RADIOACTIVE IRON AND ITS EXCRETION IN URINE, BILE, AND FECES*

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Recent experiments (3) in this laboratory gave clear evidence that the *plethoric* dog with all its iron stores well filled did not absorb radio-iron, in contrast to the *anemic* dog which took up such iron promptly and in considerable amounts from the intestinal tract. The transfer of the radio-iron to new red blood cells in anemia is very rapid.

The experiments tabulated below deal with *excretion* of radioactive iron after its intravenous injection as ferrous gluconate. It is generally accepted that the *urine* is not a factor in iron escape from the body and the radioactive iron shows this to be true in these experiments, except for a short period of iron excretion following the intravenous dose of iron.

The *feces* contain a remarkably uniform amount of radioactive iron under these conditions. Except for a short initial period of accelerated excretion following the intravenous iron we observe a steady and low output of 0.05 to 0.40 mg. per day, the larger dogs eliminating more iron, somewhat in proportion to their weight.

The *bile* contains but small amounts of radio-iron in these experiments in bile fistula dogs. The induction of blood destruction greatly increases this excretion. Obviously the capacity of the dog to eliminate iron is strictly limited under these conditions, and this fact is of importance when we attempt to visualize the metabolism of iron in the body.

Iron balance studies have always been difficult and the results uncertain to a degree because of the inability to distinguish between excreted iron and dietary iron which was unabsorbed in the gastro-intestinal tract. Iron balance studies called for the use of diets very low in iron content and very accurate iron analysis to obtain significant results. Varied results and con-

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clusions so numerous in the literature of this subject speak eloquently for these difficulties.

In this controversial field reviewed elsewhere (2) perhaps the most interesting results are those recently reported by McCance and Widdowson (6, 7) who, in a series of papers reporting tests on human subjects, arrived at the conclusion that excretion of iron is usually so small as to be negligible, and that therefore the iron content of the body is usually regulated by control of absorption. Our earlier experiments (3) with labeled iron in dogs are in accord with their results in human subjects and the experiments tabulated below are likewise in harmony with their hypothesis.

Methods

The radio-iron used in these experiments was prepared in the cyclotron at the Radiation Laboratory of the University of California using the methods described by Wilson and Kamen (10).

Only an infinitesimal fraction of the total iron in the target is rendered radioactive by this procedure. It appears certain that this radioactive fraction affords a quantitative means of labeling the whole iron sample or any portion of it, regardless of the pathway it may take in the course of its biological utilization. At present all chemical and biological evidence indicates that the increased atomic weight of the radioactive isotope would not be enough to produce any appreciable difference in utilization from that of the ordinary isotopes with which it is mixed. Thus, the total iron sample containing the radioactive iron is justifiably considered as a *labeled iron* for the purpose of feeding or injection experiments. Subsequently in this paper the terms "radio-iron," "radioactive iron," and "labeled iron" are used interchangeably to indicate the whole labeled iron sample or fraction thereof.

Preparation of the material for determination of beta-ray activity has been described in detail elsewhere (1, 3, 4). Routine care of the animals, including methods of bleeding, determination of plasma and blood volume, and hemoglobin, as well as composition of diets has been described elsewhere (4, 9).

Unless otherwise specified, radioactivity measurements are given in terms of counts per minute as recorded on our scale-of-four Geiger-Müller counter (1000 recorded counts means 4000 actual counting tube discharges per minute).

Iron for injection in the form of ferrous gluconate was prepared as follows: Radioferric oxide was reduced to metallic iron by heating at 800–900°C. in a stream of hydrogen. The radio-iron was converted to ferrous gluconate by removing the iron from the hydrogen atmosphere and rapidly adding it to a slowly boiling solution of gluconic acid (50 to 100 per cent excess) in a 125 cc. Erlenmeyer flask covered with a small watch glass. The gluconic acid was always freshly prepared by mixing equivalent amounts of hot calcium gluconate and oxalic acid solutions. The solution was boiled until the effervescence of hydrogen had almost ceased, filtered, and washed rapidly into a volumetric flask, and cooled to about 45°C. The solution was then diluted to the mark, mixed thoroughly, and a large aliquot taken for injection. The injections were carried out immediately after the preparation was ready, to minimize atmospheric oxidation. In this way 60 to 80 per cent of the radio-iron was converted to soluble form for injection.

