Rapid Induction of Iron Deposition in Spleen and Liver with an Iron-Fructose Chelate.* (27426)

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Recently we reported on the chemical properties of chelates of iron with fructose, other reducing sugars and polyols, all of which were extremely stable and soluble over a wide range of pH encountered in the gastrointestinal tract(1).

The rapid absorption of these complexes through washed, intact sections of rabbits' small intestines was measured by continuous monitoring of the radioactivity of the blood, using Fe^{59} as a tracer. Thus a new hypothesis concerning iron metabolism was indicated regulated by chelation and equilibrium binding(2,3).

Several disease states are characterized by abnormal absorption of dietary iron, *i.e.*: hemachromatosis, refractory anemia and Bantu cytosiderosis. Bantu cytosiderosis is a nutritional disease related to a high carbohydrate, low protein and a high iron diet(4). A similar cytosiderosis can be produced in rats on such a diet(5). It seemed possible that the enhanced iron absorption could be directly related to the absorption of the iron-sugar complex formed *in situ* by these diets.

The experiments presented here were designed to compare the dietary efficacy of $FeSO_4$ and ferric fructose for introducing iron into the liver and spleen of the rat.

Methods. Ferric-fructose was prepared by dissolving 1.21 g of $FeCl_3 \cdot 6H_2O$ and 80 g of fructose in about 400 ml of distilled water. The pH was adjusted to 7.0 by rapid addition of 6N KOH, and the volume taken to 500 ml with distilled water. Six-week-old male 70-100 g albino rats, of the Wistar strain, were obtained from 4 litters. Three groups of 8 animals were selected at random and placed on the following schedule:

8:00 A.M.	Withdraw food
10:00 A.M.	Introduction of 1.0 ml of solution by stomach tube
3:00 P.M.	Introduction of 1.0 ml of solution by stomach tube
4:30 P.M.	Replace food

Food was removed prior to and replaced after the iron administration in order to minimize competitive interaction of the dietary components with the metal. Food was provided *ad libitum* in the form of Wayne Lab Blox. Water was available at all times. The control animals, Group I, were stomach tubed with distilled water. Group II received a solution of $FeSO_4$ containing 0.5 mg Fe/ml, prepared under nitrogen immediately prior to each intubation. Group III received a solution of ferric-fructose containing 0.5 mg Fe/ml.

Three animals were maintained on the diet for 19 days; 5 animals for 33 days. Their weights were recorded daily to check over-all rate of growth. The animals were sacrificed under ether, and spleens and livers were removed and weighed. Total iron was determined by an adaptation of the method of Peters and Giovanniello using bathphenanthrolene as the color reagent(6). Weighed aliquots of the tissues were ground with sand in concentrated HCl prior to reduction with thioglycolate and development of the color.

Hematoxalin and eosin stained tissues were studied histologically and hemosiderin was demonstrated with the Perl's acid ferrocyanide reaction(7).

Results and discussion. The iron analyses for the various livers and spleens of the animals sacrificed at the end of 19 days and at the end of 33 days are presented in Table I. There is a striking enhancement of the deposition of iron within the livers and spleens by the ferric-fructose within the initial 19 days. At the end of 33 days the ferric-fruc-

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TABLE I. Average Iron Concentration in Livers and Spleens from Rats on Control (Distilled Water), $FeSO_4$ and Ferric-fructose Supplemented Regimens.

Condition	Days	Animals	Liver μg Fe/100 mg wet wt	Liver Δ*	Spleen μg Fe/100 mg wet wt	Spleen Δ*
Control	19	3	11.5 (± .5)		15.4 (± 3.1)	
$FeSO_4$	19	3	12.3 (± 2.4)	.8	32.9 (± 3.0)	17.5
Ferric-fructose	19	3	18.4 (± 2.0)	6.9	57.4 (± 8.2)	42.0
Control	33	5	10.1 (± 1.2)		12.5 (± 1.8)	
$FeSO_4$	33	5	19.5 (± 4.8)	9.4	28.3 (± 7.8)	15.8
Ferric-fructose	33	5	28.6 (± 5.3)	18.5	36.2 (± 4.5)	33.7

* Net increase over control (μg Fe/100 mg wet wt).

tose was noted to be more effective in depositing iron.

Table II shows the results of microscopic examination of iron-stained tissues of 33-day animals expressed on an arbitrary scale of 4+. There is obvious increase in hemosiderin which closely parallels the quantitative iron determinations by the colorimetric technic. No pathological changes similar to those observed in iron storage diseases could be detected in these animals.

The most interesting observation made in these experiments is that iron can be moved from the lumen of the intestine across the mucosal wall and ultimately into the iron stores of the liver and spleen in a relatively short period when the iron is presented to the system as a soluble low molecular weight complex. The particular chemical and physical properties of ferric-fructose are such that, once the complex is formed at pH 3.5 or above, it is extremely stable and will readily enter into the mucosal cells. $FeSO_4$ is also effectively utilized by the system since the mucosal cell secretes an endogenous ligand (as yet unidentified) which is capable of combining with Fe^{++} and not with Fe^{+++}(3). It appears from our studies that either the amount of the endogenous ligand is insufficient to effectively complex all of the Fe^{++} fed, or that the rate of its transport across the gut wall is less than that of the ferric-fructose.

TABLE II. Evaluation of Average Amount of Stainable Iron Observed in Sections of Livers and Spleens from Animals on Control, $FeSO_4$ and Ferric-fructose Regimens for 33 Days. Five animals in each group; units on arbitrary scale of 0 to 4.

Condition	Liver	Spleen
Control	.3	.8
$FeSO_4$	.8	1.0
Ferric-fructose	.9	2.0

The chemical conditions encountered in the lumen of the small intestine immediately distal to the stomach are particularly favorable for chelation, since the high ratio of carbohydrate to iron and the gradual increase of pH from the acid region to neutrality are similar to those conditions found optimal for complex formation(1). The molecular basis for the iron storage in Bantu cytosiderosis and experimental storage of iron in rats can be explained by formation of a sugar chelate which is rapidly absorbed from the intestine and deposited as hemosiderin in the tissues.

Summary. The dietary effectiveness of an iron-fructose chelate was compared with $FeSO_4$ using the criteria of total iron analyses and histologic staining technics on liver and spleen of rats. The ferric-fructose was more rapidly absorbed and deposited in the tissues indicating the importance of chelation phenomena in metal transport, and suggesting a molecular basis for Bantu cytosiderosis and experimentally induced iron storage in rats.

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