RADIOACTIVE IRON ABSORPTION BY GASTRO-INTESTINAL TRACT*‡

Influence of Anemia, Anoxia, and Antecedent Feeding Distribution in Growing Dogs

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These experiments aim toward a better understanding of the absorption of iron by the normal and anemic dog. The gastro-intestinal mucosa accepts iron readily when the iron reserve stores are depleted by chronic anemia but in a plethoric state there is very little absorption of iron. The body has no ready means of disposing of surplus iron—small amounts escape in the bile. The body protects itself normally against a large accumulation of iron within the body which may cause damage to important organs, *e.g.* hemochromatosis and Mediterranean anemia. The mechanism of this acceptance or refusal of iron is of great interest to physiologists and physicians. The experimental data below may contribute toward a better understanding of this basic property of the mucosa of the gastro-intestinal tract. We cannot believe it is a matter of membrane diffusion but rather a part of the cell metabolism probably involving cell proteins including ferments.

The term *physiological saturation* with iron as applied to mucosal epithelium may be useful and may not mean wide limits of iron concentration. Changes in the mucosal epithelium related to iron absorption are measured not in hours but in days.

The rather remote possibility also exists that excretions poured into the gastro-intestinal lumen, influenced by anemia may modify the absorption of iron.

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Methods

Unless otherwise noted the animals were fed a diet consisting of hospital table scraps. Where a diet low in iron content was desired it consisted mainly of canned salmon, white bread, dried skim milk powder, cod liver oil, and a salt mixture (10).

Hemoglobin was determined by a modification of the Newcomer method (15). Plasma volume was determined by the use of brilliant vital red dye (13) and red cell mass by the donor cell-isotope method (8). Procedures for the ashing of tissues, separation and electroplating of the iron have been taken up in preceding contributions (6, 7).

Preparation of iron for feeding was as follows: After removal of radioactive contaminants such as phosphorous, cobalt, manganese, etc., the radio-iron in hydrochloric acid solution was precipitated using sodium hydroxide, redissolved in hydrochloric acid, and made to a known volume, usually 100 cc. An aliquot (usually 2 cc.) of this material was diluted to 25 cc. and was reserved for total iron analysis and "piloting" the particular sample of the isotope. In this way a check on the purity of the radio-iron was had as well as a basis for calculating the amount of activity in any preparation fed. The remaining 98 cc. of ferric chloride was reprecipitated as hydroxide and the precipitate dissolved in citric acid with the aid of heat. Excess acid was neutralized by the addition of an excess of ammonium hydroxide and the material heated on a steam bath to remove excess ammonia. The ferric ammonium citrate was dissolved in water and made to a known volume. Aliquots of this solution were used for administration to the animals.

The calculation of the amount of radio-iron utilized by these dogs was made as follows: The concentration of the labelled iron in the red cells was determined at the time of feeding if the radio-iron had been fed at a previous date, in order to correct for residual radio-iron which had not been removed by bleeding. An estimate of the total mass of red cells in circulation was made. In some instances a direct determination of the red cell mass had been made at some previous time by the donor-isotope-red cell procedure (8). Where this had not been done the red cell mass was estimated indirectly from the plasma volume as determined by the brilliant vital red dye dilution method, as follows: Blood volume = plasma volume/100 − RBC hematocrit. RBC volume = blood volume − plasma volume. This value was multiplied by a factor of 0.75 as it has been shown that the red cell mass as determined by the indirect dye method is in error by about 25 per cent (8).

In either case the assumption was made that the red cell mass is a linear function of the venous hematocrit, this assumption being based on previously reported observations (5). On a chart in which the ordinates were represented by the jugular hematocrit values and the abscissae by red cell mass any determined values for these two components taken at the same time were plotted. The best straight line as determined by the method of least squares was then drawn. If only a single determination was available this point was connected with the origin. The product of the red cell mass and the red cell radio-iron concentration then yielded a value for total circulating radio-iron.

It has been shown that the red cell isotope concentration may rise rapidly following feeding of radio-iron only to fall again due to the dilution of the tagged red cells with

cells formed during recovery from the anemia from exogenous non-radioactive iron following complete utilization of the isotope (6). Thus the point at which complete utilization has occurred is difficult to establish accurately from the red cell concentration curve. The curve of isotope concentration in the whole blood is more uniform, however, rising to a maximum and thereafter remaining relatively constant. Therefore the latter curve was used to determine the *time* at which to determine complete utilization from the red cell isotope concentration. The corresponding value for the concentration of the radio-iron in the red cells at this time was then multiplied by the red cell mass corresponding to the jugular hematocrit and the resultant value represented the total radio-iron in circulation following feeding of the tagged iron. Subtracting the residual radio-iron present in the circulation at the time of feeding we arrive at a value for radio-iron gained in the circulation due to the feeding in question. Dividing by the amount of radio-iron fed yields the percentage utilization by red cells. The correctness of this procedure is demonstrated by the fact that the tagged iron, when fed to an anemic dog, after being completely utilized, stays in the red cells and the total circulating isotope as calculated remains constant over long periods of time despite large increases in cell mass (6).

In some experiments covering long periods and involving frequent sampling, it is advisable to correct for the radio-iron removed by bleeding. Total circulating isotope is estimated. To the latter figure is added the increments of all isotope removed previous to this time by sampling. This value then represents the total circulating isotope which would have existed had there been no sampling. It is divided by the red cell mass to give the value for corrected concentration of red cell isotope. The corrected concentration for whole blood isotope can be arrived at by multiplying the corrected red cell isotope value by the jugular hematocrit (6).

The Effect of Acute and Chronic Anemia on the Absorption of Radio-Iron

These experiments all point toward an understanding of gastro-intestinal absorption and its controlling factors. Some of the experiments are incomplete, and repeat experiments dealing with absorption from gastric, duodenal, or jejunal pouches are necessary. Because these necessary experiments cannot be done at this time it seemed best to put the experimental data on record, the work to be taken up again and expanded whenever occasion permits.

