RADIOACTIVE IRON AND ITS METABOLISM IN ANEMIA*

Its Absorption, Transportation, and Utilization

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With the production of radioactive isotopes by the physicists and the concentration of naturally occurring isotopes by the chemists, the grateful physiologists have been presented with what may prove to be the "Rosetta Stone" for the understanding and study of body metabolism. The radioactive isotopes are "marked" elements which behave precisely like their inactive replicas in the physiology of the body but can be readily recognized as distinct entities wherever found. The radioactive isotope of iron used fortunately has a long life (half life 47 days) which covers ample time for prolonged study of iron metabolism and gives assurance to the student that this iron found in tissues, bones, or fluids is the iron introduced and not some other iron coming from body storage depots, hemolysis, or red cell wastage.

The literature on iron metabolism is enormous but the net results are disappointing to say the least. Every hypothetical possibility has been championed by students but very few points have been settled to the satisfaction of all workers. One can scarcely suggest a single possibility without finding that it has been more or less vigorously supported by physiologists or physicians in the past. We feel that radioactive iron will be a means of settling many of these disputes but it is impossible at this time to review all these interesting hypotheses.

The metabolism of iron in the body at first sight appears to be simple and capable of accurate study but this illusion is promptly

dispelled when the student deals with body secretions, tissues, and bones. Iron exists in many combinations and in many places in the body—red cell hemoglobin, muscle hemoglobin, liver, spleen, and marrow tissue, to name the most important. Moreover the picture is complicated further because in nearly all tissues iron may be present in two or more entirely different forms. In the spleen for example, there is parenchyma iron which is essential to the very life of the tissue; iron in the contained blood; and storage iron, a labile reserve which may be brought into play for any of the body needs. In fact the blood iron itself consists of several entities. It is quite apparent that analysis of iron by chemical methods alone could give but little information concerning the meaning of changes in total splenic iron.

The body can mobilize its iron under certain conditions. We have little accurate knowledge as to the length of its stay in various depots. Method difficulties are very real and the magnitude of method errors is not understood by many workers in this field. The presence of calcium and phosphorus introduces serious error in the iron analyses of the feces and the bone marrow, the very places where accurate analyses are imperative.

There are recognized fractions of iron (other than in hemoglobin combination) in the plasma and red cells but the quantities of iron are very small and their determination difficult due to the fact that traces of hemolysis may confuse the picture. It is very difficult to be sure in spite of every precaution that the blood has been drawn without causing a trace of hemolysis. Study of *iron transport* is obviously of major importance and it can scarcely be approached without the aid of radioactive iron.

Iron absorption has been assumed rather than demonstrated because of method difficulties. Some years ago we (6) brought evidence to show that in the iron depleted anemic dog the iron is whisked rapidly from the intestine through the liver and marrow into the red cells. The rapidity of this movement of iron surprised us but the evidence accumulating from the use of the radioactive iron indicates that the "turnover" of iron is even more rapid than was then suspected.

When radioactive iron is fed one can readily distinguish between the

newly acquired iron and that present in the body even though the inert iron exceeds the radioactive iron by many hundred-fold. This contrasts with iron metabolism studies using inert iron where in many cases significant results depend on small differences between analytical iron values not too certain in themselves.

The *excretion* of iron has been the subject of debate by physiologists for decades and until very recently no voice was raised to question the function of the large intestine which had been accepted as the site for iron elimination. Radioactive iron should enable the student to settle this dispute beyond any reasonable doubt. Furthermore the iron metabolism of the muscle hemoglobin has never even aroused speculation but this question now can be approached with hope of ultimate success.

Absorption of iron in disease is probably abnormal and these questions call for the use of radioactive iron in the clinic.

Methods

Radioactive iron is formed by the bombardment of the Fe^{58} iron isotope with deuterons, the reaction being $Fe^{58} + H^2$——$Fe^{59} + p$. It decays with the emission of free electrons or β-rays, Fe^{59}——$Co^{59} + e$. The half life of radioactive iron is 47 days. The amount of radio-iron present in a sample is determined by a measurement of its β-ray activity using a Geiger-Müller counter. The experimental methods for doing this are basically those described by Bale, Haven, and LeFevre (1)

Briefly the method is as follows: A 2 cc. aliquot of the solution on which an activity determination is to be made or the whole sample made up to 2 cc. is placed in a glass cup the inside diameter of which is about 1 mm. greater than the outside diameter of the counter chamber, which is mounted as an inverted plunger. A rack and pinion device (adapted from a colorimeter) brings the cup up around the counter end to a point where the liquid forms a ½ mm. thick film surrounding the sensitive area of the counter. Such geometric conditions allow many β-rays to enter the counter tube producing a high sensitivity. By using equal volumes of solution and by raising the rack and pinion to the same point on a scale, the same geometric conditions are obtained for each sample and the resulting counts per minute are proportional to the radioactivity of the sample.

