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AN ANIMAL MODEL OF IRON OVERLOAD AND ITS APPLICATION TO STUDY HEPATIC FERRITIN IRON MOBILIZATION BY CHELATORS

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Abstract—Administration of 3,5,5-trimethylhexanoyl ferrocene in the diet of male Wistar rats results in a substantial increase in hepatic ferritin protein (>2-fold) and of ferritin iron (4–8-fold). The iron-loading in liver, under the conditions used, appears to be essentially in parenchymal cells rather than in reticulo-endothelial cells. It is suggested that the model represents a useful system for the study of the potential efficacy of new iron chelators for the mobilization of hepatic storage iron.

The ability of desferal® (DFO) and of a new siderophore, desferrithiocin (DFT), to mobilize hepatic ferritin iron is observed in this model of iron overload. Desferrithiocin stimulates ferritin iron mobilization, when administered either by gavage or by intraperitoneal injection, whereas desferal® is active intraperitoneally but inactive orally. Our studies lead to the conclusion that DFT merits further examinations, for its activity as an orally active iron chelator.

Iron is an element which living organisms require for their growth and maintenance. Yet despite its position as the second most abundant metal (after aluminium) and the fourth most abundant element in the earth's crust, it is only poorly biologically available. This is on account of the tendency of its oxidized form Fe^{3+}, in an aqueous milieu to hydrolyse and polymerize, forming insoluble and potentially biologically inaccessible ferric oxides and oxyhydroxides. Whereas primitive organisms must obtain iron from their environment by the excretion of iron-complexing agents (siderophores) which subsequently transport the iron into the cells by a receptor-mediated process [1], multicellular organisms must obtain their iron from dietary sources [2]. This poses a problem of homeostasis: the amount of iron which is absorbed from the diet must be compensated by the excretion of an equivalent amount of iron from the organism. And it is in this area that the human organism is limited, not only by its poor capacity to absorb dietary iron (per kg body weight only 0.1–0.2% of that in other mammals) but by its limited capacity to eliminate iron which has been accumulated (10% of that in other mammals) [3–5]. Thus, in cases of excessive iron accumulation, either by an increase in absorption from the gastro-intestinal tract (as in primary idiopathic haemochromatosis) or by the application of transfusion therapy to compensate congenital haemolytic anaemias (as in β-thalassaemia) the consequences are an accumulation of iron in the body. This accumulation of iron is the major cause of death in patients subjected to this latter treatment. Indeed in β-thalassaemia, virtually all of the iron administered by transfusion is retained in various tissues mainly in liver, heart and pancreas. The clinical manifestations of secondary iron overload appear as an iron toxicity in parenchymal compartments of these tissues [6].

To date, the only valid therapy is iron mobilization by the microbial chelating agent desferrioxamine B, produced by *Streptomyces pilosus*. The requirement for its continuous infusion [7] and its cost has stimulated the search for alternative compounds of comparable efficacy and if possible active by oral administration. A number of compounds have been studied, such as the siderophore desferrithiocin (DFT) [8] or the synthetic substances HBED (*N*,*N*′-bis(*o*-hydroxybenzyl)ethylenediamine diacetic acid), EHPG (*N*,*N*′-ethylene-bis(*o*-hydroxyphenylglycine)), PIH (pyridoxal isonicotinoylhydrazone), etc. Previous studies have tested the biologic activity of some of these compounds and their derivatives in rat models [9, 10] and have revealed that, for example, dmHBED (dimethyl ester of HBED) is a chelator absorbed from the intestinal tract, has a low toxicity and high efficiency.

We propose an iron overload model in rats, using oral administration of a ferrocene derivative, 3,5,5-trimethylhexanoyl ferrocene or HOE 117 (Fig. 1). Since the discovery of synthetic ferrocenes [11], several derivatives have been tested with respect to their absorption and metabolism. One of these, HOE 117 leads to a hepatic iron overload in normal animals and effectively increases haemoglobin synthesis in

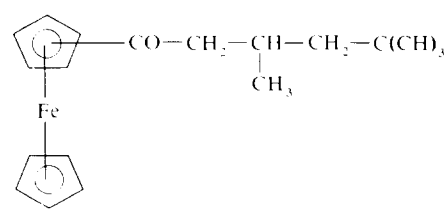

Fig. 1. Structure of 3,5,5-trimethylhexanoyl ferrocene (HOE 117).

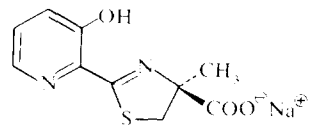

Fig. 2. Structure of desferrithiocin (DFT).

anaemic rats [12]. The *in vivo* chelating ability of DFT (Fig. 2) is evaluated using this new model of dietary iron overload.

MATERIALS AND METHODS

Male rats of the Wistar strain weighing 250–300 g were used throughout the study. All the rats were adapted to a powder diet (Aliment de Laboratoire U.A.R., Epinay, France) for days 1–14. Animals used as controls were subsequently fed the powder diet without additives whereas the iron-loaded rats received the powder diet containing 1 g of HOE 117/kg diet (this corresponds to 0.17 g of iron/kg diet). HOE 117 was supplied by Hoechst (Frankfurt, F.R.G.).

