THE IRONY OF HEMOCHROMATOSIS

Medical Grand Rounds
Parkland Memorial Hospital
April 4, 1974

Michael S. Brown, M.D.

In 1935 when Sheldon wrote his classic monograph on hemochromatosis (1), everything seemed clear. The disease was an inborn error of metabolism that resulted in massive iron accumulation and produced the classic triad of diabetes, cirrhosis and skin pigmentation. Since then, however, chaos has intervened, largely because of 3 observations: (1) it became clear that excessive administration of iron to patients with a variety of anemias could lead to all of the manifestations of hemochromatosis (2); (2) an enormously high incidence of the disease was described in the African Bantu, who ingest a large excess of both iron and alcohol (3); and (3) the studies of MacDonald seemed to indicate that anyone who consumed alcohol in excess could develop hemochromatosis (4). All of these observations led to the suggestion that hemochromatosis was not an inborn error of metabolism at all but merely a consequence of excessive alcohol and iron consumption (4).

Confusion as to the nature of hemochromatosis still prevails. I believe, however, that use of sophisticated new techniques of quantitating iron metabolism has provided enough information to once again "iron out" the problem of hemochromatosis.

This morning I will present data that I hope will convince you of three things:

(1) Idiopathic hemochromatosis is due to an inherited defect in the regulation of iron absorption.
(2) The disorder is transmitted by a single gene.
(3) The defect in heterozygotes is clinically detectable, but expression of the full syndrome requires the participation of potentiating genetic or environmental factors.

The exciting aspect of these hypotheses is that they are subject to testing by currently available techniques. If I do not convince you of the truth of these hypotheses this morning, then I hope that my review at least will serve as a conceptual framework for productive debate and, hopefully, well-designed experiments.

CASE REPORTS

Case No. 1 - [redacted] is a 25-year-old woman who was admitted to [redacted] on [redacted] 1973, because of shortness of breath. She had been well during her childhood years. At the age of 13 she underwent a normal menarche, experienced normal breast development and acquired pubic hair. However, at age 16 her menses ceased, her breasts became smaller and her pubic hair receded. At age 17 she developed diabetes mellitus and subsequently required 50-60 units of insulin for control. At age 25, 3 months prior to her [redacted] admission, she experienced the abrupt onset of substernal chest pain and shortness of breath. Shortly thereafter, she noted increasing abdominal girth and swelling of her feet. She was admitted to another hospital where the diagnosis of multiple pulmonary emboli was suspected, and she underwent ligation of her vena cava when her symptoms persisted after heparin therapy. During surgery her liver was noted to be "swollen and tense." Because of continuing dyspnea, pleural effusions, ascites and peripheral edema, she was referred to [redacted].

Physical examination revealed that she was tachypneic with marked cyanosis and severe distention of her neck veins. She had inspiratory rales at both lung bases and signs of bilateral pleural effusions. Her heart was enlarged, there was accentuation of the pulmonic component of the second heart sound and a protodiastolic gallop was audible. There was no heart murmur. There was evidence of moderate ascites and the liver span was enlarged to 15 cm. The spleen was not felt. There was marked pitting edema of the lower extremeties. Many observers commented on her profound "cyanosis" even after her hemoglobin oxygen saturation was restored to normal by oxygen administration.

Her pertinent laboratory values are given in Table I. Cardiac catherization produced findings consistent with a restrictive cardiomyopathy. Aspiration of pleural and abdominal fluid yielded material compatible with a transudate. Liver biopsy revealed marked fibrosis with loss of the normal lobular architecture and massive deposits of iron-staining material in the parenchymal cells.

Her congestive heart failure responded to treatment with digitalis and diuretics and was discharged to be followed in the Outpatient Department. It was planned that she would have 2 units of blood drawn weekly.

Case No. 2 - [redacted] is a 53-year-old [redacted] to the [redacted] from [redacted] who was admitted to [redacted] Hospital on [redacted] 1974, because of abdominal swelling. The patient said that he had consumed one quart of [redacted] wine daily for the past 30 years, and over the past 7 years had taken a teaspoonful of Geritol daily. He had been in apparent good health until three weeks before admission when he experienced progressive abdominal swelling and edema of his legs. There was no history of jaundice or past liver disease. For the past six months he has been impotent. On admission his skin was said to have a "dusky hue." Spider angiomata were present, but there was no jaundice. The neck veins were not distended, but evidence of bilateral pleural effusions was present. The abdomen was massively distended with ascites, and the liver was enlarged. The testes were small and soft. Massive pitting edema of the lower extremities was present.

Laboratory values are given in the table. Aspiration of pleural and abdominal fluid produced material consistent with a transudate. Liver biopsy showed severe fibrosis with loss of the normal lobular architecture and extensive iron deposition in the parenchymal cells. Skin biopsy also showed iron deposition. The ascites and edema responded to diuretic treatment and salt restriction. The diabetes responded to therapy with tolbutamide. The patient was discharged with plans to undergo twice-weekly phlebotomies.

Table I. Laboratory Values in [redacted] and [redacted]

	[redacted]. (46 59 90)	[redacted] (46 80 89)
Serum Iron (µg%)	236	192
Iron Binding Capacity (µg%)	236	213
Hematocrit (%)	42.4	43
Reticulocyte Count (%)	1.0	--
Fasting Blood Glucose (mg%)	361	410
Albumin (g%)	3.7	3.2
Globulin (g%)	3.8	4.6
Total Bilirubin (mg%)	1.3	0.4
Alkaline Phosphatase (KA. units)	17.5	24.5
SGOT (SMA units)	165	172 (SMA)
Prothrombin Time	12 sec. = 100%	11 sec.
Alpha-fetoglobulin	Negative	Negative
Plasma Cortisol (7:30 a.m.)	35 µg%	18 µg%
Growth Hormone	3 ng/ml	--
Thyroxine (by isotope)	6.4 µg%	11.5 µg%
ACTH	Normal	--
Metyrapone Test	Normal	--
LH	<10% of normal	--

CLINICAL PICTURE OF HEMOCHROMATOSIS

These two cases, which were fortuitously seen within a one-month period, illustrate beautifully the wide extremes of the clinical spectrum of hemochromatosis. [redacted] had the onset of her clinical disease at age 16 when her menses stopped, and one year later she developed diabetes. The onset of hemochromatosis below the age of 25 is extremely rare. Fig. 1 is taken from an extensive review of 1500 cases of hemochromatosis by Heilmeyer (5) and shows that only 3 cases in men below age 25 were seen, and none in women. On the other hand, [redacted], at age 55, falls right into the age of maximal incidence. In 1965 Perkins could only find 10 reported cases of hemochromatosis occurring before age 20, two of which were women (6). Interestingly, in these young patients the mode of presentation was almost always through congestive heart failure, in contrast to the presentation in the older age group, which is usually due to complications of liver disease or diabetes. An analysis of the causes of death in hemochromatosis by Finch (2) shows the preponderance of cardiac failure in the younger age groups, and the markedly increased incidence of hepatoma in the older age groups.

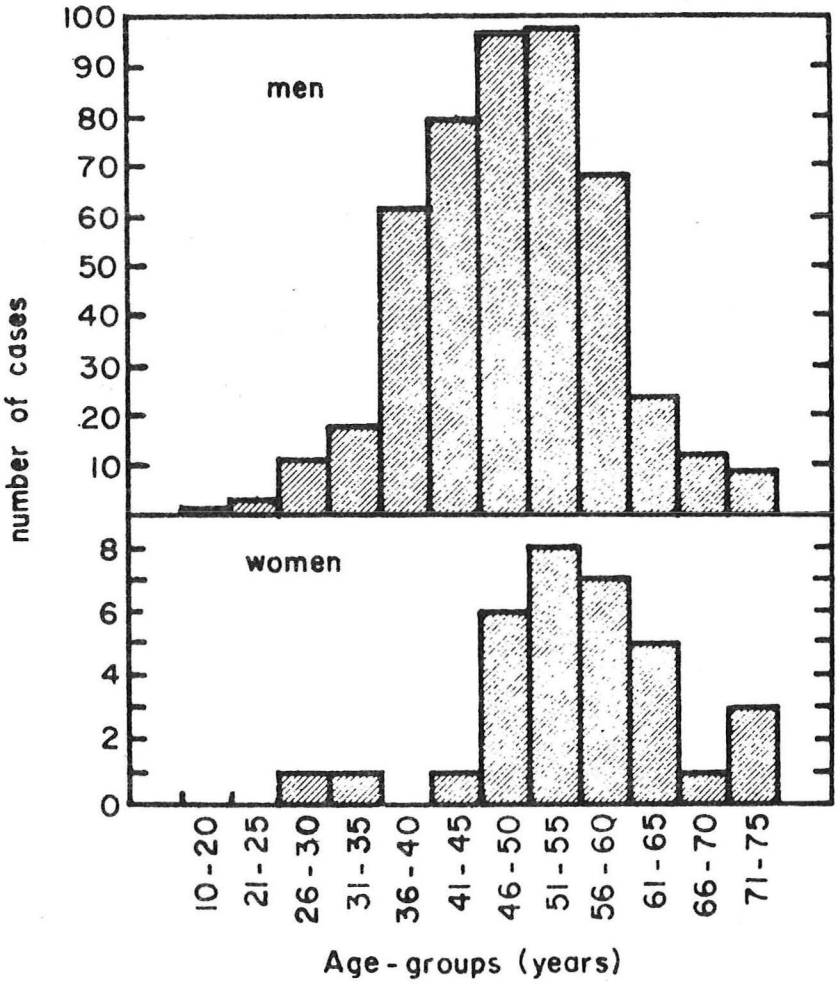
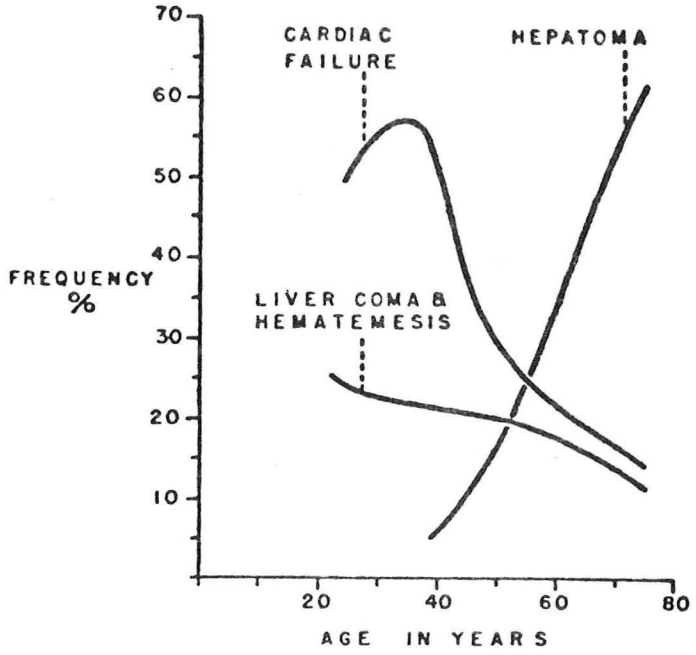


Fig. 1. Age of diagnosis of idiopathic hemochromatosis (5).

Fig. 2.

AGE DISTRIBUTION OF MAJOR CAUSES OF DEATH
IN
IDIOPATHIC HEMOCHROMATOSIS



The incidence of the various clinical manifestations and physical findings in hemochromatosis is given in Figs. 3 and 4 (2).

Fig. 3. Symptoms in patients with hemochromatosis (2).