A nearly saturated solution of potassium acetate is a convenient preparation of constant radioactivity. It is advisable to check the sensitivity of the counter at frequent intervals using this standard. Background counts (around three or four a minute for our counters) are determined on distilled water, or on acid blanks approximating the composition of the solvent for the sample being measured.

Two characteristics of radio-iron make its quantitative determination difficult. The preparations so far available have been extremely weak, compared for example with radioactive phosphorus or sodium preparations that are available. This means that in many aliquots on which activity determinations are to be made, activities are close to the minimum measurable, and experimental errors are likely to be large.

Also, the β-rays of radio-iron are of low energy and consequently easily absorbed (7). This makes necessary careful extractions of iron and in many cases use of relatively large amounts of tissue and its purification from foreign substances that would otherwise absorb the β-radiation and give too low apparent radioactive intensities. It is also necessary to make corrections in iron solutions that are not extremely dilute for its self-absorption of β-radiation. All of these factors tend to make the apparent recoveries of radio-iron in our biological experiments less than 100 per cent. This means that results in which recoveries are low should probably not, in general, be interpreted as showing a deposition of iron in unknown depots, but rather that our assay methods have still to be improved to be completely quantitative.

As has been stated above, it was found necessary to prepare the material for counting so that a relatively pure solution of iron salt was being dealt with. This minimized the absorption of β-rays by foreign materials. In the instance of plasma and red blood cell samples, as well as some of the viscera containing little calcium and phosphorus, the following procedure was employed:

The material in a conveniently sized Kjeldahl flask was wet ashed in a volume of concentrated sulfuric acid varying from 7 to 80 cc. depending upon the amount of organic material present. Perchloric acid was added as needed after charring occurred and in the event of excessive frothing caprylic alcohol was added. When the solution was clear and colorless it was cooled, diluted with an equal volume of distilled water, and cooled again. With phenol red as an indicator, the solution was brought to near neutral with 40 per cent sodium hydroxide and transferred to 100 ml. centrifuge tubes. Neutralization was completed and the precipitate centrifuged at about 2500 R.P.M. for 15 to 20 minutes. The supernatant was discarded and the precipitate dissolved in a little concentrated hydrochloric acid. If the resultant solution was not clear about $\frac{1}{2}$ to 1 gm. of ammonium chloride was added and after diluting with water and heating to dissolve the salt more indicator was added and the iron reprecipitated. After centrifuging as before, the solution was again dissolved in acid, and if clear, was made to 2 ml. and transferred to the cup for counting. If moderate amounts of calcium were present, 4 or 5 repetitions of ammonium chloride additions with subsequent precipitation often succeeded in getting rid of it. In the treatment of material high in calcium and phosphorus such as bone, food, or feces, the material was precipitated several times and the mixture of the hydroxide and extraneous salts was treated with an excess of concentrated hydrochloric acid. After evaporation to dryness, the material was transferred to a continuous extractor similar to that described by Griffith (3) by means of repeated washings with concentrated,

hydrochloric acid. The mass was extracted with ethyl ether until the acid phase showed a negative test for iron using thiocyanate. It was often necessary to add ammonium persulfate to take care of reduction occurring during extraction (presumably due to impurities in the ether) since ferrous iron is not readily extracted. The ether extract was finally washed into beakers with dilute acid and concentrated over a steam bath, and finally made up to 2 ml. and counted.

Some samples contained very small amounts of radioactive iron where the total count was very near the background and due to inefficiency of the counter tube employed, quantitative results were impossible. This was especially true if weak samples of the isotope had been fed. In such cases the self-absorptive factor as well as absorption by solvent could be partially avoided by resort to electroplating of the iron onto tin foil cylinders made to fit snugly around the counter tube. Such a procedure resulted in obtaining 6 to 8 times the number of counts as were obtained on the same material in solution. This method will be described fully in a forthcoming publication.