Table 1 shows clearly that an *acute anemia period of 24 hours does not bring about active absorption of radio-iron.* There may or may not be a slight increase above the usual iron absorption of the normal state. The change in the gastro-intestinal mucosa which permits active absorption of iron requires a certain amount of time, perhaps for mobilization or depletion of the *mucosa* iron, slight as the iron content of the mucosa may be. After the 24-hour acute anemia period in all dogs (Table 1) there is an increase in circulating red cells and a depletion of reserve iron stores. Seven days or more after the acute anemia the usual active absorption of iron in anemia is observed even with a rising hematocrit.

TABLE 1

Absorption of Radio-Iron—Acute and Chronic Anemia Compared

Dog	Weight	Condition	Hematocrit	Radio Fe fed	Iron absorption
	kg.		*per cent*	*mg.*	*per cent*
39-196	13.1	Normal	49.5	139	1.2
"	12.3	Acute anemia	25.4	139	2.3
"	13.5	Regeneration-depletion	38.9	102	10.4
41-310	11.5	Acute anemia	30.0	12	4.0
"	11.5	Regeneration-depletion	47.0	12	6.8
41-164	9.5	Acute anemia	28.0	13	2.4
"	9.5	Regeneration-depletion	37.5	13	9.9
"	10.0	Chronic anemia	23.5	14	4.4
"	10.5	" "	29.4	14.4	13.6
39-299	12.0	Normal	48.2	102	2.8
"		"			3.1

Dog 39-196, Table 1, was an adult mongrel beagle male weighing 13.0 kilos, with a normal blood picture (red cell hematocrit 49.5 per cent and hemoglobin of 17 gm. per cent). This animal was given by gavage a single dose of radio-iron consisting of 139 mg. of the metal in the form of ferric citrate. About 20 gm. of lean raw beef were fed immediately to prevent vomiting. Throughout the experimental period the dog received a kennel diet consisting of hospital table scraps. Seven days after the feeding the concentration of radio-iron in the red cells of the circulation indicated that 1.2 per cent of the administered dose was in circulation.

Acute anemia was induced 14 days after the first iron feeding by rigorous bleeding over a period of 6 hours, some of the plasma being returned. In this way about 55 per cent of the estimated blood volume was removed and the hematocrit was reduced from the pre-hemorrhage level of 45.0 per cent to 25.4 per cent 24 hours later. This 24-hour period allowed for blood volume adjustments. At the latter time the blood hemoglobin was 9.8 gm. per cent and this animal had been converted from a normal state to one of normochromic normocytic anemia. A second dose of 139 mg. of radio-iron as ferric citrate was then given by gavage, again followed by a little raw beef. Seven days later the estimated radio-iron in circulation amounted to 2.3 per cent of the administered dose (Table 1). It is obvious that the 24 hours of severe acute anemia had not changed significantly the absorption of radio-iron from the gastro-intestinal tract of this dog.

After another 7 days the hematocrit had risen to 38.9 per cent, the animal having drawn on its own reserve stores of iron to make new cells. A single dose of 102 mg. of radio-iron in the form of ferric ammonium citrate was given by gavage (Table 1). Another 7 days were allowed for complete utilization of the iron absorbed from this dose and at the end of that time the estimated amount of circulating radio-iron was 10.4 per cent of the dose fed. The hematocrit was then 43 per cent.

It must be obvious from these and other experiments in Table 1 that iron absorption is not wholly controlled by the condition of *acute anemia.* Chronic anemia with depletion of the reserve iron stores would seem to be an important factor.

Dog 41-310, Table 1, an adult mongrel male terrier weighing 11 kilos. At the start the hematocrit level was unusually high, being 61 per cent, with a hemoglobin level 29 gm. per cent. By the brilliant vital red dye method the plasma volume was found to be 480 cc. and a rough estimate of the blood volume (using the plasma volume and the venous plasmatocrit relationship) was 1200 cc.

Acute anemia was then induced. Over a period of 7 hours the animal was bled 660 cc. corresponding to more than 50 per cent of the blood volume. Twenty-two hours later the venous hematocrit was 29.9 per cent and the animal was in good clinical condition. At this time a single dose of 12 mg. of radio-iron in the form of ferric ammonium citrate was given by gavage. Blood samples were taken daily for 11 days and from the appearance of the radio-iron in the red cells it was estimated that about 4 per cent absorption had taken place. Two weeks after the iron feeding the hematocrit had risen to 47 per cent. At this time an identical dose of radio-iron (12 mg.) was administered as before and by appearance of the radio-iron in the red cells 10 days later it was found that 6.8 per cent absorption had occurred, account being taken of the labelled iron already in circulation at the time of this last feeding. The hematocrit had meanwhile risen to 55 per cent.

Dog 41-164, Table 1, an adult female English setter, weight 9.5 kilos. Initially the hematocrit was 49 per cent and the hemoglobin level was 18.5 gm. per cent. By the dye method the plasma volume was found to be 600 cc. and an estimate of the approximate blood volume was 1180 cc.

Acute anemia was then induced. About 400 cc. of blood was removed by venepuncture during a period of 5 hours. Twenty-three hours later the hematocrit was 28 per cent and the animal was in good clinical condition. A single dose of 13 mg. of radio-iron in the form of ferric ammonium citrate was given by gavage. Six days later the amount of absorption was estimated at 2.4 per cent on a basis of the red cell radio-iron level. The hematocrit had reached 32 per cent. The dog was allowed to regenerate on a kennel diet using its own iron reserve stores until 12 days after the first feeding when the hematocrit was 37.5 per cent. A dose of radio-iron (13 mg.) identical with the above was given in the same form and by the same route. Nine days later the red cell isotope showed an absorption of 9.9 per cent of the administered dose, the residual isotope concentration from the earlier experiment having been taken into account.

This dog, 41-164, (Table 1) was then bled systematically over a period of 3 months to remove all the circulating isotope and deplete the iron stores. In order to test the degree of absorption in this dog under standard conditions a single dose of 14 mg. of radio-iron as ferric ammonium citrate was given by gavage (hematocrit 23.5 per cent). Six days later it was estimated that only 4.4 per cent of the dose of radio-iron had been absorbed.

This response is so much out of line with many experiments in a considerable number of dogs that the possibility of vomiting and loss of radio-iron was considered. A repeat experiment was done after an interval of 11 days. A single bleeding had kept

the hematocrit within the anemia range, it being 29.4 per cent at the time of the last iron feeding. A single dose of 14.4 mg. of the radio-iron in the form of ferric ammonium citrate was given by gavage. Taking into consideration the amount of radio-iron in the circulation at the time of feeding the amount of the new dose absorbed and utilized as determined by the appearance of the isotope in the circulation 7 days later was 13.6 per cent of the amount fed.