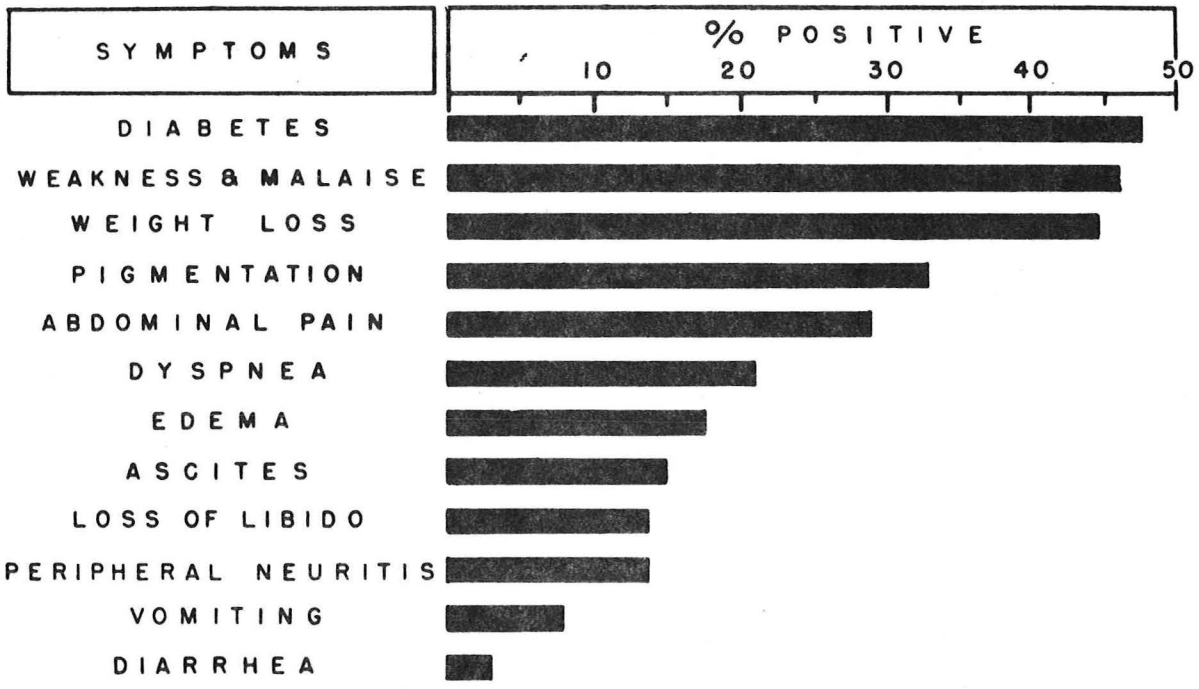
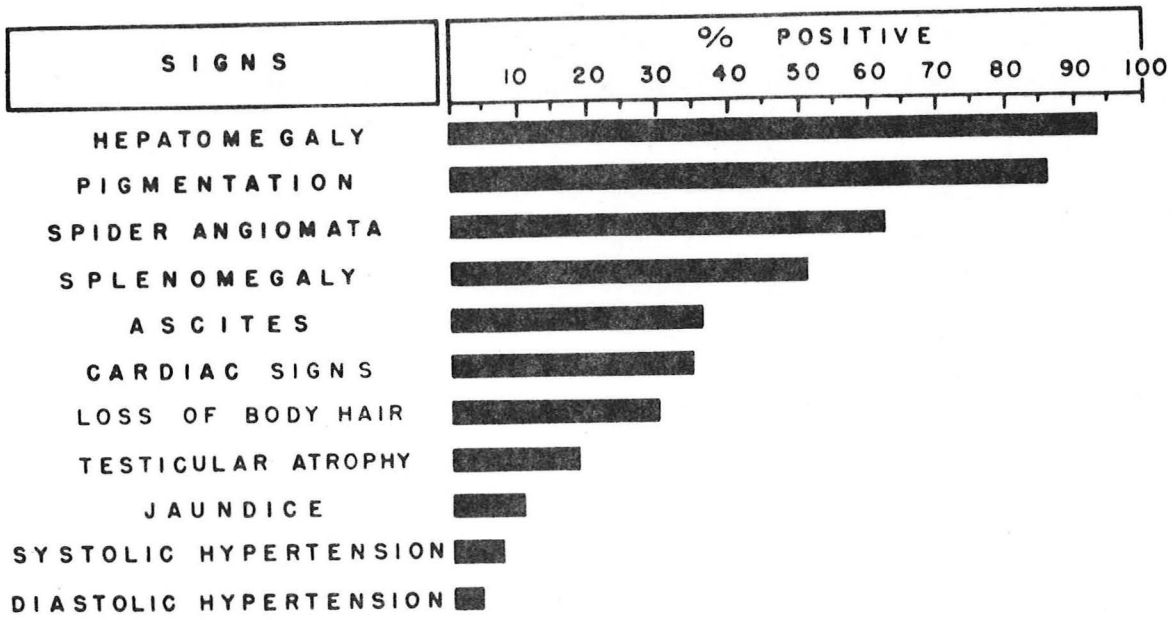
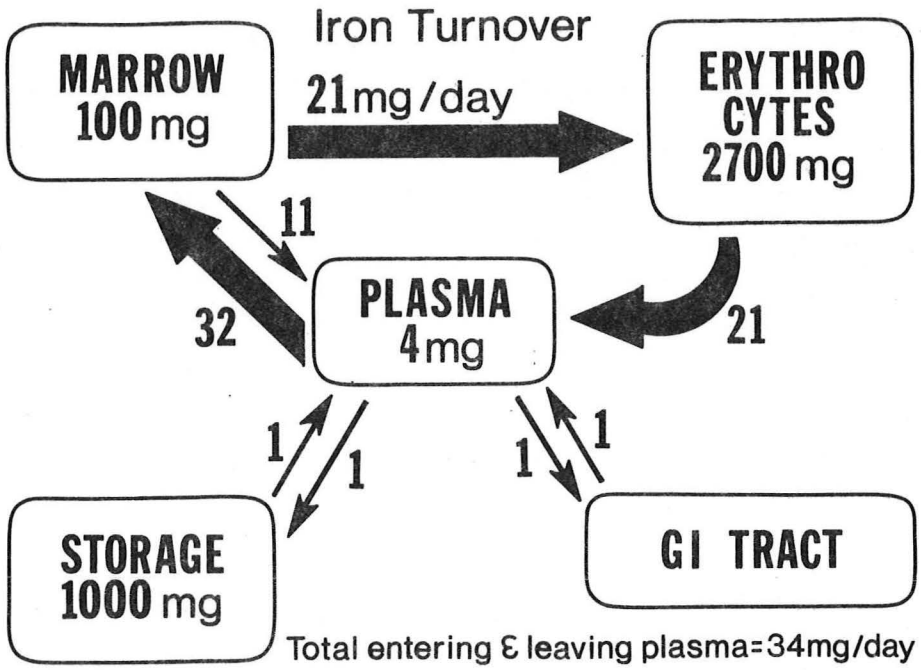


Fig. 4. Physical signs in patients with hemochromatosis (2).



In hemochromatosis there is an increased absorption of dietary iron, and this leads to secondary deposition of iron in a wide variety of tissues. Recent studies indicate that the pituitary gland is particularly susceptible (7). The amenorrhea in the first case is associated with a markedly decreased level of luteotrophic hormone. Similarly, the testicular atrophy in the second case is likely due to a loss of pituitary trophic hormones rather than to primary gonadal failure. Diabetes is seen in between 50 and 80% of all patients with hemochromatosis (2), but whether this can strictly be attributed to iron deposition in the pancreas will be discussed later at length. Cirrhosis is the sine qua non of the diagnosis of hemochromatosis and occurs, by definition, in 100% of the clinically symptomatic cases. Pigmentation of the skin is due to an increased melanin production as well as to the deposition of iron. Heart failure, usually of the restrictive cardiomyopathy type, can be strictly attributed to a toxic effect of iron, since it recedes dramatically when the iron overload is reversed by phlebotomy (8).

Fig. 5.



NORMAL IRON METABOLISM

In order to discuss the pathogenesis of hemochromatosis, one must first discuss the normal metabolism of iron in the body. In the normal man, the body contains about 4 grams of iron (9). The vast bulk of iron (2700 mg) is bound to heme in circulating erythrocytes. About 1000 mg is present in storage sites in various tissues, about half of this (500 mg) in the parenchymal cells of the liver. The bone marrow itself contains only a tiny amount of iron (100 mg). Small amounts of iron are present in various heme-containing proteins such as myoglobin and various cytochromes. In the normal subject in the steady state, the intestinal absorption of iron is severely limited. Thus, although the standard American diet contains 10-15 mg of iron ingested daily, only about 1 mg is absorbed. This absorption is just sufficient to balance the normal excretion of 1 mg of body iron, which occurs largely due to sloughing of iron-containing cells in the GI tract, as well as to the loss of a tiny amount of blood daily through a constant mild GI bleeding of about 1 ml per day. An important point is that the loss of iron from the body is fixed to a relatively narrow range. Since urinary excretion of iron is almost nonexistent, there is no way for the body to rid itself of excess iron once it gains access to the body.

Because of the unequal distribution of iron stores in the body, mechanisms must exist to facilitate the transport of iron between the various pools. This transport occurs through the plasma, where all of the iron is bound to the transport protein transferrin. The plasma contains only 4 mg of iron, yet it must deliver 21 mg of iron daily to the marrow for the production of red cells. Thus, plasma iron turns over at a rapid rate. Most of the iron entering plasma daily is derived from the destruction of red cells in the reticulo-endothelial system. Only a small amount of plasma iron exchanges with the tissue storage pools (9).

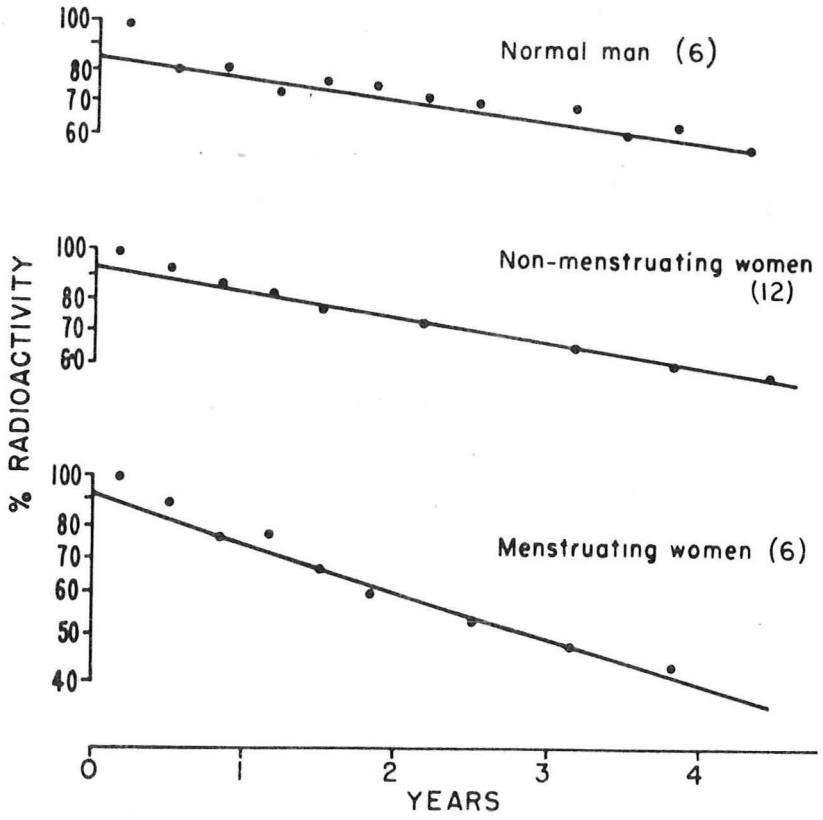


Fig. 5a. A good illustration of the slow loss of iron from the body is seen in this experiment of Finch (20). A small amount of iron was administered to normal subjects and the amount of radioactivity remaining in the circulating red blood cells was measured at intervals up to 4 years. In men and non-menstruating women, only 40% of the iron was lost over the 4-year period, which calculates to about 1 mg per day. In menstruating women, however, 60% of the iron was lost--or about 1.5 mg per day. The increased loss of menstrual iron protects women from the clinical onset of hemochromatosis and explains the 10-fold higher incidence of symptomatic hemochromatosis in men.

