THE INFLUENCE OF DIET ON IRON ABSORPTION

I. The Pathology of Iron Excess*

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Plate 6

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Excessive deposition of iron in tissues depends on either excessive absorption of iron or redistribution of iron within the body, since there is but little excretion of iron in animals or man. In dogs Hahn *et al.* (1) have demonstrated that only small amounts of injected radioiron are found in urine and stools. In man, a fraction of a milligram of iron is excreted daily and this amount cannot be increased by injections of iron (2) or by multiple transfusions (3). Attention in this study has been directed toward *excesseive absorption of iron.* The histological pattern of absorbed iron is described and its similarity to conditions of excessive iron absorption in human beings is discussed.

In hemochromatosis and in South African natives on deficient diets (4), large deposits of iron are found in the parenchymal tissue of the liver and in other organs. Gillman and Gillman (5) performed biopsies of the liver on 120 patients suffering from pellagra. They stated that 12 per cent of the adult patients had pigmentary cirrhosis with iron pigment present in these livers and that an additional 18 per cent had what they termed precirrhosis with iron pigment present in the livers in lesser quantities. These writers suggested that their work has established malnutrition in man as one cause of pigmentary cirrhosis. In 1939 Gore (6) reported a case of pellagra in which there was also hemochromatosis, but this case is the only one to be reported in this country.

There are but few reports of the production of excessive absorption of iron in experimental animals. Polson (7) fed rabbits iron for periods as long as 16 months and

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found large quantities of iron histologically in the parenchymatous cells of the liver. Mallory and Parker (8) reported the occurrence of livers of high iron content in cats, which resembled livers seen in hemochromatosis, following the feeding of high levels of copper over long periods of time. Taylor (9, 10) described excessive absorption of iron with the deposition of large quantities of hemosiderin in the livers in cats following ligation of the pancreatic ducts. This observation has recently been confirmed by Gillman in cats (11), and in dogs by the present authors (12).

Experimental work on the effect of diet on absorption of iron has been mainly directed toward iron deficiency and factors influencing the availability of the small amounts of iron normally required by the individual. It is known that alkaline diets such as the Sippy diet (13) and diets containing excessive calcium carbonate and aluminum hydroxide reduce iron absorption in rats (14). Diets high in phosphate also reduce absorption of iron apparently through the formation of insoluble iron salts (15). Phytin has a similar action as demonstrated by McCance by serum iron studies (16) and by Sharpe using radioactive iron (17).

In anemic dogs, Hahn *et al.* showed that considerable quantities of iron are absorbed but that in normal dogs in which iron reserves are adequate due to the previous feeding or injection of iron, only a little iron is absorbed (18). Normal dogs fed radioactive iron by Moore *et al.* (19) absorbed greater quantities of iron than reported by Hahn, but iron stores in these animals had not been increased by transfusion or by supplementary iron prior to the experiment. Also, the manner in which the iron was administered differed in that Moore gave the iron in solution by stomach tube while Hahn gave the iron mixed in the animals's food.

Hahn, Whipple, and their coworkers (20) concluded that the iron content of the body is regulated through absorption rather than through excretion of iron. These workers postulated a receptor compound in the intestinal mucosa capable of reversibly combining with iron, taking up limited amounts of iron from the intestinal lumen and in turn passing it on to the plasma. Granick (21) has presented evidence that ferritin, an iron-containing protein, may perform this function. In the normal animal, the acceptor mechanism was thought to be physiologically saturated with iron and therefore would not absorb additional iron from the gastrointestinal tract. Consistently with this, Copp and Greenberg (22) found that iron-depleted rats absorbed nine-tenths of the radioiron given while normal control rats absorbed less than one-third of the iron. Essentially similar results were reported by Austoni and Greenberg (23).

The work of Gillman and Gillman and the other reports mentioned above indicate that under certain conditions the mechanism described by Hahn *et al.* is either ineffective or that other factors are involved. The studies here reported show that iron absorption is excessive in animals receiving certain diets and offer an experimental approach to this problem.

