A STUDY OF IRON-INDUCED LIVER DAMAGE*

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The role of iron storage in the liver damage in the various conditions where cirrhosis and massive siderosis coexist is as yet unsettled, despite considerable accumulating evidence that such storage plays a critical role in the pathogenesis of these lesions (1, 2).

The possibility that different hepatic insults may potentiate or augment their individual effects, with a resultant damage greater than would have resulted from either alone, is often considered as a possible explanation for hepatic diseases of obscure origin. The validity of this concept, however, has seldom been critically investigated (3).

These two problems have been studied by means of examining the histochemistry of the iron-loaded liver and by determining the effect of iron loading on the response of the liver to a series of hepatic toxins whose mechanisms of toxicity are quite well known.

Materials and Methods

Mice of the Great Ormond Street strain were used. At the beginning of the injection period, which lasted approximately 3 weeks, the animals were 2 to 3 months of age. Iron was administered as iron-dextran (imferon) in divided doses to a total dose of 1000 mg/kg. Carbon tetrachloride was administered in a single dose of 0.01 ml in olive oil subcutaneously. Thioacetamide was administered in one dose of 200 mg/kg subcutaneously. Bromobenzene was administered in a single dose of 0.05 ml/100 gm body weight in olive oil after 12 hours. Intervals of sacrifice varied with toxin administered. Only among the animals given bromobenzene did spontaneous deaths occur. Animals were killed by cervical dislocation, and slices of liver were fixed in 10 per cent buffered formalin, Regaud's fluid, and Lillie's acetic alcohol formalin fixative. Other slices were frozen at −76°C for histochemical and chemical studies. The stains employed included hematoxylin and eosin, Regaud's method for mitochondria, Giemsa for cytoplasmic basophilic substance (RNA), Perls' reaction for stainable iron, periodic acid-Schiff (PAS) reaction before and after digestion with saliva, Best's carmine for glycogen, and Pearse's modification of the Ziehl-Neelsen stain (4). Enzyme histochemical procedures were those of Pearse for succinic dehydrogenase using 3-(4,5 dimethyl-thiazolyl-2)-2,5 diphenyl tetrazolium bromide (4), Scarpelli *et al.* for DPN and TPN diphorase (5), and Chiquoine (6) for glucose-6-phosphatase. Glucose-6-phosphatase was determined chemically by a modified method of Swanson (7).

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RESULTS

Animals without Added Insult.—Table I records results obtained on examination of the various histologic parameters applied to the livers of iron-loaded and control animals without additional insult.

The only differences noted between the two groups of animals were (*a*) a reduction of glucose-6-phosphatase in iron-loaded animals and (*b*) the occurrence of PAS-positive acid-fast parenchymal cytoplasmic droplets. These droplets were not seen in animals injected with comparable doses of dextran.

TABLE I

Reactions of Uninsulted Livers

Reaction	Animals examined	Control	Iron-loaded
Succinic dehydrogenase	8 × 2	Normal	Normal
TPN Diaphorase	8 × 2	Normal	Normal
DPN Diaphorase	8 × 2	Normal	Normal
Glucose-6-phosphatase	8 × 2	Normal	Diminished
Neutral fat	8 × 2	+ to ++	+ to ++
Mitochondria	8 × 2	Normal	Normal
Iron	8 × 2	0	+++
PAS droplets	8 × 2	Very rare	+

TABLE II

Response of Hepatic Glucose-6-Phosphatase to Iron Loading

Control	Iron-loaded	
6 animals, 1.27 0.717 to 1.689 (range)	16 animals, 0.566 0.151 to 1.278 (range)	0.01 *P* 0.001

P determined by t test.

The histologic depression of glucose-6-phosphatase in the livers of iron-loaded animals was confirmed chemically (Table II). Certain aspects of the dynamics of this response are interesting, and will be discussed elsewhere.

Iron-Loaded and Control Animals with Added Insult.—Qualitatively, the hepatic changes with the administration of carbon tetrachloride, thioacetamide, and bromobenzene were essentially similar to those which have been described (8–10). Quantitatively, however, one significant difference was observed between the two groups.

With carbon tetrachloride and thioacetamide, the reaction of the liver was essentially similar whether or not the organ was iron-loaded. With bromobenzene, however, a distinct difference in reaction was seen, the iron-loaded

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livers demonstrating consistently more severe damage than those in the control group (Table III).

In the iron-loaded animals the histochemical changes were much more marked than in similarly loaded animals without bromobenzene. The administration of bromobenzene also caused a decrease in the histochemical glucose-6-phosphatase activity in control animals. As Table IV indicates, however, this histochemical loss was not reflected in chemical values obtained

TABLE III

Hepatic Reactions after Bromobenzene Administration

Loss of	Animals examined	Control	Iron-loaded
Succinic dehydrogenase	6	+	++⊥
RNA	6	⊥	++
Glucose-6-phosphatase	6	+	++⊥
DPN Diaphorase	6	⊥	+⊥
TPN Diaphorase	6	⊥	+⊥

TABLE IV

Hepatic Glucose-6-Phosphatase Levels with and without Bromobenzene

	Without bromobenzene	With bromobenzene
Control	(6 animals) 1.27 0.717 to 1.689	(6 animals) 1.52 1.048 to 1.8
Iron-loaded	(16 animals) 0.566 0.151 to 1.278	(13 animals) 0.731 0.304 to 1.46

All glucose-6-phosphatase values expressed as milligrams phosphate per gram liver per hour.

from these same animals, there being in fact a slight elevation following bromobenzene administration in both groups.