This preparation was done in the laboratory of Pathology by Dr. L. L. Miller to whom we are indebted.

EXPERIMENTAL OBSERVATIONS

Dog 37-11, a small female adult mongrel, weighing 6.5 kg., was maintained on a diet low in iron (4). This animal was depleted of iron reserve stores by 2 months' continuous anemia due to bleeding. Radioactive iron was given by vein in the form of ferrous gluconate. In all, 105 mg. of iron were injected showing an activity of 4245 counts per minute. 10 days later 84 per cent of the injected iron was present in the circulating blood (hemoglobin). Urine and feces collections were made and in Tables 1 and 2 are seen the amounts of iron excretion by the kidneys and gastro-intestinal tract. It is to be noted

TABLE 1

Urinary Excretion of Radioactive Iron

Period		Total excretion during period		Excretion per day	Total net extra excretion
	duration *days*	*per cent of amount injected*	*mg.*	*mg.*	*mg.*
Dog 37-11, anemic, 6.5 kg.					
Injected 105 mg. radio-iron (4245 counts per min.) by vein					
A	2	3.7	3.9	1.9	3.8
B	2	0.015	0.016	0.008	—
C	5	0.011	0.012	0.002	—
D	6	0.008	0.008	0.001	—
Dog 37-180, non-anemic, plethoric, 9 kg.					
Injected 130 mg. radio-iron (2215 counts per min.) by vein					
E	6	0.39	0.51	0.09	0.54±
F	15	0.49	0.64	0.04	0.60±
G	30	0.00	0.00	0.00	—

that except for an initial short period of increased excretion ("spill over") the radio-iron in the *urine* amounts to only a few micrograms per day. Similarly, the first period following injection (period A, Table 2) shows an appreciable excretion of the iron in the *feces*, but in subsequent periods the amount is considerably diminished, amounting to less than 0.1 mg. per day in this animal.

Dog 37-180, a female adult terrier, weighing 9 kg., was maintained on the diet low in iron for several days before experimental use. Just previous to and during this time red blood cells suspended in saline were given (25 gm. of hemoglobin = 84 mg. of iron) by vein and three injections of colloidal iron (not radioactive) amounting to 64 mg. of iron each, were also given by vein. The animal at this time had a blood hemoglobin level of 147 per cent (13.8 gm. per 100 cc. = 100 per cent) and could be said to be normal or plethoric with a considerable iron reserve storage. An attempt was made to feed some radio-iron with the diet, but it was vomited.

Radio-iron (130 mg.) was given by vein in the form of ferrous gluconate. This material had an activity corresponding to 2215 counts per minute. Urine and feces collections were made and the results may be seen in Tables 1 and 2. Some excretion through the kidney was apparent during two periods totaling 20 days following injection. Subsequently the excretion by this route dropped to zero. The fecal excretion during the first 4 days following injection amounted to about 0.7 mg. per day but later only about one-fourth of this amount per day was excreted by the gastro-intestinal tract.

Dog 37-122, a large female English bull, weighing 19 kg., was operated upon to produce a bile fistula (sterile bag type (8)[1]) and fed a diet of standard salmon bread (9). Dog bile was given daily. Anemia was established by bleeding and maintained for 3 months to deplete the iron stores. Subsequently radio-iron was given by mouth in the form of