Methods

For the first series of experiments four diets were used (Table I). Diet I consisted solely of Purina dog chow. Diet II consisted of Purina dog chow to which 2 per cent ferric citrate (U.S.P. VIII powder) was added. Diet III was a mixture of corn grits (80 per cent) and lard (20 per cent). Diet IV was made up of 98 per cent of diet III plus 2 per cent ferric citrate. One drop

of haliver oil was given to each animal in each group each week. Analysis of the diets showed that the dog chow contained 350 μg. of iron per gm. of diet, while the corn grit diet contained only 27 μg. of iron per gm. The addition of 2 per cent ferric citrate supplied an additional 3.1 mg. of iron per gm. of diet in diets II and IV.

Adult male rats were fed the diets *ad libitum*. The animals from each group were sacrificed at 27 to 33 days. Some animals (Table I) receiving the corn grits diets were continued on the diet for 56 to 61 days. The animals were weighed at 4 day intervals and growth curves were made.

When the animsls were sacrificed, they were etherized, the thorax was opened, and as much blood as possible was removed from the heart by venipuncture. The needles, syringes, and bottles used had previously been treated with concentrated nitic acid and rinsed with redistilled water to eliminate as much iron as possible. Hemoglobin, serum iron, and iron-binding capacity of the blood plasma were determined as described below.

TABLE I

The Effect of Diet and Iron Supplements on Blood and Liver Iron

Group	Diet	No. of rats	Duration	Hemoglobin	Serum iron	I.B.P.* capacity	Liver weight	Liver iron	
			days	*per cent*	*γ/100 cc.*	*γ/100 cc.*	*gm.*	*total mg.*	*mg./100 gm.*
I	Purina	4	27–33	15.9	290	175	11.3	1.02	9.0
II	Purina + 2 per cent Fe citrate	4	27–33	15.9	245	117	12.6	1.75	13.9
III	Corn grit	4	27–33	16.2	229	225	6.5	1.26	19.4
III		4	56–61	13.5	—	—	4.3	0.45	10.4
IV	Corn grit + 2 per cent Fe citrate	4	27–33	15.2	413	50	6.8	4.65	69.6
		4	56–61	14.4	—	—	3.9	6.60	169.2

* Serum iron-binding protein capacity.

Once the blood had been collected, the vena cava was cut and the portal vein was canalized. The liver was then perfused with normal saline until the organ was homogeneous in color and until the saline leaving the hepatic vein was free from blood. This usually required perfusion for a period of 20 to 30 minutes. The perfused livers were then placed in iron-free containers for chemical analysis.

Sections were then taken from the left and right sides of the liver and from all the other organs. The sections were fixed in 10 per cent formalin and stained with hematoxylin-eosin and for iron using a modification of Perl's stain. Stains for iron were made using potassium ferrocyanide for the identification of ferric iron and ferricyanide for the identification of ferrous iron. Sections were also stained for reticulum using Foot's modification of Hortega's silver carbonate method.

Serum iron was determined by the method of Kitzes, Elvehjem, and Schuette (24). The iron-building capacity of the serum was measured colorimetrically according to a method which utilizes the production of a red pigment by the union of the β_1-globulin with iron (25) Hemoglobin was determined as oxyhemoglobin using an Evelyn colorimeter (26).

Livers were weighed immediately. The entire liver except for slices removed for pathological examination was wet-ashed with sulfuric and perchloric acid. The clear digest was diluted to

50 cc. or more when the iron content was high. A 1 cc. aliquot of this was adjusted to a pH of 4.6 using concentrated ammonia and acetic acid buffer with 1 drop of 0.1 per cent solution of paranitrophenol as an indicator. Iron was determined colorimetrically using orthophenanthroline as described in the serum iron method. Results were expressed in milligrams of iron per 100 gm. of wet tissue and as total iron per liver.

