IRON STORAGE DISORDERS

MEDICAL GRAND ROUNDS

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IRON METABOLISM

Some of the parameters of iron balance in normal adults are shown in Table 1.

	Content	Concentration
Total body iron	4.5 g	
Heme - Iron	3.0 g	
Hemoglobin	*2.6 g*	*0.25µg/ml blood*
Myoglobin	*0.4 g*	
Non-Heme (mobilisable) iron	1.5 g	
Liver	*400 mg*	*230µg/g wet weight*
Muscle	*730 mg*	*26µg/g wet weight*
Spleen	*50 mg*	*240µg/g wet weight*
Brain	*60 mg*	*10-210µg/g wet weight*
Bone marrow	*300 mg*	*2.7µg/mg protein*

Table 1. Body and tissue iron content (from Jacobs and Worwood, 1978).

The normal dietary intake of iron is approximately 15 mg per day. The normal daily losses vary from approximately 0.5 mg per day (males) to 1.5 mg per day (young women). An amount of iron equivalent to these losses is absorbed by the G.I. tract. Normal iron balance is controlled by modifying the amount of iron absorbed. There are no physiological mechanisms whereby the body can excrete an excessive iron load.

Most iron containing proteins in the body are concerned with the carriage of oxygen (hemoglobin and myoglobin) or with the utilization of oxygen in tissues (cytochromes and iron-sulphur proteins). In addition, there are proteins for iron transport (transferrin) and iron storage (ferritin and hemosiderin).

IRON ABSORPTION

There is considerable variability in the efficiency of iron absorption from different foods. Certain ligands released from digestion of food or present as such in the diet may form insoluble complexes with the iron and render it unavailable. Thus as little as 1% of the iron in some vegetables is available for absorption compared to from 10 to 25% of iron in meat. Iron absorption occurs primarily in the upper part of the jejunum and the mechanisms of its control continue to be poorly understood. Once iron gains access to the jejunal mucosal cell, it faces one of two fates. Some passes readily into plasma and the rest is bound to ferritin and stays in the cell until the cell is desquamated from the tip of the villus a few days later. This iron is not absorbed. The amount of iron absorbed is influenced by a number of factors but the mechanisms involved in controlling iron absorption are not understood. The major controlling influences seem to be:

Amount of iron presented: The absolute amount of iron absorbed is directly related to the amount of iron ingested. The more iron presented to the cells, the more will be absorbed. The *percentage* of the dose absorbed, however, becomes increasingly lower as the dose is increased.

Body iron stores: There is an inverse relationship between both the bone marrow iron content and the serum ferritin level with iron absorption. In iron deficient animals, the mucosal cells do not form ferritin but contain a protein with some characteristics of transferrin. There is no correlate between the iron content of the jejunal mucosa itself and the rate of iron absorption, however.

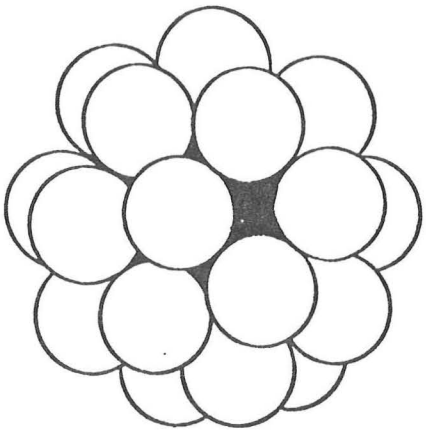
Erythropoiesis: There is a direct relationship between iron absorption and the state of marrow erythropoiesis, whether this be effective or not.

Pathological states such as hypoxia, liver disease, pancreatic insufficiency and portocaval anastomoses have all been shown to be associated with an increase in G.I. iron absorption.

IRON TRANSFER AND STORAGE

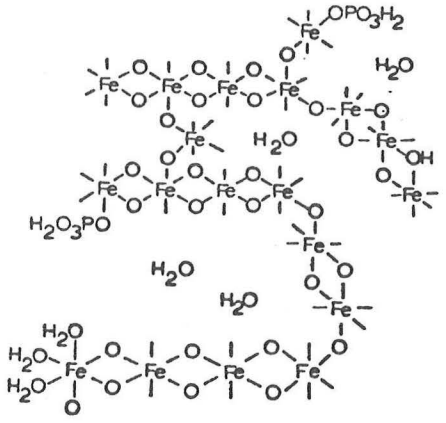
The exchange of iron through plasma and the extracellular fluid between tissues is mediated by *transferrin*. This glycoprotein has a molecular weight of 80,000 and migrates as a β globulin. It is composed of a single polypeptide chain and binds two atoms of iron at specific sites. These sites will also bind other metals (e.g. zinc) but have a much higher affinity for iron. A similar but quite distinct protein is present in milk and neutrophils and is called *lactoferrin*. Transferrin binds ferric ions (Fe^{3+}) with a bicarbonate ion (HCO_3') and will not accept ferrous ions (Fe^{2+}). It is believed that a physiological role of caeruloplasmin is to mediate the oxidation of Fe^{2+} to Fe^{3+}. The caeruloplasmin level must be extremely low, however, before iron transport is disrupted. The cellular uptake of iron requires an interaction between transferrin and membrane receptors with the subsequent release of the protein and the sequestration of the iron in the cell.