Dog 39-299, Table 1, a normal adult female terrier, hematocrit 48.2 per cent and hemoglobin level of 20 gm. per cent, was given a single dose of 102 mg. of radio-iron in the form of ferric ammonium citrate by gavage. Seven days later by the concentration of labelled iron in the red cells and the estimated cell volume it appeared that 2.8 per cent of the dose was in circulation. Depleting the body radio-iron by bleeding and determination of the total radioactivity removed showed that actually 3.1 per cent of the ingested radio-iron had been absorbed.

The Effect of Anoxia on Iron Absorption

Table 2 supports Table 1. *Anoxia* due to breathing 50 per cent normal oxygen concentration should correspond to the type of acute anemia used in Table 1 at least insofar as oxygen transfer is concerned. *Anoxia* evidently is *not an important immediate factor* controlling the absorption of iron. If anoxia is a significant factor in iron absorption then we may say that the anoxia must last more than 48 hours. Such test experiments would be interesting.

We have the belief that an absorption of 1 to 5 per cent of even small single doses of radio-iron comes within the range of normal. Actual "plethora" may push absorption figures down to minimum levels even when using very small doses of iron (Table 2). When we see values of 8 per cent to 50 per cent absorption of radio-iron we are convinced that anemia or depletion of iron stores or both are responsible.

TABLE 2

Anoxia and Iron Absorption

Dog	Condition	Weight	Hematocrit	Time in low oxygen chamber		Radio-iron	
				Before feeding	After feeding	Fed	Absorbed
		kg.	*per cent*	*hrs.*	*hrs.*	*mg.*	*per cent*
39-320	Normal	8.5	49.7–53.3	48	22	102	3.7
40-133	Plethoric*	11.0	51.5–53.8	50	8	78	4.4
40-268	"*	11.0	53.7–55.0	48	18	80	1.1

* Plethoric here means a very high hematocrit with augmented iron reserves, attained by feeding of large amounts of iron over weeks or months, as well as parenteral administration of colloidal iron hydroxide and red cells, see clinical histories below.

Dog 39-320, Table 2, a young adult female fox terrier weighing 8.5 kilos. The red cell hematocrit was 49.7 per cent and the hemoglobin level in the blood 19.2 gm. per cent. To test anoxemia as a factor in the absorption of iron from the gastro-intestinal

tract, the animal was placed in a metal chamber in which the ratio of oxygen to nitrogen as supplied was approximately 1:10 instead of 1:5. This corresponds to an altitude of approximately 17,000 feet. This was accomplished by introducing equal parts of air and nitrogen at a total rate of 4 liters of mixed gases per minute. Flowmeters were used to control the inflow of each gas. When necessary, ice was applied to the exterior of the chamber to keep the inside temperature at a comfortable level, this being maintained at about 20–22°C. The dog remained in the chamber constantly for 48 hours except for two periods of a minute or so each when she was removed for blood sampling. The hematocrit rose to 53.3 per cent and the hemoglobin level to 20.8 gm. per cent at the end of the 2nd day. At this time a single dose of 102 mg. of radio-iron as ferric ammonium citrate was given by gavage and the dog returned to the chamber for another 22 hours. The diet during this whole period was raw lean beef. The dog appeared in good clinical condition at all times except for some slight cyanosis of the buccal membranes and tongue. She was apparently comfortable at all times although somewhat restless on occasions. Seven days after the feeding the hematocrit had dropped to 46.3 per cent. From red cell radio-iron concentrations and determination of red cell mass by subsequent donor cell determinations, it was estimated that 3.7 per cent of the administered dose was in circulation.

This experiment was duplicated in two other dogs in which the iron reserves were increased before the animals were placed in the low oxygen chamber. Dog 40-133 was a female adult beagle weighing 11 kilos. She was placed on 400 mg. of iron as ferric ammonium citrate daily by mouth and was given a total of 192 mg. of colloidal iron by vein during the 2 weeks preceding the experiment. Her hematocrit was 51.5 per cent and hemoglobin was 20.6 gm. per cent when she was placed in the chamber. During the 2 days of the experiment, the O_2 content of the chamber was 10 to 11 per cent. No untoward symptoms were noted clinically. On the 2nd day the dog was given by mouth 78 mg. of radio-active iron and returned to the chamber for an additional 8 hours. The hematocrit taken 12 hours later was 53.8 per cent. By bleeding the radioactive red cells from the circulation it was found that the absorption was 4.4 per cent of the dose fed.

Dog 40-268 was a young adult mongrel weighing 11 kilos. She was given daily feedings of 400 mg. of iron as ferric ammonium citrate. She was also given 480 mg. of colloidal iron by vein as well as four injections of red cells from donor dogs over a period of 3 months preceding the experiment. Hematocrit at the beginning of the experiment was 53.7 per cent and hemoglobin 21.8 gm. per cent. During the 2 days she was in the low oxygen chamber, the concentration of oxygen was 10 to 11 per cent. No symptoms or signs were noted. On the 2nd day the hematocrit was 55 per cent. She was given 80 mg. of radio-active iron with a little food and returned to the chamber where she remained for an additional 18 hours. By the bleeding out technique it was found that 1.1 per cent of the dose fed had been absorbed.

Table 3 gives evidence that preceding doses of iron may cause some "mucosa block." When radio-iron is given 1 to 2 hours *after* the "blockage dose" we see in most experiments less than the expected absorption of radio-iron by an anemic, iron-depleted dog. The pattern is not uniform and there are adequate reasons for irregularities in absorption of the second dose.

It may be argued that some areas of the gastro-intestinal mucosa are ade-

quately exposed to the iron solution and other areas of mucosa are slightly if at all in contact with the first iron dose. The second dose (radio-iron) might then contact fresh unexposed areas in duodenum or jejunum. Our understanding of the step-by-step movement of intestinal contents would suggest this possibility. Gastric peristalsis may be accelerated or slowed by various doses of iron, by emotion, by stimuli (stomach tube), or other factors and thus influence primary or secondary iron absorption in such experiments (Table 3).