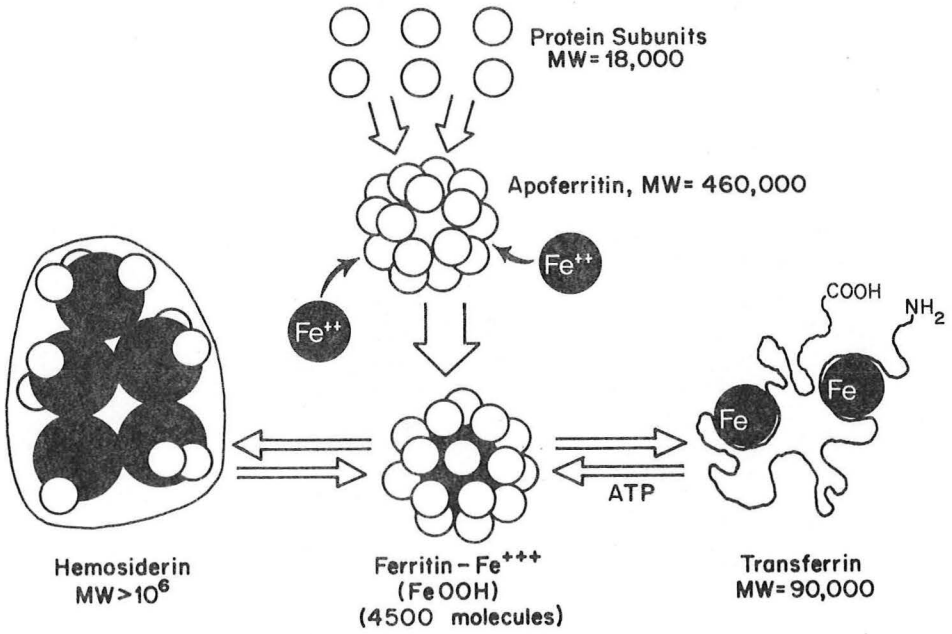
The best way to expose the regulatory controls in such a system is to perturb it. One way to perturb the system is to remove a proportion of the circulating red cells by phlebotomy. When this happens, there is an immediate increase in iron uptake from plasma by the marrow in order to provide substrate for an increased rate of erythropoiesis. The marrow is capable of expanding its red cell production maximally about 6-fold, but the maximal rate at which iron can be removed from the storage pool is about 35 mg/day, or enough to support about twice the normal rate of erythropoiesis. As iron is transferred from the stores to the plasma, the actual concentration in the plasma initially remains constant. Only when the storage depots have been exhausted does plasma iron concentration begin to fall (9). Thus, in conditions of iron depletion, a low serum iron is strong evidence for a depletion in the tissue stores of iron.

Now let us examine what happens when the system is perturbed by the accumulation of excessive iron. If the excessive accumulation comes from the GI tract, plasma iron is initially elevated, but the iron is rapidly unloaded into the storage depots, and plasma iron soon returns to its former level. If iron is administered intravenously, it tends to accumulate first in cells of the reticuloendothelial system, and again, the plasma iron level drops. Thus, in conditions of iron overload, the plasma iron concentration is a poor reflection of the iron stores in the body.

IRON-BINDING PROTEINS

In plasma, iron travels in the ferric state bound to the transport protein, transferrin (Fig. 6). This protein, which appears to consist of a single polypeptide chain of 90,000 daltons, is capable of binding two molecules of iron per molecule of protein. The classic studies of Jandl in the early 1960's

Fig. 6. Iron-binding Proteins.



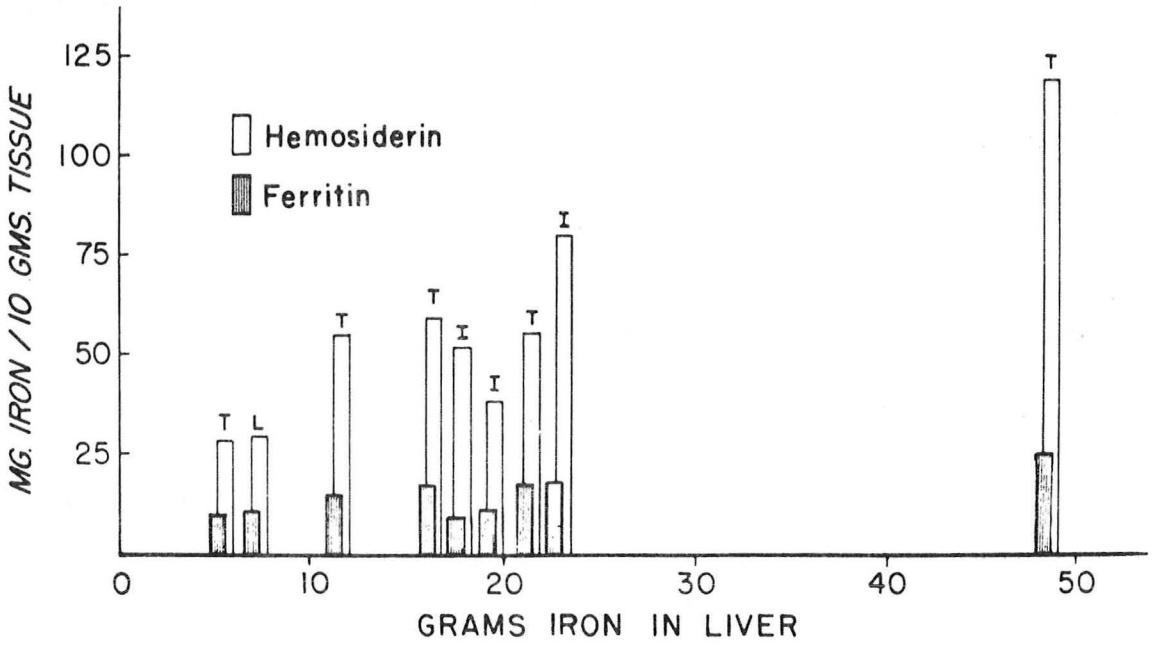
demonstrated that transferrin has the unique property of being able to bind specifically to receptor sites on reticulocyte membranes and to deliver its iron rapidly after binding to the cell surface (10). Recent studies indicate that the two binding sites for iron are not functionally identical, and that iron bound to one of the sites may be more easily transported to the reticulocyte than iron bound to the other site.

The major non-heme proteins which bind iron in the body tissues are shown in Fig. 6. Under normal conditions most storage iron is bound to ferritin. This protein occurs in all body tissues, but it is present in highest concentrations in the liver parenchymal cell. In order to produce ferritin, the liver first synthesizes a series of protein subunits, which appear to be identical and which appear to have a molecular weight of about 18,000 (11). A specific aggregation of 24 of these subunits next occurs to form the protein apoferritin, which has molecular weight of 460,000--made up of the aggregated ferritin subunits. The apoferritin subunits have the important property of being able to oxidize iron enzymatically from the ferrous state to the ferric state (12). Iron is present in the liver cytosol as a mixture of the ferrous and ferric forms, and these molecules are then taken into the core of the apoferritin particle where the iron is oxidized and stored in the form of ferric hydroxide (FeOOH). The iron center of the ferritin molecule is present as a hugh micelle, with 4500 molecules of iron in each ferritin molecule. Thus, unlike the case with transferrin, there is no true stoichiometric relation between the amount of iron and protein in the ferritin molecule, and ferritin molecules of widely differing iron contents are present at all times (13). The final particle remains completely soluble, and it has a characteristic appearance on electron microscopy.

A major conceptual advance was made by Granick, who showed that the administration of iron to rats produced an immediate increase in the number of ferritin molecules in the liver (15). Fineberg and Greenberg (16) and Munro and Drysdale (11) then demonstrated by measuring the incorporation of radioactive amino acids into protein that the administration of iron actually induced the de novo synthesis of apoferritin protein in animal liver. A similar induction of ferritin synthesis by iron has been shown recently in HeLa cells growing in vitro (17). The induction by iron of ferritin synthesis explains why the liver is able to accept enormous amounts of iron that are presented to it in the iron-loading states.

When large amounts of ferritin accumulate they are ingested by lysosomes (18). After this ingestion, the protein moiety becomes partially digested and denatured, and the iron micelles aggregate into an insoluble mass of iron. This intralysosomal iron accumulation is termed hemosiderin. Although the iron appears to be present in an amorphous wastebasket form in hemosiderin, it is still available to the body, and can be effectively mobilized when iron stores are depleted, for example, by phlebotomy.

Fig. 6a Accumulation of ferritin and hemosiderin in livers of patients with iron overload (20).



Since in idiopathic hemochromatosis there is an excessive iron accumulation in the body, and since iron is not manufactured in the body, then the deposition of iron must come from an increased absorption of dietary iron. Theoretically, this increased absorption could come from a malfunction of any of these iron-containing proteins. However, the few studies to date indicate that all of these proteins function normally in hemochromatosis. Thus, in Fig. 6a, taken from the work of Finch (20), the content of hemosiderin and ferritin was measured in livers of patients with iron overload. As the amount of iron increased, the amount of ferritin initially increased, but above 10 g iron in the liver, the amount of ferritin stopped rising and all of the additional iron was present as hemosiderin. This distribution was identical in patients with idiopathic hemochromatosis (I) and transfusion hemosiderosis (T), indicating that there was no primary defect in hepatic iron-binding proteins in hemochromatosis.

Similarly, transferrin behaves normally in hemochromatosis. Thus, in Fig. 6b, Crosby labeled normal transferrin (open circles) with Fe^{55}, and transferrin from a patient with hemochromatosis (closed circles) with Fe^{59}. He then injected a mixture of both transferrin preparations into both a normal subject and a patient with hemochromatosis. In both patients, the disappearance curve for both transferrin preparations were identical, indicating that transferrin from the hemochromatosis patient behaved normally.

Additional evidence against a primary defect in the tissue distribution of iron comes from the fact that subjects who chronically over-ingest iron (African Bantu), or who hyper-absorb iron (Thalassemia and sideroachrestic anemia) can develop a syndrome identical to idiopathic hemochromatosis (20).

Thus, the defect in hemochromatosis appears to involve a disorder in the regulation of intestinal iron absorption.