DISCUSSION

Biochemical Effect of Hepatic Storage Iron.—Under the conditions of the experiments there was no synergism, in terms of hepatic damage, between iron and either carbon tetrachloride or thioacetamide.

The findings of an apparent synergism between bromobenzene and storage iron in terms of hepatic damage is most interesting. Bromobenzene toxicity is recognized as resulting from a "conditioned deficiency" (Popper) of sulfo-

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amino acids (10). Goldberg has demonstrated that cirrhosis following chronic ethionine feeding develops much more rapidly in iron-loaded as compared to control animals (11), and evidence has been presented indicating that the hepatotoxic effect of ethionine is due to an ability to diminish available sulfo-amino acids (12). The accumulating evidence thus suggests that the ability to reduce the number of effective SH groups is a critical hepatotoxic property of storage iron. This is compatible with the demonstrated ability of iron and sulfhydryl donors each to reverse the effects of the other in a biologic system (13). It is possible that this is due to the pro-oxidant properties of storage iron which have been stressed by Goldberg (14). Since glucose-6-phosphatase is not sulfhydryl-dependent, additional as yet undefined biochemical effects of storage iron are indicated by the constant reduction of the enzyme.

The presence of a marked alteration in histochemical glucose-6-phosphatase, without a marked alteration in the chemical values for this enzyme, in animals to whom bromobenzene was given, most likely represents microsomal damage sufficiently acute and severe to allow diffusion of glucose-6-phosphatase to a level at which it was no longer histochemically demonstrable, even though chemical values had not altered. This is an example of an instance in which histochemistry is a more sensitive index of cellular integrity than chemical analysis.

Site of Toxic Iron Action.—It is well known that there is considerable specificity in the localization of biochemical activities within the liver cell. The findings in these experiments thus shed some light on the sensitivities of the various subcellular units to the toxic effects of storage iron. Negatively, the finding in the iron-loaded but otherwise uninsulted animals, of normal mitochondria (morphologically as revealed by the light microscope, and functionally as demonstrated by the normal pattern of succinic dehydrogenase, TPN diaphorase, and DPN diaphorase) and the lack of enhancement of CCl_4-induced damage in iron-loaded animals, indicates that the mitochondria are relatively resistant to any direct toxic effect of storage iron. On the other hand, considerable sensitivity of extra-mitochondrial structures is indicated by the reduction of glucose-6-phosphatase, an enzyme which is localized in the microsomes (15), and by the occurrence of periodic acid-Schiff–positive, non-digestible droplets, which are probably closely related to the lysosomes (16, 17). It is of interest that a synergist with storage iron, ethionine, also causes abnormality in an extra-mitochondrial locus, *i.e.*, the endoplasmic reticulum (18), and also that biochemical studies of the iron-loaded liver, published by Goldberg *et al.* while the present work was in progress, have suggested an effect of storage iron on extra-mitochondrial structures (19).

That storage iron may under certain conditions contribute to mitochondrial dysfunction is demonstrated in the excessive reduction of succinic dehy-

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drogenase activity in iron-loaded animals to whom bromobenzene was given. It is not possible to state whether this is a direct and primary effect on the mitochondria under the conditions of great demand on available sulfhydryl groups, or whether it represents a secondary effect due to disturbances primarily in extra-mitochondrial structures. The fact that succinic dehydrogenase loss was as great as glucose-6-phosphatase loss under these conditions suggests a direct mitochondrial effect.

Concept of Complementary Liver Insults.—The failure of a substance, in this case storage iron, to enhance the effects of certain hepatotoxins while enhancing those of others, suggests that relatively specific biochemical relationships may be requisite for a synergism between hepatotoxins. Such a concept is supported by the finding that specific hepatotoxic dietary deficiency does not appear to increase mortality secondary to virus infection (20).

Importance of Findings to Iron Storage Diseases.—The results demonstrate that storage iron is not inert in terms of an effect on the tissues in which it is stored. They also demonstrate, however, in the relative scarcity of histochemical changes, the subtle nature of short term iron-induced liver damage in the unstressed liver. This property is also indicated by the failure to find demonstrable abnormality in the respiration of slices of iron-loaded liver (11). Readily discernible effects of tissue storage iron are seen only under the added stress of a complementary insult, or, as appears to be the case in certain human diseases, when a critical level of intracellular iron is reached and maintained. It is probable that this character of iron-induced liver damage is responsible for the numerous failures to create hemochromatosis in the laboratory animal (21), 22).

If further experiments confirm that hepatic storage iron reduces the number of effective available hepatic sulfhydryl groups and demonstrate that this effect is chronically operative, it would then seem reasonable to consider that the supplementation of the sulfoamino acid pool in individuals with conditions which predispose them to hepatic siderosis would be of specific value in the arrest or prevention of the liver damage so frequently seen.

SUMMARY

The nature of short term iron-induced liver damage and its effect on the hepatic damage induced by other toxins have been studied by the use of histochemical techniques. The results suggest that a reduction of effective available sulfhydryl groups is a critical hepatotoxic property of storage iron. A reduction of glucose-6-phosphatase, consistently found in iron-loaded animals, demonstrates the sensitivity of the microsomes to the presence of storage iron. The mitochondria appear to be less sensitive, but may be affected under certain conditions. The results suggest that in order for simultaneously acting liver

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insults to result in additive damage, the mechanisms by which they act must have a critical and relatively specific relationship.

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