TABLE 2

Fecal Excretion of Radioactive Iron

Period		Total excretion during period		Excretion per day	Total net extra excretion
	duration days	*per cent of amount injected*	*mg.*	*mg.*	*mg.*
Dog 37-11, anemic, 6.5 kg.					
Injected 105 mg. of radio-iron (4245 counts per min.) by vein					
A	9	1.90	2.00	0.22	1.17 (feces)
B	6	0.50	0.52	0.09	—
C	7	0.60	0.63	0.09	—
Dog 37-180, non-anemic, plethoric, 9 kg.					
Injected 130 mg. radio-iron (2215 counts per min.) by vein					
D	4	1.40	3.00	0.74	2.24 (feces)
E	4	0.22	0.47	0.12	—
F	3	0.42	0.90	0.30	—
G	11	0.63	1.30	0.12	—
H	30	2.30	4.90	0.17	—
Dog 37-122, anemic, bile fistula, 19 kg.					
Injected 250 mg. radio-iron (6890 counts per min.) by vein					
J	6	0.54	1.30	0.22	0.052 (bile)
K	10	—	—	—	0.039 (bile)
L	8	1.30	3.20	0.40	0.059 (bile)

$FeCl_3$ but was vomited. Ferrous gluconate containing the radioactive isotope was then injected by vein during 3 days, in all 250 mg. of radio-iron being introduced (6890 counts per minute). At this time the diet was shifted to the white bread-salmon, low iron type (4). Fecal collections were carried out and the results of activity determinations may be seen in Table 2. Period J of 6 days includes the injection period, the iron having been administered on the 1st, 2nd, and 4th day of the period. During the last period L, 76 per cent of the amount of iron injected was in circulation, presumably as new hemoglobin.

Dog 38-112, an adult female mongrel, weighing 10 kg., was made *anemic* by bleeding and was maintained in this condition for 3 months in order to *deplete the iron reserve*

[1] We are greatly indebted to Dr. W. B. Hawkins of the Department of Pathology who supplied the two bile fistula dogs for these experiments.

stores. The diet during this time consisted of hospital table scraps. Ferrous gluconate containing radio-iron (122 mg. counting 13,600 per minute) was given by vein and the animal allowed to regenerate hemoglobin including this radio-iron. At the end of 10

TABLE 3

Fecal Excretion of Radioactive Iron

Dog 38-112, anemic and depleted at start, 10 kg.

Period after injection		Duration period	Hemoglobin level	Total excretion during period		Excretion per day	Total net extra excretion
	days	*days*	*per cent*	*per cent of amount injected*	*mg.*	*mg.*	*mg.*
	0	Injected 122 mg. radio-iron (13,600 counts per min.) by vein					
A	0–11	11	64 to 88	2.24	2.73	0.25	2.2 (feces)
	11–16	Acetyl-phenylhydrazine (1.3 gm. total) injected subcutaneously					
B	11–24	13	88 to 55	7.65	9.34	0.72	8.7 (feces)
C	24–29	5	55 to 78	0.33	0.40	0.08	—
D	29–34	5	78 to 76	0.17	0.21	0.04	—
	64	Operation for bile fistula (sterile bag type)					
E	70–74	4	110 to 126	0.20	0.24	0.06	—
	74–80	Acetyl-phenylhydrazine (0.8 gm. total) injected subcutaneously					
F	74–81	7	126 to 82	0.72	0.88	0.13	0.6 (feces)
G	81–88	7	82 to 86	0.15	0.19	0.03	3.9 (bile)

TABLE 4

Fecal Excretion of Radioactive Iron

Dog 38-182, anemic, 9 kg.

Period after injection		Hemoglobin level	Total excretion during period		Excretion per day	Total net extra excretion
	days	*per cent*	*per cent of amount injected*	*mg.*	*mg.*	*mg.*
	Injected 160 mg. radio-iron (6090 counts per min.) by vein					
A	7	68 to 93	1.62	2.59	0.37	2.0
B	7	93 to 105	0.58	0.91	0.13	—
	Acetyl-phenylhydrazine (1.1 gm. total) injected subcutaneously					
C	7	105 to 76	0.08	0.14	0.02	—
D	7	76 to 54	1.17	1.90	0.27	1.3
E	7	68 to 80	0.54	0.84	0.12	—

days 54 per cent of the radio-iron injected was in circulation. During this time (period A, Table 3) a total of 2.7 mg. of the radio-iron was lost in the feces, representing an increased excretion of about 2.2 mg.