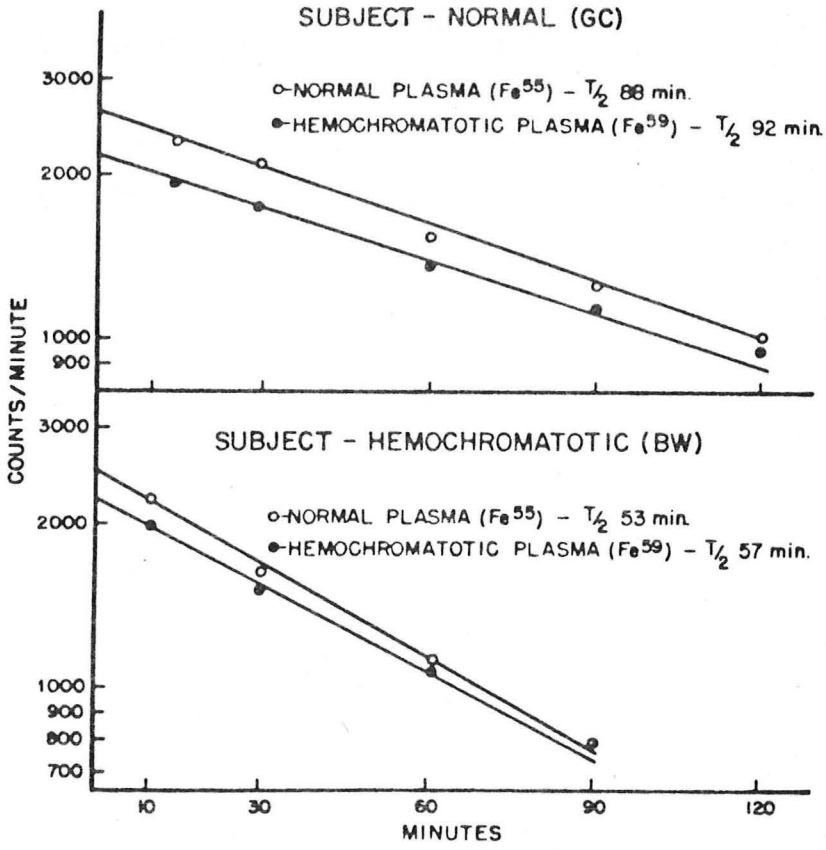
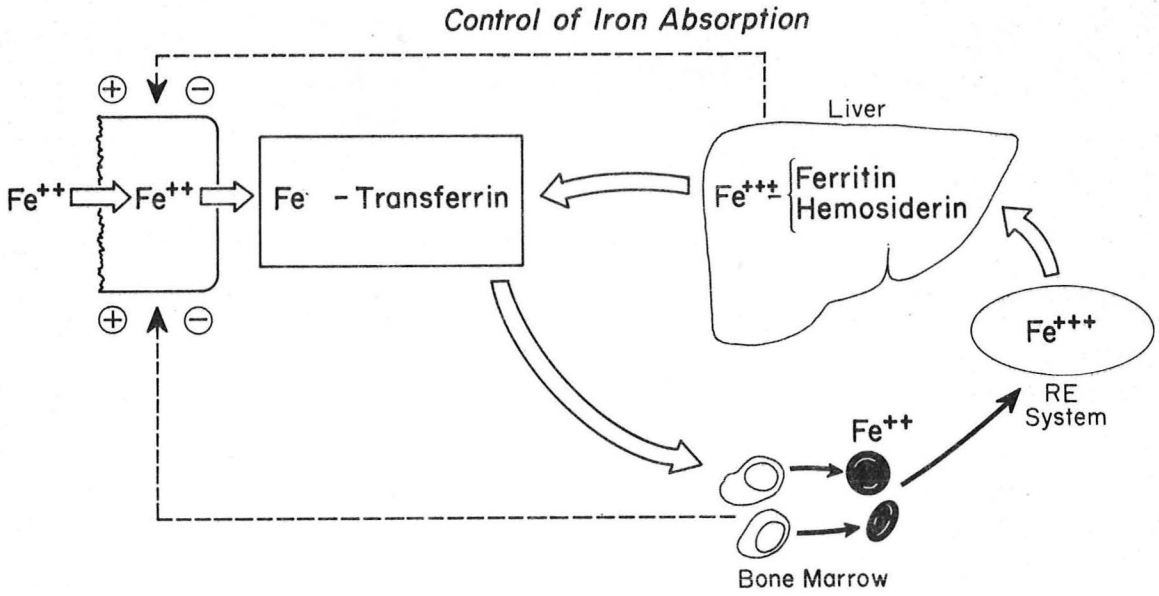


Fig. 6b Turnover of transferrin from a normal subject and a patient with hemochromatosis after administration to a normal subject and a patient with hemochromatosis.

REGULATION OF INTESTINAL IRON ABSORPTION

As a result of many studies over the past 15 years, particularly those of Crosby (19) and those of Finch (20), it is now clear that the low level of iron absorption seen in the normal state is not because of any intrinsic limitation on the behavior of the intestinal mucosa, but it is due to a precise regulation of iron absorption. Two major factors appear to work independently in regulating iron absorption (Fig. 7). First, and probably most important under physiologic circumstances, iron absorption is regulated by the iron content of the body, particularly the liver. Thus, if one raises rats on an iron deficient diet, they have a marked increase in their ability to absorb iron (20). Similarly, if one phlebotomizes human subjects and then waits until their red cells have been restored and their rate of erythropoiesis is normal, these people will continue to hyperabsorb iron from the GI tract until their iron stores are again raised to normal (21). On the other hand, if one overloads the iron stores of normal individuals with intravenous iron administration, their iron absorption is severely curtailed (21).

Fig. 7



In addition to its control by the state of the iron stores, however, iron absorption is controlled independently by the erythropoietic cells of the bone marrow (Fig. 7). Thus, in conditions of increased erythropoiesis, even in the face of iron overload, human subjects absorb elevated amounts of iron. The classical example of such a state is the disorder β-thalassemia in which homozygotes have a severe hemolytic anemia and ineffective erythropoiesis. Despite their severe iron overloading secondary to transfusions, patients with thalassemia continue to absorb excessive amounts of iron from the GI tract, and they frequently develop the full-blown picture of hemochromatosis (20). Thus, two independent processes function to regulate iron absorption--the state of iron stores in the body and the rate of erythropoiesis.

Table II

REGULATION OF BODY IRON STORES IN FOUR EXPERIMENTAL SUBJECTS

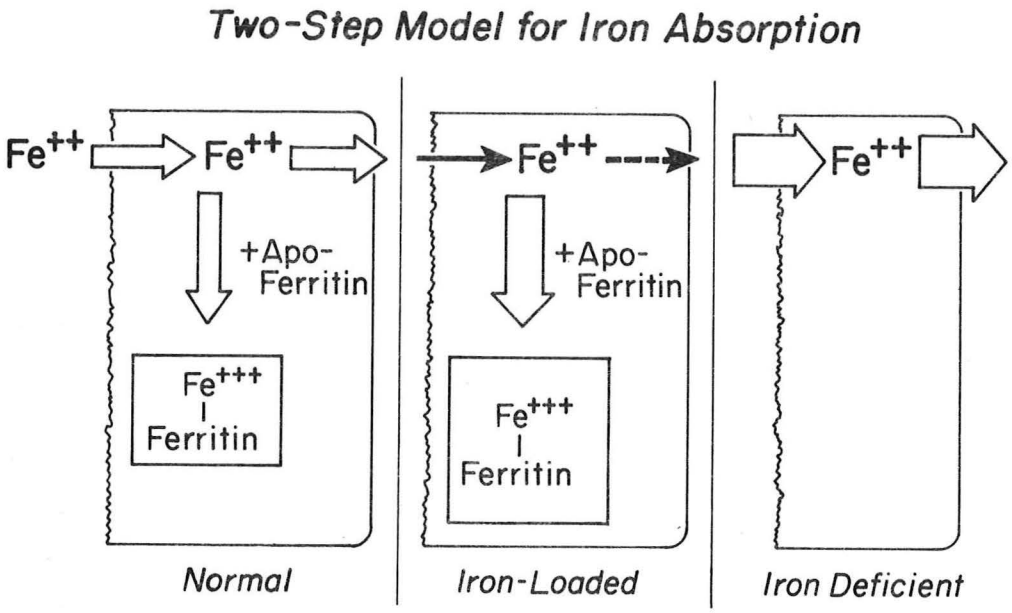
	Iron Depleted (2 subjects)	*Iron Loaded (2 subjects)*
Initial stores (mg.) (assumed)	1250	1250
Experimental alteration	−1250 (phlebotomy)	+1400 (parenteral iron)
Initial size of stores (assumed)	0	2650
Iron absorption over 2 years (determined × 3)	20%	4%
Iron recovered by phlebotomy after 2 years (mg.)	1300	2100
Calculated iron balance (mg.)	+1300	−550
Calculated absorption — loss (mg./day)	2.2	−0.1
Calculated absorption for 15 mg. diet (%)	15	0

An excellent experimental example of this regulation in humans is seen in Table II which is taken from the work of Finch (ref. 20, page 126). Two normal subjects were phlebotomized to the point of anemia with the removal of 1250 mg of iron. Two other subjects were overloaded with 1400 mg of iron administered parenterally. Two years later the subjects were again subjected to phlebotomy to determine their iron stores. The two iron-deficient patients had re-accumulated all of the iron that had been lost, and one can calculate that they absorbed 2.2 grams of iron per day (15% of a 15 gm intake). On the other hand, the iron-loaded subjects actually sustained a net loss of iron over the two year interval, so their net iron absorption was zero.

MECHANISM OF IRON ABSORPTION

When one reviews the experimental studies of iron absorption by the small intestine, one is struck by the fact that this process has not been studied by the highly sophisticated techniques of undirectional flux measurement which

Fig. 8



have revolutionized our ideas of the absorptive mechanisms for other substances such as sugars, fats and amino acids. (The work of John Dietschy exemplifies the power of these new methods.) Thus, the data on iron absorption are a bit muddled; however, the few studies that have been performed seem to suggest a two-step model for iron absorption as shown in Fig. 8. The first step in this process is the uptake of iron across the mucosal border. This step appears to require metabolic energy, and it may exhibit saturation kinetics, indicating that a carrier mechanism might be involved in the transport of iron across the mucosal surface of the cell (22). Once inside the cell, a fraction of the iron becomes bound to ferritin, and this material is unable to be further absorbed (23, 24). In order to traverse the serosal border of the cell, iron requires a second transport process. This process also requires energy, is specific for iron in the ferrous form, and competition can be shown between iron and calcium (22).

As we have previously seen, when an animal is iron-loaded, the overall absorption of iron declines. Granick initially suggested (15), and Crosby popularized (24), the theory that this so-called mucosal block was due to an increased content of ferritin in the epithelial cell, with trapping of iron so that it could not be transported. This hypothesis is not consistent with the kinetic data. If the mucosal block in iron absorption were due to trapping by ferritin, however, one would suppose that as the iron concentration presented to the cell increased, a point would be reached at which the ferritin would become saturated and iron absorption would suddenly increase. However, this is not the case. Fig. 9, taken from Finch (20), shows that in man, iron absorption is limited at any given iron concentration, but it increases in a log linear fashion as the iron concentration presented to the cell increases; there is no real evidence of any trapping mechanism. About 10% of the iron is absorbed at any level of iron. Recent studies in several laboratories, including a major study by Sheehan and Frenkel of our department (25), have further shown that the transport of iron is not limited by the ferritin content of the mucosal cell. In rats with hemolytic anemia, the ferritin content of the mucosal cells did not change, yet iron absorption was massively increased (25). The mucosal block seems

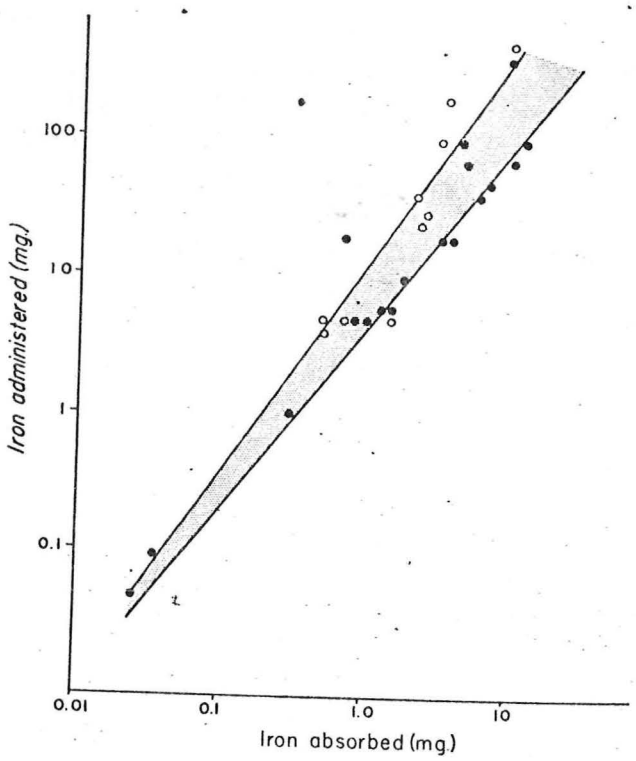


Fig. 9 Iron absorption in man as a function for the dose of iron administered

to occur because of a decreased rate of two processes; (1) the initial mucosal uptake process, and (2) the transfer step between the mucosal cell and the plasma (22, 26). It appears that when the mucosal cell is formed in the intestinal crypts, it is programmed by two messages--one tells the cell the rate of erythropoiesis, and the other tells the cell what the level of iron stores is. In response to both of these messages, the cell's level of both the uptake process and the transfer process are set, and for the three-day life span of that cell, it will only transport the amount of iron that it has been programmed to transport.

