# Distribution of Intravenously Administered Ferrioxamine-59 and Ferric $N,N'$-Ethylenebis($\alpha$ Imino-2-Hydroxy-5-Chlorophenylacetate) in Rats* (32805)

DEMETRIUS H. BAGLEY, JR., E. J. ZAPOLSKI, MARTIN RUBIN, AND J. V. PRINCIOTTO
*Department of Physiology and Biophysics, and the Department of Biochemistry, Georgetown University, Schools of Medicine and Dentistry, Washington, D. C. 20007*

The relative importance of molecular structure in comparison to quantitative iron binding capability of iron chelates has not been delineated for the problems of the membrane transport of iron, the distribution of the metal *in vivo* and its excretion (1,2). Ferrioxamine and ferric $N,N'$ ethylenebis-($\alpha$ imino-2-hydroxy-5-chlorophenylacetate) (FeCl-EDDHA) are two iron chelates of differing molecular architecture whose iron binding strength exceeds that of iron transferrin (3, 4). The present study suggests that the molecular environment of the metal is a major determinant in its biological fate.

*Materials and Methods. Preparation of tagged chelates.* Ferrioxamine and FeCl-EDDHA were prepared by metathesis from ferric chloride and a 20% molar excess of the ligand dissolved in 0.2 $M$ sodium carbonate solution. Radioactive ferric chloride was added (0.1 $\mu$C/ml) and the pH was adjusted to 7.3 with sodium carbonate. The iron concentration was 2.8 mg/ml. The solutions so prepared were intensely colored and stable to hydrolysis.

In vitro *transfer of iron to $\beta$-globulin.* Mixtures of plasma and chelate, 0.30, 0.15, 0.75, and 0.030 m$M$ chelate in plasma, were incubated at 37°C for 15 min. These solutions were the equivalent of 100, 50, 25, and 10% of the dosage administered to a rat and calculated as wholly diluted in the plasma. After incubation, the mixtures were subjected to electrophoresis in barbital buffer on cellulose acetate strips (5). The unstained strips were scanned for radio iron in a $4\pi$ detector[1] and a duplicate strip was stained for visual location of the beta-globulin fraction.

In vivo *studies.* Fasted, male Sprague-Dawley rats (150–200 gm) were used in these experiments. The animals were injected in the saphenous vein while under i.p. sodium pentobarbital anesthesia (35 mg/kg). The iron dosage was 6 mg of Fe/kg. At prescribed time intervals each rat was exsanguinated by cardiac puncture, quickly opened by an abdominal incision, and the kidneys, liver, gut, spleen, and bladder were removed. Two people working simultaneously could accomplish the entire procedure within 30 sec. Radio iron content was determined in a Baird Atomic well (model 810A). The results were compared to a counting standard representing 1% of the injected dose and the percentage of the dose present calculated for each sample. The blood volume was calculated as 6.5% of the body weight. Groups of three animals were used for each time interval studied.

*Results and Discussion.* In vitro *transfer of iron to beta-globulin.* The radioscan and integration of the strips obtained from this study showed that 15 min after incubation less than 0.5% of the radioactive iron in the iron chelate was transferred to the beta-globulin fraction, as established by the electrophoretic technique. The chelates were clearly discernable as an intense colored band on the unstained strips well in advance of the beta-globulin fraction determined by Ponceau S Staining. For ferrioxamine, these results substantiate findings reported by Keberle (4). The fact that there was no transfer of iron from chelate to transferrin in the time of observation permits some simplification in interpretation of the *in vivo* results.

The data are in keeping with the view that the transfer of a metal iron from one chelate to another may not be significant when the differences in their stability constants are of the order of $10^3$ or greater, as in the present instances (6). A modifying factor in such a transfer, however, is the relative con-

---

* This investigation was supported in part by U.S. Public Health Service Research Grant AM 04447, from the National Institute of Arthritis and Metabolic Diseases.

[1] Atomic Accessories, Inc. Radiochromatogram Scanner, Model RSC-363.