Acetyl-phenylhydrazine was then given subcutaneously daily over a period of 6 days in order to destroy a considerable number of red blood cells. As can be seen in period B, Table 3, a considerable amount of the radio-iron was excreted by the gastro-intestinal tract. Following this period the excretion dropped precipitously to what might be looked upon as a base line value of about 0.05 mg. per day.

The animal was then operated upon to produce a bile fistula (sterile bag type) and the excretion of iron in the bile and feces was determined. There was no appreciable activity due to radio-iron demonstrable in the bile samples while the dog was forming hemoglobin but the feces again showed a base line level of excretion. At this time there was 58 per cent of the amount of radio-iron originally injected in circulation.

Another course of hydrazine injections resulted in a definite increase in excretion of radio-iron in both the feces and bile, about 85 per cent of the extra excretion being accomplished through the biliary tract.

Dog 38-182, an adult beagle hound, weighing 9 kg., had been used in a feeding experiment in the study of absorption of radio-iron. All the measurable isotope had been removed by subsequent experimental bleedings of 2½ months' duration. A diet of hamburg was begun (as a low residue food). Radio-iron (160 mg.) as ferrous gluconate (6090 counts per minute) was given by vein and the animal allowed to regenerate hemoglobin including this iron. In 10 days 51 per cent of the amount injected had appeared in the circulation. Period A, Table 4, shows that about 2 mg. of extra radio-iron appeared in the feces following the injection. The base line excretion was established at about 0.09 mg. of radio-iron per day.

A course of hydrazine injections was instituted. During the week (period C) in which the drug was given there was no extra fecal excretion but about 1.3 mg. appeared during the following week (period D, Table 4).

DISCUSSION

Before considering the excretion of the labeled iron by the gastrointestinal tract it might be well to stress that in the experiments reported here we are not concerned with *total* iron excretion. Although a considerable quantity of the injected iron is incorporated in the red blood cells as hemoglobin (and in some cases for long enough periods that these cells are about midway in their life cycle (5)) and some is in the storage depots, nevertheless any fair estimate of excretion of iron arising from the breakdown of muscle hemoglobin or parenchyma iron (cellular ferments, etc.) is obviated since time for complete equilibrium may not have been sufficient. Furthermore conditions in the reported experiments are not properly controlled for differentiation as to the source of the excreted iron. Such studies are in progress and promise to throw additional light on the matter. Therefore these experiments are intended to show that iron labeled with the radioactive isotope is excreted at a low level but at a fairly constant rate in the feces; that this excretion can reach fairly high levels under certain conditions; and that the liver is involved in some manner with secretion of iron through the biliary tract.

Fecal excretion of radioactive iron calls for discussion even though our evidence is inadequate to establish many points of interest to physicians.

The output of radio-iron during long periods (excepting the week or two following iron by vein) is quite uniform when one considers the difficulties of collection and complete analysis. The daily output in the various dogs tested runs between 0.05 and 0.40 mg. and the larger dogs eliminate somewhat more. It is suggested that some of this radio-iron may derive from the epithelial wastage of the mucosa of stomach and intestine. The dog has a thick mucosa and very muscular walls and the wear and tear must be considerable. It is significant that the plethoric dog (37-180, Table 2) eliminates no more than does the anemic dog where conservation must be active. The bile probably contributes very little iron under these standard conditions (37-122, Table 2).

Fecal excretion in the week or two following injection of the radio-iron is quite definite and usually amounts to 1 or 2 mg. We have no adequate explanation to offer. The radio-iron probably remains in the circulation only a few days after intravenous injection and can hardly be the sole factor. This iron compound (ferrous gluconate) is soluble and capable of diffusion. We have noted (unpublished data) its presence in the circulating plasma for 48 hours or longer following the injection of radio ferrous gluconate. On the contrary, when iron is given by vein in the form of colloidal ferric hydroxide it is removed very rapidly by the reticulo-endothelial system and is probably all out of the circulation in a very short time. It would seem by a process of exclusion that some active process in the mucosa must be responsible.

