RADIOIRON ABSORPTION IN ANEMIC DOGS

Fluctuations in the Mucosal Block and Evidence for a Gradient of Absorption in the Gastrointestinal Tract*,‡

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The body's control over the absorption of iron from the gastrointestinal tract has remained a fascinating and somewhat elusive problem in spite of considerable study. The data collected over many years and by various investigators (12) indicate that under ordinary conditions the body carefully guards its supply of iron. The limited ability to excrete iron points to absorption controlled by some mechanism which seems to be located in the cells of the gastrointestinal mucosa. This mechanism is in some manner linked to the amount of iron in storage since acute anemia does not increase iron absorption whereas depletion of the iron stores effected by regeneration after acute bleeding, or associated with a chronic iron deficiency anemia, markedly increases it (6). In a small series of preliminary experiments (6) it was found that the absorption of an oral dose of radioiron was somewhat reduced when preceded at 1 to 6 hours by a feeding of 100 mg. of ordinary iron. It was concluded therefrom that under these conditions the mucosa could be saturated or "blocked" in a matter of hours.

A quantitative study reported here of the "mucosal block" phenomenon indicates that following the oral administration of 100 mg. of iron to chronically anemic and iron-deficient dogs there is a reduction in the absorption of a standard test dose of radioiron. The control absorption of radioiron was recorded and the decrease to 35 per cent of control values was noted after an interval of 5 hours. This block due to administration of 100 mg. of ordinary iron gradually decreased and had disappeared after about 24 hours (Fig. 1). The "mucosal block" was of considerably shorter duration than had been assumed previously.

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Observations on the absorption of radioactive iron introduced into the jejunum and colon showed a decreasing ability of the gastrointestinal tract, per unit area, to absorb iron as the more distal portions were reached. This suggests that the "mucosal block" following oral iron occurs predominantly in the stomach and duodenum.

Methods

All the dogs used were healthy adult mongrels vaccinated against distemper. When non-anemic they were maintained on a diet of hospital table scraps. During periods of red cell and iron depletion a salmon bread diet was fed. Details of the procedures for producing anemia by blood loss, and for determining hemoglobin, plasma iron, and radioiron concentrations have been previously described (10, 11). Blood volumes were measured by the dye method using T-1824 (4). All iron, whether radioactive or not, was given as an aqueous solution of $FeCl_3$. For oral administration a stomach tube was used and iron was introduced into the colon through a ureteral catheter.

TABLE A

The Influence of Prefeeding 100 Mg. of Iron as $FeCl_3$ on the Subsequent Absorption of a Tracer Dose of Radioactive Iron Fed at Various Time Intervals

Dog	Weight	Hemoglobin	Time after 100 mg. Fe	Plasma iron at time of radioiron feeding	Amount of radioiron fed	Absorption of radioiron	Per cent of control absorption
	kg.	*gm./100 ml.*	*hrs.*	*γ/100 ml.*	*mg.*	*per cent of dose*	
47–155	16.8	7.1	Control	27	4.2	66	—
47–155		7.6	5	280	4.3	23	35
47–155		7.8	12.5	19	4.5	36	55
48–129	15.9	7.0	Control	30	10.2	35	—
48–129		5.0	7	23	10.0	14	40
48–129		5.7	24	36	10.2	38	110
49–95	14.5	3.9	Control	44	9.1	60	—
49–95		3.4	12	24	9.6	40	67
49–95		5.9	16	52	8.3	52	87
49–98	14.5	6.9	Control	18	4.4	61	—
49–98		7.5	18	25	4.8	54	89

EXPERIMENTAL OBSERVATIONS

Table A contains the data pertaining to the effects of prefeeding 100 mg. of iron at various times on the depression of absorption of a subsequent test dose of radioiron. Each animal served as its own control. Absorption of the test dose was determined by measuring the amount of the isotope that ultimately appeared in the circulating red cells (7). Maximum values were usually found at 2 to 3 weeks after radioiron feeding. Some animals were allowed to return to a relatively non-anemic state and during subsequent depletion all radioiron removed was measured. In no instance was there evidence of storage of the previously introduced radioiron.