The large dose of iron (400 mg. dog 41-310) probably stimulated gastric peristalsis, was passed rapidly out of the stomach leaving the gastric mucosa "unexposed" to iron and ready for rapid absorption. The radio-iron absorption (28.4 per cent) is close to optimum absorption (8) for this amount of iron (14 mg.).

The Effect of Colloidal Iron Given Prior to Radio-Iron

Colloidal iron given by vein before the feeding of radio-iron does not significantly modify iron absorption of the large dose (126 mg.) given (dog 39-196, last experiment Table 3).

TABLE 3

Gastro-Intestinal Mucosa Block: Radio-Iron Superimposed on Feeding of Ordinary Iron

Dog	Weight	Anemia hematocrit	Ordinary iron fed	Interval between feeding ordinary and radio-iron	Radio-iron fed	Radio-iron absorption
	kg.	*per cent*	*mg.*	*hrs.*	*mg.*	*per cent*
39-196	13.8	19.8	100	6	77	7.3
"	13.9	29.8	100	1.5	14	4.8
41-310	12.2	19.0	100	1.5	14	7.7
"	12.1	19.5	400	1.5	14	28.4
39-320	8.7	22.6	100	1.3	14	7.8
39-196	13.0	20	304*	—	126	8.4–7.3

* Colloidal Fe given by vein, see history.

We are indebted to Dr. David Loeser of the Loeser Laboratories, New York City, for this colloidal iron.

Dog 39-196 (Table 3) was bled systematically over a period of 10 weeks to remove from the circulation radio-iron previously accumulated from feeding (Table 1). At the end of this time the hematocrit was 20 per cent. To test such an iron-depleted animal, given adequate *storage iron* in the form of *colloidal ferric hydroxide* the dog was injected by vein with 60 mg. of iron daily, the iron being of the non-radioactive type. In 5 days he received 304 mg. of iron, an amount well in excess of the normal reserve stores. Three days after the last injection the hematocrit had risen to 32.4

per cent and at this time 126 mg. of radio-iron in the form of ferric ammonium citrate was given by gavage. By estimates of the circulating red cell mass and the concentration of radio-iron in the red cells it was found that 8.4 per cent of the administered dose was in circulation (last experiment Table 3). The animal was subjected to rigorous bleeding to deplete him of circulating radio-iron. By determination of the total labelled iron removed it was found that the total iron absorption had been 7.3 per cent. This figure is considered more accurate than the 8.4 per cent. This series of bleedings once more reduced the animal to an iron-depleted anemic state, the hematocrit finally being 19.8 per cent.

This anemic dog, 39-196 (Table 3) was now fed 100 mg. of ordinary iron as ferrous sulfate by mouth with a little canned salmon. Six hours after the feeding when the plasma iron level presumably would be back near normal, a single dose of 77 mg. of radio-iron in the form of ferric ammonium citrate was given by gavage. Seven days later it was estimated that 7.3 per cent of the administered dose was in the circulation. Obviously the preliminary iron feeding did not block significantly iron absorption of a dose given 6 hours later.

As another test concerning the possibility of a "mucosa block," this dog 39-196 (Table 3) was given a dose of 100 mg. of ordinary iron in the form of ferrous sulfate by gavage and 1½ hours later was given a single dose of 14.4 mg. radio-iron in the form of ferric ammonium citrate. At the time of feeding the red cell hematocrit was 29.8 per cent. A week later the concentration of radio-iron in the red cells showed that 4.8 per cent of the dose had been absorbed and utilized. At this time the hematocrit had risen to 33.6 per cent.

In another experiment (dog 41-310, Table 3) following depletion of the labelled red cells (Table 1) and return to the anemic state, there was an hematocrit of 19 per cent and hemoglobin level of 8.1 gm. per cent. The dog was given 100 mg. of ordinary iron in the form of ferrous sulfate by gavage. One hour and a half following this, a single dose of 14 mg. radio-iron was given in the form of ferric ammonium citrate by gavage. Six days later it was estimated from the red cell radio-iron level that 7.7 per cent of the administered dose had been absorbed. The hematocrit meanwhile had reached 24.5 per cent.

This dog (41-310, Table 3) was bled several times to continue the anemia and when the hematocrit value had reached 19.5 per cent the above experiment was repeated using a larger initial feeding of iron. In this case 400 mg. of ordinary iron as ferrous sulfate was given by gavage and an hour and a half later a dose of 14 mg. of radio-iron as ferric ammonium citrate was given by the same route. Seven days after the feeding the amount absorbed and utilized as determined by the appearance of the radio-iron in the red blood cells was found to be 28.4 per cent of the radio-iron fed, correction being made for the labelled iron already present in the circulation at the time of feeding.

Dog 39-320, Table 3, was also tested for "mucosa block." A single dose of 100 mg. of ordinary iron as ferrous sulfate was given by gavage. One hour and 25 minutes later a dose of 14.4 mg. of radio-iron was given in the same way. At this time the red cell hematocrit was 22.6 per cent and adequate bleeding had exhausted the reserve iron stores. A week later the hematocrit having risen to 30.2 per cent the amount of the radio-iron absorbed and utilized in red cells was found to be 7.8 per cent of the amount administered.

Plasma and Serum Iron

During the past decade there has been considerable interest attached to the iron content of plasma and serum, both in health and disease, as well as plasma iron changes resulting from the administration of iron by mouth. The subject has been reviewed by a number of investigators (12, 14, 17, 18). Changes in plasma iron as an index of absorption have been studied by these workers.

TABLE 4

Radio-Iron in Plasma Following Feeding

Dog	Iron dose	Iron salt	Time of peak	Peak level per cent amount fed per 100 cc. plasma	Radio-iron absorbed
	mg.		*hrs.*		*per cent*
1-J	64	Fe ammonium citrate	1.3	0.13	1.4
37-202	55	" " "	1.0	0.36	4.2
37-202	58	Fe citrate	2.0	0.70	12.2
1-G	36	Fe ammonium citrate	1.5	0.19	14.0
4-E	48	" " "	2.0	0.11	18.4
37-227	0.2	Fe citrate	1.0	1.7	24.0
37-202	115	Fe chloride*	6.0	0.56	3.2
38-137	42	Fe sulfate*	5.7	0.41	30.0

* Mixed with food.