Bile excretion of radio-iron during standard periods appears to be insignificant, about 0.01 mg. a day (37-122, Table 3), but more evidence on this point must be produced. Blood destruction increases significantly iron elimination in the bile. What may be the optimum reaction to demonstrate this iron excretion we do not know, but the largest elimination appears to be associated with maximal red cell destruction (hydrazine) and as much as 4+ mg. of iron may escape within a week or two. When acetylphenylhydrazine was injected subcutaneously causing the destruction of a large percentage of the circulating red blood cells the attendant loss of iron by the feces is quite apparent (Tables 3, 4). It is of interest to note that most of this excretion in the experiment in which iron content of the bile was measured (periods F and G, Table 3) was by way of the biliary tract. In other experiments the amount secreted in the bile may be greater than is shown by fecal excretion since the possibility of partial reabsorption has not been excluded. This phase of the problem is under consideration and should readily lend itself to solution. The extent to which excretion may take place may be further appreciated when we consider the first experiment involving phenylhydrazine in dog 38-112 (Table 3). Before administration of the drug the radio-iron in circulation was 54 per cent of the amount injected. As a result of the red cell breakdown this was reduced to 30 per cent. The loss in milligrams was therefore from 66 to 37, or 29 mg. Of this, 8.7 mg. or 30 per cent was excreted in the feces.

In the second phenylhydrazine experiments (Table 3) with this dog the radio-iron dropped from 58 per cent of the amount injected (equivalent to 71 mg.) to 24 per cent (equivalent to 29 mg.). Of the 42 mg. liberated, 4.5 mg. or about 11 per cent was excreted in the bile and feces. The difference in the amount excreted and the probable reasons for this difference is not within the scope of this paper and will be considered at a later time. It can be readily seen, however, that the excretion by way of the gastrointestinal tract may be a matter to be reckoned with under certain conditions.

There is little need to dwell upon the excretion of iron by the kidney. It has long been recognized that under normal conditions the urinary iron was practically a negligible quantity. The evidence obtained with radio-iron bears this out (Table 1). The extra excretion immediately following injection is probably in part related to the presence of a considerable amount of diffusible iron in the plasma for a short interval. It is not a surprising finding since even hemoglobin will find its way into the urine following intravenous injections of large amounts.

Perhaps we may hazard a guess as to the capacity of the dog (anemic or plethoric) to eliminate surplus iron in comparison with the amount of iron in circulation. Dog 37-180, weight 9 kg., had a blood volume of 700 ml. and a total amount of hemoglobin in circulation amounting to 140 gm. (equivalent to 480 mg. of iron). This dog received injections of iron (or red cell equivalent of iron) amounting to 400 mg. of which 130 mg. was labeled radio-iron. The base line of radio-iron excretion (Table 2) was approximately 0.18 mg. per day. If the excretion of labeled iron is typical of the behavior of all surplus iron in this dog this would represent an iron excretion of about 0.6 mg. per day. If this excretion of assumed surplus iron represents the *normal ability of the dog to excrete excess iron* it would require about $2\frac{1}{2}$ years for the animal to eliminate excess iron, equivalent to the amount in circulation as hemoglobin—evidence of the difficulty of this task for the dog.

SUMMARY

Radioactive iron as ferrous gluconate given by vein enables us to study iron excretion in urine, bile, and feces.

There is an initial extra output in urine and feces during a few days (3 to 15 days) following the iron injection and this may total 2 to 8 per cent of the injected iron.

Following this initial reaction the *urinary* excretion of radio-iron drops to traces or even to zero.

The *feces* always contain measurable amounts of radio-iron—in five dogs receiving 100 to 250 mg. of radio-iron the fecal excretion per day settled down to 0.05 to 0.4 mg. per day.

Blood destruction (acetyl-phenylhydrazine) causes a definite increase in radio-iron eliminated in the feces (0.1 to 1.0 mg. per day). Probably most of this excess iron comes through the biliary tract (bile fistula). The bile under usual conditions contributes very little iron to the intestine (0.01 mg. radio-iron per day or less).

The body controls its iron stores by absorption or lack of it rather than by its capacity to eliminate it. The evidence is overwhelming that the dog excretes iron with difficulty and in small amounts (even in the plethoric state) by means of the biliary and gastro-intestinal tracts.

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