What are the messages that tell the epithelial cell how much iron to absorb? These remain a mystery. Many negative experiments have been performed in which investigators have transfused plasma from iron-deficient animals into iron-loaded animals (27). No humoral factor has been identified. Although erythropoietin would seem a likely candidate to transmit the message of the degree of erythropoiesis, no one has been able to demonstrate that erythropoietin plays a role in regulating intestinal iron absorption.

NATURE OF THE DEFECT IN IDIOPATHIC HEMOCHROMATOSIS

As we have seen, iron absorption is not fixed; in normal subjects it is tightly regulated by the availability of iron stores as well as by the rate of erythropoeisis. In hemochromatosis iron absorption is inappropriately elevated at a time when iron stores are high. This means that the regulation of iron absorption is faulty. The major question is: does this fault lie in the erythropoeitic tissues, the liver or the intestinal epithelial cell itself? The erythropoeitic system has been exonerated; the rate of iron utilization by red cells is normal, and when iron is removed by phlebotomy there is no inability of red cell precursors to take up iron (9). Similarly, current data, to be presented below, indicate that the intestinal epithelial cell is behaving normally; that is, it is responding normally to a signal to increase iron absorption. It is the signal that is faulty. The faulty signal that tells the intestine to keep absorbing iron even though the iron stores are overloaded appears to be coming from the liver. What is the evidence for this statement? The major evidence comes from studies of iron absorption in patients with hemochromatosis before and after phlebotomy. The response of one such patient to phlebotomy is shown in Fig. 10, taken from Finch (20). In this patient one unit of blood was drawn twice weekly as indicated. There was little change in the hematocrit or in the serum iron until nearly 20 grams of iron had been removed. In a normal subject the fall in hematocrit and serum iron would have taken place after the removal of less than a gram of iron. Now let us look at iron absorption in such subjects. Fig. 11, which is representative of a large

Fig. 10

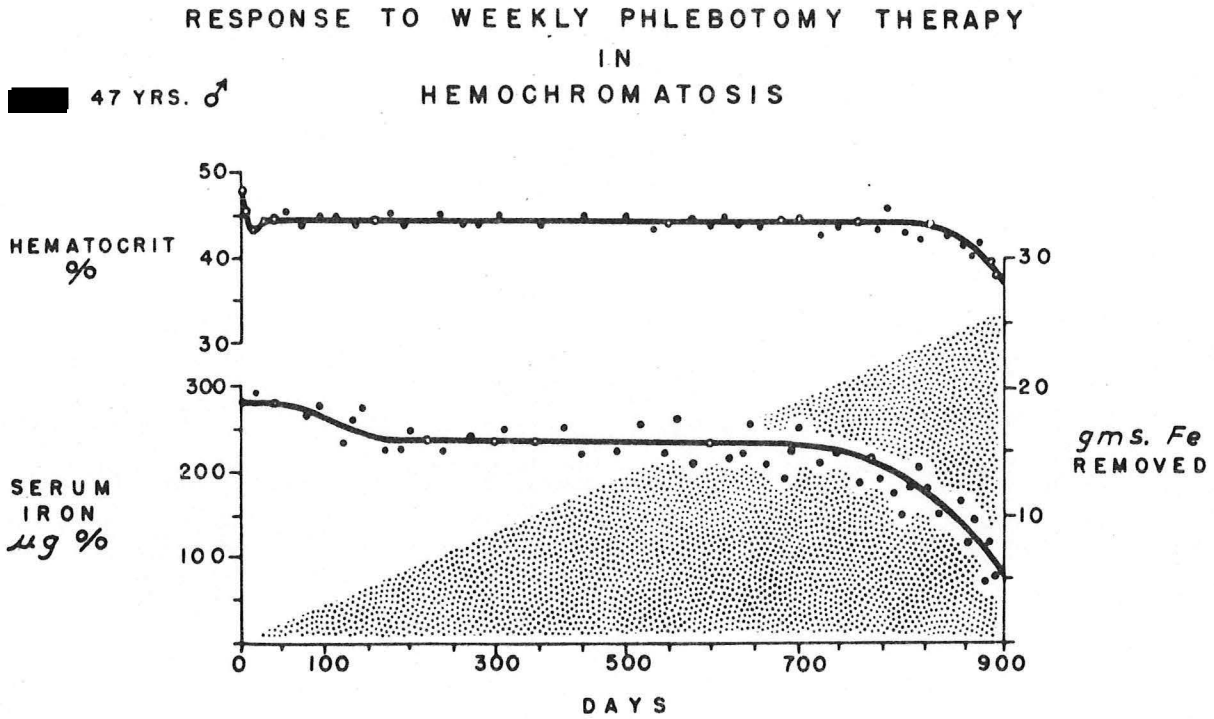
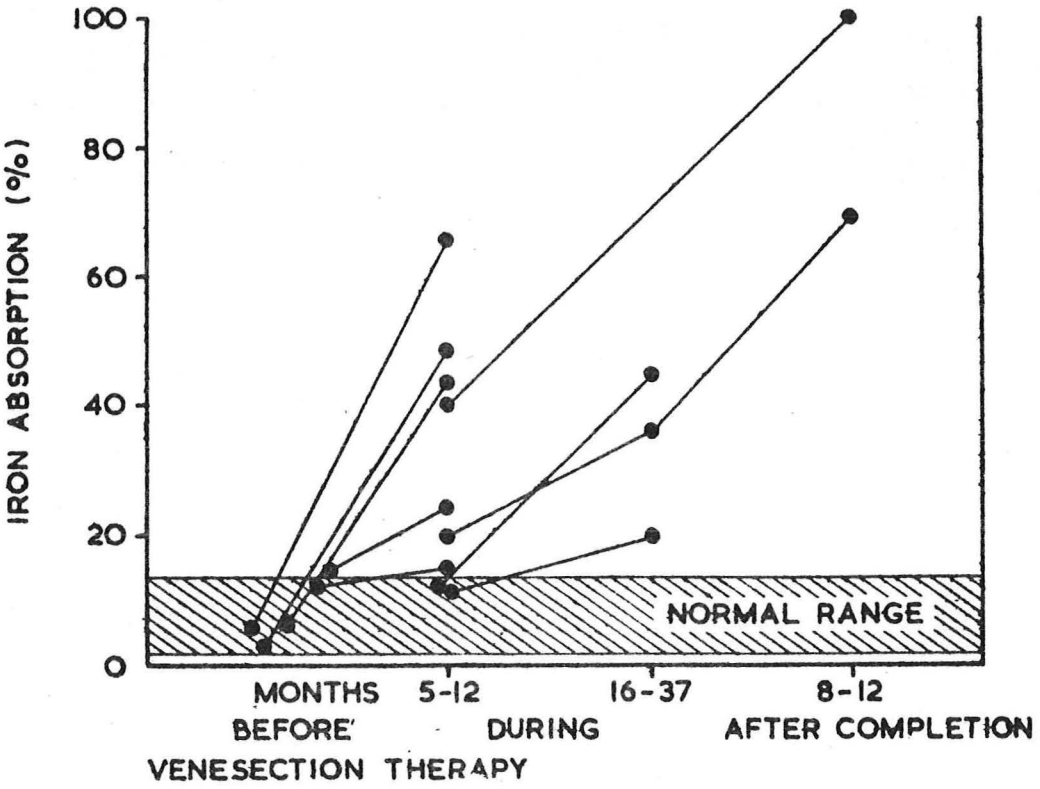
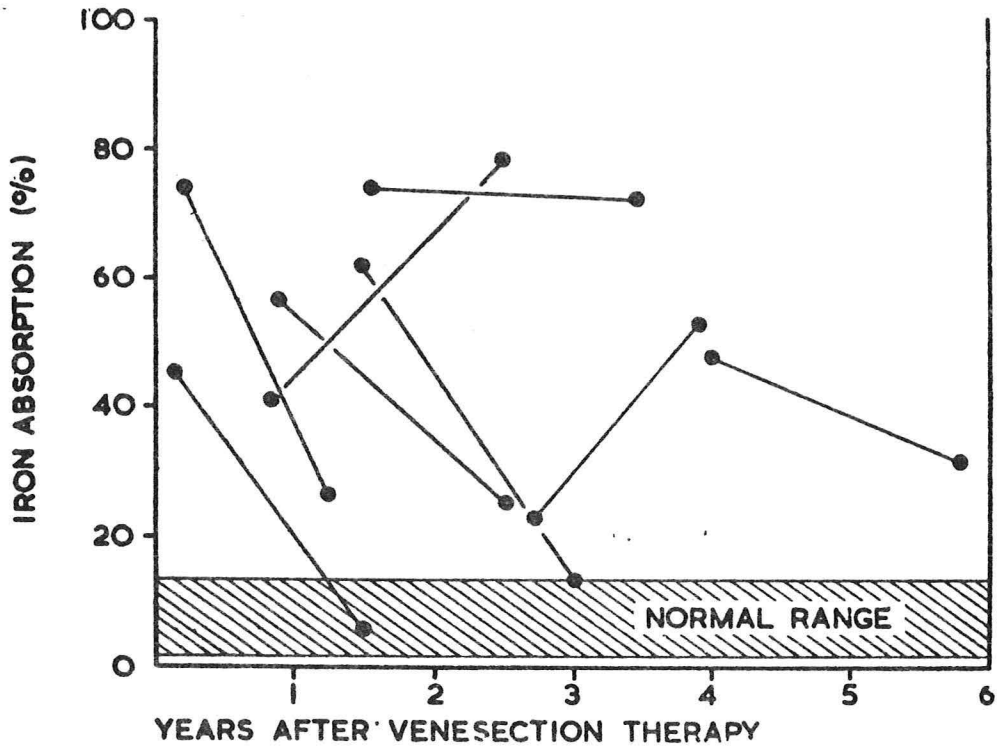


Fig. 11 Iron absorption before, during and immediately after venesection therapy in patients with hemochromatosis.



