Iron Deposition in Tissues*

J. V. KLAVINS, M.D., PH.D.

Department of Pathology,
The Catholic Medical Center of Brooklyn and Queens, Inc.,
Jamaica, NY 11432

ABSTRACT

Among clinical conditions with increased tissue iron, idiopathic hemochromatosis is unique. It is associated with increased absorption and iron deposition in all tissues. Experimental models to study iron absorption and deposition in tissues may provide information on the processes associated with excess iron uptake. Low protein high fat diets induced excess iron deposition in liver of rats. When diets contained a balanced amount of fat, 15 to 18 percent of protein was necessary for an adequate iron uptake. Part of this protein effect could be explained on the amino acid facilitated iron absorption from the gastrointestinal tract. Both excess and deficiency of methionine induced iron deposition in liver. In methionine excess, there was also iron deposition in spleen associated with hemolytic anemia. Increased amounts of iron in liver were induced also by homocystine and excess cystine and in renal tubules by excess dietary serine. Using ethionine feeding as an experimental model, it was established that increased liver iron deposition was associated with an increased tissue affinity for iron.

Such increased affinity for iron may be associated with idiopathic hemochromatosis also. While in idiopathic hemochromatosis the tissue siderosis is irreversible, the dietary liver siderosis, induced by corn grits, was reversed after feeding a balanced diet. Liver siderosis and affinity for iron in ethionine-fed rats disappeared on prolonged ethionine feeding. Although ethionine induced inhibition of protein synthesis in general, ferritin synthesis was increased. Thus the tissue affinity for iron may be a reflection of an increased ferritin synthesis. It was suggested that ferritin synthesis is induced by iron at the level of translation. The genetic defect in idiopathic hemochromatosis may be associated with cell membrane permitting increased transfer of iron.

Clinical Conditions with Increased Tissue Iron

Excess iron accumulates as a result of abundant dietary content of iron, multiple blood transfusions, hemolytic anemias, infectious and liver diseases including alcohol. In these conditions, there is no generalized iron deposition in all tissues. It is localized in the liver and spleen as ferritin and hemosiderin.

In idiopathic hemochromatosis, iron is deposited in excessive amounts in all tis-
sues. This is a genetically determined disorder with the responsible gene located on the chromosomes of the sixth pair. In homozygotes, it is present on both chromosomes and in heterozygotes, on only one of them. The mechanism of increased iron absorption and deposition in tissues in idiopathic hemochromatosis is not known. It is possible that the absence of the gastric iron binding protein-gastroferrin in patients with idiopathic hemochromatosis results in a greater availability of iron for the absorption from the gastrointestinal tract. More recently, it has been suggested that an imbalance between chelatable iron and ferritin synthesis in the epithelium might result in an increased iron absorption.

**Experimental Models to Study Iron Absorption and Deposition in Tissues**

**Effects of Dietary Fat and Protein**

Feeding rats a diet consisting of 10 percent casein and 60 percent lard for four weeks resulted in an increased iron deposition in liver. The histologically demonstrable iron in liver was present predominantly in the hepatocytes resembling that seen in idiopathic hemochromatosis. In other organs, there was no histochemically demonstrable iron. In addition, there was shrinking and distortion of pancreatic acini.

Since hemoglobin values did not differ significantly among the animals on low-protein, high-fat diets and those on control balanced diets receiving 18 percent casein and 10 percent lard, increased liver iron content in the group on low-protein high-fat diet was interpreted as an increased iron absorption. Pancreatic acinar damage or dietary factors per se could have contributed to the increased iron absorption and deposition in the liver. Therefore, the effects of dietary protein were examined further.

Feeding protein free diets did not induce an increased iron absorption and deposition in liver. On the contrary, animals on a protein free diet absorbed significantly less iron than the control rats. Approximately 15 to 18 percent of protein was necessary in the diet to maintain an unimpaired iron absorption and deposition in liver. Liver iron values reflected iron absorption except in animals on five percent protein diets without iron supplement. In this group, liver iron values were higher than in animals on 10 percent protein, while there was no significant difference in the total body iron or hemoglobin values.