RESULTS

As expected, the rats comprising group I on the control diet of Purina alone did well. They appeared sleek and well fed and showed a steady gain in weight (Chart 1). This was true for the rats in group II receiving Purina to which iron had been added, and these animals actually gained somewhat more than the animals on Purina alone.

The rats on the corn grits and lard diet (group III) showed a slight gain during the first few days on the diet but this was followed by a steady and sharp

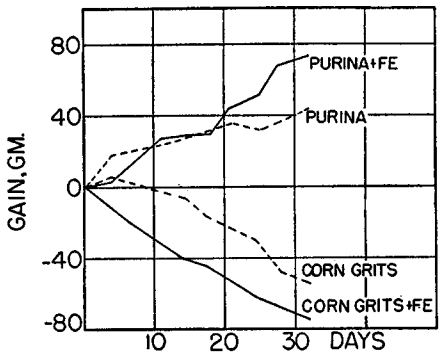

CHART 1. Average weight changes with time, on the various diets studied.

loss of weight. These animals were scrawny and unkempt with ruffled coats of poor texture. After 4 weeks on the diet, they were weak and unsteady.

The animals receiving the corn grits and lard diet supplemented by iron (group IV) did poorly. Weight loss was immediate and precipitate and the animals became weak and cachectic.

The results of the various chemical determinations are summarized in Table I. The hemoglobin levels in all animals in each group were within normal limits and there was no significant variation in the reticulocyte counts.

The differences in the values for the iron content of the livers were striking. In group I these ranged between 7.3 and 13.2 mg. per 100 gm. of liver, and the total liver iron averaged 1.02 mg. There was a slight but definite increase in the iron content of the livers of the rats in group II. There was also an increase in the iron concentration is some of the animals in group III. These determinations averaged 19.4 mg. and 10.4 mg. per 100 gm. of liver tissue for the animals which received the diet for approximately 30 days and 60 days respectively. No increase in total liver iron was found. However, a striking change was noted

in the liver iron values for group IV. These averaged 69.6 mg. per 100 gm. of liver after a month, and 169.2 mg. after 56 to 61 days. The total liver iron in these animals was four to six times that found in the first three groups.

There was no significant variation in the serum iron levels between groups I, II, and III. This was true as well for the determination of the iron-binding capacity of the serum. However, there was a marked increase in the serum iron levels for the animals in group IV. At the same time, the iron-binding capacity levels fell to zero in all but one animal.

At autopsy there were no significant gross or microscopic differences between the animals in group I and group II. The livers in both groups were smooth and light reddish-brown and following perfusion they were a homogeneous pale buff in color. The other organs were normal in size and appearance.

Microscopic examination of the livers in group I showed the expected normal histological structure. This was true as well for the livers in group II. Here, however, iron stains revealed the presence of rare Kupffer cells containing iron pigment. Comparison of iron stains of the spleens from group I and group II showed a moderate increase in the amount of stainable iron present in group II. Sections of the bone marrow, lungs, heart, adrenals, thyroid, kidneys, parathyroid, stomach, pancreas, testes, and small intestine were negative, and no deposition of iron was noted in any of these organs.

The livers of the rats in group III were mottled yellow and brown. Following perfusion, the livers were pale buff and yellow. There was a tendency for the left side of the liver to be more yellow than the right side. Histological examination of the liver showed many vacuolated liver cells which were most numerous in the periphery of the lobules. These ranged from a few involved cells in the animals on diets for a short period of time to marked involvement in those animals on the diet for longer periods. The fat appeared first in the peripheral zone and extended to the midzone of the liver lobule and still later to the entire lobule. The vacuoles were large and clear. In support of the gross appearance of the liver, the fatty changes appeared earlier and were more marked on the left than the right side of the liver. There was no necrosis and no scarring or destruction of the lobular pattern. Occasional Kupffer cells containing yellowish-brown pigment granules were found. This material gave a positive reaction with potassium ferrocyanide but did not react with potassium ferricyanide. In rare liver cells of some rats minute quantities of iron were demonstrated by the iron stains. These cells were always near portal radicles.