There are four commonly employed methods which have been used in the study of the amounts of iron absorbed following oral administration.

1. Use of isolated loops and pouches where the iron remaining in the segment was washed out after definite periods of time and the difference between the washings and that originally instilled taken as a measure of the absorbed iron. This procedure has been criticized on the grounds that not inconsiderable amounts of iron are *adsorbed to the mucosa* under these conditions and such iron is therefore erroneously reported as absorbed.

2. Iron balance experiments in which food and medicinal iron intake is compared with the combined fecal and urinary iron excretion, the difference being taken as the amount absorbed. We have cited elsewhere (7) the chief objection to this procedure as being one of method difficulty. Many reports of markedly positive balances in normal individuals who were given small or massive doses of iron orally have been recorded in the literature but we feel that loss of iron in ashing excreta or lack of complete separation of iron combined with phosphates and other compounds must have been responsible for such results since they are not at all in harmony with more recent findings. The amount of iron in the food is quite small and furthermore only a small fraction of this is absorbed so that very small errors in analysis of dietary or excretory

iron may easily lead to a false impression of absorption when such has not occurred. Most investigators have concluded that balance studies of this metal must await further method improvements or give way to other procedures.

3. Increases in serum or plasma iron have been used by some investigators in an attempt to determine the degree of absorption of administered iron. We feel that no more quantitative measure of the absorption of iron may be obtained by this method than could be done for the absorption of glucose from the shape of a sugar tolerance curve. Variation in the *rate of absorption* as well as *rate of removal* of the iron from the serum would undoubtedly play an important part in determining the character of such curves.

4. The use of the artificial radioactive isotope of iron has presented a satisfactory means of studying iron absorption in spite of the fact that it requires special equipment and that the supply of the isotope is at present time rather limited. Appearance of the radio-iron in the red blood cells affords an index of absorption which is accurate provided we assume that all of the absorbed iron is built into the hemoglobin of the red cells and not stored elsewhere in the body. That this assumption is justifiable under the conditions of an iron deficiency anemia has been shown (1, 6) and, when guarded interpretations are made, this method may be used to indicate the absorption in normal subjects (1). It is of course obvious that an estimate or measurement of the red cell circulating mass must be involved in the calculation of iron absorbed by this method.

Using the radioactive isotope of iron it is possible to distinguish between the level of iron normally present in the plasma and that increment recently introduced by feeding the radio-iron. When frequent (30-minute) samples of the plasma are taken following feeding of single doses of radio-iron, we find that there is a great lack of uniformity in the type of curve obtained (Table 4). The level of the plasma radio-iron at the peak of the curve does not seem to be related in any way to the size of the dose or the amount absorbed as subsequently determined by appearance of the radio-iron in the red cells. Sometimes we have obtained a plasma curve in which a definite plateau has been apparent whereas at other times in the same animal there is a sharp peak in the curve representing some point of maximum absorption rate or decreased removal rate from the circulation or both. Furthermore we have noted that when the iron is administered by gavage on an empty stomach there is likely to be a peak of some sort during the first 2 hours (Table 4). However when the iron is mixed with the diet there is a delayed rise in the plasma radio-iron curve and the peak is reached only after 5 or 6 hours (Table 4). This makes it clear that there must be many variables operating to modify plasma iron curves and therefore introduce error into quantitative absorption determinations calculated from such curves.

The Absorption of Radio-Active Iron from Gastric, Duodenal, and Jejunal Pouches

Iron absorption is active in all these areas. There is need of more experiments to determine the effect of duration of *mucosal exposure* to the iron, influence of size of dose, and effect of therapy or clinical abnormalities on absorption. This is a fertile field for subsequent work.

The *gastric pouch* experiment was very satisfactory and the amount of radio-iron absorption was quite high. The plasma iron curve was determined and showed a sharp peak during the 2nd hour as is the rule for iron given to a standard anemic dog with empty gastro-intestinal tract. When 188 mg. radio-iron was given the determined absorption was 10.4 per cent. The long continued contact with the gastric mucosa is probably responsible for this high degree of absorption but it is noteworthy that the plasma iron curve was falling *before* the radio-iron was washed out of the gastric pouch. Perhaps this means a temporary block of iron absorption due to physiological saturation of the mucosa by iron. Further study of this factor is needed.

The *duodenal and jejunal pouches* (dog 38-179) gave satisfactory but not quantitative evidence of iron absorption. The *duodenal* pouch in which 38 mg. radio-iron was placed, in spite of considerable leakage showed 4.8 per cent iron absorption. The *jejunal pouch* in which was placed 6.4. mg. radio-iron in spite of leakage showed 14 per cent iron absorption.

A *jejunal fistula* showed that the lower part of the small intestine absorbed 6.5 per cent of a 14 mg. dose of radio-iron in contrast to the same dose given by mouth whereupon there followed a 22.7 per cent absorption of the ingested iron.

It is to be admitted that these pouches are in some sense abnormal but they are much used in physiological experiments. Under these conditions in the anemic depleted dog there is active iron absorption in these areas of isolated mucous membrane.

Gastric Absorption of Iron.—Dog 39-198, an adult mongrel beagle hound weighing 12 kilos. To test iron absorption by the stomach alone, this dog which had been depleted previously of its iron stores by repeated hemorrhage was prepared for a gastric pouch operation.[1] The complete stomach was isolated, the cardiac end being inverted and closed by suture. The pylorus was brought out through an abdominal stab wound, the serosa sutured to the belly wall, and its opening lightly sutured. The duodenum was anastomosed to the esophagus. The resulting pouch was drained daily of accumulated gastric juice (30 to 190 cc.). Alumina gel was instilled as well as smeared about the orifice frequently during a period of 8 days. The animal was given lean raw beef divided into small individual feedings spread over the day. Once each day 200 to 300 cc. of 5 per cent glucose in normal saline were administered by vein to guard against the excessive loss of chlorides. Radio-iron was instilled in the pouch on the 8th postoperative day and at this time the plasma chlorides were at a normal level (0.114 mols per liter).

