

## Acute iron poisoning. Rescue with macromolecular chelators.

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### Research Article

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## Acute Iron Poisoning

### Rescue with Macromolecular Chelators

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#### Abstract

Acute iron intoxication is a frequent, sometimes life-threatening, form of poisoning. Present therapy, in severe cases, includes oral and intravenous administration of the potent iron chelator, deferoxamine. Unfortunately, high dose intravenous deferoxamine causes acute hypotension additive with that engendered by the iron poisoning itself. To obviate this problem, we have covalently attached deferoxamine to high molecular weight carbohydrates such as dextran and hydroxyethyl starch. These macromolecular forms of deferoxamine do not cause detectable decreases in blood pressure of experimental animals, even when administered intravenously in very large doses, and persist in circulation much longer than the free drug. These novel iron-chelating substances, but not deferoxamine itself, will prevent mortality from otherwise lethal doses of iron administered to mice either orally or intraperitoneally. Further reflecting this enhanced therapeutic efficacy, the high molecular weight iron chelators also abrogate iron-mediated hepatotoxicity, suppressing the release of alanine aminotransferase. We conclude that high molecular weight derivatives of deferoxamine hold promise for the effective therapy of acute iron intoxication and may also be useful in other clinical circumstances in which control of free, reactive iron is therapeutically desirable.

#### Introduction

Iron intoxication is an important cause of poisoning in young children. In 1987 there were 2,910 cases of poisoning by oral iron preparations and 14,000 additional cases of poisoning with iron-containing vitamins reported to the American Association of Poison Control Centers (1). The lethal oral dose of elemental iron is estimated to be 200–250 mg/kg. Because supplementary iron tablets contain up to 105 mg of elemental iron, the ingestion of several tablets can result in severe poisoning in a small child. The precise mechanism of acute iron toxicity is unknown but may derive from the known oxidative

reactivity of the metal. Indeed, the ingestion of iron and ascorbate, the combination found in many supplemental iron pills, may generate hydroxyl radicals in vivo (2). Hydroxyl radicals are extremely reactive and cause indiscriminate damage to biomolecules.

In the therapy of iron poisoning, the ingested iron must be removed by chelation because the human body has no efficient means of excreting absorbed iron. The current drug of choice for acute iron poisoning is deferoxamine (Desferal). Deferoxamine (DFO)<sup>1</sup> is a potent hydroxamate-type iron chelator isolated from *Streptomyces pilosus*, and is administered both intravenously and by gavage after acute iron poisoning (3). Historically, DFO represented a tremendous improvement over previous therapies for iron poisoning; the mortality associated with iron poisoning dropped from almost 50% to ~ 1% with the introduction of DFO treatment (4). Removal of unabsorbed iron from the gastrointestinal tract by orally administered DFO prevents further accumulation in the body. Intravenous infusion of DFO results in the formation of an iron chelate that is catalytically unreactive (5) and is more rapidly excreted by the kidneys. However, the dose of DFO which can be administered intravenously is limited by its adverse effects on blood pressure (6, 7). High dose bolus infusion of DFO has been associated with hypotension, apparently mediated by histamine release (8). In experimental animals, somewhat larger doses of DFO cause hypotension and death. Since iron poisoning per se also leads to hypotension (9), the additional hypotensive effect of high concentration DFO is cause for concern. In an effort to minimize the toxicity of deferoxamine, we have synthesized new, high molecular weight forms of this drug, by covalent attachment of DFO to polymeric carbohydrates such as dextran and hydroxyethyl starch. The efficacy of these new forms of DFO in alleviating the effects of acute iron poisoning has now been evaluated in vivo.

#### Methods

A model of acute iron poisoning was established in male Swiss-Webster mice (25–30 g) obtained from Biolabs, St. Paul, MN. DFO was obtained as the mesylate salt (Desferal; Ciba-Geigy, Inc., Basel, Switzerland); dextran as Rheomacrodex (a 10% wt/vol solution in normal saline; Pharmacia Fine Chemicals, Piscataway, NJ); hydroxyethyl starch as a 10% wt/vol solution in normal saline (Dupont Critical Care, Waukegan, IL).

