EFFECT OF ETHIONINE-INDUCED PANCREATIC DAMAGE
ON IRON ABSORPTION

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It is generally agreed that the iron equilibrium of the body is maintained
by regulation of absorption of iron, since it has been clearly demonstrated
that very little iron is excreted by the body (1, 2). Ingested iron may be ab-
sorbed and temporarily stored as ferritin (3) in the epithelial cells of the
intestinal mucosa, principally in those of the upper third of the duodenum.
The iron then enters the metabolic pool of body iron by way of the serum
iron-binding protein. Under most circumstances it has been assumed that
the mucosal cells are limited in their ability to produce ferritin and conse-
quently to absorb iron, and so serve as a "mucosal block" to the absorption
of iron. However, it is possible to overcome this so called mucosal block by
altering the composition of the diet (4, 5), by giving extremely large doses of
iron (5) and by ligation of the pancreatic ducts (6, 7).

Since there is so little information available regarding the possible influence
of the pancreas upon the absorption of iron, it was felt that this problem
deserved further investigation. For this reason rats were fed diets containing
dl-ethionine (α-amino-γ-(ethylmercapto)-butyric acid) in quantities suf-
ficient to damage the exocrine cells of the pancreas. It was found that iron absorption
was excessive in those animals in which there were demonstrable pan-
creatic lesions.

Materials and Methods

Experiment 1.—Male albino rats of the Sprague-Dawley strain weighing approximately
220 gm. each were used. The rats were divided into 4 dietary groups of 7 to 8 animals each
and fed ad libitum. They were housed in groups of 3 to 4 per cage. A control group (group
I) was fed a diet of a purified type, referred to as the basal diet.

The basal diet had the following percentage composition: glucose 67, vitamin-free casein
18, salt mixture 4 (8), corn oil 11 (containing 0.001 cc. haller oil). Crystalline vitamins
were added to supply the following amounts per 100 gm. of diet: thiamine chloride 400 μg.,
pyridoxine hydrochloride 400 μg., riboflavin 800 μg., calcium pantothenate 1.5 mg., and
nicotinic acid 2.5 mg. Group II was fed the basal diet to which was added 0.5 per cent dl-
ethionine. Group III was fed the basal diet plus 2 per cent ferric citrate, while group IV
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was fed the basal diet supplemented by 0.5 per cent dl-ethionine and 2 per cent ferric citrate. An additional group, group V, was fed the basal diet, but in limited quantities in order to match the weight loss in the experimental groups. The animals in the latter group were housed individually. All rats were weighed twice a week and were killed at the end of 28 days. At autopsy portions of the various organs were fixed in formalin for histologic examination. They were stained with hematoxylin and eosin and for iron, using a modification of Perl's stain. Each pancreas was also stained with Mallory's connective tissue stain. Livers were stained, in addition, for fat and reticulum.

The livers were weighed immediately after the animals were killed. The livers, except for the slices removed from the right and left sides for histologic examination, were wet-ashed with nitric, sulphuric, and perchloric acids. The clear digest was diluted to 50 cc. One cc. aliquots of this were 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 by the method of Kitzes, Elvehjem, and Schuette (9). Results were expressed in milligrams of iron per 100 gm. of wet tissue and as total iron per liver. In addition, the livers were rated 0 to 5 plus on the basis of iron demonstrated histologically.

Experiment 2.—Two groups of male albino CFW mice were used. Each mouse weighed approximately 20 gm. at the start of the experiment. The basal diet was ground Purina dog chow with 1 per cent iron citrate. Mice in group A were fed the basal diet alone. Mice in group B were fed the basal diet supplemented with 0.5 per cent dl-ethionine. The experiment was continued for 28 days, at which time all animals were sacrificed. Food was withheld for 24 hours before death, in order to reduce the amount of iron in the gastrointestinal tract. In addition, the gastrointestinal tract was opened and thoroughly rinsed with distilled water.

In group A, 15 mice were analyzed for iron and five were autopsied and studied. Twelve group B mice were analyzed for iron and five were autopsied. Tissues from the autopsied mice were fixed in 10 per cent formalin and stained with hematoxylin and eosin as well as for iron. In the animals that were analyzed for iron, the livers, gastrointestinal tracts, and the remainder of the carcasses were wet-ashed separately with nitric, sulfuric, and perchloric acids. The clear digest of each was diluted to 50 cc. A 1 cc. aliquot of each specimen was then treated in a similar way as the digest from the rat livers in Experiment 1. The total iron content was determined for each animal from these values.