Radio-iron, 188 mg., in the form of ferric ammonium citrate was instilled in the gastric pouch and allowed to remain for 2 hours. At this time the red cell hematocrit was 21.0 per cent and the hemoglobin level 7.3 gm. per cent. The animal was sampled at intervals while the isotope was in the pouch and after it was washed out and the

[1] We are indebted to Dr. E. B. Mahoney for this operation.

appearance of the radio-iron in the plasma was determined. The peak of the plasma iron level was very sharp at 1.5 hours after iron instillation, *i.e. before the removal* of the tagged iron from the stomach. Seven days after the instillation by estimation of the red cell mass and the concentration of the radio-iron in the red cells it was found that about 10.4 *per cent of the radio-iron had been absorbed* and utilized. This is as much and possibly more than the expected absorption of radio-iron in the same animal under these conditions of iron depletion and anemia even with an intact gastro-intestinal tract.

Autopsy, dog 39-198. In general the autopsy findings 3 months later are not related to this experiment. The marrow showed normal hyperplasia. Spleen and kidneys normal. Liver some fatty change. *Gastric mucosa* was in all respects normal. There were only slight cell accumulations in the muscle coats about the old sutures.

Duodenal and Jejunal Absorption of Iron.—Dog 38-179 was an adult female mongrel shepherd weighing 18.0 kilos. A Thierry-Vella (jejunal) fistula and later a duodenal pouch had been produced in this dog. Over a period of 7 weeks the reserve iron stores were depleted by repeated bleeding and the hematocrit at the end of this time was 22.8 per cent with a hemoglobin level of 8.3 gm. per cent. The diet was changed from one of hospital table scraps to one low in iron, consisting chiefly of white bread, salmon, and dried skim milk (10).

Some *radio-iron* was introduced into the *duodenal pouch* but the abdominal movements accompanying respiration caused a large part of the material to be expressed. The instilled material contained 38 mg. of iron and later 4.8 per cent was found in the circulating red cells. However in view of the leakage of the radio-iron solution mentioned above this was considered to be only a *qualitatively positive absorption* experiment.

After depletion of the circulating radio-iron by repeated bleedings an attempt was made to instill a small dose of 6.4 mg. of the radio-iron in the form of ferric citrate *in the jejunal loop.* Peristaltic action as well as respiratory movements again resulted in the loss of a considerable fraction of the material from the loop to the outside. Approximately 14 per cent of the instilled dose appeared in the circulation by the end of 7 days. Again the results must be looked upon as a *qualitatively positive absorption* experiment.

As a type of control the remainder of the gastro-intestinal tract of this animal (38-179) was tested. After most of the residual isotope in circulation from the preceding experiments had been removed by bleeding, the hematocrit was 23.2 per cent and the hemoglobin level 7.6 gm. per cent. A single dose of 55 mg. of radio-iron in the form of ferric ammonium citrate *was fed with the diet* and a week later 31 *per cent of this dose* was estimated to be in the circulating red cells. Since this seemed an unusually active absorption of a dose of this size, the experiment was repeated.

After most of the circulating radio-iron containing red cells had been removed by hemorrhage, the hematocrit was 22.7 per cent and the hemoglobin level 7.6 gm. per cent. A single dose of 98 mg. of radio-iron in the form of ferric ammonium citrate *was fed with the diet.* Allowing for the residual isotope concentration of the red cells at the beginning of the experiment *36 per cent of the fed dose* was found in the circulation 7 days later. By repeated bleeding and determination of the radio-iron removed

it was found that the *actual absorption was 30 per cent*, indicating that no absorption without utilization had occurred but that the estimate of the red cell mass had been too high, having been done by the dye procedure (13). This gastro-intestinal absorption (exclusive of the loops) was unexpectedly high but we may say that absorption was perhaps changed by operative procedures which may have modified peristalsis and retarded the flow of material through the stomach and intestine. The dog's capacity to *form* new hemoglobin was that of the standard anemic animal.

Inasmuch as the absorption of this animal seemed quite high in single dose experiments, calculation of the *hemoglobin production* was made during the above periods and studies of the pigment production made under specified conditions (16) by methods used in this and other laboratories. On the diet of salmon and white bread which is low in iron content (10) it was found that there were about 18 gm. of hemoglobin produced per week. When the diet was changed to one of hospital table scraps, the production rose to about 75 gm. per week. Reverting to the low iron diet and re-establishing the basal production level, 400 mg. of non-radioactive iron daily were fed with the diet for a 2 week period and from the extra production during that time and an after-period it was found that there was about 5 per cent of the extra iron utilized for hemoglobin production. This is approximately what would be expected as the reaction to such a feeding as determined in standard anemia studies (16).

Jejunal and Ileal Absorption of Iron.—Dog 1-J was an adult mongrel bull terrier weighing 16 kilos. An operation was done to establish a *jejunal fistula*[2] for use in studies of absorption. The jejunum was cut about 25 cm. below the ligament of Treitz and the lower portion was brought to the surface, the serosa being sutured to the abdominal wall. The upper cut end of the jejunum was joined to the lower by an end to side anastomosis. This resulted in peristalsis of each segment being maintained toward the ileum. The animal was bled repeatedly over a period of 3 months to exhaust the reserve iron stores and was then placed on a diet low in iron, the bleeding being continued for a month until a hematocrit of 18 per cent was reached. By this time the fistula opening was scarcely larger than 1 mm. in diameter.

A single dose of 14 mg. of radio-iron in the form of ferric ammonium citrate was instilled into the jejunal fistula and did not leak appreciably. A week later the concentration of isotope in the circulating red cells indicated an absorption of 6.5 per cent of the instilled dose. The hematocrit had risen to 26 per cent.

The animal was subjected to three bleedings totaling 700 cc. and this reduced the hematocrit to 22.7 per cent. Another single dose of radio-iron of the same size and in the same form was *administered by gavage.* Taking into account the isotope circulating at the time of feeding the fraction of the dose administered absorbed and utilized as determined by the appearance of the radio-iron in the circulating red cells was 22.8 per cent. This is in keeping with the expected response to a feeding of this amount of iron to an intact dog (7, 9). One can speculate as to how much of this 22.8 per cent absorption took place in the stomach but at least there is clear indication that the lower jejunum and possibly the ileum can absorb iron.

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[2] We are indebted to Dr. E. S. Nasset for this operation on 38-179 and 1-J.