Routine care of these animals including methods of bleeding; determination of plasma and blood volume; and hemoglobin, as well as the composition of the diet low in iron, have been described in detail (6, 11). Blood volumes were done weekly by the brilliant vital red dye procedure. Hemoglobin was determined by the acid hematin procedure (13.8 gm. per 100 cc. taken as 100 per cent). Viviperfusion is also described (10) but in brief it consists of bleeding the dog under ether anesthesia at the same rate at which a modified Locke's solution is given intravenously. This leaves the tissues practically free of red cells excepting the spleen and bone marrow.

Values determined from single samples of plasma or red cells are expressed as total amounts in circulation by applying plasma and blood volume corrections respectively. Marrow samples were corrected roughly to indicate total body marrow tissue according to figures for marrow volume given by Fairman and Whipple (2).

EXPERIMENTAL OBSERVATIONS

Over a period of years investigations have been carried out in the Rochester laboratory relative to the absorption, storage, and utilization of iron in dogs which had been previously depleted of all reserve iron stores by continued anemia and subsistence on diets low in iron (6, 8). The limitations of these procedures have been outlined in detail (4). It seemed advisable to repeat some of the typical experiments using iron containing the radioactive isotope to test the accuracy of this method under actual laboratory conditions.

Dog H-9, a young female terrier, weighing 4.5 kilos (Table 1) was placed on a diet low in iron, consisting chiefly of white bread and canned salmon (6). It was

made anemic by bleeding and the anemia was maintained for 3 weeks. Radio-iron was fed (in the form of $Fe_2(SO_4)_3$) at a level of about 55 mg. daily (showing 192 counts per minute) for 4 days. At the time of the first feeding the blood hemoglobin level was 39 per cent (13.8 gm. hemoglobin per 100 cc. = 100 per cent). 20 hours after the last feeding the animal was subjected to viviperfusion to render the viscera blood free (10) and the perfusate was collected in several fractions. The final hematocrit at the end of this procedure was 0.4 per cent in contrast to 17 per cent at the beginning.

TABLE 1

Radioactive Iron Content of Tissues

Tissue or Organ Content = Per Cent of Total Amount Fed

	Anemic						Normal		
Dog No.	H-9	H-8	37-116	37-227	37-204	37-202	37-77	37-144	37-214
Number of feedings.	4	2	1	1	1	1	18	5	1
Blood volume.	330	350	500	370	440	770	480	630	700
Plasma volume.	260	260	390	250	330	620	240	360	400
Iron fed in mg.	220	66	130	84	300	115	650	103	60
Counts per minute as fed.	770	464	5,730	21,500	6,590	13,000	600	2,120	14,240
Hb. level per cent when fed.	39	62	53	68	61	56	178	138	114
Hours after last feeding.	20	20	23	4 | 75	11 | 26	6	84	23	7
Radio-iron found									
Liver.	0.4	0.4	0.5	— | —	— | —	—	0.2	0.03	0.02
Spleen.	0.0	0.0	0.1	— | —	— | —	—	0.0	0.02	0.01
Marrow.	0.2±	3.0±	2.0±	— | —	— | —	—	0.0	0.03±	—
Plasma.	0.0	0.3	0.1	0.7 | 0.1	0.8 | 0.2	3.5	0.0	0.01 ⎫	⎫ 0.05
Red cells.	8.7	9.0	1.4	0.9 | 4.6	0.0 | 0.4	0.2	0.04	0.06 ⎭	⎭
Total radio-iron.	9.3±	12.7±	4.1±	1.6 | 4.7	0.8 | 0.6	3.7	0.24	0.15±	0.08

Radioactivity measurements on the ashed viscera and blood show (see Table 1) that a total of 9.3 per cent of the amount fed was absorbed during the feeding periods. Most of this (8.7 per cent) was already in the red blood cells at the end of 5 days. The liver and bone marrow (estimated total) contain the remainder (0.6 per cent). The colon and feces accounted for about 63 per cent of the amount fed. The remainder (26 per cent) represents the inefficiency of recovery of material at this time.

Dog H-8, a female mongrel fox terrier puppy, aged about 6 months, and weighing 4.2 kilos, was made anemic by bleeding and placed on a white bread-salmon diet low in iron. The anemia was continued for 9 weeks to remove all iron reserves. Iron containing the radioactive isotope was fed with the diet in the form of $Fe_2(SO_4)_3$, on 2 successive days at a level of 33 mg. per day. The count of each dose was 232 per minute. 20 hours following the last feeding, viviperfusion was carried out.