TABLE I. $^{59}$Fe Distribution after an Intravenous (6 mg Fe/kg) Dose of $^{59}$Ferrioxamine.[a]

| Time: (min) | 0.5 | 1 | 1.5 | 2.5 | 5 | 10 | 15 | 20 | 25 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|
| Kidneys | 1.83 | 2.71 | 3.25 | 2.84 | 1.40 | 1.25 | 0.76 | 0.98 | 0.64 | 0.57 |
| Spleen | 0.24 | 0.09 | 0.07 | 0.08 | 0.04 | 0.05 | 0.03 | 0.03 | 0.03 | 0.03 |
| Liver | 0.87 | 1.15 | 1.06 | 1.06 | 0.64 | 0.66 | 0.63 | 0.77 | 0.70 | 0.55 |
| Gut | 0.33 | 0.31 | 0.28 | 0.25 | 0.17 | 0.20 | 0.20 | 0.20 | 0.18 | 0.12 |
| Bladder | 0.04 | 0.04 | 0.24 | 1.12 | 3.33 | 8.13 | 8.33 | 8.51 | 12.1 | 11.0 |
| Blood | 11.8 | 7.8 | 6.0 | 4.7 | 1.84 | 1.94 | 1.73 | 1.48 | 1.33 | 1.05 |

[a] Percentage of administered dose.

TABLE II. $^{59}$Fe Distribution after an Intravenous (6 mg Fe/kg) Dose of $^{59}$Ferric Cl-EDDHA.[a]

| Time: (min) | 0.5 | 1 | 1.5 | 2.5 | 5 | 10 | 15 | 20 | 25 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|
| Kidneys | 8.52 | 8.2 | 3.9 | 7.11 | 5.80 | 4.45 | 2.68 | 3.40 | 2.54 | 3.39 |
| Spleen | 0.34 | 0.65 | 0.30 | 0.39 | 0.28 | 0.09 | 0.12 | 0.15 | 0.10 | 0.10 |
| Liver | 16.2 | 19.0 | 7.4 | 23.3 | 25.6 | 26.6 | 18.2 | 21.5 | 28.1 | 21.4 |
| Gut | 0.78 | 1.10 | 0.99 | 1.30 | 2.20 | 8.66 | 19.9 | 27.1 | 12.3 | 14.2 |
| Bladder | 0.09 | 0.42 | 0.03 | 0.99 | 1.21 | 9.51 | 17.1 | 19.0 | 7.4 | 13.0 |
| Blood | 59.4 | 35.5 | 33.0 | 18.1 | 16.7 | 8.32 | 6.90 | 5.6 | 5.1 | 4.6 |

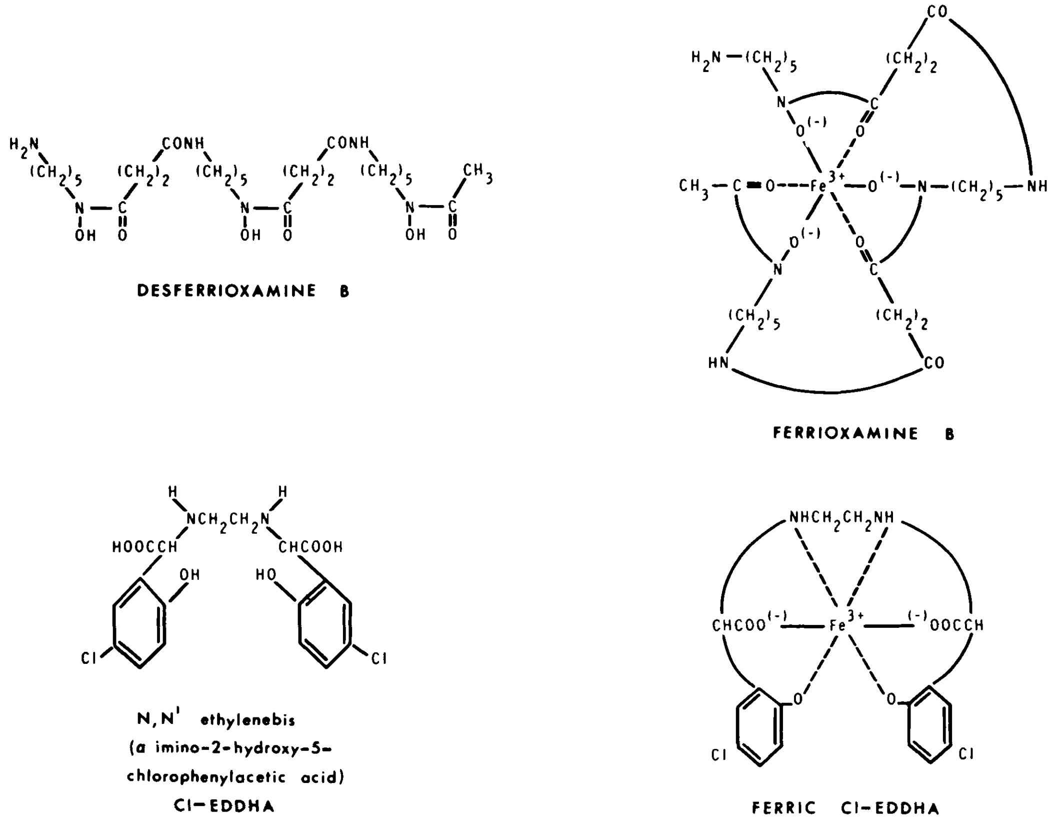
[a] Percentage of administered dose.