*Preparation of conjugated DFO.* The complete description of the preparation and characterization of the DFO conjugates is reported

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1. *Abbreviations used in this paper:* ALT, alanine aminotransferase; CPK, creatine phosphokinase; DFO, deferoxamine; FO, ferrioxamine; HES, hydroxyethyl starch.

elsewhere.<sup>2</sup> Briefly, dextran or hydroxyethyl starch (in 10% solution wt/vol) was oxidized with 0.1 M sodium metaperiodate to yield reactive dialdehydes. After removal of low molecular weight reaction products using dialysis or diafiltration, the activated polysaccharides were reacted with 0.1 M DFO. The Schiff bases formed between the terminal amino group of the chelator and aldehyde groups on the polysaccharide were reduced with sodium cyanoborohydride. Remaining aldehyde groups were reduced with sodium borohydride. The high molecular weight conjugate was separated from reaction products by a second dialysis or diafiltration step. The presence of free DFO in the final preparation was determined by high pressure liquid chromatography (FPLC System; Pharmacia Biotechnology) using a Superose 6 gel permeation column. The concentration of free DFO in the final preparations of the polysaccharide-DFO conjugate was < 2%. The conjugates used in this report were dextran containing ~ 25% by weight of bound DFO and an hydroxyethyl starch derivative containing 15% by weight of bound DFO.

**Murine model of acute iron toxicity.** To establish the lethal intraperitoneal dose of iron in mice, ferrous sulfate was dissolved in 0.15 M sterile NaCl and varying doses (0.25–1.5  $\mu\text{mol/g}$  body wt) were injected in a volume of ~ 1 ml. Based on the results of these studies, 1.25  $\mu\text{mol/g}$  (the approximate LD<sub>90</sub>) was chosen as the intraperitoneal dose for acute iron toxicity. To evaluate the efficacy of various chelators, this dose of iron was followed within 2 min, or in the case of the delayed administration experiments 60 min, by an intravenous injection (via the tail vein) of ~ 1 ml of either polymer (10% solution of dextran or hydroxyethyl starch in normal saline), free DFO with polymer, or the polymer-conjugated DFO in normal sterile saline. The dose of free DFO, which was limited by the toxicity of the drug, was 0.15  $\mu\text{mol/g}$  body wt. Higher doses of DFO given as a bolus injection caused mortality even in the absence of iron. The conjugated DFO preparations were given at doses of 1.4  $\mu\text{mol/g}$  (DFO equivalents) in the case of dextran-DFO and 0.9  $\mu\text{mol/g}$  (DFO equivalents) in the case of hydroxyethyl starch-DFO.

For oral iron administration, ferrous sulfate was dissolved in deionized water and ~ 1 ml was administered by gavage. A dose of 10  $\mu\text{mol/g}$  (associated with ~ 70% mortality) was chosen as the appropriate dose for this model. This dose is in agreement with a previously reported LD<sub>50</sub> for oral iron in mice (10). The doses of polymer, free DFO and polymer or conjugated-DFO in the oral iron model were the same as previously described above for the intraperitoneal model. All injections were done in the morning, after which the animals were returned to their cages and observed at intervals throughout the day.

**Tissue injury assessed by serum alanine aminotransferase and creatine phosphokinase levels.** Serum levels of alanine aminotransferase (ALT) and creatine phosphokinase (CPK) were determined according to well established methods (11, 12). Serum ALT was measured by a linked enzyme assay in which the conversion of alanine to pyruvate by ALT was followed by determining the rate of pyruvate generation. CPK activity in serum was assessed by determining the rate of ATP generation from creatine phosphate and ADP.

**Statistical analyses.** The significance of differences in survival between groups 48 h after administration of iron was determined by Chi-square ( $\chi^2$ ). Significance of differences among the group's mean serum levels of ALT and CPK ( $\pm\text{SD}$ ) was determined using a one-way ANOVA. A nonpaired t test was used to assess the differences between groups (13). Statistical significance was set at  $P < 0.05$  and values are reported in the figure legends.