Ferritin is the major iron storage protein in mammalian tissue. It may be present in all cells of the body. It is very large with a molecular weight of 450,000 daltons and each molecule can bind up to 4000 atoms of iron. The protein (apoferritin) is composed of 24 smaller sub-units which are arranged as a shell around a central core wherein lies the iron in polynuclear complexes.



Arrangement of ferritin molecule

Figure 1



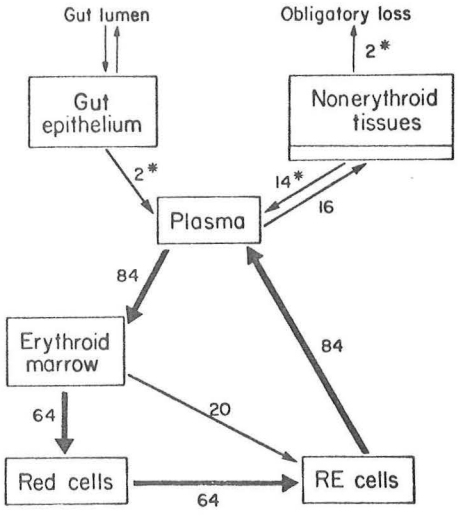
Complexes of iron present in ferritin

Figure 2

Variations in the composition of the polypeptide subunits of the molecule contribute to the tissue specific isomers (isoferritins) which can be separated and identified by physico-chemical and immunological methods. Apoferritin synthesis is stimulated by the presentation of iron to any cell or tissue. Such synthesis is well maintained in the face of protein deprivation (cf. albumin and transferrin). The incorporation of iron into the ferritin "micelle" requires that Fe^{2+} be oxidized to Fe^{3+}. Release of ferritin iron from storage involves the reverse step. Ferritin is the major form of non-heme iron in normal animals and most of it is in hepatic parenchymal cells. The ferritin is distributed throughout the cytosol in soluble form. As the iron content of the cell increases, more and more of the ferritin becomes incorporated into lysosomes and ferritin polymers begin to occur. This may be the first step in the formation of hemosiderin.

Hemosiderin consists of huge insoluble aggregates of iron and protein. The precise relationship between ferritin and hemosiderin is not clear but it is believed that hemosiderin develops as the result of denaturation and partial digestion of the protein shell of ferritin molecules occurring within lysosomes of iron loaded cells. As the degree of iron loading in a tissue increases, so does the ratio of hemosiderin to ferritin in that tissue. These hemosiderin granules are large enough to be visible on light microscopy.

The majority of the body's iron is in the form of heme and most of this is in hemoglobin of red blood cells. As these cells become effete, they are removed by the R.E.S. and the contained iron is released and returned to the bone marrow via transferrin. The major circuit of body iron, therefore, is the return from the R.E.S. of scavenged iron to the bone marrow for erythropoiesis. The contribution of iron absorbed from the G.I.T. is very small.



Iron turnover in normal adult

Figure 3

Jacobs, A. and Worwood, M. Normal iron metabolism in metals and the liver. L. W. Powell, ed. M. Dekker Inc., page 3, 1978.

Halliday, J. W. and Powell, L. W. Ferritin metabolism and the liver. In: Metals and the Liver. L. W. Powell, ed. M. Dekker Inc., page 53, 1978.

Walters, G. O., Jacobs, A., Worwood, M. Iron absorption in normal subjects and patients with idiopathic hemochromatosis. Relationship with serum ferritin concentration. Gut 16:188, 1975.

Biggs, J. C., Davis, A. E. Relationship of diminished pancreatic secretion to hemochromatosis. Lancet (ii):814, 1963.

IRON TOXICITY

While there has been dispute in the direct role of iron deposition in causing hepatotoxicity in states of chronic iron overload, there has been agreement that acute iron intoxication leads directly to liver cell injury.

Acute iron poisoning. This is overwhelmingly a problem that follows the inadvertent ingestion of large amounts of iron compounds by small children. It is seen occasionally in adults, however, after attempts at suicide with such compounds. Within a few hours of ingestion, the patient becomes acutely ill with vomiting, diarrhea, G.I. hemorrhage and vascular collapse. This frequently leads to severe cidosis. Lethargy, coma and death results in as many as 50% of the patients. There is often evidence, chemically, of liver injury and this is confirmed pathologically where the striking features are necrosis and inflammation of the G.I. tract and the liver. If the patient survives, late complications may include small bowel obstruction from strictures induced by the G.I. lesions.

The serum iron levels in these patients are extremely high with plasma levels well in excess of 1000μg/dl. The mechanism of the cellular injury is not established but it is believed that the amount of free iron present in cells exceeds the cells' usual mechanism for handling the metal (i.e. forming ferritin) and the excess free iron induces membrane lipid peroxidation (possibly by free radicle formation). If this process affected lysosomal membranes, hydrolytic enzymes would be released into the cells and would cause necrosis and subsequent inflammation.

Chronic Fe toxicity. The evidence that iron itself is toxic to cells in states of chronic iron overload is circumstantial but, on the whole, convincing. Tissue injury is related to the degree of parenchymal iron and not to total liver iron content. Thus, the amount of iron present in the liver in patients with transfusion siderosis may be equal to that in idiopathic hemochromatosis but because the iron is largely confined to the R.E. cells, no cellular damage results. The failure after many attempts to produce an animal model of hemochromatosis by the administration of large amounts of iron over time can be explained by the predominant sequestration of such iron in cells of the R.E. system. Lisboa did manage to produce a pigmentary cirrhosis in dogs given iron sorbitol parenterally for months on end but the value of such a model is questionable.