Analysis of the viscera of this animal for radioactivity showed 0.4 per cent of the amount fed in the liver (Table 1), approximately 3 per cent in the marrow, and 9 per cent in the red blood cells. The plasma showed a count of 0.3 per cent corresponding to 0.2 mg. of iron. About 70 per cent was recovered from the colon and feces.

Dog 37-116, a female mongrel adult, weighing 6 kilos, was made anemic by bleeding and placed on the usual diet low in iron. The anemia was continued for 5 weeks to deplete the reserve. For a month preceding administration of radioactive iron there had been a leucocytosis which persisted to the date of perfusion. The animal was given a single dose of radio-iron as $Fe_2(SO_4)_3$ mixed with the diet, consisting of 130 mg. of iron which counted 5,730 per minute. The animal had to be coaxed to eat and spent one-half hour eating most of the food. The remainder was given by gavage. Blood samples were taken 3 and 5 hours after start of feeding and showed hematocrits of 20.9 and 19.8 per cent, respectively. Viviperfusion was carried out 23 hours following feeding (see Tables 1 and 2). The hematocrit at the beginning was 19.9 per cent and at the end 1.1 per cent.

At autopsy the organs appeared well perfused. Heart, lungs, kidneys, liver, and spleen appeared to be normal, in gross. There was gross and histological evidence of a moderate grade of chronic endometritis. The other viscera were ashed *in toto* for analysis.

The absorption of iron is much less in this experiment than in the two preceding—a total found of only 4 per cent. This is due to the feeding of a large single dose of iron which is known to lessen the percentage absorbed (optimum percentage absorption follows 20 to 40 mg. iron per day). Absorption of 30 per cent may be found in anemic dogs (8) with doses of 30 to 40 mg. iron daily, but absorption of only 5 per cent may be found with doses of 400 mg. iron. The red cells here contain nearly one-third of the absorbed iron even after 23 hours following rather reluctant and prolonged ingestion of the iron.

TABLE 2

Radioactive Iron as Found in Body

Figures in Table = Per Cent of Iron Given

Dog 37-116. Female adult mongrel, 6 kilos. Fed single dose of 130 mg. of radioactive iron (counting 5,730 per minute).

	Plasma*	Red cells*
Sample at 3 hrs.	1.06	0.23
Sample at 5 hrs.	1.36	0.24

†Fractions from viviperfusion 23 hrs. after feeding	Plasma total	Red cell ½ total	Red cell protein†	Red cell protein-free filtrate†
Fraction 1 (170 cc.)	0.063	0.305	0.36	0.013
Fraction 2 (220 cc.)	0.028	0.26	0.24	—
Fraction 3 (170 cc.)	0.016	0.08	0.10	0.005
Fraction 4 (175 cc.)	0.005	0.03		—
Total 735 cc.	0.11	0.68	0.70	0.02

Liver (153 gm.)....0.53%   Whole blood.......1.41%   Stomach and small
Spleen (16 gm.)....0.12%   intestine.........0.46%
Marrow...........2.0±%   Colon.............52.0%

* The 3 and 5 hour plasma samples were corrected to represent the iron in the total circulating plasma, and the red cell samples for total circulating cells, using plasma and blood volumes respectively as determined beforehand.

† Red cell fractions of the perfusate were divided into 2 equal parts. One-half was subjected to ashing and counting directly. The other was laked in each case and the proteins precipitated with trichloracetic acid and the precipitates and filtrates ashed separately.

Dog 37-227, an adult male poodle, weighing 4.5 kilos, was made anemic by bleeding and kept anemic for 5 weeks. During this time the diet was of hospital table scraps. The low iron diet was then begun and 4 days later a sample of iron containing the radioactive isotope was given in the form of $FeCl_3$. 84 mg. were given, which counted 21,500 per minute (see Chart A).

Samples were taken at periods after feeding of 1, 2, 4, 12, and 24 hours. In each case the plasma and cells were separated by centrifugalization. One-half of the red cells were laked in distilled water and run into 40 cc. of 10 per cent trichloracetic acid. The plasma samples were treated similarly. With frequent shakings, all samples were allowed to stand for 15 minutes. After filtration the precipitates and filtrates were each ashed and prepared for counting.