## Results

The relative toxicity of iron, DFO, ferrioxamine (FO), conjugated-DFO and conjugated-FO are compared in Table I. Note

2. Hallaway, P. E., J. W. Eaton, S. S. Panter, and B. E. Hedlund. 1989. Modulation of deferoxamine toxicity and clearance by covalent attachment to biocompatible polymers. *Proc. Natl. Acad. Sci. USA*. In press.

Table I. Acute Toxicity of Iron and DFO Preparations Administered to Mice by Various Routes

Agent	Route	Dose	Mortality
		$\mu\text{mol/g}$	%
Fe <sup>2+</sup>	Oral	10	80
Fe <sup>2+</sup>	IP	1	50
DFO*	IV	0.4	50
FO*	IV	0.9	50
Dextran-DFO*	IV	1.4	0
Dextran-FO*	IV	1.4	0

\* Data from footnote 2.

that, on a molar basis, intravenous DFO is approximately twice as toxic as intraperitoneal iron. It is also important that the DFO:iron chelate, FO, is itself quite toxic. By contrast, lethal doses for the dextran-DFO conjugate and for the iron-saturated dextran-DFO conjugate could not be established; both were well tolerated at doses up to 1.4  $\mu\text{mol}$  (DFO equivalents) per gram body weight. In fact, the amounts of these conjugates which cause mortality are so high that they may be lethal by virtue of causing acute hypervolemic and hyperoncotic effects.

When ferrous sulfate is administered intraperitoneally to mice, a sharp increase in mortality occurs at doses > 0.75  $\mu\text{mol/g}$  (Fig. 1 A). Based on these results, we elected to use an intraperitoneal dose of 1.25  $\mu\text{mol/g}$  (approximately the LD<sub>90</sub>) in further experiments. The dose of iron for acute oral toxicity was set at 10  $\mu\text{mol Fe/g}$  body wt (approximately the LD<sub>75</sub>) (Fig. 1 B).

The ability of conjugated-DFO to prevent mortality due to iron administered intraperitoneally is shown in Fig. 2. At an iron dose causing 90% mortality of animals given polymer or free DFO with unmodified polymer, treatment with conjugated DFO resulted in 100% survival. None of the animals exhibited any morbidity or mortality until sacrifice 1 wk later.