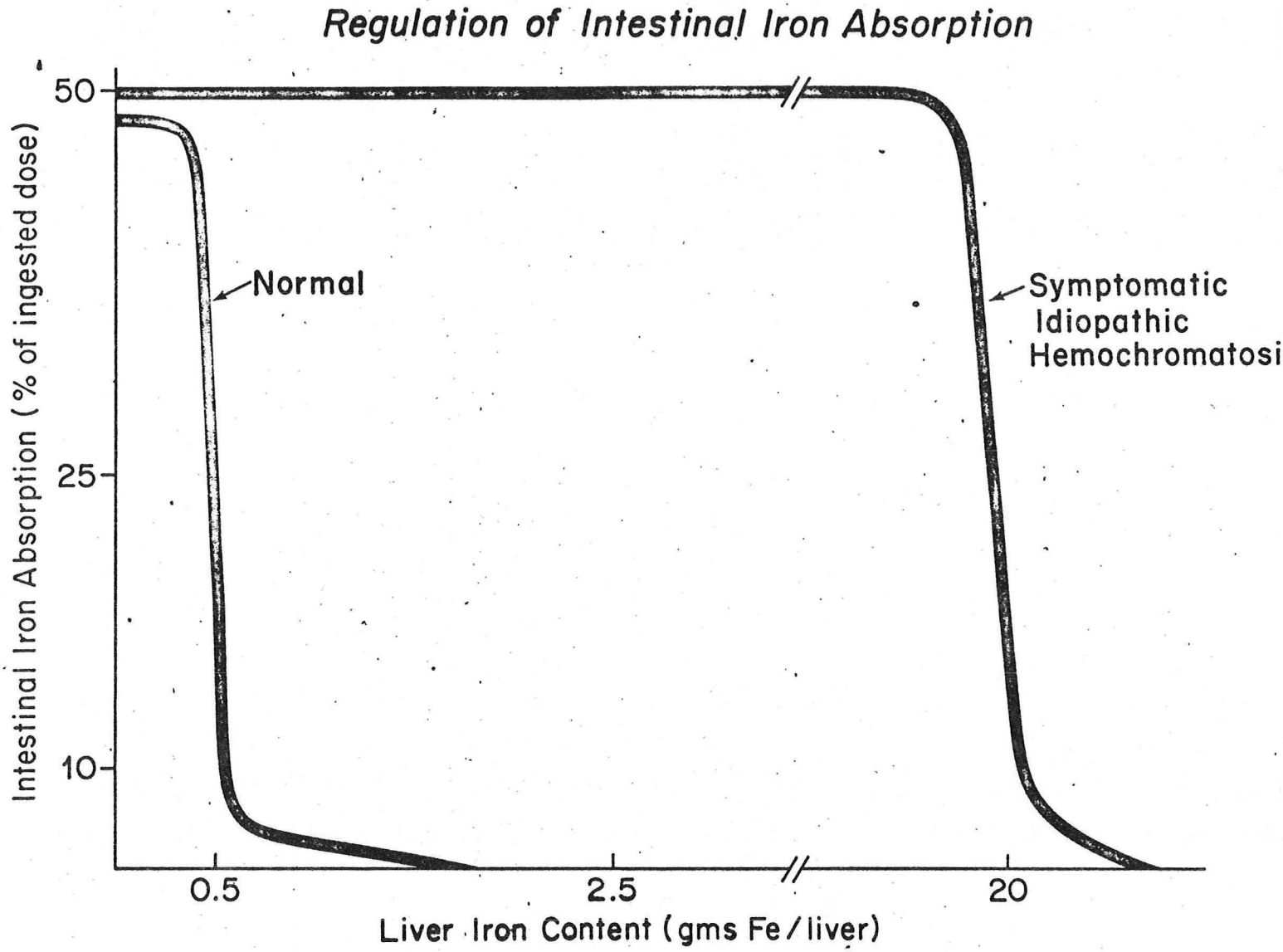
number of similar studies, comes from the work of Williams in London (28). In these five subjects with hemochromatosis, iron absorption was "normal" when the patients were originally seen. However, after the removal of only a small amount of iron, at a time when the hematocrit was unchanged, and when the serum iron remained elevated and the iron stores remained considerably enlarged, iron absorption became markedly elevated above normal. Even more striking were the findings when these subjects were studied over a period of years after the completion of venesection therapy (Fig. 12). At these time points the subjects had already re-accumulated a marked excess of iron, but their stores had not yet reached their former high levels. Since they were no longer undergoing phlebotomy, their rates of red cell production were normal. Nevertheless, these patients continued to absorb massive amounts of iron. If left untreated, they would eventually accumulate iron to their original levels, at which point iron absorption would again diminish. These results have been confirmed in several laboratories, using several different techniques (9, 29).

Fig. 12 Iron absorption after completion of venesection therapy in patients with hemochromatosis.



A graphic depiction of the meaning of these findings is shown in Fig. 13. When normal subjects have less than half a gram of iron in their livers, they absorb iron from the diet until they accumulate half a gram, and then iron absorption is reduced. In patients with clinical hemochromatosis, this regulatory mechanism is present, but it is set at a higher level. That is, these subjects continue to hyperabsorb iron until they accumulate 25 grams in their livers, and then they, too, shut off their iron absorption. Thus, hemochromatosis is an inherited disease in which the regulation of iron absorption is faulty. Further insights into the nature of this defect come from genetic studies.

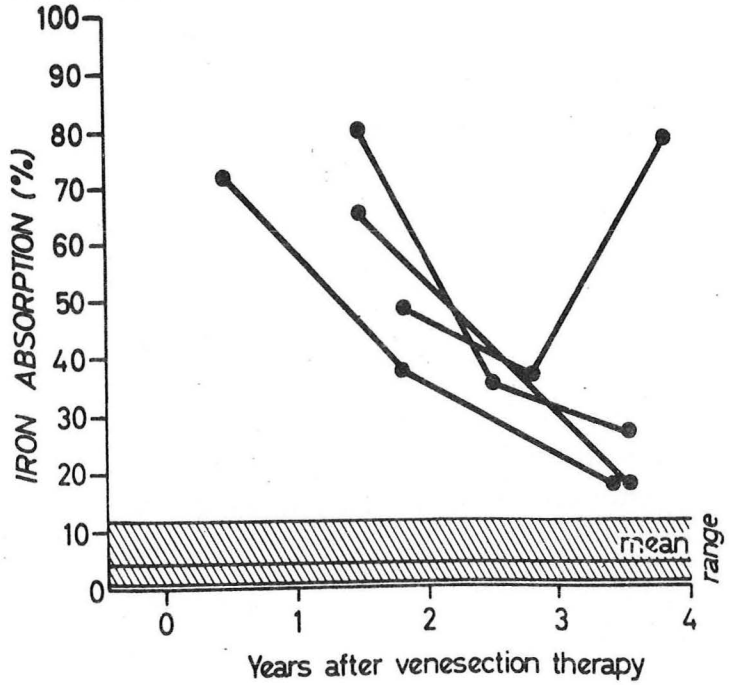
Fig. 13


Regulation of Intestinal Iron Absorption
50
25
10
Intestinal Iron Absorption (% of ingested dose)
Normal
Symptomatic
Idiopathic
Hemochromatosis
0.5
2.5
20
Liver Iron Content (gms Fe/liver)

GENETIC STUDIES IN HEMOCHROMATOSIS

Proceeding pari passu with these studies on the defect in patients with the clinical syndrome of hemochromatosis, have been family studies which help enormously to clarify the defect. The original studies in this regard were undertaken by Williams and Sherlock who performed liver biopsies on first-degree relatives of 16 patients with hemochromatosis (30, 31). They found that in about 50% of these relatives iron stores, as measured by histologic staining, were elevated above those found in controls. These investigators then followed up these studies by measuring iron absorption in relatives of patients with hemochromatosis (32). They found that iron absorption was elevated above normal levels in 50% of the relatives. The most exciting result, however, came when these investigators tested the response of the relatives to phlebotomy (Fig. 14). Just as in the affected patients, iron absorption rose after venesection therapy, but unlike in their affected relatives who had 25 grams of iron in the liver, these asymptomatic relatives began to show signs of iron deficiency when only 2.5-5 grams of iron had been removed. Thus, they had accumulated a level of iron that was intermediate between the 0.5 gm present in normals and the 25 gm present in the clinically symptomatic relatives. When the phlebotomies were stopped, the asymptomatic relatives continued to hyperabsorb iron for a number of years; however, their absorption values began to decline toward normal much faster than in the clinically affected relatives. Iron absorption declined toward normal values after 2-5 grams of iron was absorbed. Thus, 50% of the asymptomatic relatives of hemochromatosis patients appear to have a partial defect in the regulation of iron absorption--they accumulate iron until 2-5 grams of iron is stored in the liver, and then once again they limit their iron absorption and achieve a steady state.

Fig. 14 Iron absorption in relatives of hemochromatosis patients after venesection.



-Serial readings of iron absorption in 4 relatives examined after completion of venesection therapy.

If 50% of the relatives of hemochromatosis patients have increased iron absorption and elevated iron stores, why don't these relatives have clinical hemochromatosis? A partial answer to this question comes from studies of the effect of alcoholism on iron absorption.

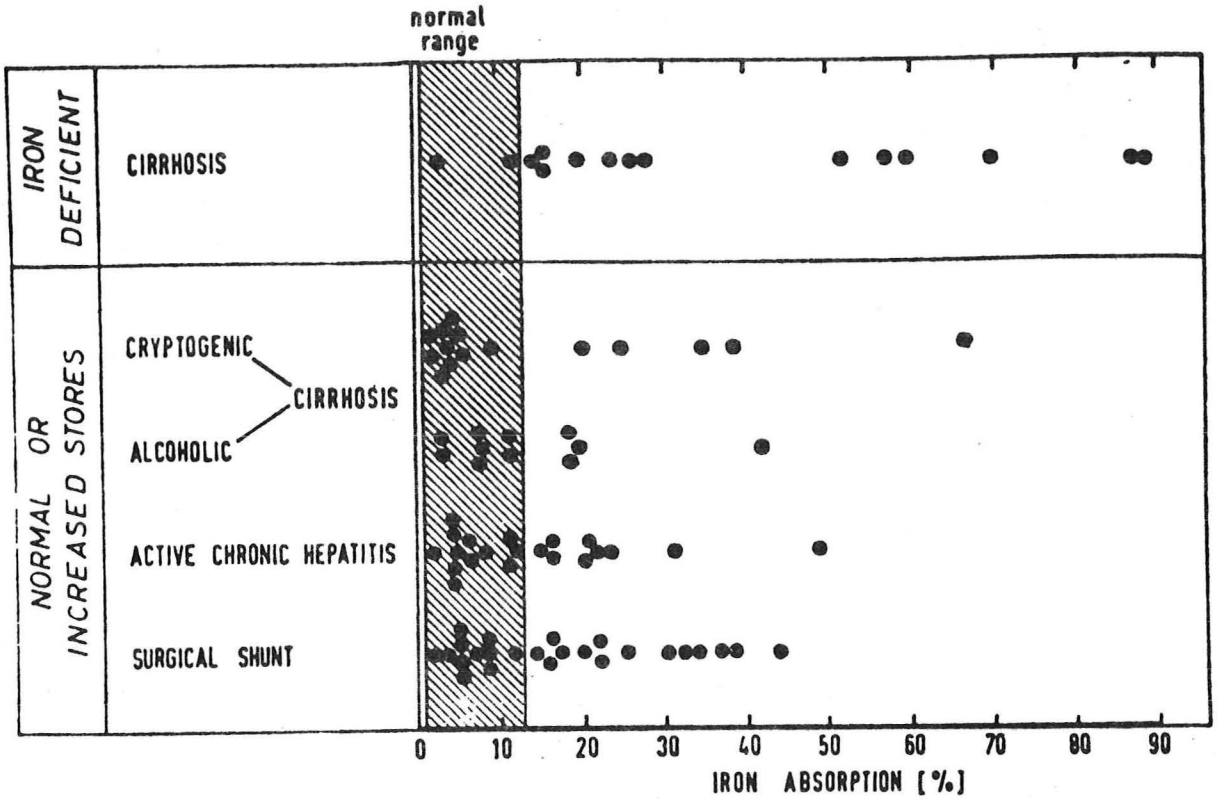
DISTINCTION BETWEEN HEMOCHROMATOSIS AND ALCOHOLIC CIRRHOSIS WITH HEMOSIDEROSIS

The studies of MacDonald clearly showed that as many as 80% of alcoholics with Laennec's cirrhosis have excessive iron deposits in the liver (4). MacDonald erred, however, in confusing these minor iron deposits with the massive deposition of iron that occurs in hemochromatosis. Because he never quantitated these deposits, but relied only on histology, MacDonald reached the false conclusion that clinical hemochromatosis was merely one end of a spectrum of alcoholic liver disease associated with secondary hemosiderosis (4). Other, more quantative studies, however, have now established without a doubt that the severe degree of iron deposition seen in hemochromatosis is rarely, if ever, seen in alcoholics who do not have the gene for hemochromatosis. Thus, Zimmerman (33) quantitated iron histologically in liver specimens from 116 patients with Laennec's cirrhosis and found only eight cases in which the severity of iron deposition approached that seen in hemochromatosis. These patients most likely had genetic hemochromatosis.