It was found that nearly 80 per cent of the iron in the plasma was either not in protein combination in so far as it appeared in the trichloracetic acid filtrates, or that the latter acid has split it from loose combination. The radio-iron of the red cells was almost entirely in the fraction precipitated by the trichloracetic acid, only traces occurring in the filtrates of the precipitated laked cells.
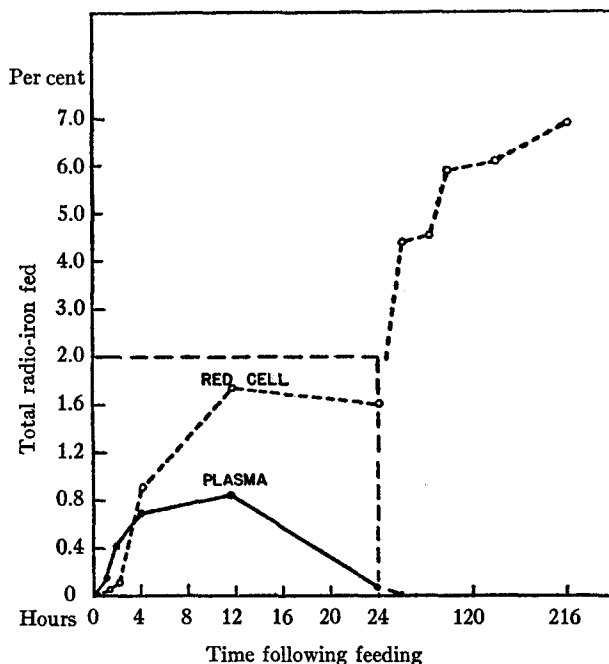

CHART A. Plasma and red blood cell radio-iron. Single feeding experiment. Dog 37-227.

The result can be seen in Chart A. This animal was studied for a long period and gradual depletion of the radio-iron in circulation was effected.

Dog 37-204, a male adult poodle, weighing 4.5 kilos, was made anemic and placed on a diet low in iron. It might be noted that this animal had practically no reserve iron stores. After an anemia period of 3 weeks, 300 mg. of iron containing the radioactive isotope was mixed in the diet in the form of $FeCl_3$. This amount counted 6,590 per minute. About 80 per cent of the diet was eaten immediately and the rest later in the day. A blood sample was taken 2 hours following feeding and others at 11 and 26 hours.

No radio-iron was detectable in the 2 hour samples of plasma or red cells of this animal. None was detectable in the 11 hour sample of red cells but the total blood plasma contained 0.8 per cent of the amount fed (Table 1). The 26 hour sample of plasma showed 0.2 per cent, while 0.4 per cent of the amount fed was present in the circulating red blood cells. 6 days after the feeding there was 3.8 per cent in the circulating red cells.

The feces collected during a 9 day period following the feeding showed 63 per cent of the amount of radio-iron fed. The low absorption of 3.8 per cent was probably referable to the high level at which the iron was fed (300 mg.).

Dog 37-202, an adult female mongrel spitz, weighing 10 kilos, was placed on a diet low in iron and made anemic by bleeding. After 4 months on this régime a sample of radioactive iron was given in a part of the diet, representing 115 mg. and counting 13,000 per minute. Samples were taken at 1, 4, 6, 8, 10, 12, 15, 19, and 24 hours and daily thereafter for several days.

Absorption in this animal as indicated by both the plasma iron determinations shortly after feeding and by the ultimate amount appearing in the red cells was quite low, amounting to only about 3.7 per cent of the iron fed. However it might be stated that the peak of absorption as shown by the plasma radio-iron occurred between the 5th and 7th hours reaching a value at the end of the 6th hour of about 4 times that in the preceding or following hour. Thus in Chart A the curve may not show accurately the changes in plasma radio-iron. More complete data are being compiled concerning this point and will be presented in detail. Practically all of the iron (95 to 98 per cent) in the plasma was in the filtrate following precipitation of the proteins with trichloracetic acid. A duplicate sample of plasma taken 12 hours after feeding was dialyzed in a cellophane membrane against running tap water for 24 hours. The resultant material was ashed and showed no activity.

Control Non-Anemic or Plethoric Dogs.—Dog 37-77, an adult female bull terrier, of 5 kilos weight, was placed on a diet of hospital table scraps, supplemented by 400 mg. of iron in the form of ferric citrate, daily for 12 days. In addition, a series of whole blood transfusions were given over a period of a week, during which 53 gm. of hemoglobin, equivalent to 178 mg. of iron, were injected

by vein. As the animal was normal at the start, it was felt that there was no doubt that the iron stores were abundant. Before the transfusions, the hematocrit was 48 per cent with hemoglobin level of 120 per cent. At the end of the whole blood administration the hematocrit was 63 per cent and the hemoglobin level was 178 per cent. One transfusion of whole blood (equivalent to 5.4 gm. hemoglobin) followed this determination.