The mechanism by which cellular damage results in patients with chronic iron overload is not known but it is likely that similar mechanisms to those in acute iron intoxication are operative. In these states of chronic iron overload, enzymic analyses have shown increased activities of acid hydrolases and enhanced lysosomal fragility in liver homogenates. These changes are reversed by phlebotomy and suggest that lysosomal damage (?by free radicle-induced lipid peroxidation) may be present. The conversion of Fe^{3+} to Fe^{2+} entails a one-electron transfer and catalyses many redox reactions in which free radicles are formed. Finally, the observation that extensive hepatic and pancreatic fibrosis may develop in the absence of much overt cell necrosis has raised the question as to whether iron itself may stimulate collagen synthesis directly. Little information is available on this question.

Jacobs, J., Greene, H., Gender, B. R. Acute iron intoxication. N. Engl. J. Med. 273:1124, 1965.

Lavender, S., and Bell, G. Iron intoxication in an adult. B. M. J. 2:406, 1970.

Powell, L. W., Basset, M. L., and Halliday, J. W. Hemochromatosis: 1980 update. Gastroenterology 78:374, 1980.

Grace, N. W. Evidence for hepatic toxicity of iron. In: Metals and the liver. L. W. Powell, ed. Marcel Dekker Inc., page 131, 1978.

Peters, T. J., and Seymour, C. A. Acid hydrolases activities and lysosomal integrity in liver biopsies from patients with iron overload. Clin. Sci. Mol. Med. 50:75, 1976.

Lisboa, P. E. Experimental hepatic cirrhosis in dogs caused by chronic massive iron overload. Gut 12:363, 1971.

HEMOCHROMATOSIS

DEFINITION

The term "hemochromatosis" refers to a group of disorders in which there is a progressive increase in total body iron stores with deposition of iron in the parenchymal cells of the liver, heart, pancreas and other organs. The increase in total body iron results from iron absorpbtion inappropriate to the level of body iron stores, either alone or in combination with parenteral iron loading. Parenchymal deposition of iron eventually results in cellular damage and functional insufficiency of the organs involved.

Increased R.E. deposition of iron is innocuous and disorders associated with this sort of iron overload (e.g. transfusional iron overload) are not included in the definition. Terms such as "hemosiderosis" are best avoided. The causes of hemochromatosis are listed below.

1. *Idiopathic (hereditary) hemochromatosis*

2. *Secondary hemochromatosis*
   a) Ineffective erythropoiesis (e.g. Thallassemia, Sickle Cell Disease, etc.)
   b) Liver disease (Cirrhosis, post P-C shunts)
   c) High oral intake (medicinal iron, "Bantu siderosis")

The criteria that allow the diagnosis of idiopathic hemochromatosis are not always easily met. They consist of:

(i) a demonstrated increase in total body iron stores, distributed principally in parenchymal rather than R.E.S. cells
(ii) a family history of iron storage disease
(iii) the demonstrated absence of other known causes of iron overload.

In clinical practice, one may have to accept a presumptive diagnosis with the demonstration of marked increase in hepatic parenchymal iron storage in the absence of known causes for same.

Distinction between idiopathic hemochromatosis and alcoholic liver disease. This probably represents the greatest problem of differential diagnostic difficulty in that some patients with alcoholic liver disease have increased stainable iron on liver biopsy and some (perhaps up to 40%) of patients with idiopathic hemochromatosis are heavy drinkers. The essential point is that there is a very poor correlation between the amount of stainable iron seen on a liver biopsy and the total body iron stores. Much of the past confusion has arisen because any stainable iron was considered abnormal. It is clear that alcoholic cirrhosis is associated with an increase in stainable iron in as many as 30% of patients. The iron stores of these patients, however, are essentially normal unless they have had a portocaval shunt, have a significant hemolytic anemia or have had a major increase in oral iron ingestion (medicinal iron, red wines, etc). Even then they seldom have the huge body iron stores seen in patients with hemochromatosis.

The high incidence of alcoholism in symptomatic patients with idiopathic hemochromatosis remains unexplained and strongly suggests that this external environmental factor may play a significant role in the determination of the clinical expression of the disease. There is no evidence, however, that alcohol per se influences the absorption of iron from the G.I. tract and so the influence that alcoholism plays is presumably exerted by more indirect mechamisms.

METHODS OF ASSESSING IRON STORAGE

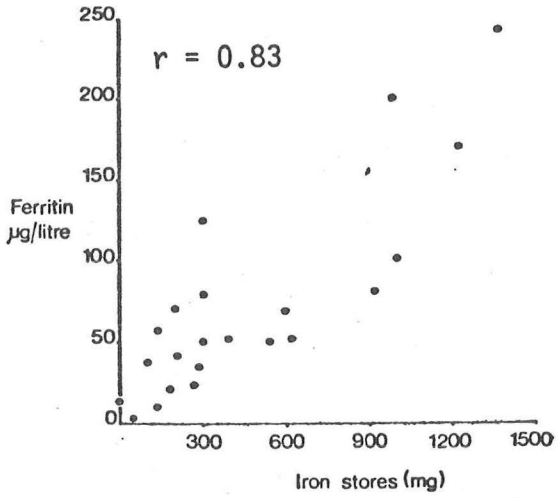
Serum Iron and Iron binding capacity (T.I.B.C.)