RESULTS

Experiment 1.—There was striking damage to the pancreas in all the rats on a diet supplemented with 0.5 per cent dl-ethionine, i.e., groups II and IV. The pancreatic changes were similar to those described by others (10-15). They consisted of interruption of architecture, acinar degeneration, atrophy and destruction, decrease of basophilia, cytoplasmic vacuolization, pyknosis and loss of nuclei of acinar cells, regeneration of acini, fibroblastic proliferation, fibrosis, and lymphocytic infiltration (Fig. 1). No morphologic changes were observed in the islets of Langerhans except for occasional slight cytoplasmic vacuolization.

Lesions in the livers also were noted in the rats in groups II and IV. The changes consisted of degeneration and necrosis of liver cells as well as regeneration of hepatic cells, bile duct and fibroblastic proliferation, and fibrosis
There was also lymphocytic and polymorphonuclear leukocytic infiltration in the damaged areas. There was no destruction of reticulum which, in fact, was slightly increased in the areas of fibrosis. There was a decrease in the fat content. These findings are in essential agreement with that of other workers (10-12, 14, 15).

There was no histologic evidence of iron in the livers of the rats in group I. In those animals fed the basal diet supplemented with iron (group III) and those on the limited basal diet (group V), little or no iron could be demonstrated histologically. The iron content of the liver of the rats fed the basal diet plus 0.5 per cent dl-ethionine (group II) was markedly increased and the histologic score varied from 2 to 4+ with an average of 3+. (Table I).

There were even greater concentrations of iron in the livers of the rats which received both iron and dl-ethionine supplement (group IV) (Fig. 2). These livers were graded 4 to 5+ with an average of 4.4+ (Table I).

The results from chemical analysis of the livers were in striking agreement with the other observations (Table I). The average total liver iron was 1.6 mg. with a range of 1.3 to 1.9 mg. in the control group on the basal diet, while it was 2.9 mg. with a range of 1.9-4.1 mg. in group II receiving the basal diet supplemented by ethionine. The average liver iron value was 2.1 mg. with a range of 1.6 mg. to 2.8 mg. in group III receiving the basal diet supplemented by 2 per cent ferric citrate, whereas it was 5.7 mg. with a range of 4.6 to 6.7 mg. in group IV receiving the same diet but to which was added the ethionine. Group V, which received the same basal diet as group I but in limited quantities, had average liver iron values of 1.6 mg. with a range of 1.4 to 2.0 mg.

Varying degrees of testicular degeneration were noted in all animals receiving dl-ethionine (16). In addition, the kidneys in 8 out of 16 animals showed degeneration and necrosis of the epithelium lining the convoluted tubules, while 6 out of 16 showed tubular regeneration. The renal lesions were similar to those described by Wachstein and Meisel (17).
Experiment 2.—There was no evidence of damage to the liver or pancreas in the group A mice which were fed the basal diet. The livers in this group were graded from 0-2+ for iron on histological examination and the average grade was 0.8+. The results of chemical analysis were in agreement with these findings (Table II). The average total liver iron was 0.5 mg. with a range of 0.4 to 0.6 mg. The average total iron for the entire body including liver was 1.9 mg. with a range of 1.5 to 2.1 mg.

In the mice in group B which were fed the basal diet supplemented with ethionine, the changes in the pancreas and liver were similar to those found in the rats fed ethionine. In contrast to the mice in group A, all livers were graded 5+ for iron (Fig. 3). The average liver iron value was 0.9 mg. with a range of 0.5 to 1.1 mg. The average total iron value for the entire mouse was 2.3 mg. with a range of 2.0 to 2.8 mg. (Table II).

TABLE II  
Effect of Ethionine on Liver Iron and Total Body Iron in the Mouse

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>No. of mice</th>
<th>Liver iron</th>
<th>Total body iron</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mg./100 gm</td>
<td>mg. mg.</td>
</tr>
<tr>
<td>A .... Basal ..................................</td>
<td>15</td>
<td>32.4</td>
<td>0.5</td>
<td>8.0</td>
</tr>
<tr>
<td>B .... Basal + 0.5 per cent dl-ethionine ....</td>
<td>12</td>
<td>79.0</td>
<td>0.9</td>
<td>5.0</td>
</tr>
</tbody>
</table>