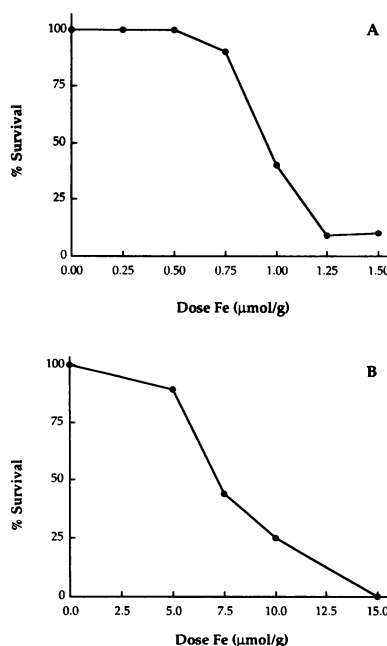
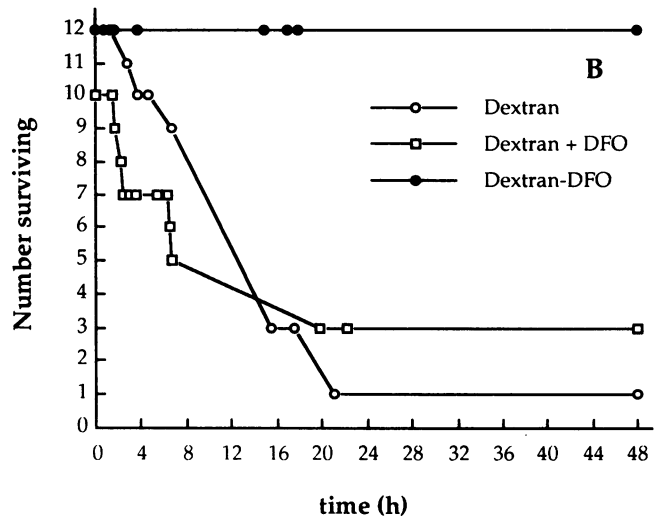
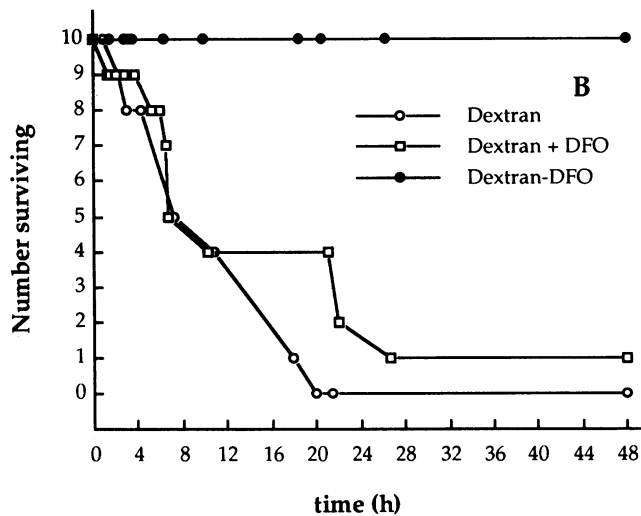
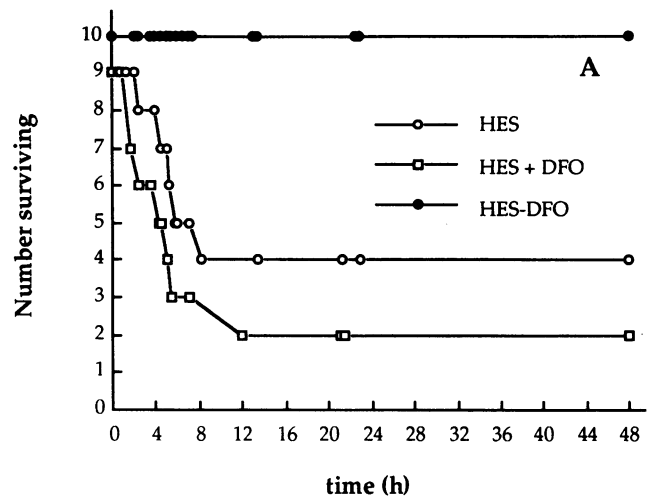
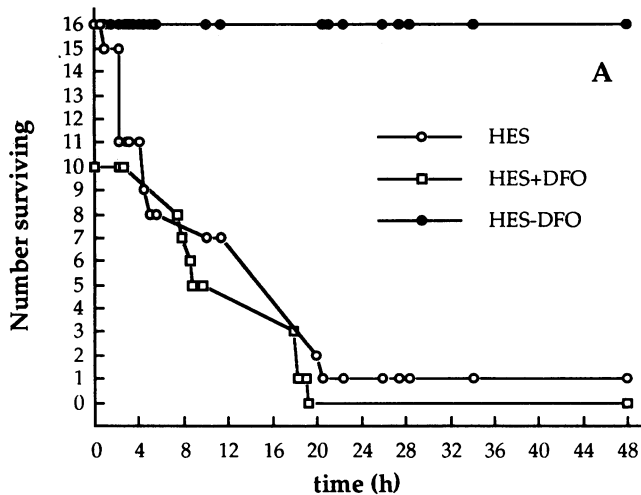


Figure 1. Percentage survival of mice 24 h after intraperitoneal (A) or oral (B) administration of the indicated doses of ferrous sulfate dissolved in deionized water for oral administration and in sterile isotonic saline for intraperitoneal injection. All doses were given in a volume of ~ 1 ml. Each point represents values obtained with 10 animals.