The appearance of livers of the rats fed diet III for a period of 56 days did not vary significantly from that of the livers of the animals on the diet for 33 days. Moderate quantities of hemosiderin were present in the secondary nodules of the follicles. An occasional macrophage containing pigment was found in sections of the femoral marrow. Examination of the remainder of the organs was negative.

The livers of the rats in group IV were a homogeneous dark brown and this

color persisted after perfusion. Fatty changes in the liver cells were similar to those noted in the rats in group III. Considerable quantities of yellowish-brown granular pigment were present. This was noted in the Kupffer cells throughout the liver (Fig. 1). The liver cells in the peripheral zones contained large amounts of similar pigment (Fig. 2). In the livers in which the pigment deposition was most marked, the liver cells in the midzonal area were also involved. This pigment reacted positively with potassium ferrocyanide and did not react with potassium ferricyanide. The iron stains demonstrated the presence of iron-positive material in the wall of the portal vein and in the lumen of the bile canaliculi.

The pigment, as far as could be determined, was not present in any greater quantities in liver cells containing fat than in the non-vacuolated liver cells. There was no significant difference in iron deposition between the right and left sides of the liver.

The appearance of the livers of the animals fed diet IV for 56 days was similar to that of the livers of the rats fed the diet for 33 days except that greater quantities of pigment were present both in the liver cells and the Kupffer cells. Hemosiderin was present in the spleen in greater quantities than in any of the above groups. The pigment was confined to the splenic pulp and was not seen in the germinal centers of the malpighian corpuscles. For the most part, the pigment was contained in macrophages.

Examination of the gastrointestinal tract showed the presence of iron-staining material in the lumina of the stomach, small bowel, and large intestine. However, with the exception of the duodenum no iron-positive material was present in the mucosa. In the duodenum the lining epithelium took a faint blue cast with the iron stains and occasional iron-positive particles could be identified. Large mononuclear cells containing pigment were present in the stroma and lacteals of the villi of the duodenum.

Considerable pigment was demonstrated by the iron stains of the femoral marrow, which was in macrophages. The marrow was otherwise not remarkable.

Iron stains demonstrated the presence of minute quantities of iron in an occasional lining cell in the renal tubules. Also, rare interstitial cells in the adrenals and testes contained iron.

No iron or other abnormalities were noted in sections of the brain, lung, heart, pancreas, thyroid, or parathyroid.

DISCUSSION

Under the experimental conditions described, excessive iron was deposited in the tissues of rats. Since there is ample evidence that the excretion of iron is negligible, this must represent either an increase in absorption of iron or a redistribution of body iron. Large losses in body weight may result in increased iron concentration of the tissues because of a decrease in tissue size and a transfer of iron from the diminished red cell mass.

The animals which received the corn grit diet without iron lost an average of 52 gm. of body weight, and demonstrate the effect of weight loss on iron metabolism. As expected the concentration of liver iron was somewhat increased and some hemosiderin was observed histologically. However, the total liver iron remained within normal limits. Serum iron values were not elevated and the iron-binding capacity was normal.

In contrast to these results, the animals which received the same diet plus iron showed (*a*) massive deposition of hemosiderin in the tissues, (*b*) four to six times as much total iron in the livers, and (*c*) markedly elevated serum iron and nearly complete saturation of the iron-binding protein. No anemia was observed in these animals. These results can be explained only by an absolute increase in body iron of the animals receiving the corn grit and iron diet.[1]

The most significant histological finding was the large amount of iron in the hepatic parenchyma. The hemosiderin deposits were heaviest in the peripheral zone of the liver lobule (Fig. 1). Hemosiderin was discernible when the liver iron had increased to 15 to 20 mg. per 100 gm. of wet tissue. Although there was a concomitant increase in hemosiderin in the reticuloendothelial tissue of the spleen, liver, and bone marrow, the deposition of iron in the hepatic parenchyma was more conspicuous. Of interest was the presence of iron in mononuclear cells in the villi and the mucosal cells of the duodenum in the animals of group IV. Hemosiderin was not found in any other portion of the gastrointestinal tract. Further, these changes were not found in the duodenums of the rats on the Purina diets. When the iron was present in lesser amounts in the liver, it appeared to be deposited in the Kupffer cells. The first hepatic cells to contain iron were invariably in the portal areas.