The reason for the shift of iron to the liver in animals on low protein diets was not apparent. The process of protein enhanced iron absorption and deposition in tissues is also not known. A significant factor could be the amino acids released from the dietary protein. They facilitate significantly the absorption of iron from the gastrointestinal tract.

**Effects of Amino Acids**

Single amino acids and $^{59}$FeSO$_4$ were introduced in isolated loops of small intestine to measure the uptake of iron in serum and liver in rats. All eight amino acids studied (L-methionine, L-proline, L-phenylalanine, L-serine, L-glutamic acid, L-asparagine, L-histidine and L-glutamine) induced an increase of serum iron and deposition in liver. These effects were interpreted as an increased absorption and not as a shift in iron distribution, since both the serum and liver iron values were elevated. $^{59}$FeSO$_4$ activity in liver was four to five times larger when iron was administered with an amino acid then with phosphate buffer. The amino acids may facilitate the absorption of iron by chelation.

Dietary deficiency of methionine was associated with the shift of iron to the liver. In methionine deficient rats, mean
total liver iron was 1.35 mg, while in pair-fed control animals on a balanced diet this value was 0.78 mg. Histochromically, increased iron deposits were demonstrated diffusely in livers of all rats on methionine deficient diets. The mean total body iron excluding gastrointestinal tract in methionine deficient animals was 3.98 mg and in controls 5.04 mg, indicating impaired iron absorption in animals on methionine deficient diets. The shift of iron to liver in methionine deficient animals could be attributed to a decrease in hemoglobin synthesis since the hemoglobin concentration was significantly lower than in the control rats.

Dietary excess of sulfur containing amino acids induced iron deposition in liver or in liver and spleen. Increase of tissue iron in these conditions was associated with anemia. Therefore, this iron deposition reflects a shift to tissues of unutilized iron for hemoglobin synthesis or iron from the breakdown of the red blood cells.

In methionine, excess increased amounts of iron were demonstrated histochromically in spleen and in Kupffer cells, predominantly in portal areas. There was no evidence of increased iron absorption. In addition to histologic alterations of the pancreas, gastrointestinal tract, salivary glands, kidneys, spleen, thymus, thyroid and adrenal glands, the rats on methionine excess diets developed a hemolytic anemia. Thus the shift of iron to the liver and especially to the spleen can be attributed to hemolysis. Excess iron deposition in the spleen of methionine fed rats was diminished when arginine or glycine or both were added to the diets. These amino acids did not modify iron deposition in the liver. Serine had no effect on liver or splenic iron of rats on methionine diets.

Homocystine, the intermediary metabolite of methionine induced histochromically demonstrable increased iron deposition in liver cells adjacent to portal areas. The iron deposits in the hepatocytes were concentrated around the bile canaliculi. Only a few Kupffer cells around the central veins contained iron granules. There was no excess iron deposition in the spleen in contrast to rats fed methionine excess, when large amounts of iron were present in splenic tissues. It is possible that iron deposition in liver in homocystine fed rats is the result of a different hematologic disorder than in that associated with methionine excess. Equimolar amounts of serine added to homocystine containing diets prevented significantly the iron deposition in the liver.

Excess serine containing diets induced iron and calcium deposition in the distal portion of proximal convoluted tubules of kidneys. The deposits were partially prevented by equimolar administration of homocystine. It is possible that homocystine and serine interactions lead to the formation of cystathionine, thus eliminating singular effects of these compounds on iron deposition and tissue damage.

Cystine excess was associated with some periportal iron deposition in hepatocytes and macrophages.

Tissue Affinity for Iron

Xanthine oxidase activity has been related to release of iron from ferritin, the iron storage protein. Increased iron absorption and deposition in liver was associated with the decreased liver xanthine oxidase activity when rats were fed 0.5 percent ethionine containing diet. However, the changes in xanthine oxidase activity could not be correlated with all the liver iron values. It was demonstrated by us that in lowering liver xanthine oxidase activity by sodium tungstate there was no alteration in iron content of liver.

