Excretion and Distribution of Iron During Chronic Deferoxamine Therapy

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In order to evaluate the consequences of chronic chelation therapy on iron overload, the cumulative excretion and tissue distribution of iron were analyzed in a child given deferoxamine (DFO) for transfusion hemosiderosis. During 5 yr of treatment, DFO induced a mean urinary loss of 13.0 mg iron per 24 hr per 500 mg injected. Of the 32 grams of iron administered as transfused red cells during the treatment period 30% was recovered in the urine. Mapping of the tissue distribution of iron was facilitated by splenectomy after 41 mo of chelation therapy. Despite an abundance of iron in Kupffer cells and bone marrow reticulum cells, the spleen was depleted of iron. The heterogeneous distribution of reticuloendothelial iron suggests that the spleen contains a reservoir of metabolically active, chelatable iron, whereas those parts of the reticuloendothelial system less active in heme catabolism store iron in a metabolically dormant, nonchelatable pool. The capacity of DFO to chelate spleen iron in the face of severe erythroid hypoplasia suggests that transferrin-prompted flow of iron across cell membranes is not necessary for chelate mobilization.

The survival of children with congenital hypoplastic anemia depends on the lifelong transfusion of red blood cells. Because there is no physiologic mechanism for the excretion of iron administered as transfused red cells, children with congenital erythroid hypoplasia succumb to the lethal complications of iron overload, usually during the second decade of life. In recent years the iron-chelating agent deferoxamine (DFO) has been used extensively in patients with hemosiderosis. Although the use of this drug for states of chronic iron overload is theoretically sound, no evidence has been presented to indicate that the long-term administration of DFO improves the survival of children with lifelong transfusion requirements. The extent to which DFO can effect a decrease of tissue iron in the face of continuing iron infusions is not well established. Moreover, the data that pertain to the relative availability of myocardial, hepatic, and reticuloendothelial iron to this chelating agent are incomplete.

The purpose of this report is to relate our experience with the administration of DFO over a 5-yr period to a child with congenital hypoplastic anemia (Blackfan-Diamond syndrome). Our observations (1) permit an evaluation of
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the efficiency of DFO-induced iron excretion relative to continued iron administration and (2) demonstrate preferential mobilization of splenic iron by DFO.

**Case Report**

The patient is a 10-yr-old Caucasian boy who was first seen at the University of Missouri Medical Center at 7 wk of age for evaluation of severe anemia. He had been born at term following a pregnancy that was complicated in the third trimester by a 3-day episode of gastroenteritis, for which the mother received a single dose of chloramphenicol (100 mg). Pallor was noted at 4 wk of age. Neither blood loss nor jaundice was observed. Both parents and an older sister were well and there was no family history of anemia. The infant presented with anicteric pallor, a pulse of 180 per min, respirations of 50 per min, lid edema, and a liver edge 6 cm below the right costal margin. No somatic anomalies were present.

The hemoglobin was 2.0 g/100 ml, volume of packed red cells 10%, and reticulocytes fewer than 0.1%. The peripheral blood demonstrated normocytic, normochromic red cells and numerous normoblasts. A bone marrow aspirate revealed a cellular marrow with 9% pronormoblasts and 3% basophilic normoblasts. No normoblasts beyond the basophilic stage were observed. (Subsequent marrow aspirations demonstrated progressive erythroid hypoplasia with selective depletion of mature normoblasts. By 5 yr of age the myeloid: erythroid ratio was 20:1). Myeloid and megakaryocytic morphology was normal and peripheral blood granulocytes and platelets were quantitatively normal. Serum bilirubin levels and fecal excretion of urobilinogen demonstrated no evidence for increased pigment production. Hemoglobin electrophoresis at 1 yr of age demonstrated a single band, which migrated with hemoglobin A. Hemoglobin F was 0.9%. Serum erythropoietin level (assayed in posthypoxic polycythemic mice) was 48% 59Fe RBC incorporation per milliliter of serum. (Normal in our laboratory is less than 1% incorporation/ml serum). Acid hemolysis (Ham test) and sucrose hemolysis tests were normal.

A transfusion program, designed to maintain the circulating hemoglobin between 4 and 8 g/100 ml, was initiated. In addition, the child received therapeutic trials of the following agents in pharmacologic doses: crude liver extract, folate, vitamin B12, pyridoxine, prednisone, and oxymethalone (4 mg/kg/day for 5 mo). None of these preparations affected changes in transfusion requirements, bone marrow morphology, or numbers of peripheral blood reticulocytes.