Preparation of iron chelators and administration. Different dilutions of all of the chelators were prepared and a final volume of 0.5 ml was administered. Desferrioxamine B methane sulfonate (DFO, MW 656, Ciba-Geigy) was dissolved in distilled water. Solutions of the sodium salt of desferrithiocin (DFT, MW260, Ciba-Geigy) were prepared in the same manner. Two molecules of DFT are needed to form a complex with ferric ion while only one molecule of DFO is necessary. Before each administration these solutions were filtered through acrodisc filters of 0.2 μm pore size (Gelman).

For the final experiment, DFT monohydrate was dissolved in 60% PBS and 40% DMSO, ferrithiocin (FT) in 25% DMSO and 75% H_2O, and ferrioxamine B (FO) in distilled water.

The chelator-treated rats received the chelators either by intraperitoneal injection or by gavage for days 15–28. Drug administration was every 2 days corresponding to 7 injections or gavages.

On the 28th day all of the animals were sacrificed and the liver was removed. Electron microscopy was used for examination of samples removed directly from the liver of rats. The remains were stored at −20° prior to biochemical analysis.

Isolation procedure for rat liver ferritin. Individual liver homogenates were centrifuged at 12,000 *g* for 30 min. The supernatant was subjected to a heat denaturation at 70° for 10 min. Thereafter the solution was cooled on ice and centrifuged at 12,000 *g* for 30 min. After adding solid $(NH_4)_2SO_4$ to 35% saturation, the solutions were left at 4°.

After 12 hours at this temperature, centrifugation at 12,000 *g* for 30 min yielded a pellet which contained the ferritin fraction. The pellet was dissolved in a minimum of distilled water. The ferritin solutions were dialysed for 2 days against frequent changes of distilled water (pH 8) prior to further analysis. Polyacrylamide gel electrophoresis in denaturing and non-denaturing conditions indicate that the protein is >95% ferritin.

Determination of protein. Protein was estimated by the method of Lowry [13]. Bovine serum albumin was used to obtain a calibration curve.

Determination of ferritin iron. Iron concentration in ferritin samples was determined by reaction with α,α'-bipyridyl after reduction of ferric iron by Na_2SO_3 [14]. A calibration curve was obtained with a solution of Fe(II) as ferrous ammonium sulfate.

Electron microscopy. Blocks of liver were fixed in 2.5% glutaraldehyde, postfixed in osmium tetroxide (1%), dehydrated with a graded series of ethanol and embedded in propylene oxide.

Sections of 1 μm were stained with toluidine blue and viewed by light microscopy. Thereafter representative areas were sectioned for EM. Ultrathin sections were stained with uranyl acetate (2%) and lead citrate. Certain sections were examined unstained.

Statistical methods. All data are expressed as mean ± S.E.M. Significance of differences was assessed by the Student's *t*-test or the Cochran's test [23].

RESULTS

Positive examples of iron-overloaded hepatocytes have been observed already when HOE 117 was given by gavage in ground-nut oil [12]. When this compound was administered in the powder diet to normal rats hepatic ferritin protein and ferritin iron of untreated overloaded rats increased significantly compared to controls (Tables 1–3). There was an increase of hepatic ferritin protein of 110–310% and of ferritin iron of 370 to 805%. The iron/protein ratio was increased by a factor of 2.25 to 2.75. The morphological observations were in accordance with the biochemical results. Electron microscopy showed an increase of quantity of iron stocked in ferritin and haemosiderin of liver parenchymal cells. At low magnification (Figs 3 and 4) we could observe secondary lysosomes in iron loaded rat hepatocytes.

At higher magnification (Fig. 5), these lysosomes corresponded to siderosomes which were full of iron-rich ferritin iron cores. Ferritin particles could be seen scattered throughout the cytoplasm. There was no evidence of an increase of these iron storage forms in reticulo-endothelial cells. No morphological anomalies seemed to be associated with HOE 117 administration (Fig. 6).

The diminution of hepatic iron overload following treatment with chelators was evaluated by analysis of liver ferritin protein and iron content at the end of the treatment compared to that of untreated iron-loaded controls.

By intraperitoneal injections (Table 1) DFT at a dose of 100 mg/kg provoked a reduction of total ferritin iron of 38% compared to 23% for the same dose of DFO; the effect of DFT at 30 mg/kg was similar to that of DFO at 100 mg/kg ($P > 0.1$). Even at 10 mg/kg DFT was active and a diminution of 14% was observed ($P < 0.005$). No statistically significant effect on ferritin protein content was found for any of the chelator treatments ($P > 0.1$) although the iron/ferritin protein ratio was reduced by 14% for DFT at 10 and 30 mg/kg, 35% for DFT at 100 mg/