centration of the reactive components. In the present study the concentration of FeCl-EDDHA and ferrioxamine was varied from the maximum that would be present at injection if the entire dose were concentrated in the plasma volume to one tenth of this value. Iron was not transferred to β-globulin at any concentration ratio.

*Metabolism of ferrioxamine.* Although a number of studies have delineated the catabolism of desferrioxamine (1, 4), there is less information about the fate of the iron saturated ligand, ferrioxamine. Keberle pointed out that the presence of iron in the complex resulted in metabolic stabilization of the molecule. Five hours after intravenous administration of an 8.5 mg Fe/kg dose to dogs, 95% was excreted unchanged in the urine (4). On the other hand, Brown *et al.* were able to detect a small, but measurable, increase in gut iron following desferrioxamine induced mobilization of tissue iron in humans (1). Whether this biliary iron excretion was associated with the desferrioxamine ligand or its metabolites was not established.

Data in the present study is restricted in time to 30 min following injection, a period during the *in vitro* studies demonstrated the absence of nonmetabolic transfer of iron from ferrioxamine to other naturally occurring ligands in plasma. If metabolic transfer of iron had indeed taken place during the period of experimental observation, *in vivo*, the pathway of the tracer iron would be similar to that following the injection of inorganic iron. Consideration of the data (Table I) reveals striking differences. In contrast to injected ferric chloride (7), the rapid decrease in blood radio iron is concomitant with a large excretion of the metal by way of the kidney and bladder. The rapidity of the blood clearance is suggested by the buildup of bladder iron in the 30 min period. On the other hand, the low level of liver and gut iron establishes that for ferrioxamine the pathway of biliary excretion is minimal in rats.

*Metabolism of ferric Cl-EDDHA.* We have previously reported on the unusual distribution of $^{59}$Fe Cl-EDDHA (8). In longer term studies than those described here, iron injected as $^{59}$Fe Cl-EDDHA was partitioned between the renal and biliary excretory routes. For both kidney and liver (Table II) the initial high levels represent iron in transit to excretory channels rather than tissue deposition. The marked influence of the ligand structure on the transport of iron across cellular membranes was further established in



DESFERRIOXAMINE B

FERRIOXAMINE B

N,N' ethylenebis
(a imino-2-hydroxy-5-chlorophenylacetic acid)
Cl-EDDHA

FERRIC Cl-EDDHA

FIG. 1. Structure of desferrioxamine and Cl-EDDHA ligands and their ferric chelates.

studies on iron incorporation by reticulocytes (3). The mechanism was shown to be different than that utilized by the cell for iron incorporation from transferrin-$^{59}$Fe (9). The present work confirms and extends our earlier reports. The FeCl-EDDHA is indeed remarkably stable to nonspecific scission of iron in normal animals and follows the unique metabolic pathways, for iron, of urinary and biliary excretion.

The structure of the iron chelate is balanced between hydrophylic and hydrophobic characteristics. Its excretion by the biliary route as indicated by the liver and gut accumulation (Table II) and by biliary cannulation as previously reported, is not unexpected for a nonpolar compound. On the other hand its small molecular weight and aqueous solubility undoubtedly promotes its elimination by the kidney. Our earlier studies demonstrated that biliary ligation resulted in enhanced excretion in the urine (8). An increase in polarity of the generalized EDDHA structure, as by the introduction of carboxylic or sulfonic acid groups, shifted the excretory route almost completely to the urine.

As has been pointed out by Keberle (4), ferrioxamine (Fig. 1) is characterized by partial negative charges on the nitroso groups with a balancing positive charge locus on the iron atom. It may also be expected that the terminal amino group will be protonated at physiologic pH. Thus, ferrioxamine is a relatively polar iron chelate. Its distribution *in vivo* is in accord with this structure.