Serum iron is measured by colorimetric methods. Serum transferrin is measured as the total iron-binding capacity of serum after the addition of excess iron. The amount of circulating iron is a balance between iron entering and leaving the plasma and does not directly reflect the amount of storage iron in tissues. The correlation between both serum iron levels and transferrin saturation and total body iron stores is quite poor. The synthesis of

transferrin which is stimulated by iron deficiency is affected by many factors other than iron (protein loss, protein deprivation, catabolism, inflammation etc.). Erythropoiesis becomes limited by the supply of iron when transferrin saturation is less than 16% due either to iron deficiency or to malignant or inflammatory conditions. High serum iron with reduced T.I.B.C. but a high transferrin saturation is found in patients with hemochromatosis but also in patients with hemolytic anemia and ineffective erythropoiesis.

Serum ferritin

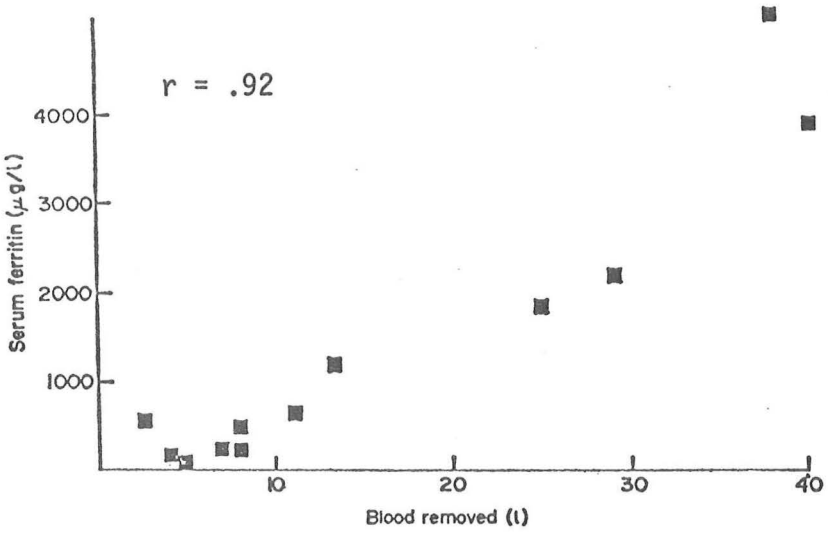
The serum ferritin level has been shown to bear a close relationship with the total body iron stores (judged by phlebotomy) in normal patients and in normal patients rendered iron deficient.



Iron stores and serum ferritin concentration.

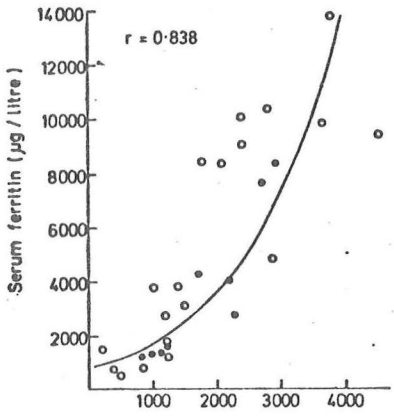
from Walters et al, J. Clin. Path. 1973
Figure 4

In infants and children, the concentration of serum ferritin has been shown to parallel the known changes in iron stores during normal development. All children with iron deficiency were identified as having a serum ferritin less than 10 ng/ml. No causes of spuriously low serum ferritin have been identified. A good correlation between serum ferritin and iron stores in patients with idiopathic hemochromatosis has also been reported.



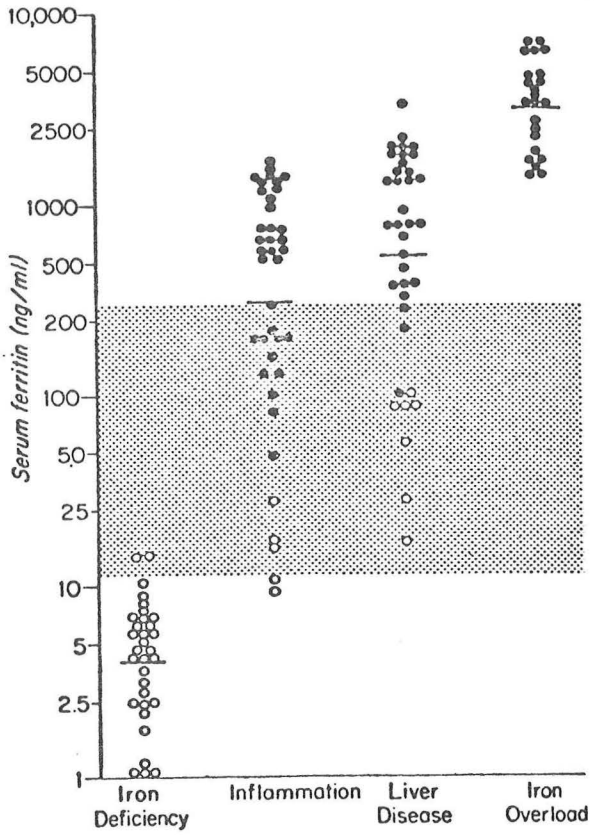
Ferritin and iron stores in hemochromatosis
from Beamish et al, B. J. Hem. 1974
Figure 5

This has been confirmed in patients with other causes of iron overload as well.