Radio-iron, as recovered from other experiments, was fed nearly every day over a period of 5 weeks. The total iron given during this time approximated 650 mg. and counted about 600 per minute. 84 hours after the last feeding, the animal was perfused and the organs, all of which appeared normal in gross, were ashed for counting.

The colon contained 0.9 per cent of the total amount of iron fed, the remainder of the gastro-intestinal tract only 0.03 per cent. None was found in the vertebral bodies, plasma, or spleen. The liver contained 0.2 per cent and the combined red cells 0.04 per cent of the amount fed. It is apparent that very little iron was absorbed (see Table 1).

Dog 37-144, a female adult beagle mongrel, weighing 8 kilos, was given several transfusions of whole blood (21 gm. of hemoglobin equivalent to 70 mg. of iron). White bread-salmon diet, with a supplement of 400 mg. of iron daily was given for 5 weeks. During this time occasional injections of colloidal Fe(OH)$_3$ were given by vein, 64 mg. at a time. The total amount of colloidal Fe injected in this way was 394 mg. The animal originally had an hematocrit of 50 per cent and a hemoglobin level of 138 per cent, so the iron storage depots could be assumed to be full. Radio-iron (20 mg.) recovered from another experiment was fed with the diet and 2 days later 40 mg. of the same material were given. On each of the next 2 days, 21 mg. of a new sample were given in the diet. The total radio-iron intake was 103 mg. and counted 2,120 per minute. 23 hours later the animal was perfused.

The viscera of this animal appeared normal and were ashed for counting. The activity can be seen in Table 1. The stomach contained 0.11 per cent of the amount fed, the combined jejunum and ileum 0.13 per cent, and the colon, with contained feces, 82 per cent. It is to be noted that less than 0.2 per cent was absorbed as determined by analysis of blood and tissues.

Dog 37-214, an adult female mongrel spitz, weighed 7 kilos. This animal was vaccinated for distemper but did not receive the virus because of some coughing. There was no nasal secretion. A single transfusion of whole blood (8 gm. of hemoglobin equivalent to 27 mg. of iron) was given. Eight injections of 64 mg.

each of iron as colloidal Fe(OH)$_3$[1] were given at various times over a period of 10 weeks to insure excess iron storage. The initial hematocrit was 37 per cent with a hemoglobin level of 105 per cent. At the end of injections the hematocrit was 42 per cent and the hemoglobin 114 per cent.

A sample of radio-iron recovered from a previous experiment was fed, which contained about 60 mg. of iron and counted 14,240 per minute. Perfusion followed feeding by 7 hours.

The very small amounts of radioactive iron found in the viscera of this animal can be seen in Table 1. The marrow sample was lost. The whole gastro-intestinal tract contained 84 per cent of the amount fed.

It is important to compare this dog 37-214 with the anemic dog 37-202. Both dogs were given about the same amount of radioactive iron as measured by the counts per minute. The anemic dog received 115 mg. Fe in contrast to the control non-anemic dog which received 60 mg. Fe. This would favor more absorption (in per cent) in the control. Yet the anemic dog showed 3.7 per cent in the blood in contrast to 0.05 in the non-anemic dog—an impressive ratio of over 70 to 1 for the absorption in anemia.

DISCUSSION

The apparent ability of the dog to discriminate physiologically as concerns the amount of iron absorbed is of immediate interest (5). When there is a distinct need for the element (anemia) a fair quantity will pass from the gastro-intestinal tract into the blood stream. When the body reserves of iron are ample, very little is assimilated (Table 1). The mechanism for this reaction is not known. That it may be dependent upon a concentration gradient existing between the gastro-intestinal contents and the mesenteric blood is very doubtful since the iron in the blood (in a form other than hemoglobin) is present in very small amount in any case as compared with the amount in the gastro-intestinal tract itself.