DISCUSSION

It has been known for years that pancreatic damage commonly is present in hemochromatosis and Sheldon, in his review, states that “a cirrhosis of the pancreas occurs in at least 90 per cent of the cases” (18). In this connection it is of interest to note that Althausen et al. (19) found pancreatic function tests to be abnormal in six of fifteen cases of hemochromatosis. It has been generally assumed that pancreatic damage was one of the end results of hemochromatosis and was due either to (a) portal hypertension, consequent to hepatic fibrosis; or (b) destruction of pancreatic cells following the deposition of iron pigment. The possibility that excessive iron absorption might be the result rather than the cause of the pancreatic fibrosis has not been considered. Taylor and his coworkers (20), while studying the effects of pancreatectomy in cats, noticed that the livers of these animals contained large quantities of iron. This group then ligated the pancreatic ducts of cats (6) and concluded that iron was absorbed in large quantities because of damage to the epithelium
of the duodenum which they attributed to lack of vitamin A. Recent work indicates that in dogs iron is absorbed more readily when the pancreatic ducts are ligated (7). In these experiments the duodenal mucosa was intact.

In the present experiments there was a significant increase in the deposition of iron in the liver following damage to the exocrine cells of the pancreas. This increase occurred in the rats receiving dl-ethionine alone, since the average iron content was 35.3 mg. per 100 gm. of liver (group II), as contrasted to the control group (group I) in which the iron content was 14.6 mg. per 100 gm. of liver. When iron as well as ethionine was added to the diet (group IV) the liver iron values averaged 55.8 mg. per 100 gm. of liver. It is felt that the increase of liver iron after ethionine administration is not due to the liver damage, since the liver damage in group IV was not greater than in group II, yet the difference in the amount of liver iron in these two groups is striking.

In order to eliminate the possibility that the increase in iron in the liver was due to shift in body iron following loss in weight, seven rats (group V) were kept upon reduced rations so their weight would closely conform to those rats receiving ethionine. There was no increase in total liver iron in rats in this group. This indicated that the increase in liver iron following ethionine feeding was not from endogenous sources on the basis of weight loss.

The experiment using mice corroborated the results obtained in rats. There was an increase in the amount of iron in the livers of the mice suffering from pancreatic damage. The average iron content of the livers of the mice on the basal diet was 32.4 mg. per 100 gm. of liver, while the mice fed the same basal diet supplemented by ethionine had livers in which the iron content averaged 79.0 mg. per 100 gm. of liver.

The mouse experiment was designed primarily to determine if the elevation of the liver iron value was due to an actual increase in the amount of iron absorbed or was the result of a shift in iron to the liver from other body stores brought about by the ethionine. The results indicate that the increase in liver iron in the animals with pancreatic damage is due to increased absorption of iron and not to a shift in iron from endogenous sources (Table II). Chemical analysis showed that the mice receiving a diet lacking in ethionine had an average of 1.9 mg. of total body iron (8 mg. per 100 gm.) whereas the mice on ethionine supplemented diet contained on the average 2.3 mg. of iron (12.7 mg. per 100 gm.). It follows, then, that the increase in total body iron was due to increased absorption of iron from the gastrointestinal tract.

**SUMMARY**

Groups of rats were fed a diet of the purified type which was supplemented by dl-ethionine to produce pancreatic damage. It was shown that animals with pancreatic damage absorbed more iron from the gastrointestinal tract than control animals without pancreatic damage. When iron was added to the
diet the animals with pancreatic damage absorbed even greater quantities of iron. These findings in rats were corroborated by similar findings in mice.

BIBLIOGRAPHY


EXPLANATION OF PLATES

PLATE 20

Fig. 1. Pancreas. Rat received 0.5 per cent dl-ethionine in diet for 28 days (group II). Note the decrease in the number of acini and the damage to the remaining acini. Hemotoxylin and eosin. X 185.

Fig. 2. Rat liver stained for iron. Basal diet with 0.5 per cent dl-ethionine and 2 per cent ferric citrate for 28 days (group IV). Note the marked pigment deposition in the liver cells and Kupffer cells. This was graded 5+. Prussian blue reaction. X 185.
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PLATE 21

Fig. 3. Mouse livers stained for iron. Prussian blue reaction, × 278. Fig. 3 A. Control on basal diet for 28 days (group A). Note absence of histologically demonstrable iron. Fig. 3 B. Basal diet with 0.5 per cent ethionine for 28 days (group B). Note abundant pigment in parenchymal and Kupffer cells.