The degree of anemia present during each experiment is indicated by the hemoglobin concentrations listed in column 3, Table A. Time intervals between

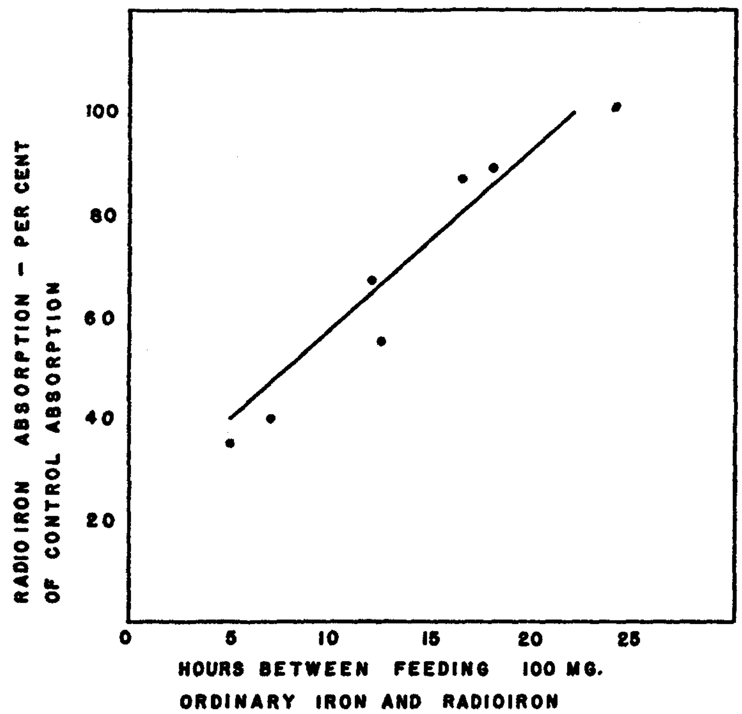

Fig. 1. Graph showing that the suppression of the gastrointestinal absorption of radioiron fed after 100 mg. of ordinary iron falls off progressively as the interval between the two feedings is increased. No suppression can be expected after an interval of 22 hours.

feeding ordinary iron and radioactive iron are shown in column 4, and it will be noted from column 6 that each dog received a comparable dose of the isotope at each interval tested although the dose varied somewhat from animal to animal. The figures in the final column represent the per cent of the control values to which the absorption of radioiron was reduced by a prior feeding of 100 mg. of ordinary iron.

After 5 hours, the shortest interval, absorption was reduced to 35 per cent of the control value, after 18 hours to 89 per cent only, while after 24 hours no reduction was observed. Fig. 1 demonstrates that the degree of reduction of labeled iron absorption appears to bear a direct relationship to the time after feeding 100 mg. of ordinary iron. The portion of the curve at less than 5 hours is difficult to explore. The percentage absorption of a large amount of iron is always less than that following a small one (7). At short time intervals the tracer dose might mix with the prefed iron and one would then be measuring the absorption of an unknown quantity of iron rather than that of 5 to 10 mg., hence a small absorption might well be expected. The last portion of the curve strikes the 100 per cent level at about 22 hours which represents the approximate duration of the "mucosal block" induced by 100 mg. of iron.

Plasma iron concentrations in all the dogs at the time of feeding the large amount of ordinary iron ranged between 13 and 50 γ per 100 ml., the low levels usually encountered in anemic, iron-deficient dogs. Administration of 100 mg. of iron resulted in a prompt and brief elevation of the plasma iron concentrations varying from 210 to 400 γ per 100 ml. In all but one instance the plasma iron had fallen to base line levels before the tracer iron was fed (column 5, Table A). Dog 47-155 with a sustained elevation of plasma iron at about 300 γ per 100 ml. for 5 hours received 4.3 mg. of radioiron at the end of that time. The plasma iron level of 280 γ per 100 ml. continued to fall in spite of the appearance of radioiron in the plasma, which reached a maximum of 35 γ per 100 ml. 1 hour after the tracer feeding.