Distribution of Radio-Iron in the Tissues of Pups

Distribution of radio-iron in the tissues of growing pups following its absorption from the gastro-intestinal tract is of interest to all students of iron metabolism. The experiments shown in Tables 5 and 6 are only the first of what should be a large series. We hope to continue these studies as time and material permit. The two pups were growing rapidly and had a normal blood picture. The radio-iron gave high counts so that the determinations of radio-iron in the tissues are accurate and suitable for careful comparison. No total iron analyses were done but the average values for normal dogs are given and in general the radio-iron runs 1 to 3 per cent of the expected organ tissue iron.

The presence of a considerable amount of radio-iron in the *spleen* is of much interest. One can hardly explain this radio-iron as coming from destroyed red cells rich in radio-iron as only 39 days elapsed between the start of the iron feeding and death. The normal life cycle for the red cell in the dog is not less than 100 days (11). Evidently the spleen acts as a depot for iron coming in through the gastro-intestinal tract on its way to general body use (new organ cells, new hemoglobin in red cells).

TABLE 5

Recovery of Fed Radio-Iron

Percentage of Fed Iron in Blood and Tissues

	Pup 1-F	Pup 2-F
Blood and perfusate	6.8	6.9
Viscera	1.3	1.1
Striated muscles	1.2	0.9
Bone marrow	19±	29±
Total recovered	28±	38±
Weight at start, *kg.*	4.3	3.4
" " end, *kg.*	6.6	6.1
Hematocrit at end, *per cent*	38.3	36.1
Hemoglobin level at end, *gm. per cent*	15.1	14.8

The *gastro-intestinal tract* shows an interesting *gradient*, the high values for the duodenum being particularly significant. Perhaps the Brunner's glands of this region are responsible for higher iron concentration in duodenum as compared with the jejunum and stomach (compare with the high values for the pancreas). Values for ileum and colon are relatively low. The normal dog shows no significant gradient and in general the iron content of the gastro-intestinal tract of the normal adult dog is 1 to 2 mg. per cent. Whether one

chooses to explain the gradient on the basis of a more active and bulkier mucosa or due to more absorption or excretion of iron in the upper gastro-intestinal tract may be a matter of debate.

Voluntary muscle is quite uniform in its radio-iron content. Attempts to immobilize a fore leg caused no difference in radio-iron deposit. Cardiac muscle contains about 3 times as much of this iron as do voluntary muscles. It is to be recalled that in dogs of this type the muscle hemoglobin of heart and voluntary muscle run about 200 to 300 mg. per cent, the heart being a little ahead of the voluntary muscle at this age (19). In the adult the muscle hemoglobin contains about one-half of the cell iron. Evidently the demand for iron of this growing heart at this stage was more intense than that of the voluntary muscles.

Pancreas tissue in normal dogs shows the base line for tissue iron—frequently 1 mg. per cent, yet in these growing pups it competes with the heart and is one-half to one-third as active as the liver in storing radioactive iron. This may mean very active growth of these cells or perhaps accumulation of enzymes.

Bone marrow shows surprisingly high values which mean among other things that the demand for new red cells during growth is not as acute as in anemia and the radio-iron piles up in reserve. This deserves further study.

TABLE 6

Distribution of Radio-Iron in Perfused Tissues of Growing Pups

Tissue	Fed radio-iron—mg. per 100 gm. fresh tissue		Total iron in normal adult dogs mg. per 100 gm. tissue
	Pup 1-F	Pup 2-F	Limits*
Liver	0.202	0.183	15–30
Spleen	0.430	0.397	20–50
Pancreas	0.056	0.079	1–2
Heart	0.098	0.088	3–5
Diaphragm	—	0.032	
Foreleg	0.033	0.028	
Left hind leg (bound)	0.034	0.027	3–4
Right hind leg	0.028	0.026	
Stomach	0.020	0.020	1–2
Duodenum	0.031	0.028	
Jejunum	0.023	0.017	1–2
Ileum	0.016	0.016	
Colon	0.017	—	1–2

* These values taken from perfused tissues of normal dogs, Bogniard and Whipple (2).

Dogs 1-F and 2-F were litter mates, 12 weeks old mongrel collie pups. They were both active and healthy. They show steady weight gain (Table 5). The left hind leg of each was strapped with adhesive in an attempt to minimize movement in that limb during the course of the growth experiment. The diet was of the white bread-

salmon variety (10) low in iron. The *radio-iron* was administered mixed with the food at a level of *7 mg.* per dose on *nine occasions* (total 63 mg. radio-iron) during the course of 27 days. At this low dosage of iron it was expected that maximal absorption of the metal would take place. Twelve days after the last feeding of the isotope the animals were both perfused under ether as described elsewhere (10) to determine how much radio-iron had been absorbed and deposited in the body cells, including the erythrocytes.

The following assumptions were made in determining the total activity in the marrow of these animals. (*a*) That rib samples taken were representative of the bones with reference to the marrow content. (*b*) That 36 per cent of the bone mass is represented by marrow (3). (*c*) That the mass of marrow in the body is about two-thirds of the weight of the liver (10). Thus in dog 1-F the calculations were as follows:—

Counts per gm. of whole rib = 388

$\frac{388}{0.36}$ = counts per min. per gm. marrow = 1075

Marrow mass = ⅔ liver mass = 319 × ⅔ = 210 gm.

Activity in marrow = 210 × 1075 = 226,000 counts per min.

$$\text{Per cent amount fed in marrow} = \frac{226{,}000}{1{,}180{,}000\ \text{(counts per min. fed)}} = 19 \text{ per cent}$$

In Table 6 is shown the distribution of the radio-iron in the other tissues. Unlike the experiments we have reported in which the isotope was fed to iron-depleted, anemic dogs (7) a considerable fraction of the absorbed iron is found in tissues other than the circulating red cells.

DISCUSSION

Considerable variation in percentage absorption of *single doses of radio-iron* is illustrated in the tables and experiments above. When iron is fed during a 2-weeks period and the hemoglobin production of the anemic dog measured, the absorption as determined by the new formed hemoglobin is more constant. To explain these facts we may suggest that the absorption of iron even in an anemic dog with an empty gastro-intestinal tract is never 100 per cent—rather 50 per cent as maximal for small doses and 5 per cent for large doses (400 mg.). Iron absorption takes place in stomach and small intestine—perhaps more in the stomach and duodenum. Contact duration of the solution of iron with the mucosa is important and here come in many variables to make for lack of uniformity of absorption—time of stay in stomach due to mechanical irritation or emotion may vary greatly—speed of passage through duodenum and jejunum certainly must vary widely due to many things beyond our control. These day to day variables largely vanish when the experiment is carried through the standard 2 weeks of anemia.

