abnormal with toxemia, pre-eclamptic. Gestation 7 months; therapeutic abortion. She complained of ankle edema, headaches, nausea, and vomiting. The patient was markedly obese. Blood pressure was 230/130. Glomerular filtration 31 per cent. Albuminuria was marked. She was given 122 mg. of radio iron and vomited 1½ hours later. At the time of feeding, blood studies showed red blood count 3,600,000; hemoglobin 12.8 gm. per cent; hematocrit 37.2 per cent. One day later, labor was induced. Subsequent blood samples showed 2.5 per cent of the amount fed in the circulation.

Case 27. E. A. (Hospital No. 177796.) Age 24 years; para 1. Normal pregnancy, contracted pelvis. Gestation 8.5 months; cesarean section. Blood studies: red blood count 3,250,000; hemoglobin 12.5 gm. per cent; hematocrit 38 per cent. 3 hours before delivery she was given 1.9 mg. of radio iron; 16.4 per cent of the amount fed was estimated to be in the circulation.

Case 28. L. K. (Hospital No. 169989.) Age 21 years; primipara, full term, normal pregnancy. At the time of feeding, red blood count was 3,070,000; hemoglobin 9.8 gm. per cent; hematocrit 24.8 per cent. 63 mg. of radio iron was given and spontaneous delivery occurred 10 days later. The blood samples revealed 3.9 per cent of the amount fed in the circulation.

Case 29. E. S. (Hospital No. 173986.) Age 30 years; primipara, full term. Normal pregnancy. Blood studies: red blood count 4,100,000; hemoglobin 13.1 gm. per cent; hematocrit 31.7 per cent. 33 hours following feeding of 0.9 mg. of radio iron, the patient was delivered spontaneously. 3.3 per cent of this dose was the estimated amount found in the circulation.

Case 30. E. M. (Hospital No. 148022.) Age 32 years; para 2, term. Normal pregnancy. Blood studies: red blood count 4,000,000; hemoglobin 11.5 gm. per cent; hematocrit 37.5 per cent. She was given 5 mg. of radio iron, with delivery occurring spontaneously 15 minutes later. The maternal blood on subsequent sampling showed 2.9 per cent of the dose in circulation.

Case 31. E. W. (Hospital No. 145651.) Age 23 years; para 2, full term. Funnel pelvis, cesarean section. Blood studies: red blood count 4,100,000; hemoglobin 10.6 gm. per cent; hematocrit 34.2 per cent. She was given 93 mg. of radio iron and 20 hours later was delivered by cesarean section. 2.2 per cent of the amount fed was estimated to be in the circulating red cells.

TABLE 2

Radioactive Iron in Pregnancy

Case	Gestation	Weight	Estimated red cell mass	Hematocrit at feeding	Hematocrit at sampling	Interval between feeding and sampling	Dose iron	Per cent amount fed per 100 ml. red cells	Per cent amount fed circulating estimated
	mos.	*kg.*	*ml.*	*per cent*	*per cent*	*days*	*mg.*	*per cent*	*per cent*
21. M. C.	2	59.4	1490	43.3	41.7	150	16	0.18	2.7
22. P. F.	3	88.6	1820	37.5	34.3	10	53		4.9
23. J. F.	4	77.6	2070	43.7	44.3	200	122	0.20	4.2
24. E. L.	4.5	64.8	1440	42.0	37.0	17	62	0.26	3.2
25. V. G.	5	90.6	2100	38.6	38.5	150	16	0.15	3.2
26. J. T.*	7	100.4	2480	37.2	41.1	200	122	0.37±	2–5
27. E. A.	8.5	63.0	1490	38.0	39.5	45	1.9	1.1	16.4
28. L. K.	Term	74.8	1510	24.8	33.6	22	63	0.25	3.9
29. E. S.	"	73.6	1710	31.7	38.7	44	0.9	0.19	3.3
30. E. M.	"	62.0	1420	37.5	38.2	6	5.0	0.20	2.9
31. E. W.	"	68.2	1655	34.2	40.6	10	93	0.13	2.2
32. J. B.	"	57.6	1200	40.3	34.8	43	20	1.87	22.4
33. A. M.	"	84.4	2380	43.4	46.9	7	20	0.07	1.6
34. A. C.	"	65±	1125±	32.1	28.8	13	14	2.46	27.7

* Vomited 1.5 hours after feeding.

Case 32. J. B. (Hospital No. 178277.) Age 35 years, primpara, full term. Normal pregnancy. Blood studies: red blood count 4,150,000, hemoglobin 12.4 gm. per cent, hematocrit 40.3 per cent. She was given 20 mg. of radio iron and was delivered spontaneously 40 minutes later. Of this dose, 22.4 per cent was estimated to be in the circulation.