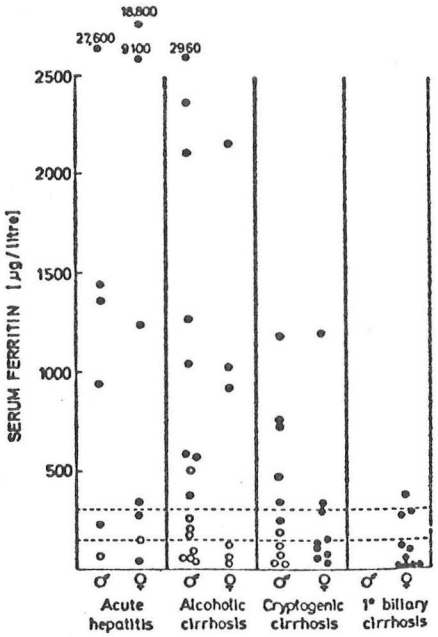
Ferritin and liver iron in iron overload
from Prieto et al, Gastroenterology 1975
Figure 6

Unfortunately, there are other "spurious" causes for an elevated serum ferritin which do not correlate with total body iron stores and which limit the usefulness of the test. These include acute infections, acute leukemia, Hodgins disease and various liver diseases.



Serum ferritin as a measure of iron stores
from Lipschitz et al, N. Engl. J. Med. 1974
Figure 7

Many of these patients with "inflammation" had a hemolytic anemia which may have helped to explain the high ferritin levels but liver disease of almost any cause is associated with an artefactually high serum ferritin.



Ferritin in liver disease

(open circles indicate patients with recent hemorrhage)

from Prieto et al, Gastroenterology 1975

Figure 8

This occurs, presumably, because of parenchymal cell injury with release of the contained ferritin. Under normal circumstances, too, serum ferritin is cleared almost entirely by the hepatic parenchyma rather than the R.E.S. Impairment of the liver's ability to clear ferritin (presumably derived mainly from the R.E.S. even in states of iron overload) may contribute to the elevated serum levels observed in these various liver diseases.

A more serious limitation on the value of serum ferritin as a predictor of increased iron stores was identified by Wands et al who studied family members of 2 patients with idiopathic hemochromatosis and found no correlation between the serum ferritin levels and the iron loads of the relatives. In fact, all but one had a normal serum ferritin despite evidence of marked increase in iron stores in seven of them. This was explained by suggesting that the R.E. system was not able to synthesize ferritin effectively and hence the low serum level, the increased iron absorption and the well documented sparing of macrophages in the iron overloaded state of idiopathic hemochromatosis. It was suggested, further, that when parenchymal liver damage resulted from the iron load, ferritin from the parenchymal cells was released and reached the blood and caused the very high levels seen in symptomatic disease. Halliday et al, on the contrary, found the serum ferritin a very useful screen in relatives of patients with idiopathic hemochromatosis, identifying 98% of those with increased iron stores by an elevated serum ferritin. These authors argued that idiopathic hemochromatosis may represent a number of disorders, all of which result in increased iron absorption but which vary in the site at which the regulation of iron absorption is impaired.

Beamish, M. R., Walker, R., Miller, F. et al. Transferrin iron, chelatable iron and ferritin in idiopathic hemochromatosis. Brit. J. Haem. 27:219, 1974.

Siimes, M. A., Addiego, J. E., and Dallman, P. R. Ferritin in serum: Diagnosis of iron deficiency and iron overload in infants and children. Blood 43:581, 1974.

Walter, G. O., Miller, F. M. and Worwood, M. Serum ferritin concentration and iron stores in normal subjects. J. Clin. Path. 26:770, 1973.

Lipschitz, D. A., Cook, J. D., Finch, C. A. A clinical evaluation of serum ferritin as an index of iron stores. N. Engl. J. Med. 290:1213, 1974

Paieto, J., Barry, M., Sherlock, S. Serum ferritin in patients with iron overload and with acute and chronic liver diseases. Gastroenterology 68:525, 1975.

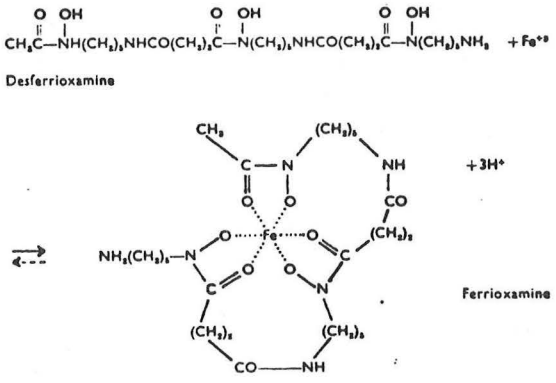
Wands, J. R., Rowe, J. A., and Mezey, S. E. Normal serum ferritin concentrations in precirrhotic hemochromatosis. N. Engl. J. Med. 294:302, 1976.

Halliday, J. W., Russo, A. M., Cowlishaw, J. C. et al. Serum ferritin in diagnosis of hemochromatosis. Lancet (ii):621, 1977.

Beaumont, C., Simon, M., Fauchet, R. et al. Serum ferritin as a possible marker of the hemochromatous allele. N. Engl. J. Med. 301:169, 1979.