**Figure 2.** Mortality after injection of 1.25  $\mu\text{mol/g}$  body wt i.p. of ferrous sulfate immediately (within 2 min) followed by intravenous injection of hydroxyethyl starch (A) alone ( $\circ$ ,  $n = 16$ ), in combination with free DFO ( $\square$ ,  $n = 10$ ) or as DFO conjugates ( $\bullet$ ,  $n = 16$ ), or dextran (B) alone ( $\circ$ ,  $n = 10$ ), in combination with free DFO ( $\square$ ,  $n = 10$ ) or as DFO conjugates ( $\bullet$ ,  $n = 10$ ). Control animals were injected with 1 ml of either dextran or hydroxyethyl starch in sterile normal saline. Free DFO accompanied by dextran or hydroxyethyl starch in sterile normal saline was administered at a dose of 0.15  $\mu\text{mol DFO/g}$  body wt. Hydroxyethyl starch-DFO or dextran-DFO conjugates were injected at a dose of 0.9 and 1.4  $\mu\text{mol/g}$  i.v., respectively (DFO equivalents). At 48 h, the  $\chi^2$  for HES vs. HES + DFO = 0.65,  $P = 0.42$ ; HES vs. HES-DFO = 28.24,  $P < 0.0001$ ; HES + DFO vs. HES-DFO = 26,  $P < 0.0001$ . At 48 h, the  $\chi^2$  for dextran vs. dextran + DFO = 1.05,  $P = 0.3049$ ; dextran vs. dextran-DFO = 20,  $P < 0.0001$ ; dextran + DFO vs. dextran-DFO = 16.36,  $P < 0.0001$ .

**Figure 3.** Mortality after oral administration of 10  $\mu\text{mol/g}$  body wt of ferrous sulfate immediately (within 2 min) followed by intravenous injection of hydroxyethyl starch (A) alone ( $\circ$ ,  $n = 9$ ), in combination with free DFO ( $\square$ ,  $n = 9$ ) or as DFO conjugates ( $\bullet$ ,  $n = 10$ ), or dextran (B) alone ( $\circ$ ,  $n = 12$ ), in combination with free DFO ( $\square$ ,  $n = 10$ ) or as DFO conjugates ( $\bullet$ ,  $n = 12$ ). Ferrous sulfate was dissolved in deionized water and administered by gavage. Control animals were injected with 1 ml of either dextran or hydroxyethyl starch in sterile saline. Free DFO in hydroxyethyl starch or dextran in sterile normal saline was administered at a dose of 0.15  $\mu\text{mol DFO/g}$  body wt. Hydroxyethyl starch-DFO or dextran-DFO conjugates were injected at a dose of 0.9 and 1.4  $\mu\text{mol/g}$  i.v., respectively (DFO equivalents). At 48 h, the  $\chi^2$  for HES vs. HES + DFO = 1,  $P = 0.3173$ ; HES vs. HES-DFO = 12.3,  $P = 0.0004$ ; HES + DFO vs. HES-DFO = 7.54,  $P = 0.006$ . At 48 h the  $\chi^2$  for dextran vs. dextran + DFO = 1.25,  $P = 0.2636$ ; dextran vs. dextran-DFO = 18.28,  $P < 0.0001$ .

In the case of oral iron intoxication, once again the macromolecular forms of DFO are uniquely effective in preventing mortality (Fig. 3). Hydroxyethyl starch-DFO (Fig. 3 A) or dextran-DFO conjugates (Fig. 3 B), injected at a dose of 0.9 and 1.4  $\mu\text{mol/g}$  i.v. (DFO equivalents), respectively, completely prevented the mortality associated with oral administration of toxic amounts of iron. In contrast, treatment with polymers alone had no significant effect. Furthermore, the combinations of free DFO and polymers did not significantly improve sur-

vival. Mortality after treatment with DFO and hydroxyethyl starch or DFO and dextran was 78 and 70%, respectively. The effectiveness of the DFO-containing polymers is underscored by the fact that, in a total of 48 animals given iron orally or intraperitoneally and treated immediately with conjugated-DFO, there were no deaths.

