Absorption of Hemoglobin Iron by the Rat. (30670)

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The largest source of iron in man's diet is found within a porphyrin ring as a constituent of myoglobin or hemoglobin. Thus, it is important to study the absorption of the element ingested in this form. In the past, the majority of studies dealing with the metabolism of iron have been performed with test doses of iron in the form of a salt(1,2,3,4). Since the rat was the animal commonly used in these studies we decided to investigate the absorption of hemoglobin iron in comparison to ferrous sulfate in this species.

Methods. Male albino rats (Walter Reed Carworth Farm) weighing 225-250 g were used in this study. Solutions of hemoglobin tagged with iron^{59} were prepared from hemolysates of rabbit erythrocytes. A male rabbit was injected with 300 microcuries of ferrous^{59} citrate. Seven days later the animal was bled and red cells washed 3 times in saline. The cells were lysed with distilled water and the hemolysate separated from the stroma by centrifugation. The solution was adjusted with distilled water or a nonradioactive hemoglobin solution in such a way that each test dose of hemoglobin iron was contained in a volume of 0.5 to 0.75 ml and had an activity of 0.5 microcurie of iron^{59} hemoglobin. Iron in form of a salt was given as ferrous^{59} citrate with a carrier of ferrous sulfate with volume and specific activity comparable to that of the hemoglobin iron^{59} solution. Hemin was isolated from a hemoglobin solution by crystallization and then dissolved in 0.1 N NaOH(5).

The test dose for absorption studies was administered in a 0.5 ml volume through an olive tipped 17-gauge endoesophageal tube to rats fasted for 16 hours. Whole-body radioactivity ($0.8 \rightarrow \infty$) was measured in a small animal whole body liquid scintillation detector 3 hours and 7 days after dosing to determine the percentage of the test dose absorbed by the rats. The reliability of this technique has been reported(6).

Blood letting was performed under light ether anesthesia by insertion of a heparinized capillary tube into the retro-orbital venous plexus. Plasma iron and total iron binding capacity were performed by the Ramsay methods(7,8). Whole livers were homogenized in a Virtis-tissue homogenizer, and the nonheme iron content was determined on an aliquot by the method of Brückman and Zondek(9).

The animals were fed a standard rat and mouse diet (G. L. Baking Co., Frederick, Md.). The iron concentration was assayed in our laboratory and found to be 80 μg per gram of food. The only source of porphyrin iron in this diet was from fish and accounted for less than 20% of the total iron. The experimental diet was made from iron-free sucrose, casein, Wesson oil and salt mix to give the same distribution of calories as the standard diet. The iron content of this mix was less than 5 μg per gram. A hemoglobin hemolysate solution was added as the only source of iron: final iron concentration was 80 μg per gram of food.

Results. A. *Absorption of hemoglobin iron* vs *ferrous sulfate in normal animals.* The absorption of iron in ferrous sulfate and hemoglobin was determined in normal rats. Seven animals were placed in each group. The dose of elemental iron ranged from 0.0275 to 0.44 mg. As the dose of hemoglobin iron or ferrous sulfate was increased, the percentage of absorption decreased (Fig. 1). However, at any level of dosage the absorption of ferrous sulfate was approximately 7-fold that of hemoglobin iron.

B. *Effect of phlebotomy on iron absorption.* To ascertain the relationship of the effect of the stimulus of increased erythropoiesis on the absorption of hemoglobin iron a group of animals were each bled 5 ml. Five days later the absorption of hemoglobin iron

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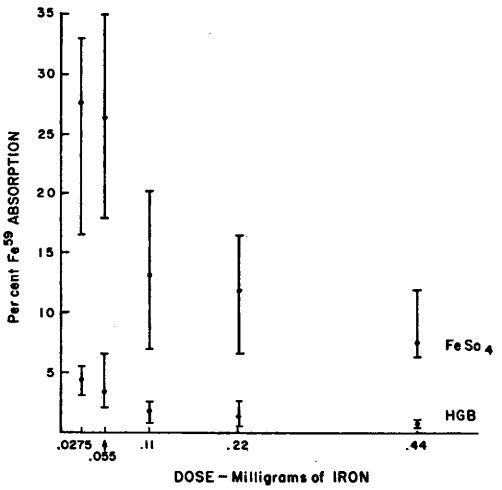

FIG. 1. Absorption of varying doses of $Fe^{59}SO_4$ and hemoglobin iron[59] in the rat. The bar represents the range and the dot the mean value of each group of seven animals.

and ferrous sulfate was measured. Each dose contained 0.2 mg of iron. There was a 3-fold increase in the absorption of ferrous sulfate but no significant increase in absorption of hemoglobin iron (Table I) when compared to normal animals receiving the same test doses.