In an even more quantitative study, Sherlock's group evaluated 161 patients with various forms of cirrhosis (30). In an average of 50% of these patients iron stores were elevated by histologic staining as compared with controls. Even more striking, iron absorption could be shown to be elevated in one-third of patients with Laennec's cirrhosis despite the elimination of patients who had iron deficiency (34) (Fig. 15). Increased iron absorption was also seen in a high proportion of patients with active chronic hepatitis, even though these patients had increased iron stores. Thus, liver disease in any form can cause excessive iron absorption, and subsequent excessive deposition of iron in the liver. However, in these conditions, the actual amount of iron deposited is quantitatively very small. When Sherlock's group phlebotomized one alcoholic patient who appeared to have a moderate degree of iron overload, they were able to remove only 1.2 grams of iron before signs of iron deficiency developed.

The conclusion of these studies is that in liver disease of any etiology, increased iron absorption can occur, once again indicating that the liver plays an important role in regulating iron absorption. However, this defect is mild and leads only to the accumulation of 1-5 grams of excessive iron in the liver. This minor degree of iron overload does not, in itself, cause liver disease. However, in perhaps 1-2% of patients with alcoholic cirrhosis, massive iron overload in the range of 25 grams occurs. I believe that these latter patients are heterozygotes for hemochromatosis.

Fig. 15 Iron absorption in patients with liver disease.



EXPRESSION OF THE HEMOCHROMATOSIS GENE IS GOVERNED BY ENVIRONMENTAL AND GENETIC FACTORS

Let us return now to the question as to why only some of the patients with the hemochromatosis gene develop clinical hemochromatosis.

One answer to this question can be seen in the interesting family reported by Balcerzak (35) (Fig. 16). In this family, 2 members of the second generation (II-1 and II-3) had full-blown hemochromatosis with mobilizable iron stores of 17 g and 10 g respectively. Two sisters and one brother, as well as the mother and 2 children of the propositus, had moderately elevated iron stores. In this family three generations were affected, suggesting that the gene was transmitted in an autosomal dominant fashion. Each abnormal member of the family has a single dose of the hemochromatosis gene, yet only 2 developed clinical hemochromatosis. These 2 patients were also the only ones in the family who were alcoholics.

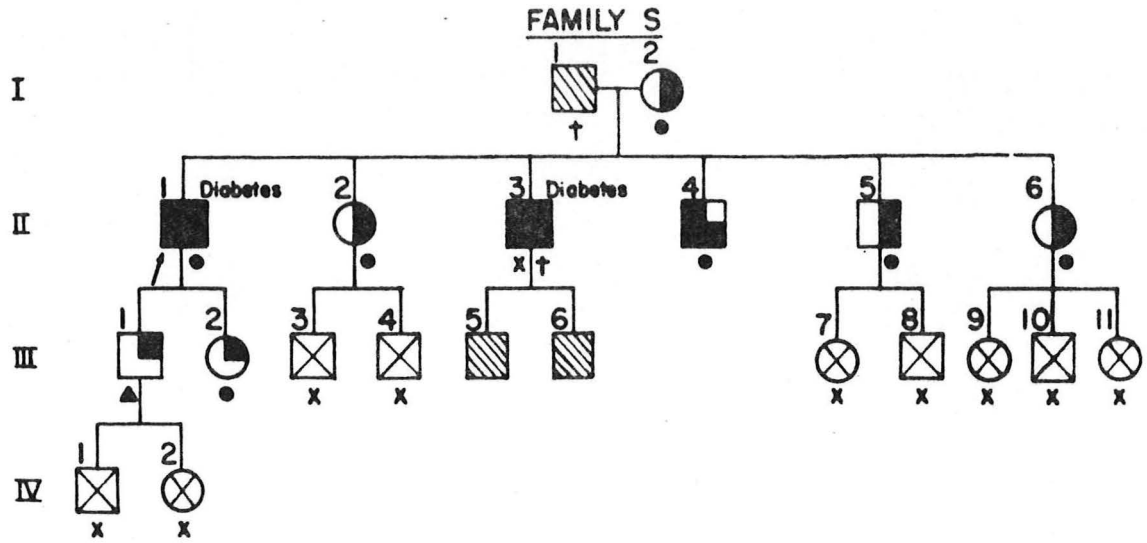
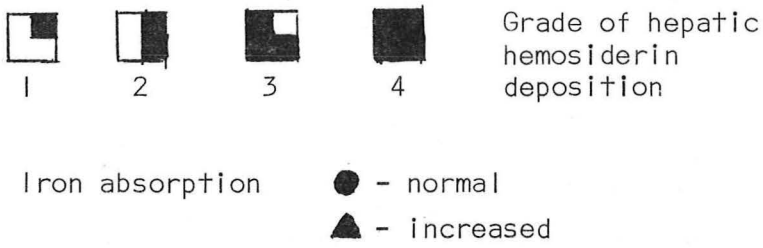


Fig. 16 Iron absorption and hepatic iron deposits in family members of patients with hemochromatosis.



Only about 5% of the general population are alcoholics, yet 30-50% of patients with genetic hemochromatosis are alcoholics. It is clear that there is a massive enrichment in alcoholism among patients with fully expressed genetic hemochromatosis. Yet much less than 1 in 250 alcoholics develops hemochromatosis. This situation can be explained when one examines families such as the one discussed above. If a subject inherits the gene for hemochromatosis, he will usually sustain only a mild increase in hepatic iron stores and remain asymptomatic (as in family members II-2, II-4, II-5, and II-6 in Fig. 16). However, such subjects have a 5% chance of becoming alcoholic--and the consumption of alcohol appears to potentiate the genetic defect. Alcoholism alone increases iron absorption, as does one dose of the hemochromatosis gene--when both factors are present together they produce the massive iron overload that results in the full-blown clinical picture of hemochromatosis.

ALCOHOLISM AND HEMOCHROMATOSIS

5% of the general population are alcoholics.

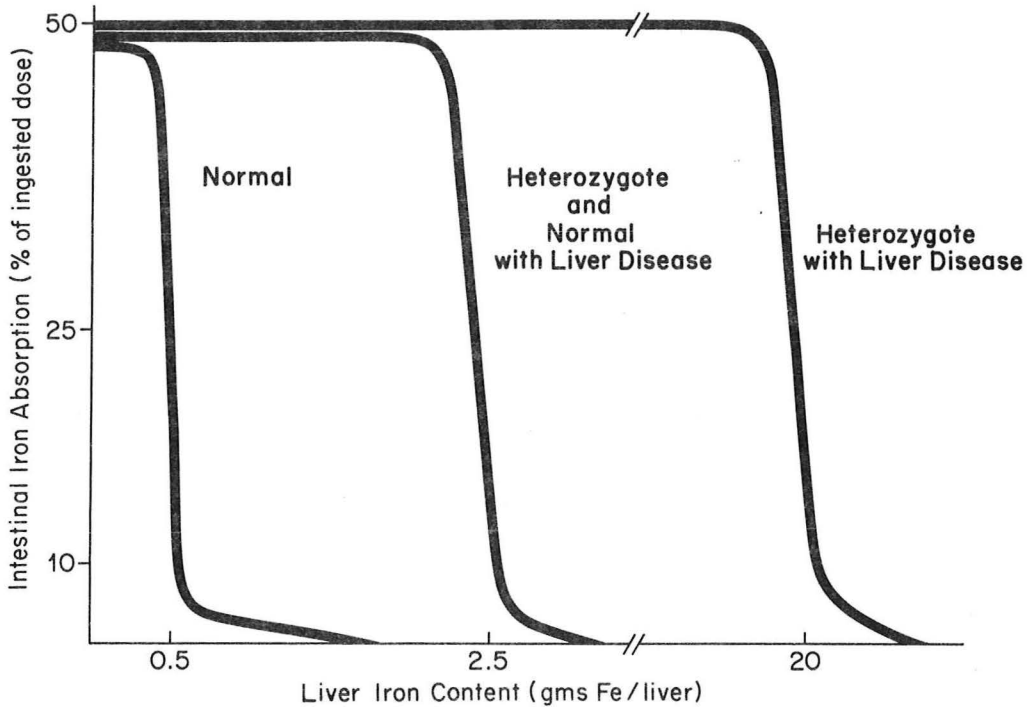
30-50% of patients with clinical hemochromatosis are alcoholics.

<1% of alcoholics have hemochromatosis.

The manner in which the hemochromatosis gene interacts with the environmental factor of alcoholism is shown in Fig. 16a. Again, normal subjects suppress their iron absorption when 0.5 g of iron accumulates in the liver. Heterozygotes with hemochromatosis, and patients with liver disease, appear to have a similar mild defect in that they accumulate up to 5 g iron in the liver before absorption is suppressed. When both factors are present, however, they potentiate each other. In this circumstance, 20 g of iron must accumulate in the liver before absorption is suppressed.

Fig. 17

Regulation of Intestinal Iron Absorption



What about the 50-70% of clinically symptomatic patients with hemochromatosis who are not alcoholics? One major factor that appears to act like alcohol in facilitating the expression of hemochromatosis is genetic diabetes.

INTERACTION OF THE DIABETES GENE AND THE HEMOCHROMATOSIS GENE

In most series, about 60% of patients with clinical hemochromatosis have clinical diabetes (2, 5, 36). Since all patients have an extensive deposition of iron in the pancreas, the diabetes has generally been felt to be secondary to iron-induced damage to this organ. This should be viewed with skepticism, however, since pancreatic exocrine deficiency, which usually precedes endocrine deficiency in diffuse pancreatic disease, is not seen in hemochromatosis. Recently, several studies have challenged the view that diabetes is secondary to iron-induced damage to the pancreas (36, 37, 38). These studies indicate, instead, that patients with hemochromatosis develop diabetes because they have independently inherited the diabetic gene. In the most extensive of these studies, Williams' group in England showed that in 115 cases of hemochromatosis, 63% had diabetes (36). In families of the patients with diabetes, there was a 25% incidence of clinical diabetes. In contrast, in the families of hemochromatosis patients without diabetes, there was only a 4% incidence of diabetes--which was the same as the incidence in the general population. Strikingly similar results were obtained by Balcerzak, who showed that diabetes in family members could not be correlated with increased iron stores as measured by phlebotomy, thus confirming the independent transmission of the diabetes gene (37).

FREQUENCY OF DIABETES IN RELATIVES OF PATIENTS WITH HEMOCHROMATOSIS

Author	Criterion for Diagnosis	Frequency in Family Members of Propositi With Diabetes	Frequency in Family Members of Propositi Without Diabetes
Balcerzak (36)	Glucose Tolerance Test	47%	—
Dymock (37)	Glucose Tolerance Test	25%	4%
Saddi (38)	Glycosuria	9.1%	0%

Other evidence that the diabetes of hemochromatosis is the same as genetic diabetes comes from studies of the complications of diabetes. Thus, Williams' group found that 22% of hemochromatosis patients with diabetes had retinal capillary microaneurysms. Neuropathy and nephropathy were also seen in a substantial number of cases (36).

Table III

CAPILLARY BASEMENT MEMBRANE WIDTH IN PATIENTS WITH HEMOCHROMATOSIS

Patient	Diabetes	Basement Membrane Width (Angstroms)
[redacted]	+	1709
	+	1932
	+	1705
	+	2680
	+	1455
	+	1522
	+	2058
[redacted]	–	1181
Normal Controls	–	1080 ± 27

Data of Raskin and Siperstein

A striking confirmation of the existence of genetic diabetes in hemochromatosis has come from studies of capillary basement membrane thickness in quadriceps muscle biopsies carried out by Drs. Raskin and Siperstein of this department (Table III). In seven patients with hemochromatotic diabetes, the basement membranes were thickened in all, whereas in one patient with hemochromatosis and no diabetes, the basement membranes were normal. Two of the cases of hemochromatotic diabetes are the ones presented today.

In dealing with diabetes and hemochromatosis, we seem to have a situation analogous to the one with alcohol. Thus, while only a tiny fraction of patients with diabetes have hemochromatosis, at least 60% of the patients with clinical hemochromatosis have genetic diabetes. Again, the best explanation for this enrichment is that the two conditions potentiate each other--thus, a patient who has the gene for hemochromatosis may remain clinically asymptomatic with only mild iron-loading unless he also has inherited the gene for diabetes. If he has both the diabetes gene and the hemochromatosis gene, he will develop massive iron loading--resulting in cirrhosis and worsening the hyperglycemia.