By 5 yr of age evidence of iron overload was clearly established: skin pigmentation was increased, the liver edge measured 5 cm below the right costal margin, serum iron was increased (234 µg/100 ml) and smears of marrow aspirates stained with potassium ferrocyanide demonstrated large, confluent masses of iron in reticulum cells. A program of DFO administration was instituted. Between 5 and 8 yr of age, the patient received 2 injections per day (500 mg DFO per injection), 6 days per wk. During this 3-yr period the patient was given three rest periods of 5 mo each. Between 8 and 10 yr of age the frequency of injections was decreased to one per day, 6 days per week. In the first 5 yr of chelation therapy, the patient received 490 g of DFO.

Following an episode of congestive heart failure at 7 yr of age, the transfusion program was modified in order to maintain the circulating hemoglobin concentration between 10 and 15 g/100 ml. Venous pressure remained normal subsequent to intensification of the transfusion schedule. During his first decade of life, the patient received 180 units of red blood cells.

Because of progressive splenomegaly and evidence for splenic sequestration of transfused erythrocytes (T ½ of 51Cr-labeled homologous red cells 12 days), the spleen was removed at 8 yr of age. Currently, at 11 yr of age, the patient participates in all phases of an active school curriculum. He has enjoyed normal social development and has achieved average scholastic standing.

**Materials and Methods**

Urinary losses of iron were monitored during periods of rest as well as during periods
Table 1.—Urinary Iron Excretion

<table>
<thead>
<tr>
<th>Treatment Period</th>
<th>No DFO</th>
<th>Per 500 mg DFO</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.2 (± 0.2)*</td>
<td>13.6 (± 2.7)</td>
</tr>
<tr>
<td>II</td>
<td>0.8 (± 0.1)</td>
<td>10.4 (± 2.0)</td>
</tr>
<tr>
<td>I and II</td>
<td>0.4 (± 0.3)</td>
<td>13.0 (± 2.8)</td>
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</tbody>
</table>

Treatment period I, 5–8 yr of age, before splenectomy; treatment period II, 8–10 yr of age, after splenectomy; DFO, deferoxamine.

* Mean ± standard deviation.

of drug administration. The total urine output for 24 hr was voided directly into acid-washed glass jugs. Urinary iron was quantitated colorimetrically with aa-dipyridyl after reduction with sodium hydrosulfite.

A portion of the spleen was ashed with sulfuric, hydrochloric, and perchloric acids. The iron content of the digest was quantitated colorimetrically with o-phenanthroline.

RESULTS

During the 5-yr study period, the patient’s basal urinary excretion of iron increased from 0.2 ± 0.2 mg per 24 hr (mean ± standard deviation) to 0.8 ± 0.1 mg per 24 hr (Table 1). This increase paralleled expansion of body iron stores. With chelation therapy, the mean 24-hr iron loss increased to 13.0 ± 2.8 mg per 500 mg DFO injected. In order to evaluate the effect of splenectomy on the efficiency of iron chelation, iron excretion data obtained prior to splenectomy (Treatment Period I) are compared with post-splenectomy data (Treatment Period II). Despite an increase in basal iron losses, DFO-induced iron excretion decreased following splenectomy (from 13.6 ± 2.7 to 10.4 ± 2.0 mg/24 hr/500 mg DFO). This decrease is significant (p < 0.01, Wilcoxon two-sample rank sum test).

Calculated iron balance measurements are presented in Fig. 1. Prior to the institution of chelation therapy at 5 yr of age, an estimated 12 g of iron had been administered as hemoglobin. Between 5 and 10 yr of age, an additional 32 g of iron was given in the form of red cell transfusions. Urinary losses of

Fig. 1.—Iron balance during deferoxamine treatment. Iron in, iron administered as transfused red cells; Iron out, iron recovered in the urine.
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Fig. 2.—Photomicrographs of (A) liver × 200, (B) spleen × 200, and (C) bone marrow aspirate × 500. Stained with potassium ferrocyanide and counterstained with safranin.

Iron prior to 5 yr of age are assumed to have been negligible (less than 0.3 g). During the 5-yr study period, measured losses of iron totaled 9.8 g. Without DFO, it is estimated that 0.7 g would have been excreted during this period. In the 5 yr of chelation therapy, 30% of the iron given as hemoglobin was recovered in the urine. Since extrarenal losses of iron were not monitored, these figures should be regarded as minimal estimates of iron excretion.

The morphologic appearance of iron in the liver, bone marrow, and spleen during the fourth yr of chelation therapy is illustrated in Fig. 2. The liver
biopsy demonstrates massive accumulation of iron in Kupffer and parenchymal cells. Likewise, marrow reticulum cells are replete with prominent masses of iron. In contrast, no stainable iron can be identified in the spleen. Chemical analysis demonstrated 0.13 mg iron/g spleen (wet weight). The 330-g spleen contained only 42.8 mg iron.