C. *Absorption of hemoglobin iron and hemin iron.* To determine if globin interferes with the absorption of iron from heme a group of normal rats were dosed with 0.2 mg of iron as hemin and compared to a similar group receiving the same dose of iron as hemoglobin. The retention of iron[59] was 0.9% in those animals receiving hemin and 1.9% in the animals receiving hemoglobin (Table II).

D. *Effect of the source of dietary iron on hemoglobin iron absorption.* A group of newly weaned rats were raised on a diet wherein the only source of iron was hemoglobin. The content of carbohydrate, fat, protein, vitamins, salt and iron was equal to that of the standard laboratory chow which was fed to the control group of weaned animals. The rate of growth of the animals fed hemoglobin was slightly below that of the control group and at the end of 8 weeks when the animals weighed approximately 230 g there was a weight differential of 20 g. Hematologic studies revealed a hypochromic microcytic anemia in the hemoglobin-fed animals. The hemoglobin concentration averaged 11.9 g% in contrast to the control value of 14.5 g% (Table III). The serum iron levels (58 μg *vs* 211 μg per ml) and the content of nonheme iron in the livers (14 μg *vs* 84 μg per g) also reflected the presence of iron deficiency in the hemoglobin-fed animals (Table IV). Ten animals from each group were dosed by mouth with 0.3 mg of iron as ferrous sulfate or hemoglobin iron. The absorption of hemoglobin iron was low in both groups: 2.8% in the hemoglobin-fed rats and 1.8% for the controls (Table V). In contrast the animals fed the hemoglobin diet had a 2½-fold increase (29.5%) in the absorption of ferrous sulfate as compared to the controls (13.8%).

TABLE I. Effect of Phlebotomy on Absorption of Iron[59] in Hemoglobin and Ferrous Sulfate.

	% hemoglobin iron absorption		% ferrous sulfate absorption	
	Normal	Bled	Normal	Bled
Mean	2.1	3.0	11.2	30.0
S.E.	.3	.5	1.3	2.8
Range	1.3-3.5	1.5-4.6	8.0-15.9	17.4-40.0
No. of animals	7	7	7	7

TABLE II. Absorption of 0.2 mg of Iron[59] in Heme and Hemoglobin.

	% heme iron absorption	% hemoglobin iron absorption
Mean	.90	1.92
S.E.	.13	.34
Range	.71-1.75	1.17-3.77
No. of animals	8	8

Discussion. The results of these studies indicate that the rat does not absorb the porphyrin iron as well as it does a comparable dose of iron in the form of ferrous sulfate. That the discrepancy is not due to the inability of the rat to split off the heme ring from the globin was demonstrated by the relatively poor absorption of hemin iron. Similar results in man suggest that the protein in some way may aid the absorption of porphyrin iron(10). As the size of the carrier dose of hemoglobin iron is increased the percentage absorbed decreases. A similar relationship is noted with iron salts and suggests the presence of a rate limiting mechanism in both processes(11).

Iron deficiency does not improve the absorption of hemoglobin iron. Following the

TABLE III. Hemoglobin Determinations and Red Cell Indices in Rats Raised on a Diet with Hemoglobin as the Only Source of Iron.

	HMCT†	HGB†	RBC†	MCV†	MCH†	MCHC†
			Normal diet*			
Mean	45.5	14.5	7.39	61.5	19.7	32.0
S.E.	.4	.1	.13	.6	.3	.2
Range	43.0-48.0 %	13.9-15.1 g %	7.07-7.98 million/cu mm	60.2-63.8 cu u	18.9-20.7 u u	31.6-32.4 %
			Hemoglobin diet*			
Mean	39.0	11.9	7.59	51.5	15.7	30.4
S.E.	.8	.3	.17	1.5	.6	.1
Range	35.5-41.0 %	10.7-12.6 g %	7.18-7.96 million/cu mm	47.8-56.2 cu u	14.4-17.2 u u	29.3-30.9 %

* 6 animals in each group.
† HMCT—hematocrit, HGB—hemoglobin, RBC—red blood cells, MCV—mean corpuscular volume, MCH—mean corpuscular hemoglobin, MCHC—mean corpuscular hemoglobin concentration.

stimulus of acute phlebotomy there is no significant increase in the absorption of hemoglobin iron. This is in contrast to the 3-fold rise in absorption of ferrous sulfate.