DIABETES AND HEMOCHROMATOSIS

5% of the general population has diabetes.

60-80% of patients with clinical hemochromatosis have diabetes.

<1% of diabetics have hemochromatosis.

Table IV.

CAUSES OF SYMPTOMATIC IRON-OVERLOAD
(>10 g. iron in liver)

1. Idiopathic Hemochromatosis

   A. Homozygotes (very rare)
   B. Heterozygotes with potentiating factor (Alcoholism, Diabetes, Hepatitis, malnutrition, ? other)

2. Secondary Hemochromatosis

   A. Massively increased iron intake (Bantu)
   B. Increased intestinal absorption secondary to increased erythropoiesis (Thalassemia, sideroachrestic anemia, etc.)

EXPRESSION OF HEMOCHROMATOSIS

In addition to alcoholism and diabetes, other agents that damage the liver may also elicit the expression of the hemochromatosis gene. Thus, in the 1500 cases of hemochromatosis reviewed by Heilmeyer, 15% had a history of hepatitis and 10% had a history of chronic malnutrition. Of the 16 cases reported by Sherlock, two had been held in prisoner-of-war camps with attendant malnutrition. The factors affecting the expression of the hemochromatosis gene in heterozygotes are listed in Table IV.

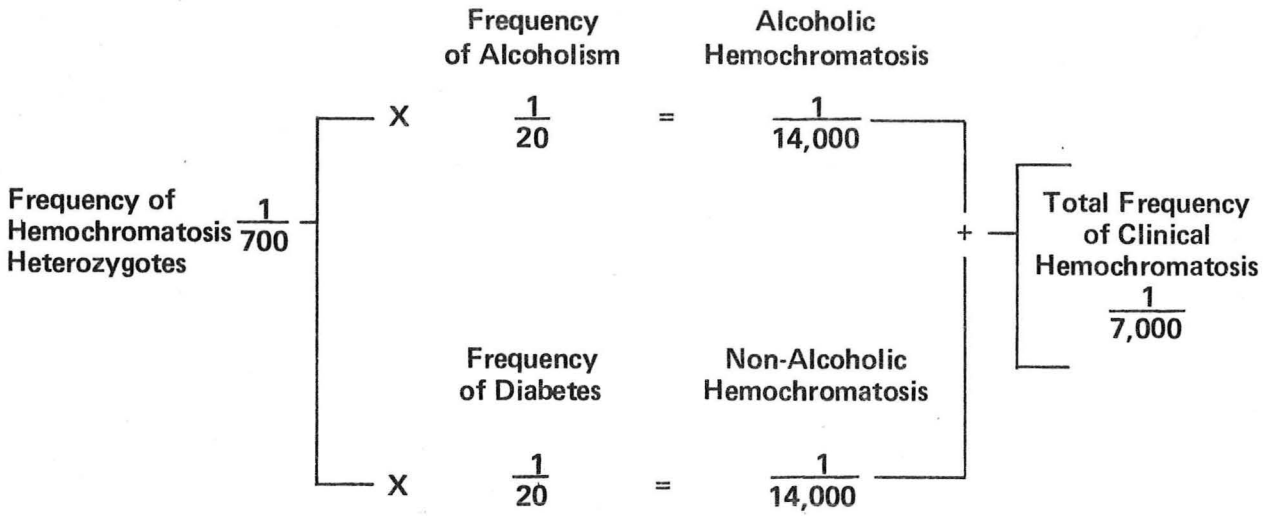
CAUSES OF MILD HEMOSIDEROSIS
(1-5 g. iron in liver)

1. Idiopathic hemochromatosis (heterozygotes without potentiating factor).

2. Alcoholic Cirrhosis (without hemochromatosis gene).

3. Other Liver Disease (chronic active hepatitis, cryptogenic cirrhosis).

POPULATION FREQUENCY OF CLINICAL HEMOCHROMATOSIS



If one puts together all of the above facts, one can make a rough calculation of the true frequency of the hemochromatosis gene in the population (Fig. 17). The reported incidence of the full-blown hemochromatosis syndrome at autopsy is 1:7000 males (2). If half of these are alcoholics (2), and if the population frequency of alcoholism is 1:20, one can calculate that about 1 in 700 people carry the gene for hemochromatosis. One reaches the same figure if one correlates the incidence of diabetes in hemochromatosis with the incidence of diabetes in the general population. Thus, one can calculate that the hemochromatosis gene is fully expressed only in one-tenth of the heterozygotes who carry it.

HOMOZYGOTES FOR HEMOCHROMATOSIS

If 1:700 people are heterozygous for the hemochromatosis gene, then one can calculate that about one in two million in the population will inherit two abnormal genes for hemochromatosis, and thus become homozygotes. I believe that Case No. 1 (Brenda DeLord) is a homozygote for hemochromatosis. Evidence indicates that such patients will accumulate iron without any regulation at all, and thus will develop hemochromatosis at a very early age without any predisposing factors (39). If Brenda is a homozygote, then both her parents must be heterozygotes. However, since neither is an alcoholic, and since neither has diabetes or a history of hepatitis or malnutrition, they have not expressed the gene. Nevertheless, the theory presented today would predict that if liver biopsies were performed on the parents, they would disclose mild iron overload in the liver. Phlebotomy should disclose iron stores in the neighborhood of 2-5 grams. After phlebotomy, iron absorption should be elevated until the iron stores again reached 2-5 grams. Thus, the genetic theory is immediately susceptible to direct testing. I hope that soon I will be able to tell you that it is confirmed.

1. Sheldon, J.H. *Hemochromatosis*, London, Humphrey Milford, 1935.

2. Finch, S.C. and Finch, C.A. Idiopathic hemochromatosis, an iron shortage disease. *Medicine* 34:381, 1955.

3. Bothwell, T.H. and Bradlow, B.A. Siderosis in the Bantu. *Arch Pathol* 70:279, 1960.

4. MacDonald, R.A. and Mallory, G.K. Hemochromatosis and hemosiderosis. *Arch Intern Med* 105:686, 1960.

5. Heilmeyer, L. Pathogenesis of hemochromatosis. *Medicine* 46:209, 1967.

6. Perkins, K.W., McInnes, I.W.S., Blackburn, C.R.B. and Beal, R.W. Idiopathic haemochromatosis in children. *Am J Med* 39:118, 1965.

7. Stocks, A.E. and Powell, L.W. Pituitary function in idiopathic haemochromatosis and cirrhosis of the liver. *Lancet* 2:298, 1972.

8. Easley, R.M., Schreiner, B.F., and Yu, P.N. Reversible cardiomyopathy associated with hemochromatosis. *N Engl J Med* 287:866, 1972.

9. Pollycove, M. Hemochromatosis in *The Metabolic Basis of Inherited Disease*. Stanbury, J.B., Wyngaarden, J.B. and Fredrickson, D.S., eds, New York, McGraw-Hill, pp. 1051-1084.

10. Jandl, J.H. and Katz, J.H. The plasma-to-cell cycle of transferrin. *J Clin Invest* 42:314, 1963.

11. Munro, H.N. and Drysdale, J.W. Role of iron in the regulation of ferritin metabolism. *Fed Proc* 29:1469, 1970.

12. Macara, I.G., Hoy, T.G., and Harrison, P.M. The formation of ferritin from apoferritin. *Biochem J* 126:151, 1972.

13. Richter, G.W. On ferritin and its production by cells growing in vitro. *Lab Invest* 12:1026, 1963.

14. Linder, M.C. and Munro, H.N. Metabolic and chemical features of ferritins, a series of iron-inducible tissue proteins. *Amer J Pathol* 72:263, 1973.

15. Granick, S. Iron metabolism. *Bull N Y Acad Med* 30:81, 1954.

16. Fineberg, R.A. and Greenberg, D.M. Ferritin biosynthesis: Acceleration of synthesis by the administration of iron. *J Biol Chem* 214:107, 1955.

17. Chu, L.L.H. and Fineberg, R.A. On the mechanism of iron-induced synthesis of apoferritin in HeLa cells. *J Biol Chem* 244:3847, 1969.

18. Trump, B.F., Valigorsky, J.M., Mergner, W.J. and Kinney, T.D. The relationship of intracellular pathways of iron metabolism to cellular iron overload and iron storage diseases. *Amer J Pathol* 72:295, 1973.

19. Crosby, W.H. Intestinal response to the body's requirement for iron. *JAMA* 208:347, 1969.

20. Bothwell, T.H. and Finch, C.H. *Iron Metabolism*, Boston, Little, Brown and Co., 1962.

21. Pirzio-Biroli, G. and Finch, C.A. Iron absorption. III. The influence of iron stores on iron absorption in the normal subject. *J Lab Clin Med* 55:216, 1960.

22. Manis, J.G. and Schachter, D. Active transport of iron by intestine: Features of the two-step mechanism. *Am J Physiol* 203:73, 1962.

23. Charlton, R.W., Jacobs, P., Torrance, J.D., and Bothwell, T.H. The role of the intestinal mucosa in iron absorption. *J Clin Invest* 44:543, 1965.

24. Conrad, M.E.,and Crosby, W.H. Intestinal mucosal mechanisms controlling iron absorption. *Blood* 22:406, 1963.

25. Sheehan, R.G. and Frenkel, E.P. The control of iron absorption by the gastrointestinal mucosal cell. *J Clin Invest* 51:224, 1972.

26. Wheby, M.S., Jones, L.G. and Crosby, W.H. Studies on iron absorption. Intestinal regulatory mechanisms. *J Clin Invest* 43:1433, 1964.

27. Beutler, E. and Butterweiser, E. The regulation of iron absorption. I. A search for humoral factors. *J Lab Clin Med* 55:274, 1960.

28. Williams, R., Monenti, F., Williams, H.S. and Pitcher, C.S. Iron absorption in idiopathic haemochromatosis before, during, and after venesection therapy. *Lancet* 2:78, 1966.

29. Smith, P.M., Godfrey, B.E. and Williams, R. Iron absorption in idiopathic haemochromatosis and its measurement using a whole-body counter. *Clin Sci* 37:519, 1969.

30. Scheuer, P.J., Williams R. and Muir, A.R. Hepatic pathology in relatives of patients with haemochromatosis. *J Path Bact* 84:53, 1962.

31. Williams, R., Scheuer, P.J. and Sherlock, S. The inheritance of idiopathic haemochromatosis. *Q J Med* 31:249, 1962.

32. Williams, R., Pitcher, C.S., Parsonon, A. and Williams, H.S. Iron absorption in the relatives of patients with idiopathic haemochromatosis. *Lancet* 1:1243, 1965.

33. Zimmerman, H.J., Chomet, B., Kulesh, M.H. and McWhorter, C.A. Hepatic hemosiderin deposits. *Arch Int Med* 107:494, 1961.

34. Williams, R., Williams, H.S., Scheuer, P.J., Pitcher, C.S., Loiseau, E. and Sherlock, S. Iron absorption and siderosis in chronic liver disease. *A J Med* 36:151, 1967.

35. Balcerzak, S.P., Westerman, M.P., Lee, R.E. and Doyle, A.P. Idiopathic hemochromatosis: A study of three families. *Am J Med* 40:857, 1966.

36. Dymock, I.W., Cassar, J., Pyke, D.A., Oakley, W.G. and Williams, Roger. Observations on the pathogenesis, complications and treatment of diabetes in 115 cases of haemochromatosis. *Am J Med* 52:203, 1972.

37. Balcerzak, S.P., Mintz, D.H. and Westerman, M.P. Diabetes mellitus and idiopathic hemochromatosis. *Am J Med Sci* 255:53, 1968.

38. Saddi, R. and Feingold, J. Personal communication.

39. Felts, J.H., Nelson, J.R., Herndon, C.N. and Spurr, C.L. Hemochromatosis in two young sisters. *Ann Intern Med* 67:117, 1967.