The main point brought out by the experiments (Table 1) in which the otherwise normal animals were rendered acutely anemic would seem to be that

the condition of anemia *per se* is not the factor controlling the acceptance or refusal of iron from the gastro-intestinal tract. The spontaneous partial recovery of the circulating hemoglobin presumably by withdrawal of iron from the reserve depots resulted in the absorption of iron several times that which occurred in the animal when normal or when in a state of acute normochromic normocytic anemia of 24-hour duration due to hemorrhage. Most stores of iron appear to be concentrated in the liver, spleen, and to some extent in the bone marrow. It would tax one's credulity to suppose that depletion at these remote points could *directly* affect the condition of the mucosa of the alimentary tract. It has been pointed out that most tissues contain, over and above the indispensable iron, some iron which can be depleted by long continued anemia (2, 10). This general depletion of iron involves the gastro-intestinal tract and presumably is a factor in absorption of the metal.

The possibility of a *plasma iron concentration* as the controlling factor in absorption has been advocated by some investigators. It is found that individuals with iron deficiency diseases have a total plasma iron level definitely below normal, whereas in the anemia conditions in which there is no iron deficiency such as uncomplicated pernicious anemia or hemolytic anemias the serum iron is elevated. Furthermore after administration of specific liver therapy in pernicious anemia the plasma iron returns to normal levels.

Assuming the true facts of plasma iron concentration to be given as above, the method by which low plasma iron operates to promote iron absorption is not apparent. If the magnitude of the concentration gradient between the intestinal lumen and blood plasma were of primary importance in promoting iron absorption it might be expected that the larger doses of iron, with the resultant establishment of a greater differential between the iron content of the lumen and plasma, would favor the more efficient absorption of the metal.

Such is not the case since the smaller doses of iron are more *efficiently* absorbed and as the dosage is increased the total percentage amount of iron absorbed does not increase proportionately (9, 16). This plasma gradient may have an influence on the reserve stores of iron of the mucosa and therefore an indirect effect on iron absorption just as is true for the general reserve stores of the liver, spleen, and bone marrow.

Further speculation may be permitted. We recognize a *mobile* hemoglobin in the red cells concerned with the transfer of oxygen, and a fixed hemoglobin (myohemoglobin) which we assume has to do with oxygen uptake in the muscles. Let us postulate a compound in the mucosa which is capable of combining lightly and reversibly with iron. Such an acceptor in the mucosa cells might be a protein, or more specifically a material such as ferritin or apoferritin (4) which latter is capable of stoichiometrically taking up iron. This acceptor would be capable of taking up limited amounts of iron from the intestinal lumen and in turn passing it on to the plasma when the iron level there was lowered. Such a mechanism would explain in part the limited ability

of the body to accept iron and the relatively greater efficiency of absorption of small doses of iron. In the normal animal with a normal plasma iron, the acceptor mechanism would be physiologically saturated with iron and incapable of picking up more from the gastro-intestinal tract.

The question might be raised why the reverse action of transfer of iron from plasma to the acceptor to the lumen of the gut might not act in an excretory capacity in instances in which the plasma iron is elevated (pernicious anemia, familial hemolytic icterus, etc.) and result in the production of a complicating iron deficiency anemia over protracted periods. This might be explained by the suggestion that iron in transport in the plasma is combined with protein (probably a globulin) which form of combination is not sufficiently loose to allow transfer of the iron in the reverse direction to the acceptor. Thus this acceptor together with the action of the plasma might act as a valve mechanism allowing the body to obtain iron when needed and conserve what it has for future needs. In lieu of a demonstration of such a complex mechanism in the mucosa epithelium we can only say that it offers a reasonable hypothesis for the experimental facts of iron metabolism as we know them today.

The experiments (Table 3) in which the non-radioactive colloidal iron was administered intravenously before feeding of the radio-iron show that iron stores of this character are not a determining factor. Iron given in this form is taken out of the circulation by the reticulo-endothelial system and probably under such circumstances does not involve the concentration of iron in the mucosal epithelium. This colloidal iron when needed can contribute to the building of new hemoglobin in anemia (16).

SUMMARY

Iron absorption is a function of the gastro-intestinal mucosal epithelium. The normal non-anemic dog absorbs little iron but chronic anemia due to blood loss brings about considerable absorption—perhaps 5 to 15 times normal. In general the same differences are observed in man (1).

Sudden change from normal to severe anemia within 24 hours does not significantly increase iron absorption. As the days pass new hemoglobin is formed. The body *iron stores are depleted* and within 7 days iron absorption is active, even when the red cell hematocrit is rising.

Anoxemia of 50 per cent normal oxygen concentration for 48 hours does not significantly enhance iron absorption. In this respect it resembles acute anemia.

Ordinary doses of iron given 1 to 6 hours *before radio-iron* will cause some "*mucosa block*"—that is an intake of radio-iron less than anticipated. Many variables which modify peristalsis come into this reaction. Iron given by vein some days before the dose of radio-iron does not appear to inhibit iron absorption.

Plasma radio-iron absorption curves vary greatly. The curves may show

sharp peaks in 1 to 2 hours when the iron is given in an empty stomach but after 6 hours when the radio-iron is given with food. Duration time of curves also varies widely, the plasma iron returning to normal in 6 to 12 hours.

Gastric, duodenal, or jejunal pouches all show very active absorption of iron. The plasma concentration peak may reach a maximum *before* the solution of iron is removed from the gastric pouch—another example of "mucosa block."

Absorption and *distribution* of radio-iron in the body of *growing pups* give very suggestive experimental data. The spleen, heart, upper gastro-intestinal tract, marrow, and pancreas show more radio-iron than was expected.

The term "physiological saturation" with iron may be applied to the gastro-intestinal mucosal epithelium and explain one phase of acceptance or refusal of ingested iron. *Desaturation* is a matter of days not hours, whereas *saturation* may take place within 1 to 2 hours. We believe this change is a part of the complex protein metabolism of the cell.

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