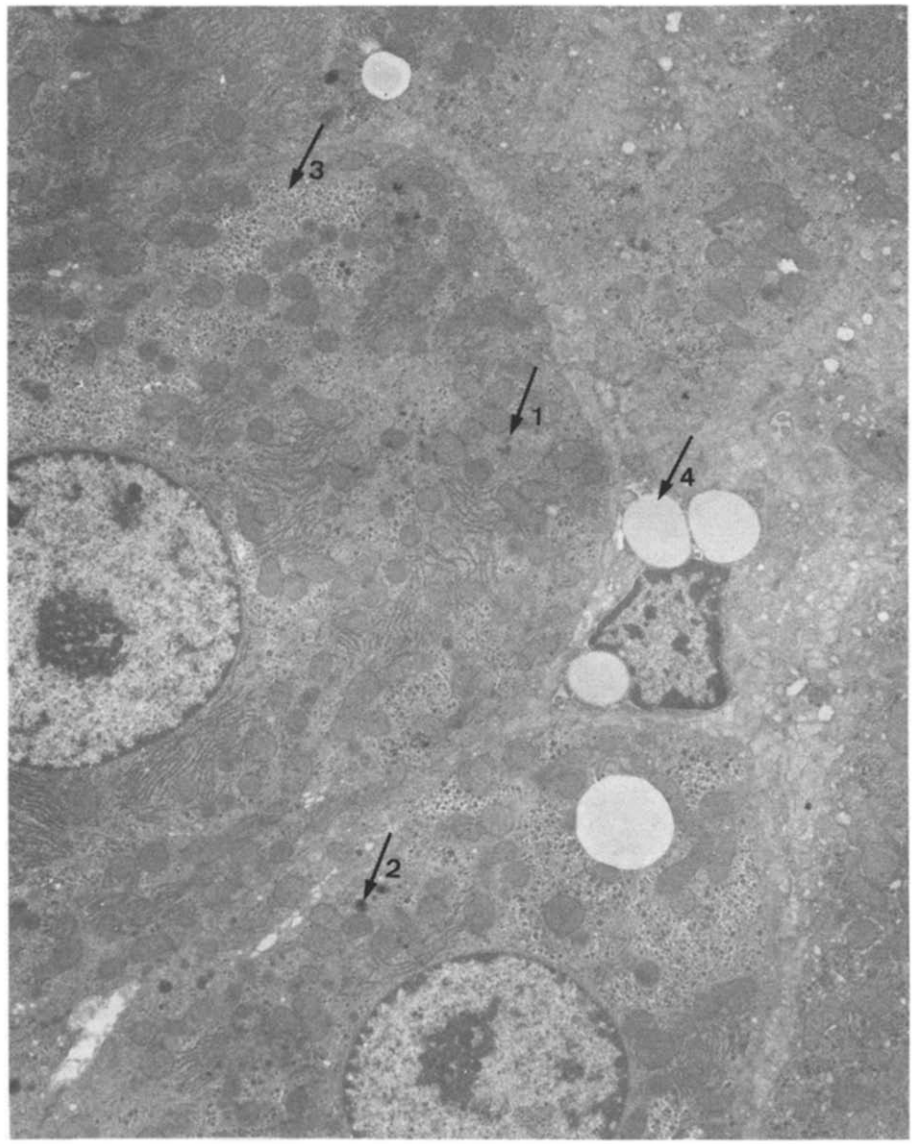

Fig. 3. Electron microscopy of normal rat hepatocytes. Uranyl acetate and lead citrate stains (×2000): 1. primary lysosome; 2. secondary lysosome; 3. glycogen; 4. fat bodies.