Case 33. A. M. (Hospital No. 166168.) Age 23 years, para 2, full term. Normal pregnancy. Breech delivery. Blood studies: red blood count 4,670,000; hemoglobin 13.5 per cent; hematocrit 43.4 per cent. 20 mg. of radio iron was given. 20 minutes later she was delivered spontaneously. 1.6 per cent of the dose fed was estimated to be in the circulation.

Case 34. A. C. (Hospital No. 180768.) Age 27 years; primipara, full term. Normal pregnancy with small pelvis; cesarean section. 110 minutes after feeding 14 mg. of radio iron, cesarean section was done. Of this dose, 27.7 per cent was estimated to be in circulation.

Table 2 and the related brief clinical histories indicate that as a rule the pregnant woman absorbs more radio iron than normal controls. Two exceptions are noted with normal iron absorption (cases 29 and 33) where we assume that iron stores were abundant in spite of the demands of the fetus. Three cases show maximal or high absorption (cases 27, 32, and 34) but the red cell hematocrits do not correspond as a moderate grade of anemia is present in case 34 but not in cases 27 and 32. We may say that most of these pregnant women show from 2 to 10 times the absorption of radio iron one might expect in a normal individual.

DISCUSSION

To evaluate the results summarized in Tables 1 and 2 it is necessary that several points be kept in mind. The unavoidable variation in dosage would be expected to result in different percentage utilization even in the same individual under the same conditions. This was pointed out by Whipple and Robscheit-Robbins (12) in studying the effect of feeding iron over periods of 2 weeks on the hemoglobin production of anemic dogs. They found that increasing the iron dosage 10 times (*i.e.* from 40 mg. per day to 400 mg. per day) resulted in the utilization of about twice as much of the metal. This meant a drop in percentage utilization from 30 to 35 per cent to 5 to 7 per cent. Employing the radioactive isotope of iron, Hahn, Ross, Bale, and Whipple (8) showed that the per cent utilization ranged from 60 per cent with a single dose of 1.2 mg. of radio iron to 3.2 per cent with a dose of 115 mg. when iron depleted anemic dogs were studied. Since the time of the last mentioned report considerably more data have accumulated and it is possible to employ this experience in the proper evaluation of the effect of single doses even though there is appreciable variation in the amount of iron used.

It is reasonably certain that the healthy adult woman must absorb more iron than the healthy male, as we cannot assume that she excretes less iron through the intestinal tract than the male and she must replace the menstrual hemoglobin loss which averages 6 to 9 gm. hemoglobin or 20 to 30 mg. Fe per month. Whether this excess iron intake could be demonstrated by careful study of groups of healthy males and females by use of radio iron remains for the future.

The *pregnant woman* at least in the late months of pregnancy does show *increased intake of radio iron,* although there are great variations and a few cases of normal iron intake (Table 2). This intake of iron bears no relation to the red cell hematocrit as we observe maximal absorption in cases with low, and again, with high hematocrit (cases 32 and 34, Table 2). We note a normal absorption with a low red cell hematocrit (case 29) and with a high red cell hematocrit (case 33).

Such evidence as we have in this laboratory, published and unpublished, indicates that the reserve *stores of iron are usually low* in the liver and presumably in other body stores (spleen and marrow) late in pregnancy. It is reasonable to suppose that the insistent demands of the growing fetus deplete the maternal stores even without any significant anemia. Such cases should absorb radio iron readily and almost certainly do so. On a different dietary régime the pregnant woman might keep her reserve stores at an adequate level and absorb only the normal amount of radio iron, that is approximately 1 per cent or less of the iron fed.

The evidence from this group of pregnancy cases is in harmony with other clinical evidence (Table 1) and experimental evidence in the dog—that the reserve stores of iron are of more importance than the hemoglobin level in determining the amount of iron absorption. The intestinal mucosa has the power of discrimination and can take iron or leave it, but the mechanism of this peculiar capacity is obscure.

SUMMARY

Radio iron is a tool which makes iron absorption studies quite accurate in dogs and reasonably satisfactory in human beings. This method is vastly superior to others previously used.

Normal human pregnancy without significant anemia may show active radio iron absorption—16 to 27 per cent of iron intake. The pregnant woman as a rule shows 2 to 10 times the normal absorption of radio iron.

Diseased states in which *iron stores* are known to be very *abundant*—pernicious anemia, hemochromatosis, familial icterus, and Mediterranean anemia—show very little absorption, probably less than normal. This is in spite of a severe anemia in all conditions except hemochromatosis.

Chronic infections in spite of anemia show no utilization of radio iron, whether it may be absorbed or not.

Leukemia shows little utilization of radio iron in red cells in spite of absorption (autopsy), probably because of white cells choking the red marrow.

Polycythemia shows very low values for iron absorption as do normal persons. Two pregnant women showed only normal iron absorption.

We believe that *reserve stores of iron* in the body, rather than anemia, control iron absorption. This control is exerted upon the gastro-intestinal mucosa which can refuse or accept iron under various conditions.

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