CHELATABLE IRON (DESFERRIOXAMINE TEST)

Desferrioxamine B was developed initially as an antibiotic formed by *streptomyces pilosus* and was found to react with remarkable affinity with Fe^{3+} and form ferrioxamine B, a compound which is readily soluble in water and is excreted in urine.



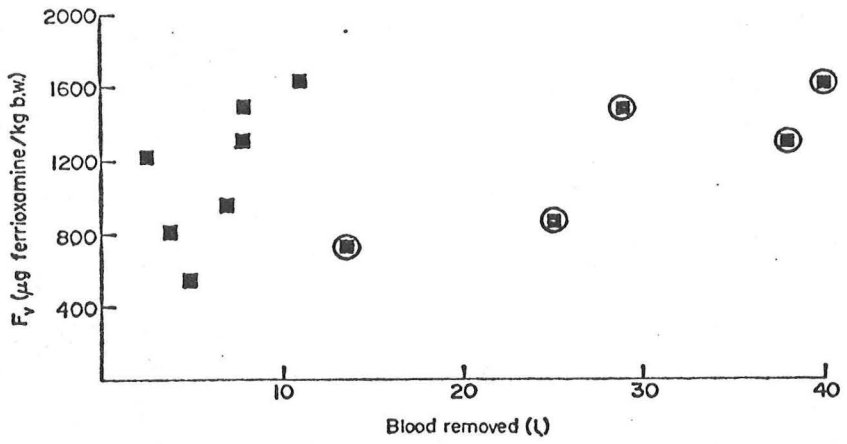
from Fielding and Braunström
J. Clin. Path. 1964

Figure 9

This binding action of desferrioxamine is selective for iron and there is little effect on calcium, copper or other metals. Use has been found for this chelating agent in treating acute iron toxicity, chronic iron overload in situations where phlebotomy is contraindicated (e.g. Thallasaemia) and as a test to assess the body's iron stores.

The differential ferrioxamine test is performed by injecting intramuscularly a solution containing 500 mg desferrioxamine plus 50 mg of ferrioxamine labelled with $2\mu c\ ^{59}FeCl_3$ and then collecting urine for 6 hours and measuring both the radioactivity and, by chemical methods, the amount of ferrioxamine excreted. The urinary ferrioxamine is derived in part from the administered ferrioxamine and, in part, from ferrioxamine produced endogenously from desferrioxamine. Since some of the ferrioxamine is not excreted in urine, the total *in vivo* chelation can be calculated by simple proportions from the isotope excretion data. This total *in vivo* chelation is referred to as Fv and is expressed as µg ferrioxamine base formed per kg body weight. The results in normal controls are approximately 100 to 500 µg/kg. In patients with hemochromatosis, values as high as 4000µg/kg are recorded.

Many authors have shown a reasonable correlation between the results of this test and the documented iron stores in normal patients and patients with untreated hemochromatosis. When patients have been rendered iron deficient, however, and iron re-accumulation begins, high values for chelatable iron have been recorded even though iron stores are now normal.



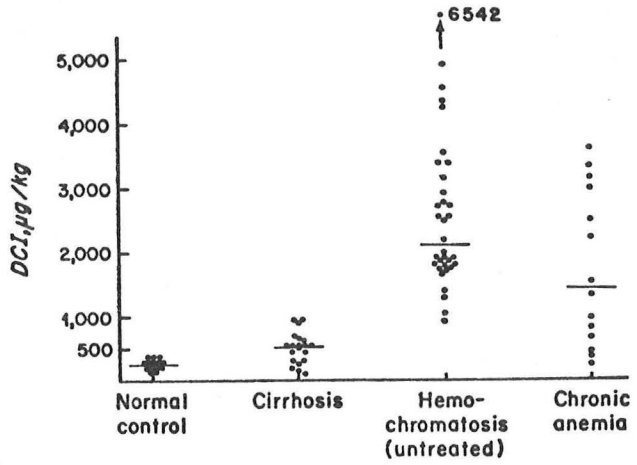
■ = previously treated patients

Chelatable iron and iron stores in hemochromatosis

from Beamish et al, B. J. Hem. 1974

Figure 10

Elevated levels of ferrioxamine formation are also found in a variety of different forms of liver disease which do not have elevated total body iron stores.



Chelatable iron in patient groups
from Baldus et al, Mayo Clin. Proc. 1978
Figure 11

The test does reliably assess the iron stores in patients with different forms of hemolytic anemia, many of whom do have increased iron stores.

Fielding, J., Braunstrom, G. M. Examination of ferrioxamine and deferrioxamine in urine. J. Clin. Path. 17:395, 1964.

Smith, P. M., Studley, F., Williams, R. Assessment of body-iron stores in cirrhosis and haemochromatosis with the differential ferrioxamine test. Lancet (i):133, 1967.

Balcerzak, S. P., Westerman, M. P., Heinle, E. W. et al. Measurement of iron stores uring desferrioxamine. Ann. Int. Med. 68:518, 1968.

Barry, M., Carter, G., Sherlock, S. Differential ferrioxamine test in haemochromatosis and liver disease. Gut 10:697, 1969.

Smith, P. M., Lestas, A. N., Miller, J. P. G. et al. The differential ferrioxamine test in the management of idiopathic hemochromatosis. Lancet (ii):402, 1969.