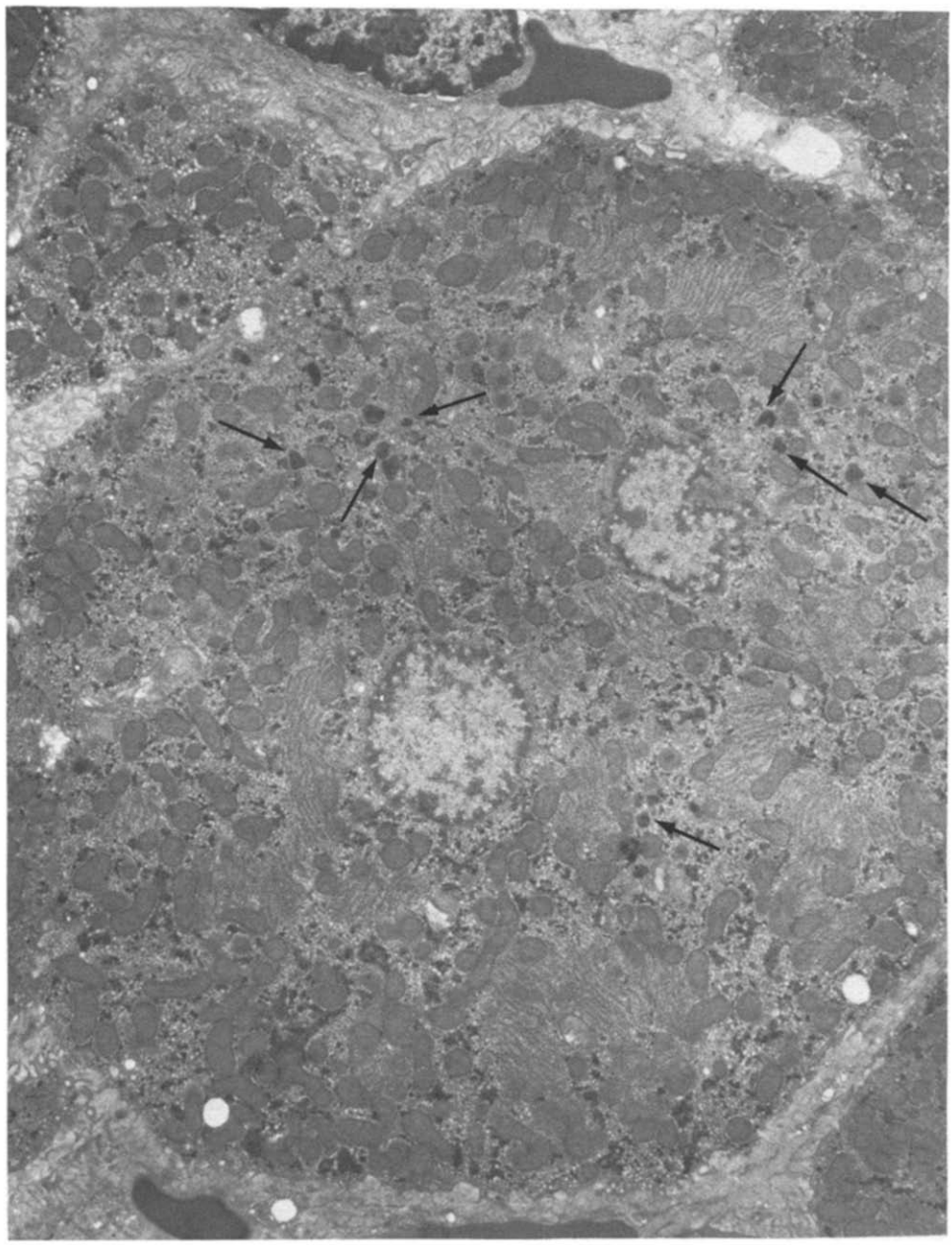

Fig. 4. Electron microscopy of rat hepatocytes after administration of HOE 117. Uranyl acetate and lead citrate stains (× 2000) ↙ siderosomes.