Toxicity from DFO was not observed. Serial slit-lamp examinations failed to demonstrate ocular changes. Hepatic and renal function remained unaltered throughout the period of treatment.

**DISCUSSION**

Calculations of chelate-induced iron losses based on urinary iron excretion underestimate the efficiency of DFO as an iron-chelating agent. Bile is rich in metabolic products of DFO, and enhancement of fecal losses of iron by DFO has been demonstrated in animals and man. Chelate-induced fecal iron losses in patients with iron overload has been observed to total 37 to 100% of urinary losses. Since stool iron was not monitored in the patient herein reported, an accurate assessment of iron balance cannot be made. The curve of iron loss in Fig. 1 represents a minimum estimate. If fecal losses approximated 40% of the urinary losses, an estimated 43% of the iron given as red cells between 5 and 10 yr of age was excreted. On the other hand, as much as 61% of transfused iron may have been mobilized and excreted if fecal losses equalled urinary losses.

Theoretically, each 500 mg of DFO is capable of binding 45 mg of iron. When given to our patient, 500-mg injections of the drug induced a mean increase in urine iron of only 12.6 mg/24 hr. This disparity between theoretical and observed in vivo chelation is in concert with the experience of others. Mean urinary iron losses reported in five series of patients with iron storage diseases range from 4.8 to 14.6 mg iron/24 hr/500 mg DFO.

A number of factors are known to influence in vivo iron chelation. The magnitude of iron stores is clearly a decisive factor in determining chelate efficiency. The observed correlation between iron stores and chelate-induced iron excretion serves as the basis for the use of DFO in the diagnosis of iron storage diseases. In addition, the organ distribution of iron appears to influence the availability of iron to chelate. A study of iron mobilization in patients with different tissue patterns of iron overload prompted Harker, Funk, and Finch to conclude that DFO preferentially chelates liver parenchymal iron. Subjects whose iron overload was confined to the reticuloendothelial system (defined as an increase in marrow reticulum cell iron with little or no hepatic parenchymal iron) experienced little increase in iron excretion in response to DFO. The saturation of transferrin with iron has been cited as a significant factor for in vivo chelation. However, the failure of DFO to chelate transferrin iron in vivo and the lack of correlation between transferrin saturation and DFO-induced iron excretion suggest that the levels of serum iron and transferrin are not primary determinants of chelation. The contribution of the spleen to chelatable body iron has not been investigated. Assuming metabolic similarity of splenic and marrow reticulum cell iron, Harker et al. suggested that splenic iron stores contribute relatively little to
chelate-induced iron excretion. The data summarized in Table 1, however, suggest that the intact spleen may have enhanced the efficiency of DFO. Despite progressive expansion of total body iron, urinary losses of iron were less after splenectomy than they were prior to splenectomy. Although the difference is statistically significant, variables other than the status of the spleen obviate firm conclusions. The treatment periods differed with respect to magnitude of iron stores, intensity of transfusion programs, and schedules of chelate administration.

The tissue distribution of iron in transfusion hemosiderosis is determined by sites of erythrocyte trapping and catabolism. Since senescent red blood cells are removed from the circulation by the reticuloendothelial system (RES), the macrophages of spleen, liver, and bone marrow bear the main burden of iron excess in transfusion hemosiderosis. In contrast, primary hemochromatosis is associated with predominant accumulation of iron in hepatic parenchymal cells. The spleen in transfusion hemosiderosis is characterized morphologically by prominent aggregates of iron and chemically by an increase in tissue iron. Cappel, Hutchison, and Jowett observed 0.2 to 8.0 g of iron/100 g dry weight of spleen (0.4 to 28 g per spleen) from patients with transfusion hemosiderosis and aplastic anemia. In contrast, no stainable iron was identified in the spleen removed from our patient. Quantitative analysis yielded a sparse 0.043 g of iron in the entire organ. This is considerably less than the iron content of a single unit of transfused blood. Unlike spleen macrophages, the Kupffer cells of the liver and reticulum cells of the marrow were engorged with confluent masses of iron. Selective exhaustion of spleen iron by chelation therapy is a new observation which affords a fresh insight into the reticuloendothelial processing of iron and modification of current concepts of determinants of in vivo iron chelation.