It was proposed that a separate pathway for absorption of the heme ring was present in the rat intestinal epithelium but did not develop in these laboratory animals because of the lack of significant amounts of heme iron in manufactured rat chow. This hypothesis was tested by raising newly weaned rats on a diet wherein the only source of iron was hemoglobin. However, after 8 weeks the rats still could not absorb hemoglobin iron as well as they did ferrous sulfate. Indeed these animals were iron deficient with hypochromic microcytic anemia, low serum iron, decreased liver iron and an increased ability to absorb ferrous sulfate. The continual feeding of hemoglobin as a part of the diet is a more physiologic approach to determine an animal's ability to utilize iron in this form than is the measuring of absorption following a single test dose while fasting. The effects of various intraluminal digestive processes stimulated by the ingestion of food and the possible enhancement or impairment of heme absorption by substances in the diet may play a role in the final retention of the iron in hemoglobin. Furthermore the day to day variation in the metabolic state of the animal is an important factor in the single dose experiments, but not when the hemoglobin is fed continuously.

In the diet of meat-eating humans the major source of iron is in the porphyrin ring of hemoglobin or myoglobin. Hemoglobin iron[59] absorption studies in man with a single test dose while fasting(10,12,13) or multiple doses with meals(14) have shown that iron in this form is absorbed as well as or better than a dose of iron salt. By giving radioactive hemoglobin iron with meals it was

TABLE IV. Serum Iron and Liver Iron Determinations in Rats Raised on a Diet with Hemoglobin as the Only Source of Iron.

	Serum iron (μg per 100 ml)		Liver iron (μg per g)	
	Hemoglobin diet	Controls	Hemoglobin diet	Controls
Mean	58	211	14	84
S.E.	7	16	.5	7
Range	32-83	156-268	13-16	66-106
No. of animals	6	6	6	6

TABLE V. Absorption of Ferrous Sulfate and Hemoglobin Iron[59] in Animals Raised on a Diet with Hemoglobin as the Only Source of Iron.

	% hemoglobin iron absorption		% ferrous sulfate absorption	
	Hemoglobin diet	Controls	Hemoglobin diet	Controls
Mean	2.8	1.8	29.5	13.8
S.E.	.32	.29	2.2	2.8
Range	1.5-4.8	1.0-4.0	18.0-42.7	9.0-26.2
No. of animals	10	10	10	10

demonstrated that absorption is enhanced in iron deficiency and depressed in iron overload(14). The failure of phytate and desferrioxamine, chelaters of dissociated iron, to reduce the absorption of heme iron as it does the salt has led others to suggest that in man there are two different pathways for the absorption of iron, one for heme, the other for ionized iron. The results of our experiments could mean that the rat lacks the first pathway. Such an observation must reinforce our caution in interpreting the results of experiments on rats in terms of human physiology.

Our finding that hemoglobin iron is poorly absorbed by rats even when they are iron deficient is in conflict with Bannerman's recent report that hemoglobin iron is well absorbed by rats(15). The reason for the discrepancy is not evident.

Summary. In the rat the absorption of a test dose of hemoglobin or hemin iron^{59} was significantly less than a comparable dose of iron^{59} as $FeSO_4$. That this is of physiologic significance was demonstrated by producing a state of iron deficiency in animals raised on a diet with hemoglobin as the only source of iron. These findings are in contrast to recent observations in man and suggest a difference between species as well as a difference in the epithelial cells' ability to sequester iron as the salt or in the heme ring.

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Phagocytosis of Heterologous Red Cells in Mice Injected with Isologous Microsomes.* (30671)

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Earlier findings of increased reticuloendothelial (r.e.) phagocytosis during restoration of liver tissue after partial hepatectomy(1) and changes observed in the phagocytic activity of tumor-bearing rats(2) and mice(3) furnished the incentive for investigating phagocytosis in mice after administration of subcellular tissue fractions. This approach was expected to provide some insight into cellular events associated with processes of physiologic or pathologic tissue breakdown, and subsequent repair and regeneration. In planning these experiments, it was essential to exclude, as much as possible, interfering factors related to isoimmunization, *i.e.*, introduc-

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