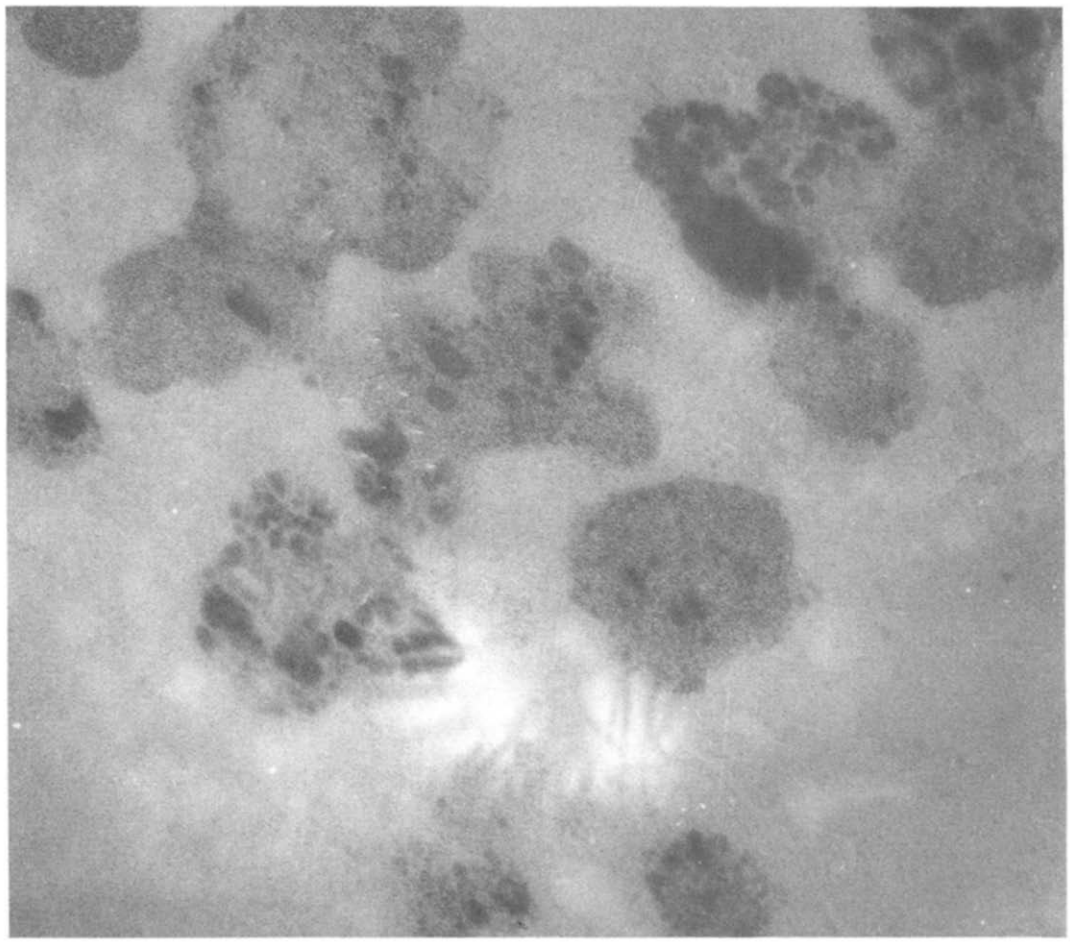

Fig. 5. Electron microscopy of rat hepatocyte cytoplasm after administration of HOE 117 (unstained, ×20,000). Siderosomes and ferritin particles in cytoplasmic matrix.

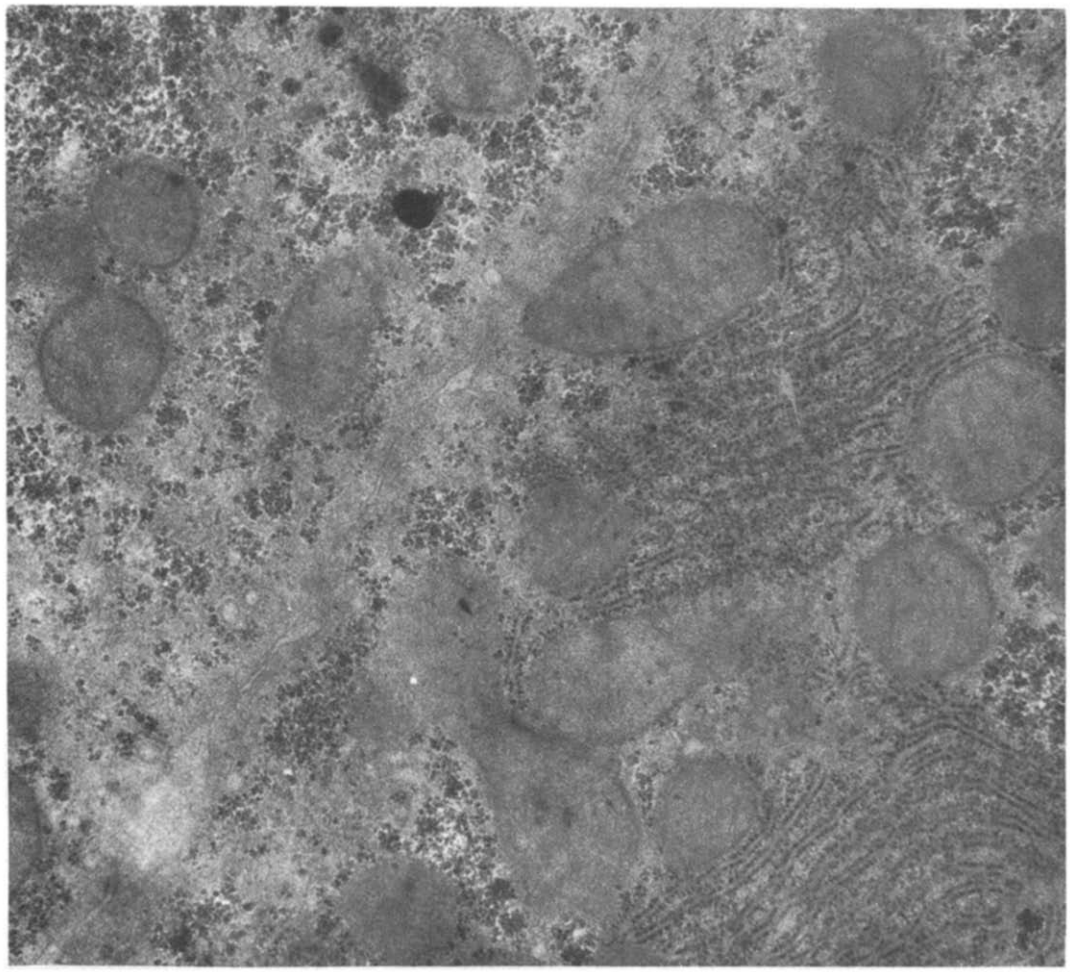

Fig. 6. Electron microscopy of rat hepatocyte cytoplasm after administration of HOE 117. Uranyl acetate and lead citrate stains (×10,000).