Jejunal and Ileal Absorption of Iron

Dog 48-113 was an adult mongrel terrier weighing 10.5 kilos. A condition of moderate anemia and iron depletion was produced by repeated bleeding over an 8 week period. A fistula was then made between the jejunum, at a point a few centimeters below the duodenum, and the anterior abdominal wall by means of a double-flanged, metal and plastic tube (9) which could be readily opened and closed and permitted easy access to the lumen of the intestine.[1] One month after the operation when the hematocrit reading was 33.6 per cent and that of hemoglobin 10.0 gm. per cent a dose of 9.85 mg. of radioiron was introduced through the fistula into the distal limb of the jejunum. The absorption of radioiron, as measured by incorporation into the circulating red cells at 7 and 14 days amounted to 7.0 per cent of the dose. One week later, 4 days after a final bleeding of 110 ml., the hematocrit reading was at 30.6 per cent and the hemoglobin 9.25 gm. per cent. On this day 9.85 mg. of radioiron was given by stomach tube. 2 weeks after this oral radioiron the red cells contained 14.0 per cent of the dose after taking into account the amount of isotope circulating at the time of feeding.

The absorption from the entire gastrointestinal tract in this test was twice that from the small intestine and colon. Only a moderate grade of anemia was maintained during it because of the tendency for the metal button to slough out in the presence of severe anemia.

Colonic Absorption of Iron.—Absorption of radioiron through the colon was determined in two dogs after introduction of the isotope as $FeCl_3$ dissolved in a small volume of water through a ureteral catheter which had been

[1] We are indebted to Dr. John A. Schilling for this operation. It was performed under ether anesthesia.

introduced rectally for a distance of 30 to 50 cm. Initial experiments were performed while the animals were non-anemic. Subsequently anemia and iron deficiency were induced by repeated bleeding and all radioiron removed was measured. This radioiron plus the amount circulating at the end of the bleeding period was taken as the total amount absorbed. Absorption of comparable doses of radioiron by the colon was then measured while the dogs were anemic. Data pertaining to these experiments are shown in Table B.

Dog 49-98 when non-anemic defecated about ½ hour after the introduction of 5.0 mg. of radioiron into the colon. An additional 4.3 mg. was immediately administered and the final dose represents the sum of these two less the amount of radioiron found in the feces. In all other experiments the radioiron was retained for at least 24 hours. Dog 49-98 was rendered anemic and iron-deficient by repeated bleedings totalling 2007 ml. over a 7 week period. Dog 43-141 was bled 2078 ml. over a period of 8 weeks between the two experiments.

TABLE B

Absorption of Radioiron from the Colon

Dog	Weight	Hematocrit reading	Hemoglobin	Radioiron dose	Per cent of dose absorbed
	kg.	*per cent*	*gm./100 ml.*	*mg.*	
49–98	14.5	55.8	18.3	7.99	0.25
49–98	14.5	28.7	6.0	8.06	1.00*
43–141	10.0	52.7	14.8	4.38	0.81
43–141	10.0	31.1	8.9	5.01	1.00*

* Red cell incorporation of radioiron in 3 weeks.

While no significant increase in colonic iron absorption resulted from the production of anemia in dog 43-141, a fourfold increase was noted in dog 49-98 with a somewhat greater degree of anemia. In all instances, however, the radioiron absorption from the large intestine was very small when compared with the anticipated absorption after an oral dose of similar size.

The much higher colonic absorption of radioiron observed by Copp and Greenberg (1) in anemic rats was probably related to the poor intestinal tone encountered in their experimental animals which permitted exposure of the mucosa to iron for periods up to 2 days. It seems obvious that dogs made anemic by bleeding and rats rendered anemic by inadequate diet are not comparable, so different are the experimental conditions.

DISCUSSION

In a consideration of iron absorption a variety of factors must be kept clearly in mind. The normal dog with a 50 per cent red cell hematocrit reading will usually absorb 1 to 5 per cent of standard small doses (5 to 10 mg.) of radioiron by mouth. The anemic and iron-depleted dog will usually absorb 40 to 80 per cent of standard small doses of radioiron. *Sudden anemia* due to large bleedings does not change the iron absorption of a previously normal dog, at least during the first 24 hours (6).

Feeding of iron by mouth a few hours before the administration of the standard dose of radioiron causes a block in the usual absorption by the anemic dog (Table A). Because the non-anemic dog requires a week of bleeding to attain maximal iron absorption it was suspected that the "mucosal block" due to iron feeding might last 6 to 7 days. The experiments recorded above (Fig. 1) indicate that the "mucosal block" due to iron feeding lasts only about 20 hours and that in anemic dogs it is at most an incomplete effect since even with a short interval of 5 hours between feedings the amount of radioiron absorbed is at least 4 to 5 times greater than that found in normal animals.