There is little question but that under normal conditions the absorption of dietary iron is regulated by the need of the body for iron. Both Hahn (20) and Copp and Greenberg (22) have demonstrated by the use of radioiron that in iron deficiency iron absorption is increased several fold. Granick has offered the explanation that the ferritin stores of the intestine govern absorption (21), and Gillman (11) has demonstrated hemosiderin deposits within the mucosal cells of the intestine following iron ingestion. That there is normally some block to iron absorption is confirmed in our animals on a Purina diet plus iron. Very little absorption occurs despite a large dietary iron intake. On the other hand, this

[1] The same conclusion is evident from (*a*) studies presented in the following paper of this series which demonstrates the accumulation of iron in the livers of animals receiving diets which allowed a gain in body weight; (*b*) data showing that animals which received the corn grit and iron diet and then were allowed to recover their normal weight upon an adequate diet still had large amounts of iron in their livers; and (*c*) complete carcass analysis of a few animals after removal of the liver and perfusion of the body to remove blood. Animals which received the corn grit plus iron diet had approximately 10 times as much iron in the livers as the controls and approximately 3 times as much in the remaining carcasses. No difference was found in the iron recovered in the perfusate. Studies upon the distribution of body iron under various conditions are now being completed.

gastrointestinal block may be overcome as shown by the group IV animals on a corn grit and iron diet.

Possible explanations for this increased absorption would be: (1) an increased availability of the iron presented to the gastrointestinal mucosa; (2) alteration in the mechanism by which iron is absorbed; and (3) abnormal tissue affinity for iron. The availability of iron in the intestinal tract for absorption is dependent on solubility of iron and its reduction since most evidence would indicate that iron is absorbed better if not exclusively in the ferrous state. In a subsequent report the rôle of blocking substances will be discussed as the probable mechanism in production of excessive iron absorption. There is no evidence to suggest any abnormality of the mechanism of iron absorption or storage. Should there be an increased tissue affinity for iron as happens in infection, serum iron would be depressed. The completely saturated iron-binding protein and greatly elevated serum iron suggest that iron is being absorbed at a greater rate than the tissues are able to convert it to storage iron. With marked iron excess, it appears that a saturated serum iron-binding protein is a reflection of the enlarged tissue stores (25). It will be observed that the serum iron does not rise higher than is consistent with its transport by the iron-binding protein, making unlikely the possibility that any other carrier mechanism may be in operation. It would thus appear that with the increased availability of iron, mucosal uptake is increased, serum iron is increased, and storage occurs predominantly in the liver.

SUMMARY

Rats placed on a corn grit diet and added iron absorbed large amounts of iron in contrast to control groups.

The histological picture was that of progressive hemosiderosis of the hepatic parenchyma and of the reticuloendothelial system. On chemical analysis, the iron content of the liver was found to be greatly increased. This supports the concept that the liver represents the chief storage organ for iron so absorbed.

These data indicate that a normal block for iron absorption may be overcome under certain circumstances.