Table 1. Analysis of hepatic iron overload. Experiment I: Effect of the administration of two chelators by intraperitoneal injection

				DFT			DFO
		Controls N = 6	Overloaded N = 6	10 mg/kg N = 7	30 mg/kg N = 8	100 mg/kg N = 9	100 mg/kg N = 8
Body weight (g)	$\bar{X}$	312	337	303	332	299	310
	s	±36	±26	±11	±14	±9	±34
Liver weight (g)	$\bar{X}$	11.2	12.2	10.9	12.1	11.1	11.6
	s	±1.2	±0.9	±0.9	±1.0	±2.9	±1.4
Liver ferritin	$\bar{X}$	700	1,824	2,066	1,745	1,961	1,623
(μg/g of liver)	s	±48	±225	±117	±208	±479	±207
Total hepatic ferritin (μg)	$\bar{X}$	7,760	22,380	22,485	21,173	20,984	18,600
	s	±396	±3,228	±2,543	±2,551	±2,266	±2,140
Ferritin iron (μg/g of liver)	$\bar{X}$	69	412	404	335	279	337
	s	±4	±18	±61	±53	±65	±71
Total hepatic ferritin iron	$\bar{X}$	771	5,040	4,357	4,096	3,107	3,891
(μg)	s	±116	±204	±324	±587	±721	±735
Ratio iron ferritin/protein		0.099	0.226	0.196	0.192	0.145	0.208

Table 2. Analysis of hepatic iron overload. Experiment II: Effect of the administration of two chelators by gavage

				DFT			DFO
		Controls N = 6	Overloaded N = 8	10 mg/kg N = 7	30 mg/kg N = 9	100 mg/kg N = 7	100 mg/kg N = 7
Body weight (g)	$\bar{X}$	336	330	344	298	278	338
	s	±28	±36	±6	±26	±18	±26
Liver weight (g)	$\bar{X}$	13.2	12.9	13.9	10.0	8.9	12.6
	s	±1.2	±1.7	±0.9	±1.6	±1.8	±1.5
Liver ferritin	$\bar{X}$	582	1,243	1,664	1,650	1,435	1,203
(μg/g of liver)	s	±64	±245	±201	±287	±242	±158
Total hepatic ferritin (μg)	$\bar{X}$	7,587	15,843	23,030	17,944	12,780	15,225
	s	±1,058	±1,214	±1,904	±3,418	±3,314	±2,138
Ferritin iron (μg/g of liver)	$\bar{X}$	52	258	220	190	159	253
	s	±9	±44	±47	±36	±27	±21
Total hepatic ferritin iron	$\bar{X}$	699	3,300	3,056	2,045	1,397	3,212
(μg)	s	±111	±156	±710	±400	±222	±236
Ratio iron ferritin/protein		0.089	0.208	0.132	0.115	0.111	0.210

kg and 7% for DFO. At the highest dose of DFT used 2 animals out of 14 did not survive the treatment.

When the chelators were administered by gavage (Table 2) DFT showed an increased mobilization of ferritin iron compared to the comparable experiments at doses of 30 and 100 mg/kg (respectively 38% and 58%). No significant decrease in ferritin iron was observed for DFT at 10 mg/kg or for DFO at 100 mg/kg ($P > 0.1$).

This latter result confirms that in the animal model used in this study DFO, as in man, is poorly if at all effective by oral administration. However, at 10 mg/kg DFT provoked a marked increase of ferritin protein (45%), and the effect of DFT on the ferritin iron/protein ratio was consistently reduced at all three doses used (from 36% to 47%). The toxicity of DFT at 100 mg/kg was even more pronounced and led to the loss of 5 animals out of a total of 12.

In order to compare DFT by oral and intraperitoneal administration, a third experiment was carried out (Table 3). The results confirm those obtained in the earlier studies, with some notable differences. Ferritin iron was reduced by 22% ($P < 0.05$), 26% and 34% by intraperitoneal administration at doses of 10, 30 and 100 mg/kg, and by 26% by gavage at 30 mg/kg ($P < 0.005$). However, at 10 mg/kg by gavage DFT increased ferritin iron by 32% and ferritin protein by 29% (although this latter effect is not statistically significant ($P > 0.1$)). This was reflected in the ferritin iron to protein ratio which remained essentially unaltered for DFT at 10 mg/kg by gavage compared to controls, but which were reduced by 19–26% in all of the other treatments. However, in this experiment a much higher mortality was observed at 100 mg/kg by intraperitoneal injection (60%).

One explanation for the results obtained by gavage with DFT at 10 mg/kg might be that the chelator was moving iron from the gastro-intestinal tract to the liver, and thereby provoking a stimulation of hepatic apoferritin synthesis, which would explain the increase in ferritin protein content observed as well as the stabilization (Table 2) or net increase in hepatic ferritin iron (Table 3). To confirm this hypothesis two experiments were carried out. In Table 4 the results of an experiment with normal rats is presented. At 10 mg/kg by gavage DFT provoked an increase in ferritin protein of 34% ($P < 0.005$) but no significant increase of ferritin iron ($P > 0.1$). The iron to protein ratio diminished by 10%.

In a final experiment DFT and its iron chelate FT were administered at doses equivalent to 10 mg/kg of DFT by gavage and intraperitoneal injection, as well as DFO and FO by intraperitoneal injection to normal rats (Table 5). Only DFT provoked a significant increase in hepatic ferritin iron ($P < 0.01$) but no significant increase in ferritin protein ($P > 0.1$).