It is interesting to speculate concerning these data however. If a normal animal absorbs iron only in proportion to the need and the anemic animal utilizes very efficiently (4, 6) what is absorbed then the

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[1] We are indebted to Dr. David Loeser of the Loeser Laboratories, New York, for the colloidal iron used in these experiments.

expected excretion requirement in either case would be very small. This is pretty much what is found by means of bile fistula dogs (to be published later). The same conclusion has been reached by Widdowson and McCance (12) who recently carried out a series of iron balance studies on human subjects. They found that iron injected in normal individuals did not result in an appreciable iron elimination and concluded that the excretory power of the gastro-intestinal tract was very limited. In a patient with an ileostomy stoma and isolated colon, Welch, Wakefield, and Adams (9) showed that feeding of large amounts of iron by mouth did not result in its excretion by the colon.

Some iron is excreted in the bile and under abnormal conditions (unpublished data) it may be increased several fold, but it does not account for the total iron excretion of the gastro-intestinal tract under normal conditions and probably for less than one-third of the total.

One may choose to believe that the *iron absorption* is largely a concern of the small intestine and furthermore that the *mucosa* is the tissue responsible for its acceptance or rejection. It may be possible to show that the epithelium of the mucosa is conditioned by the anemic state of the circulating blood so that absorption of iron takes place. At any rate the curve of iron absorption by the anemic dog indicates that the peak absorption (4 to 8 hours after feeding) takes place when the food materials are largely in the *small intestine*. At the end of 18 to 24 hours the radioactive iron is practically all in the colon and no significant absorption of iron is demonstrable. It would seem that the colon is not concerned with iron absorption.

That the plasma is the site of *transportation of iron* from the intestinal tract to the point or points at which it is further utilized is indicated by the above data (Tables 1 and 2). Further evidence along this line will be presented later but like many other materials the absorption, as indicated by plasma iron changes, increases shortly after feeding to a peak and drops off quite rapidly (Chart A). Absorption from a given dose of iron probably is complete in the dog at the end of 18 hours.

The rapidity with which the radio-iron appears in the red blood cell is worthy of comment at this time. If an anemic dog is fed neutral iron the earliest time in which it appears in the blood stream as shown by the red cell surge into the circulation, is about 3 to 5 days. How-

ever we see in dogs 37-227, 37-204, 37-116, and 37-202 (Tables 1 and 2) that the radio-iron has found its way into the red cells much sooner; in fact appreciable amounts are demonstrable in a few hours. Further studies of the transfer of iron to the red cells and the form in which it occurs there are being made.

As has been pointed out (6) the spleen and marrow do not lend themselves to complete removal of blood by viviperfusion. About one-third of the iron found on analysis of a perfused spleen from an anemic depleted dog is due to the presence of hemoglobin in the red cells contained in the sinusoids (6). The question might arise concerning the radio-iron found in the spleen of dog 37-116; whether it was present as true storage iron or represented radio-iron in the contained blood. Calculation shows that less than 10 per cent of the radioactive isotope present (0.12 per cent of the total amount fed) could be accounted for as hemoglobin iron. The remainder of the radioactive iron in the spleen presumably was in the reticulo-endothelial cells as a reserve store of readily mobilizable iron.

Discrepancies in iron balance studies in human subjects have sometimes been explained as due possibly to *adsorption* of iron to the mucosa of the gastro-intestinal tract leading to a false impression of positive balance. It is of interest to note that in both dogs 37-116 and 37-144 where viviperfusion followed the last feeding of radio-iron by 23 hours, practically all of the iron had been swept through the upper gastro-intestinal tract and had either been eliminated or was in the colon. The stomach and small intestine of 37-116 contained only 0.46 per cent of the total isotope fed whereas the colon contained 52 per cent. The upper gastro-intestinal tract of 37-144 contained 0.24 per cent of the amount of radio-iron fed while the colon and contained feces contained 82 per cent. The material fed in each case consisted of iron in the ferric form which would be expected to show adsorption (if present) to a greater extent than ferrous iron compounds.

SUMMARY

Artificially produced radioactive iron is an extremely sensitive agent for use in following iron in the course of its changes in body metabolism, lending itself to studies of absorption, transport, exchange, mobilization, and excretion.

The need of the body for iron in some manner determines the absorption of this element.   In the normal dog when there is no need for the element, it is absorbed in negligible amounts.   In the anemic animal iron is quite promptly assimilated.

The plasma is clearly the means of transport of iron from the gastro-intestinal tract to its point of mobilization for fabrication into hemoglobin.

The speed of absorption and transfer of iron to the red cell is spectacular.   The importance of the liver and bone marrow in iron metabolism is confirmed.

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