To assess the effect of chelation therapy on acute tissue injury after iron poisoning, we measured the serum levels of ALT (a reflection of hepatic damage) and CPK (which pre-

dominates in cardiac, skeletal muscle, and brain). The animals were anesthetized with diethylether and the blood was removed by axillary incision 2–3 h after administration of iron and the appropriate drug or carrier. Serum ALT increased on average 72-fold after administration of intraperitoneal iron and intravenous injection of the dextran carrier (Fig. 4). Although treatment of iron-poisoned animals with free DFO slightly moderated this damage, administration of the dextran-DFO conjugate prevented most of this increment in serum ALT (Fig. 4). Interestingly, acute iron poisoning caused relatively little cardiac toxicity; serum CPK levels increased only two to threefold 2 h after iron administration (data not shown).

Because patients are rarely treated immediately after iron ingestion, the efficacy of the delayed administration of DFO-dextran 1 h after IP iron was determined. There was a significant improvement in survival at 48 h of the DFO-conjugate-treated animals when compared to dextran or free DFO and dextran administered without a delay (Fig. 5).

## Discussion

Acute iron intoxication is an important cause of life-threatening accidental poisoning in children. The sequelae of acute iron poisoning include hemoptasis, gastrointestinal bleeding, hypotension, renal failure, and liver damage (4). Currently, Desferal is the drug of choice for treatment of acute iron poisoning. Unfortunately, the utility of DFO is limited by the hypotensive effects of this drug, effects which are additive with those of iron itself. In fact, on a molar basis, IV DFO is even more toxic than iron given intraperitoneally (see Table I). Because both iron and free DFO cause hypotension, any therapy for acute iron intoxication which did not cause iatrogenic hypotension would be an improvement over the current treatment. These considerations prompted us to examine the possible therapeutic efficacy of a new class of iron chelators comprised of DFO covalently attached to high molecular weight

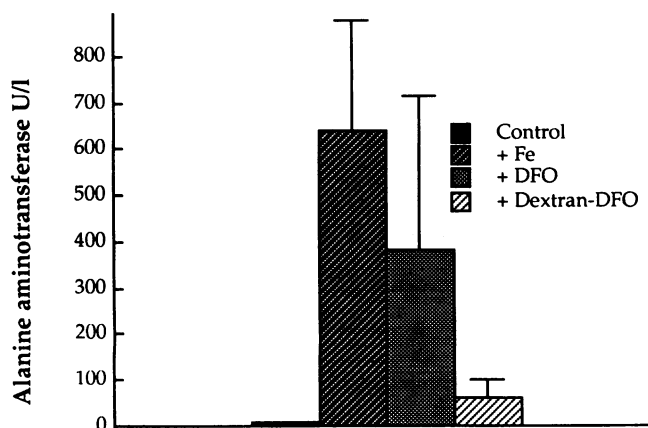


Figure 4. Serum levels of alanine aminotransferase in control mice and in iron-intoxicated mice treated without drug or with free DFO or dextran-DFO. One-way ANOVA was used to assess the differences among the groups' means,  $F = 7.621$ ,  $P < 0.003$ . Unpaired  $t$  test: Fe vs. control,  $P = 0.002$ ; Fe vs. dextran + DFO,  $P = 0.221$ ; Fe vs. dextran-DFO,  $P < 0.001$ ; dextran + DFO vs. dextran-DFO,  $P = 0.0004$ .