*Summary and Conclusions.* This study of the metabolism of ferric Cl-EDDHA and ferrioxamine B, for the period up to 30 min after iv administration in rats has demonstrated that the unique distribution of these iron chelates is singularly dependent on their molecular architecture. These results point to the importance of ligand structure on the metabolism of iron.

We wish to thank Dr. M. Weiner of the Geigy Co., Ardsley, N. Y. for generously supplying the Cl-EDDHA used in this study, and Dr. Westland of the CIBA Co., Summit, N. J. for his supply of Desferal mesylate. We thank Mr. Thomas Rosevari for his technical assistance.

---

1. Brown, E. B., Hwang, Y., and Allgood, J. W., J. Lab. Clin. Med. **69**, 382 (1967).

2. Rubin, M. and Princiotto, J. V., Agr. Food Chem. **11**, 98 (1963).

3. Princiotto, J. V., Rubin, M., Shashaty, G. C., and Zapolski, E. J., J. Clin. Invest. **43**, 825 (1964).

4. Keberle, H., Ann. N. Y. Acad. Sci. **119**, 758 (1964).

5. "Rapid Electrophoresis with Sepraphore III," Manual 70176-B, Gelman Instrument Co., Ann Arbor, Michigan, 1966.

6. Schubert, J., Ann. Rev. Med. Sci. **5**, 369 (1955).

7. Nagarajan, B., Sivaramakrishnan, V. M., and Brahmandam, S., Biochem. J. **92**, 531 (1964).

8. Haddock, E. P., Zapolski, E. J., Rubin, M., and Princiotto, J. V., Proc. Soc. Exptl. Biol. Med., **120**, 663 (1965).

9. Aserkoff, B., Rubin, M., Zapolski, E. J., and Princiotto, J. V., Federation Proc. **23**, (Pt. 1): 470 (1964).

---

Received Oct. 20, 1967. P.S.E.B.M., 1968, Vol. 127.

# Meconium Proteins and Mucoproteins in Meconium Ileus (32806)

R. E. Knauff and J. A. Adams (Introduced by R. C. Baldridge)

*Cystic Fibrosis Research Institute Laboratories, Temple University School of Medicine, Philadelphia, Pennsylvania 19140*

Information about the composition of meconium in meconium ileus (MI) is accumulating (1–4). An early report (1) indicated that MI meconium contained similar water, 13 times more trichloroacetic acid (TCA)-insoluble nitrogen, and 4 times less carbohydrate than is found in normal meconium. Other reports describe the presence of serum proteins by immunologic detection (2–4) and mucoids by isolation (4). Serum albumin was detected also in fibrocystic meconium (5).

Previous studies have not provided comprehensive and quantitative information about the protein and mucoprotein fraction of the MI meconium. The protein and mucoprotein fraction is probably of primary significance relative to the development of the pathophysiological state.

This report (a) presents a comprehensive quantitation of meconium proteins and mucoproteins and (b) correlates this quantitation with existing segments of information.

*Methods and Materials.* The individual meconium samples were frozen immediately and stored at —20°C for several days. Frozen raw meconium (1 gm) was suspended in chilled distilled water (10 ml; 4°C) and was homogenized (Vir-Tis model 23; speed setting of 30 for 4 min) in an ice-water bath. The homogenate was centrifuged at 1,500*g* for 30 min in a refrigerated centrifuge (2°C). The supernate was harvested and used without further processing. Solids were determined on the centrifugate and on an aliquot of the supernate.

The meconium supernates were electrophoresed on paper at 4°C by use of the Beckman/Spinco model R assembly. Beckman accessories and procedures were utilized. Protein was determined with the bromophenol blue method (Procedure B); and mucoprotein was determined with the periodic acid Schiff method (Beckman Technical Bulletin 6095A). All quantitation was by Analytrol scan with human serum albumin (4 times crystallized) and $\alpha$-globulin (Cohn fraction IV-I) from the Nutritional Biochemicals Corp. as color standards for protein and mucoprotein, respectively.

All substances being measured in the protein and mucoprotein methods would not react identically to the standards, of course, on a weight basis. It is not possible to quantitate such a broad range of substances on an absolute basis, but the use of albumin and $\alpha$-globulin as standards allows an approximate quantitation of the same types of substances