The effects of increased iron absorption and deposition in tissues can be attributed to an increased affinity of tissues for iron. This was demonstrated in an experimen-
tal model feeding rats with a diet containing 0.5 percent DL-ethionine. Intravenously administered $^{59}$Fe was deposited significantly more in the livers of the rats fed ethionine for one week then in the control animals. This increased iron deposition was associated with an increase of diastase resistant periodic acid-Schiff (PAS) positive material in hepatocytes.

Serum iron changes resembled those of idiopathic hemochromatosis. There was an increase in serum iron, a decrease in total iron-binding capacity and an increased saturation of transferrin. The increased liver uptake of iron was not a shift as a result of saturated transferrin. It was a result of an increased tissue affinity for iron.

There was an increased uptake of iron in vitro when liver slices of ethionine treated rats were incubated with $^{59}$Fe. Tissue affinity for iron represents a significant factor in the rate of iron deposition with a secondary absorption from the gastrointestinal tract.

**Reversibility of Tissue Iron Content**

Body iron content reflects the absorption and very little iron is excreted. However, hepatic siderosis can be reversed in several conditions. Dietary hepatic siderosis was induced by feeding rats for six weeks with 79 percent corn grit, 19 percent lard and two percent iron citrate containing diet. When this diet was replaced with Purina Chow after five months, the liver iron values reversed to normal.

Reversal of hepatic siderosis was observed in rats fed ethionine. This occurred after 15 months of ethionine feeding and coincided with the pattern of the hepatic affinity for iron. There was an increase of liver iron and the affinity for it after the first week of ethionine administration. Between three and four weeks, these values decreased to the level of control animals. By the end of the 15 months experimental period, the liver affinity for iron and content of it decreased in ethionine fed rats below the values of the control animals. It is possible that some of the iron may leave liver by migration of iron containing Kupffer cells into the hepatic vein. Iron laden cells were observed in centrilobular and hepatic veins at six weeks of ethionine feeding when the liver iron values decreased from previous intervals.

**Ferritin**

Iron storage in tissues occurs predominantly in two forms, a water insoluble hemosiderin and water soluble ferritin. The nature of hemosiderin has not been clearly defined, while some properties of ferritin are well known.

Water soluble fraction, presumably ferritin, constituted 78 percent of the total liver iron of ethionine fed rats. The amount of liver iron was significantly higher than in control animals. Thus, it was suggested that while ethionine may inhibit general protein synthesis, the ferritin synthesis may be uninhibited or even stimulated. The presence of ferritin in ethionine fed rat livers was confirmed electron microscopically. Most of the ferritin was localized in aggregates surrounded by a single membrane adjacent to bile canaliculi. These siderosomes corresponded to histochemically demonstrable pericanalicular iron granules in a diastase resistant periodic acid-Schiff positive (D-PAS) material.

In a preliminary report, it was indicated by us that ethionine induced an increased ferritin synthesis. For six days, six male albino rats were fed a synthetic diet containing 0.5 percent DL-ethionine. At the end of the experiment, three animals received one mg of iron intraparitoneally, as ferric ammonium citrate, per 100 g of body weight. Corresponding control animals received a basal diet without the ethionine supplement. Average liver iron
values in ethionine fed animals were significantly higher than in control rats.

In animals receiving iron intraperitoneally, ethionine containing diet did not induce significantly greater iron deposition in liver than in control rats (table I). Iron administration did not significantly increase its content in liver of ethionine fed rats, while in control animals this increase was significant. Evidently ethionine exerts maximal iron concentration in the liver cells and additional iron does not stimulate additional deposition. In all animals, 50 percent or more of the total iron was present as ferritin (table I).