Evidently there is a difference between the partial "mucosal block" due to iron feeding in anemia (a 24 hour period) and the "mucosal block" found in the normal dog, which is uninfluenced by acute anemia and requires at least several days of anemia and iron depletion for its complete clearance. There seems to be no reasonable doubt that the control of iron absorption normally resides predominantly in the mucosa of the gastrointestinal tract, even though it has been shown that iron absorption may be increased in some anemias without iron deficiency (2) and also under certain extreme conditions by dietary factors in the absence of anemia (8). Since Kinney, Hegsted, and Finch (8) describe profound metabolic changes, indicated by weight loss and cachexia, as result of the deficient diet in their experimental animals, some marked functional abnormality of the mucosal cells of the gastrointestinal tract related to iron absorption cannot be excluded.

The low plasma iron levels encountered in iron deficiency anemia, reflecting the low iron stores and the rapid removal of all iron available for hemoglobin synthesis, suggest that under these conditions the mucosal iron repletion due to iron feeding, though rapidly drained off *via* the plasma, is the factor responsible for the decrease in iron absorption. The curve of plasma iron due to absorption of small or large oral doses reaches a maximum in 1 to 2 hours and even after large doses returns to the base line in about 6 hours. However, in these anemic dogs the wave of iron absorption related to the gastrointestinal tract takes about 24 hours to run its course, as indicated by interference with the absorption of subsequent doses of radioiron. In the normal animal, on the other hand, the iron content of the gastrointestinal mucosa probably remains at a constant high level, in equilibrium with the normal body iron in storage or transport.

Further suggestions relating to sites of maximum iron absorption and probable duration of the "mucosal block" have appeared in the literature, based on direct observation of the mucosal cells after iron feeding.

Granick (5) using a semiquantitative method determined the distribution of ferritin in the gastrointestinal mucosa of rapidly growing guinea pigs at intervals after feeding 20 mg. of ferrous ammonium sulfate. In the duodenum, which always showed the greatest amount of ferritin, an increase was noted in 4 hours and a maximum was reached in 8 hours. 20 hours after feeding, some ferritin was generally observed in the pyloric region of the stomach but diminished rapidly in amount below the duodenum. Small quantities of ferritin were present occasionally in the large intestine. Within 5 to 7 days the ferritin content of the mucosa throughout the digestive tract had returned to approximately control levels.

More recently Endicott *et al.* (3) studied radioautographs of the gastrointestinal tract of guinea pigs and rats at various time intervals after an oral test dose of radioiron. Densities diminished smoothly from a maximum near the pylorus to a minimum in the proximal jejunum. Highest densities in normal and anemic animals were observed at 2 hours. Failure to detect radioiron in the lower intestine was probably due to amounts too low to affect a photographic plate.

The very low colonic absorption of radioiron, in the experiments here reported, modified little if at all by anemia, and the high percentage absorption by the stomach and duodenum compared to the remainder of the intestinal tract, when considered in relation to the surface area, point to a ***gradient in the capacity of the gastrointestinal tract to absorb iron.*** This is in accord with the direct observations of Granick and Endicott and suggests that the effectiveness of the "mucosal block" mechanism is likewise graded.

SUMMARY

The control of iron absorption appears to reside in the mucosa of the gastrointestinal tract.

The normal dog absorbs very little iron, but the anemic iron-depleted dog may absorb 10 to 20 times as much.

This "mucosal block" of the normal dog probably is due largely to iron stores in the mucosa.

"Mucosal block" can be effected in the anemic iron-depleted dog by feeding of iron salts, but the degree of "mucosal block" under these conditions never reaches the high degree of "mucosal block" in the normal dog.

Rapid movement of iron through the mucosa may explain the short duration of "mucosal block" due to iron feeding in the anemic iron-depleted dogs (18 to 20 hours, Fig. 1).

The colon absorbs very little iron under the conditions described. The stomach and duodenum seem to be most active in its absorption. This suggests the existence of a *gradient* in the capacity of the gastrointestinal tract to absorb iron.

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