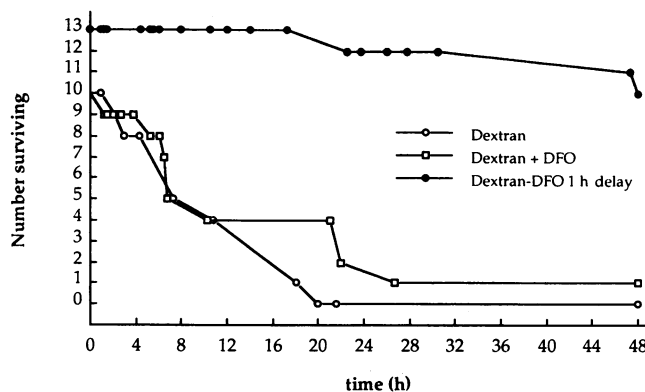


Figure 5. Mortality after administration of  $1.25 \mu\text{mol/g}$  body wt i.p. of ferrous sulfate followed by intravenous injection of dextran alone ( $\circ$ ,  $n = 10$ ), in combination with free DFO ( $\square$ ,  $n = 10$ ) or with a 1-h delayed injection of the DFO-dextran conjugate ( $\bullet$ ,  $n = 13$ ). Control animals were injected with 1 ml of dextran in sterile saline. Free DFO and free dextran in sterile normal saline was administered at a dose of  $0.15 \mu\text{mol DFO/g}$  body wt. 1 h after the administration of iron, the dextran-DFO conjugate in sterile saline was injected at a dose of  $1.4 \mu\text{mol/g}$  i.v. (DFO equivalents). At 48 h, the  $\chi^2$  for dextran vs. dextran + DFO = 1.05,  $P = 0.3049$ ; dextran vs. dextran-DFO = 13.609,  $P < 0.0002$ ; dextran + DFO vs. dextran-DFO = 10.145,  $P = 0.0014$ .

carbohydrate polymers. In this form, the chelator has unaltered affinity for iron, does not cause detectable hypotension in experimental animals and is much less toxic than the free drug when given intravenously.<sup>2</sup> Furthermore, these high molecular weight forms of DFO persist in circulation much longer than the unconjugated chelator,<sup>2</sup> perhaps conferring additional therapeutic advantage.

Our results indicate that the macromolecular DFO-conjugates are far more effective in the treatment of acute iron toxicity than is the free drug. Although the proximate cause of death in iron poisoning is not known, severe hypotension and injury to the liver are two likely contributors. The liver ordinarily generates activated oxygen species (14). The accumulation of much of the excess iron by the liver may result in increased levels of activated oxygen species (15). As an indicator of acute hepatic toxicity, we measured the release of ALT after iron administration. Serum levels of ALT may rise as much as 100-fold in humans with viral hepatitis or certain other necrotic liver diseases (although elevations 30–50 times normal are more frequently observed) (11). Affirming the likely hepatotoxic effects of iron, we find that serum levels of this enzyme show very large increases 2–4 h after intoxication with the metal. This increment in serum ALT activity is only slightly blunted by administration of the maximum tolerable dose of free DFO, reflecting the inability of the unmodified chelator to rescue animals given lethal amounts of iron. By contrast, the iron-mediated elevations in ALT activity are greatly diminished in animals given high molecular weight preparations of DFO, reaffirming the greater therapeutic efficacy of this modified form of the chelator. We should emphasize that these results do not prove that the iron is directly hepatotoxic. Hypotension arising from iron intoxication (or additively from both iron and free DFO infusion) may contribute to hepatic necrosis. There is evidence, however, that the liver is selectively damaged; only minor (two to threefold) in-

creases in serum CPK levels after iron poisoning suggest that the heart, skeletal muscle, and brain, organs rich in CPK activity, are not as susceptible to the injurious effects of acute iron poisoning.

The DFO-conjugate is therapeutically effective when administered after a 1 h delay after giving iron IP. This demonstrates that the DFO-conjugate still affords protection when administered in a fashion that more closely resembles the situation of acute iron poisoning in children. Nonetheless, it is obvious that there are important differences between our model and acute iron poisoning in children. First, the doses of iron used in this model were uniformly high and second, our treatment consisted of a single injection of chelator, whereas patients receive chelator orally as well as intravenously.

Overall, our results indicate that high molecular weight DFO-conjugates are able to prevent tissue damage, most importantly, hepatic injury and death, after iron poisoning. The therapeutic utility of modified forms of DFO may extend beyond the special case of acute iron intoxication. For example, in tissue damage arising from ischemia and reperfusion, reactive iron has been implicated as one potentiating element, and administration of DFO may moderate the extent of this damage (16–21). In this and, perhaps, other clinical circumstances, the greatly diminished toxicity of high molecular weight forms of DFO may permit the administration of much larger and more effective doses of this useful, but potentially toxic, iron chelator.

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