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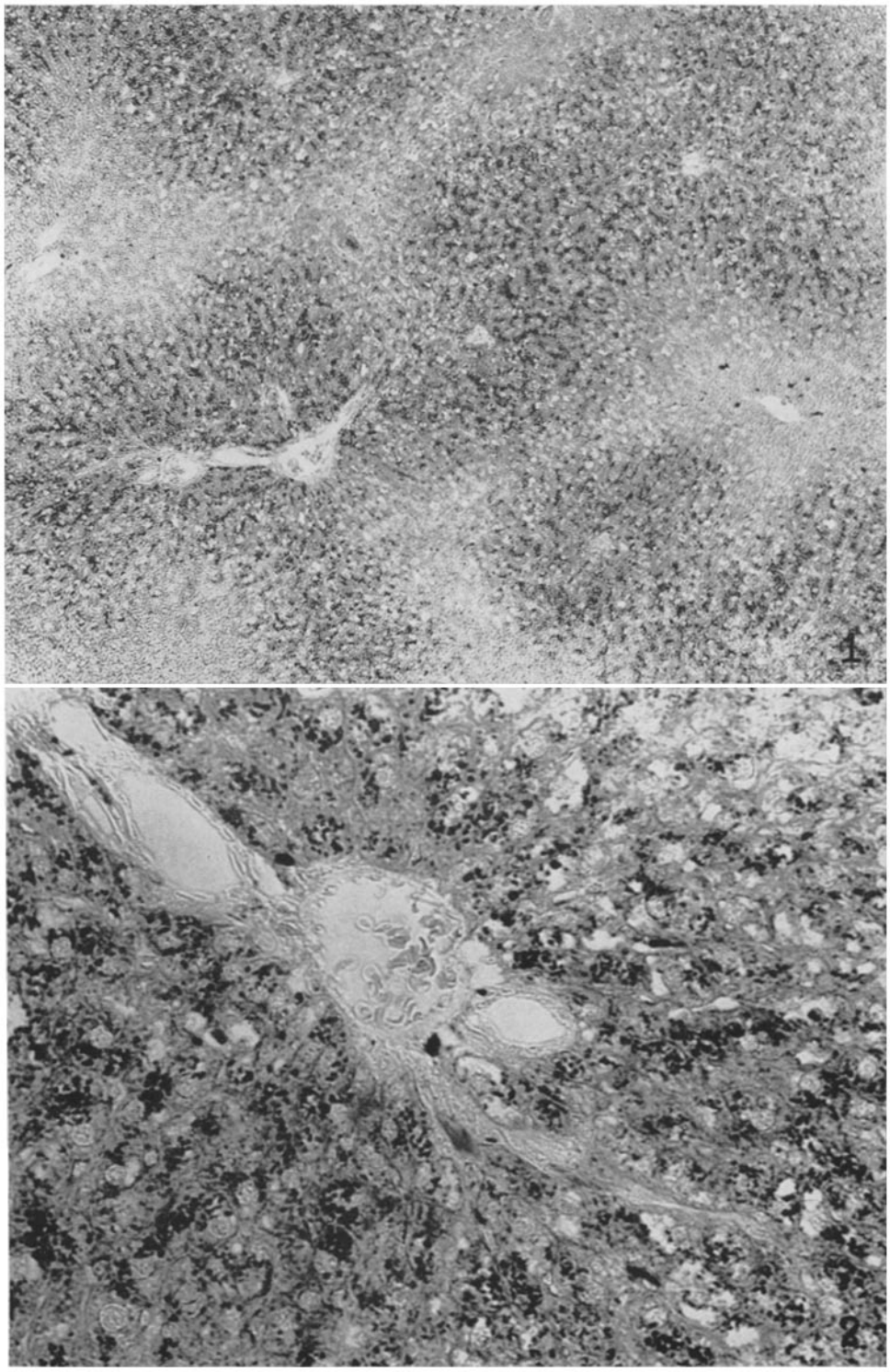
EXPLANATION OF PLATE 6

FIG. 1. Low power photomicrograph of liver stained for iron to illustrate the pattern of iron distribution. The pigment is present in greatest quantities in liver cells in the peripheral zone of the liver lobules and in Kupffer cells throughout the lobules. Prussian blue reaction. × 130.

FIG. 2. Higher magnification of peripheral zone of liver lobule showing heavy deposition of pigment in liver cells. Prussian blue reaction. × 475.



(Kinney *et al.:* Influence of diet on iron absorption. I)