DISCUSSION

The biochemical and morphological results suggest that overload in hepatic cells is directly caused by oral administration of HOE 117 to normal rats. This derivative of ferrocene, a synthetic compound which masks an iron atom by two aromatic groups [11], is well absorbed by gastrointestinal cells (see Tables 1–3) and is thereafter metabolized in the liver where it releases its iron in parenchymal cells, represented mainly by hepatocytes. Iron so released leads to apoferritin synthesis and to an increase of iron incorporation in ferritin. When the degree of incorporation reaches a very high value, ferritin particles enter lysosomes to be converted into haemosiderin and form siderosomes [15]. In liver, iron can be stored as ferritin or haemosiderin in two cell types, reticulo-endothelial and parenchymal cells. Clinical correlations indicate that iron excess is harmless in reticulo-endothelial cells and responsible for most of the clinical manifestations of haemochromatosis, when localized in parenchymal cells [6].

In the investigation of new iron chelators, a preliminary biological activity must be evaluated. This new model of dietary iron overload appears particularly useful for this purpose, since in contrast to

Table 3. Analysis of hepatic iron overload. Experiment III: Effect of the administration of DFT by gavage or by intraperitoneal injection

		Controls N = 6	Gavage Overloaded N = 6	Gavage 10 mg/kg N = 8	Gavage 30 mg/kg N = 10	Intraperitoneal injection 10 mg/kg N = 8	Intraperitoneal injection 30 mg/kg N = 8	Intraperitoneal injection 100 mg/kg N = 4
Body weight (g)	$\bar{X}$	333	344	360	310	348	325	323
	s	±17	±19	±16	±20	±16	±13	±47
Liver weight (g)	$\bar{X}$	11.7	12.8	14.0	11.2	14.0	12.8	12.8
	s	±0.9	±1.3	±1.0	±1.7	±2.0	±1.1	±4.0
Liver ferritin (μg/g of liver)	$\bar{X}$	609	2,256	2,657	2,334	1,995	2,112	2,047
	s	±87	±501	±934	±754	±249	±309	±681
Total hepatic ferritin (μg)	$\bar{X}$	6,948	28,493	36,653	26,730	27,648	26,710	25,060
	s	±753	±5,111	±5,482	±7,350	±3,505	±3,691	±6,256
Ferritin iron (μg) (μg/g of liver)	$\bar{X}$	33	275	329	230	196	204	182
	s	±4	±34	±42	±50	±33	±31	±57
Total hepatic ferritin iron (μg)	$\bar{X}$	387	3,504	4,611	2,576	2,736	2,590	2,315
	s	±49	±454	±446	±609	±601	±131	±392
Ratio iron ferritin/protein		0.054	0.122	0.124	0.097	0.098	0.097	0.091

Table 4. Analysis of hepatic ferritin. Experiment IV: Effect of the administration of DFT by gavage to normal rats

		Controls N = 6	DFT 10 mg/kg N = 4
Body weight (g)	$\bar{X}$	333	350
	s	±17	±22
Liver weight (g)	$\bar{X}$	11.7	13.1
	s	±0.9	±1.0
Liver ferritin (μg/g of liver)	$\bar{X}$	609	714
	s	±87	±120
Total hepatic ferritin (μg)	$\bar{X}$	6,948	9,288
	s	±753	±1,300
Ferritin iron (μg/g of liver)	$\bar{X}$	33	34
	s	±4	±7
Total hepatic ferritin iron (μg)	$\bar{X}$	387	437
	s	±49	±57
Ratio iron ferritin/protein		0.054	0.048

other models that measure iron excretion, it provides direct biochemical information on ferritin storage iron, and permits testing of the capacity of these compounds to enhance the mobilization of clinically relevant parenchymal storage iron.

In other models, hypertransfusion of rats and mice has often been used [16, 17, 18] and proposed as a basis for screening iron chelators. Hypertransfusion precedes the selective radiolabelling of reticuloendothelial cells and parenchymal cells in the liver [19, 20]. The models permit the study of chelators during a short time interval and give preliminary data on the biological efficacity of the tested compounds. They complement the described biochemical model by measuring excretion. Bacon *et al.* have used two models of iron overload [21]. One of these, using dietary supplementation with carbonyl iron introduces iron in hepatocytes mainly as ferritin and haemosiderin.

The model of iron overload presented here presents a number of advantages compared to those which have been proposed previously [16, 17, 19, 20].

(1) The mobilization of iron from hepatocyte ferritin represents the objective of any chelator-mediated iron mobilizations—this is precisely the parameter which is measured.

(2) Not only is hepatic ferritin iron determined, but the precision of the analysis is such that one can compare different experiments on different groups of animals.

(3) On the basis of our EM studies, it seems likely that the present model represents a model of predominantly parenchymal iron overload, which is precisely the model of iron overload that we would want to have to study potential new iron chelating agents.