There was a significantly increased ferritin synthesis in the liver of ethionine fed rats (table II), while the synthesis of total liver proteins was inhibited. There was no further induction of ferritin synthesis by iron in ethionine fed rats. Total protein synthesis in these animals was inhibited by an additional iron administration.

The finding that total liver protein synthesis in ethionine fed rats is inhibited by iron administration (table II) raises the repeatedly expressed views concerning tissue iron toxicity. It is possible that iron may exert toxic effects in pathologically altered conditions, while in the normal organism, excess iron does not produce damaging effects. In control rats, there was no inhibition of protein synthesis when additional iron was administered intraperitoneally.

These experiments demonstrate that increased iron levels in liver in ethionine fed animals cannot be attributed to shift of body iron. Hemoglobin and hematocrit values by the end of the six day experiment were significantly higher in ethionine fed animals than in the control rats (table III). It is not known whether or not there was some hemococoncentration in ethionine fed animals. They ate less and lost body weight although liver weights (table III) remained unaltered. During the same period, the control rats fed the basal diet ad libitum gained weight. Increase of liver iron appears to be the result of an increased ferritin synthesis.

This is an additional example in the regulation of protein synthesis where all the proteins do not follow uniformly the inhibition or stimulation of their synthesis by different agents. The inhibition of protein synthesis in general and stimulation of ferritin synthesis by ethionine suggest that ferritin may be synthesized on a relatively stable m-ribonucleic acid (m-RNA).

The tissue affinity for iron in rats fed ethionine appears to be associated with increased ferritin synthesis. It is possible that in the idiopathic hemochromatosis, the genetic disorder is associated with increased affinity of tissues for iron, an

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Mean Liver Iron Values</th>
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<tbody>
<tr>
<td><strong>Group, Diet and Number of Rats</strong></td>
<td><strong>Total Liver Iron mg/liver/100 g of Body Weight</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Fe Injected i.p.</strong></td>
</tr>
<tr>
<td>I Ethionine</td>
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<tr>
<td>II Basal</td>
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*Indicates lines separating significantly different values (p < 0.05).

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Mean Values of Protein Synthesis (cpm/liver/100 g Body Weight x 10^-3)</th>
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<tbody>
<tr>
<td><strong>Group, Diet and Number of Rats</strong></td>
<td><strong>Ferritin</strong></td>
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<td>I Ethionine</td>
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<td>II Basal</td>
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*Indicates lines separating significantly different values (p < 0.05). Two hours after rats were injected with iron all animals received intraperitoneally 5 μC of DL-leucine -l-14C per 100 g body weight. Two hours later, all rats were killed by a blow on the head and the radioactivity determined in isolated proteins.
increase of ferritin synthesis and iron deposition in tissues with a secondary increased iron absorption.

Ferritin synthesis is associated with free ribosomes. Ferritin molecules did not appear within the cavities of the endoplasmic reticulum immediately after induction of ferritin synthesis by iron administration. They were localized outside of these profiles. In this respect, ferritin belongs to the group of proteins which are not transferred outside of the cells in any appreciable amount.

It is attractive to speculate that ferritin synthesis is induced at the unbound relatively stable m-RNA by iron affecting the nascent peptide. The free m-RNA was estimated to measure 1600 Å to accommodate the codons for 195 amino acid residues of a ferritin subunit. This length of m-RNA can accommodate at one time no more than seven ribosomes. For all subunits, if they were to be synthesized on a single m-RNA, polysomes of 140 ribosomes should be present. Such polysomes have not been observed.

If ferritin synthesis is dependent only on the presence of iron at the sites of translation of m-RNA, then the excess iron deposition of tissues would depend on the transfer of iron across the cell membranes. Idiopathic hemochromatosis, therefore, could be associated with a defect in cell membranes permitting transfer of excessive amounts of iron.

### TABLE III

<table>
<thead>
<tr>
<th>Group, Diet and Number of Animals</th>
<th>Hemoglobin</th>
<th>Hematocrit</th>
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*Indicates lines separating significantly different values (p < 0.05).

References