The observed variation of chelating susceptibility of iron in the macrophages of spleen, liver, and bone marrow suggests that the metabolism of iron within the reticuloendothelial system is not uniform. Heterogeneity of reticuloendothelial response is apparent in certain pathologic conditions as well. Whereas mobilization of iron from most reticuloendothelial cells is accomplished with facility, iron recruitment from pulmonary and dural macrophages may be unsuccessful even in the face of systemic iron starvation. As a result, idiopathic pulmonary hemosiderosis and subdural hematomas in young children are commonly complicated by iron-deficiency anemia. The physiological basis for diversity of iron metabolism within the RES may rest with the functional state of iron rather than with its anatomic locus. Iron kinetic studies disclose two functionally distinct iron pools in the RES: a metabolically dormant pool and a dynamic pool with a rapid turnover of iron. The former pool probably corresponds to iron stores, present as hemosiderin and ferritin in iron-replete individuals. The metabolically active pool is composed of iron salvaged from heme catabolism. Iron in this pool is not obliged to compete with storage iron for transferrin. Therefore, it is rapidly mobilized by the transport protein and delivered to sites of hemoglobin synthesis. Recently acquired iron is preferentially utilized, whether that iron is put into the RES as transferrin iron or as hemoglobin.

Studies with synthetic chelates sug-
suggest that they, too, preferentially tap a pool of metabolically active iron. Expansion of the metabolically active pool enhances iron mobilization by DFO. Deferoxamine-induced iron excretion is greater in patients with increased heme catabolism (hemolytic disease) than would be anticipated from iron stores. Likewise, phenylhydrazine-induced hemolysis augments DFO-provoked iron excretion in subjects with iron overload. These observations suggest that availability of tissue iron to native or exogenous chelators is determined by the functional state of the iron.

A proposed model for the reticuloendothelial phase of iron metabolism is presented in Fig. 3. Iron removed during hemoglobin catabolism enters a labile pool within reticuloendothelial cells. This ready reserve is susceptible to chelation by apotransferrin or by DFO. To the extent that the input into this ferruginous lake exceeds output, iron is diverted into metabolically stable stores. Although less vulnerable to chelation than iron in the labile pool, iron in the metabolically dormant pool may be recruited into the chelatable pool if needed. Thus, iron stores can be depleted by sustained negative iron balance. Sudden expansion of the labile pool enhances the ease of iron mobilization. Acute hemolysis is associated with an increase in transferrin iron and with an increase in DFO-induced iron excretion. On the other hand, reduction of the labile pool by accelerated transfer of iron to the erythroid marrow decreases DFO efficiency. It would appear that the synthetic chelate is a less successful competitor for chelatable iron than is apotransferrin. DFO, in addition to promoting iron excretion, may facilitate redistribution of ionic iron. From 10 to 20% of iron infused as feroxamine into normal subjects is incorporated into the red cell mass and tissue stores. Since reticulocytes do not accept iron bound by DFO, erythroid uptake of feroxamine must be mediated by transferrin. Redistribution of tissue iron may also be indirectly mediated by DFO. Cumming, Miller, Smith, and Goldberg demonstrated significant DFO-induced ferritinemia in 13 subjects with iron overload. Their data suggest that DFO, in addition to chelating metabolically labile iron, provokes mobilization

Fig. 3.—Proposed pathways of reticuloendothelial iron. RBC, red blood cell; RE, reticuloendothelial.
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of intact ferritin molecules. The liberated ferritin may be transported in the circulation to remote storage sites.

The observed selective depletion of splenic iron suggests that this organ is a repository for labile iron. Because Harker et al. were unable to demonstrate a relationship between the iron content of marrow reticulum cells and chelate-induced iron excretion, these investigators concluded that reticuloendothelial iron is not significantly bound by DFO. Spleen iron was not evaluated in their study. In view of the heterogeneity of the RES, one cannot deduce the status of iron metabolism in the system as a whole from the appearance of iron in one of its member satellites.

It is of interest that mobilization of splenic iron by chelate was accomplished in the face of little or no flow of iron from spleen to erythroid marrow. Because DFO is able to bind iron in the presence of apotransferrin, it has been suggested that iron is chelated by DFO at sites of iron exchange between transferrin and cell membranes—that is, during transfer of iron from reticuloendothelial cell to transferrin, from transferrin to normoblasts and reticulocytes, from mucosal cells to transferrin, and from transferrin to storage sites. Reticuloendothelial iron release is closely coordinated with marrow iron requirements. In erythroid aplasia or hypoplasia, therefore, one would anticipate little movement of iron from the splenic pool of labile iron to transferrin. Despite our patient's lifelong erythroid hypoplasia, chelation therapy successfully mobilized iron delivered to the spleen by transfused red cells. One may conclude that transferrin-prompted movement of iron across cell membranes is not a requisite for in vivo DFO chelation.

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REFERENCES

10. Moeschlin, S., and Schneider, U.:


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