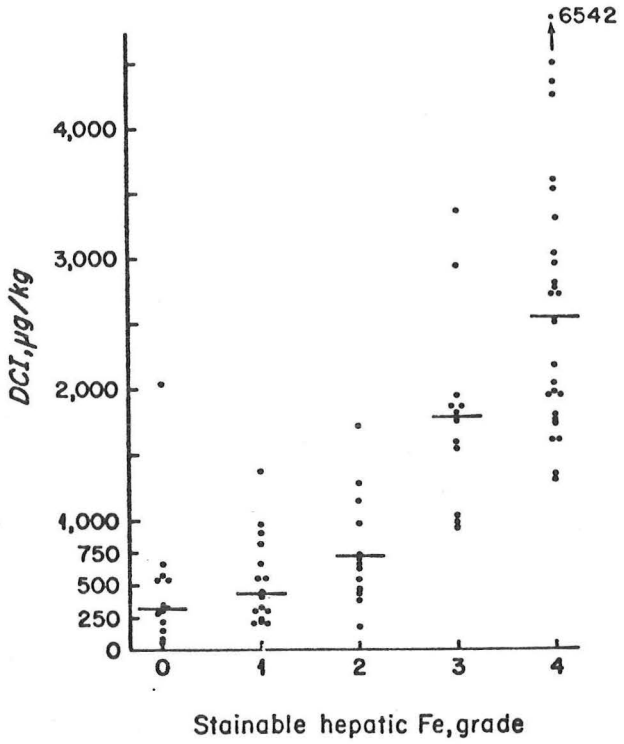
Beamish, M. R., Walker, R., Miller, F. et al. Transferrin iron, chelatable iron and ferritin in idiopathic hemochromatosis. Brit. J. Hematol. 27:219, 1974.

Baldus, W. P., Fairbanks, V. F., Dickson, E. R. et al. Deteroxamine - chelatable iron in hemochromatosis and other disorders of iron overload. Mayo Clin. Proc. 53:157, 1978.

LIVER BIOPSY

a) Stainable iron:

There is basically poor agreement between the amount of stainable iron seen on a liver biopsy and the measured body stores of iron. What appears to be a massive iron load microscopically may turn out to be rather trivial when the iron stores are actually measured.

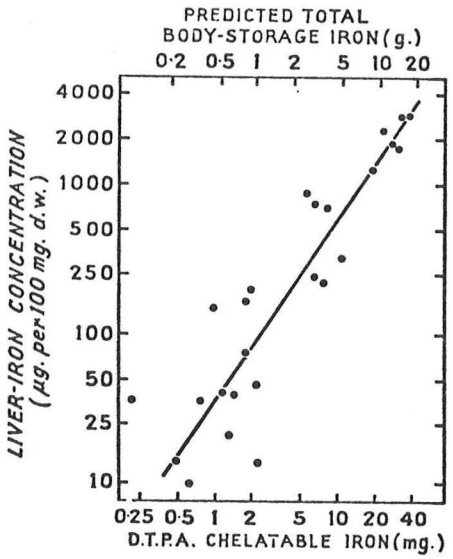


from Baldus et al, Mayo Clin. Proc. 1978

Figure 12

b) Chemical iron estimation:

In 1971, Barry described a simplified method for measuring tissue iron concentration on liver biopsy samples. He demonstrated a close correlation between chemically measured iron concentration and the total body stores of iron predicted by chelation with diethylenatriamine penta-acetic acid (D.T.P.A.) across a broad range of iron loads.



Relation between liver-iron concentration and D.T.P.A.-chelatable iron.

from Barry and Sherlock, Lancet 1971

Figure 13

Other authors have confirmed this relationship between tissue iron concentration in patients with hemochromatosis and iron stores as determined by phlebotomy. Such studies have also confirmed the poor correlation between stainable iron and iron content in patients with cirrhosis and porphyria cutanea tarda. Patients with hemochromatosis usually have liver iron concentration in excess of 1 mg/100 mg dry weight and in patients with symptomatic disease, this value is often greater than 2 mg/100 mg dry weight. Such high values are only found in patients with markedly increased total body iron stores. The major reservation with this assay is the concern of a significant sampling error in patients with cirrhosis where the assayed sample may consist largely of fibrous tissue rather than iron laden parenchymal or Kupffer cells. This concern appears to be more theoretical than real.

Zimmerman, H. J., Chomet, B., Kulesh, M. H. et al. Hepatic hemosiderin deposits. Arch. Int. Med. 107:494, 1961.

Weinfeld, A., Lundin, P. and Lunovall, O. Significance for the diagnosis of iron overload of histochemical and chemical iron in the liver of control subjects. J. Clin. Path. 21:35, 1968.

Barry, M. and Sherlock, S. Measurement of liver-iron concentrations in needle-biopsy specimens. Lancet (i):100, 1971.

Valberg, L. S., Ghent, C. S., Lloyd, D. A. et al. Diagnostic efficacy of tests for the detection of iron overload in chronic liver disease. C. M. A. Journal 119:229, 1978.

REPEATED PHLEBOTOMIES

The actual quantitation of iron stores by repeated phlebotomies until the patient is iron deficient remains the gold standard in determining the iron status of patients. To the extent that this combines effective therapy with diagnostic accuracy it remains the most effective method of defining iron overload in patients or family members of patients in whom the question is raised. One unit (500 cc) of blood removes approximately 250 mg of iron. When iron load is very high (>20 g), weekly phlebotomies may be required for more than 2 years before the iron stores are reduced to normal.