We might conclude from the results presented in Table 1 that DFT is a much more effective chelator of hepatic ferritin iron at an equivalent dose than is DFO. Further in the present animal model it appears considerably more effective at 100 mg/kg when administered by gavage than by the intraperitoneal route (Table 2) on a percentage basis. However, when we consider the total amount of ferritin iron

Table 5. Analysis of hepatic ferritin. Experiment VI: Effect of the administration of DFT, FT, DFO and FO to normal rats

			Gavage		Intraperitoneal injection			
		Controls N = 7	DFT 10 mg/kg N = 7	FT 10.7 mg/kg N = 6	FT 10.7 mg/kg N = 6	DFT 10 mg/kg N = 7	FO 12.7 mg/kg N = 5	DFO 12.8 mg/kg N = 6
Body weight (g)	$\bar{X}$	347	347	372	341	344	353	341
	s	±26	±29	±9	±14	±25	±28	±14
Liver weight (g)	$\bar{X}$	12.5	13.7	14.8	12.7	12.7	13.9	13.4
	s	±1.2	±1.0	±1.3	±1.3	±2.3	±1.0	±0.8
Liver ferritin (μg/g of liver)	$\bar{X}$	608	693	366	349	616	633	705
	s	±75	±144	±79	±45	±58	±67	±105
Total hepatic ferritin (μg)	$\bar{X}$	7,662	9,018	5,434	4,312	7,765	8,870	9,482
	s	±406	±1,737	±878	±690	±1,127	±1,318	±1,188
Ferritin iron (μg/g of liver)	$\bar{X}$	26	31	23	29	29	22	23
	s	±2	±4	±5	±3	±9	±4	±6
Total hepatic ferritin iron (μg)	$\bar{X}$	320	418	342	365	359	332	332
	s	±20	±57	±39	±67	±51	±35	±69
Ratio iron ferritin/protein		0.043	0.045	0.063	0.083	0.047	0.035	0.033

mobilized in the two experiments (1933 μg iron in experiment I and 1903 μg iron in experiment II (Table 6) we can conclude that the same amount of iron is mobilized in both studies, the percentage difference being attributable to the more important extent of the iron load obtained in the first experiment. At this dose level some 8% of the total amount of the DFT administered is required to account for the ferritin iron mobilized (the corresponding value for DFO is 7%). When we examine the total ferritin iron mobilized in the first three experiments (Tables 1–3) by DFT at 30 mg/kg, we arrive at the conclusion (Table 6) that some 13% of the chelator administered was utilized to account for the iron mobilized.

In contrast the results at low doses of DFT are rather different depending on the mode of administration. By intraperitoneal injection at 10 mg/kg 680 and 770 μg of iron are mobilized and the efficacy increases to the remarkably high value of 28–29% of the cumulative dose administered engaged in complexation of ferritin iron. By gavage, the results with DFT are extremely variable at 10 mg/kg, in one case no decrease being observed, in the other an important increase in ferritin iron. However, one factor remains remarkably constant, namely the increase in ferritin protein, which increases by 7188 μg and 8160 μg respectively (however, the standard errors render these values poorly significant in statistical terms).

They are nonetheless consistent with the increase in ferritin iron observed when DFT is administered to normal rats (Tables 4 and 5) where an increase in ferritin iron and ferritin protein is observed (respectively 50 μg and 98 μg for ferritin iron and 2340 μg and 1350 μg for ferritin protein). We can conclude from these results that, in iron replete rats there is a marked increase of the hepatic ferritin protein, with a variable effect on ferritin iron (which in any event masks the effects observed by intraperitoneal administration of DFT at the same dose). In normal rats the effect on ferritin protein and on ferritin iron are statistically poorly significant.

We conclude from these studies that DFT is not only toxic at a cumulative dose of around 200 mg, but that its efficacy as a chelator of hepatic ferritin iron increases exponentially as a function of decreasing cumulative dose administered (Table 6). This might suggest a saturation phenomenon with regard to the amount of hepatic ferritin iron which can be mobilized by the chelator. This is in accord with studies on cultured rat hepatocytes, which indicate not only that DFO mediated iron mobilization attains a plateau at around 100 μM, but that DFO is apparently taken up and released from hepatocytes by a process of passive diffusion [22].

From the data presented in Table 6 we can also conclude that at a dose of 30 mg/kg DFT is more effective by oral administration than by intraperitoneal (mean total ferritin iron mobilized 1173 μg compared to 929 μg). In view of the extraordinary efficacy of the chelator at low doses (where the toxic effects observed at doses of 100 mg/kg are not observed at all), it would seem reasonable to pursue studies on DFT which, by virtue of its potential for oral administration compared to DFO, seems to

Table 6. Hepatic ferritin iron mobilized and efficacy of chelator as a function of the administered dose

Chelator—Experiment	Dose (mg/kg)	Cumulative dose (mg)	Iron mobilized (μg)		Efficacy (%)
			i.p.	Gavage	
DFO—I	100	217	1149	—	6.45
DFT—I	100	209	1933	—	8.25
DFT—II	100	185	—	1903	9.28
DFT—I	30	70	944	—	12.14
DFT—II	30	62.8	—	1255	17.84
DFT—III	30	68.3	914	—	11.93
DFT—III	30	65.1	—	928	12.67
DFT—I	10	21.2	683	—	28.77
DFT—III	10	24.4	768	—	28.07

represent a considerable advance in the development of iron chelators for the treatment of secondary iron overload.

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