PATHOGENESIS

The ultimate abnormality in hemochromatosis is an absorption of iron from the G.I. tract inappropriate to the body iron stores already present. This could result from abnormalities in the G.I. lumen, the G.I. mucosa, an increase in tissue affinity for iron, or abnormalities elsewhere in the mechanisms controlling iron absorption. Extensive studies of G.I. secretions and intestinal mucosal function have not revealed any consistent abnormality so it seems likely that the fundamental metabolic defect lies elsewhere. It seems that the actual mechanisms of absorption are normal but that they respond to an inappropriate or abnormal signal. Nor is there evidence of an abnormality affecting either transferrin itself or a peculiar affinity of parenchymal tissue for iron molecules.

In the normal person, iron is moved from one tissue to another by transferrin. The major donor of iron to transferrin is normally the R.E. cell. The erythroid marrow is the major receptor for transferrin iron. Iron is also exchanged between parenchymal stores and transferrin. When transferrin saturation is high, iron moves into parenchymal cells and when saturation falls iron is mobilized from parenchymal stores. There is evidence that there is a defect in the ability of R.E. cells to store iron in idiopathic hemochromatosis. This would lead to saturation of transferrin and the movement of iron into parenchymal cells. The evidence to support this hypothesis is varied and not conclusive as much as suggestive:

(i) Patients with hemochromatosis are described who, despite massive iron overload, show no stainable iron in the R.E. cells of the marrow and spleen.
(ii) Circulating monocytes in idiopathic hemochromatosis have fewer and smaller iron granules than those seen in transfusion-induced iron overload states.
(iii) The jejunum contains many fewer iron-laden macrophages in idiopathic hemochromatosis than it does in the transfusion counterpart.
(iv) In iron depleted patients with hemochromatosis, serum iron and transferrin saturation is elevated well before the iron reaccumulation increases the body's total iron stores above normal.
(v) The ferritin which circulates normally in plasma is derived mainly from the R.E. system. Little of the plasma ferritin comes from the liver. Patients with hemochromatosis who have been iron depleted and who are allowed to reaccumulate iron demonstrate a lag between the rise in serum ferritin and the amount of chelatable iron. These studies suggest there is a deficit in the R.E. system ability to synthesize ferritin.

The implications of such a defect in R.E. function are far reaching. The degree of iron absorption from the G.I. tract is closely related to the R.E. storage iron in the marrow. The inappropriate increase in iron absorption in patients with hemochromatosis may be related to a failure to store iron in R.E. sites that normally regulate iron absorption. These "empty" stores may continue to signal for increased G.I. absorption of iron despite heavy deposition in parenchymal cells.

CLINICAL FEATURES. The triad of liver disease, diabetes and skin pigmentation have been emphasized in clinical descriptions of the disease but many other organs are also involved. Of particular importance are the myocardial and pituitary dysfunction seen frequently in these patients and the arthropathy which has been under-appreciated until quite recently.

HEPATIC INVOLVEMENT. Liver disease is a major component of the syndrome of hemochromatosis. It is very commonly present in patients who are symptomatic from the disease but is usually silent clinically until late in the course unless there is superimposed alcohol-induced liver injury.

Pathology. The earliest finding in the liver is the accumulation of hemosiderin granules within lysosomes in pericanalicular regions of periportal hepatocytes. With increasing accumulation, the iron remains essentially in parenchymal cells but extends to involve pericentral cells, too. Ultimately, cell injury results and iron released from damaged hepatocytes is taken up by Kupffer cells and macrophages present in the portal triads. Fibrous tissue begins to be deposited, expanding the portal tracts and spreading on into the parenchyma. The fibrous strands begin to link up and connecting bands of fibrous tissue, some quite broad, come to surround individual lobules or small groups of lobules in a monolobular pattern similar to that seen in biliary cirrhosis. The progression of this process leads finally to an established macronodular cirrhosis. At this stage, a large amount of iron may be seen in the R.E. system but parenchymal iron overload still predominates and hemosiderin granules may also be seen in mucosal cells of the biliary system. Evidence of acute cell necrosis with inflammation is usually quite mild in these patients unless they are alcoholic, in which case the whole pathological spectrum of alcoholic hepatitis may be apparent as well.

Clinical Manifestations. The characteristic clinical feature of the liver disease of hemochromatosis is its unobtrusiveness. Thus, hepatomegally is almost universally present at the time of presentation, and 50% of patients have splenomegally as well. Nonetheless, symptoms and signs of hepatic dysfunction are unusual until late in the disease. The liver disease is slowly progressive however, and late in the course of untreated disease jaundice, edema, ascites, hepatic encephalopathy and variceal hemorrhage are much more frequent. As many as a quarter of the deaths recorded from this disease were the direct result of liver failure. Abdominal pain is a relatively common hepatic symptom in these patients and while specific causes such as cholelithiasis, hepatoma or peptic ulcer disease are sometimes responsible, in the majority of instances, the explanation for such pain remains elusive.

In 1971, Powell et al published a comparative analysis of 414 patients with histological evidence of cirrhosis either at autopsy or on biopsy. As can be

seen from Fig. 14, 45 of these patients had hemochromatosis and there was the usual heavy male predominance.

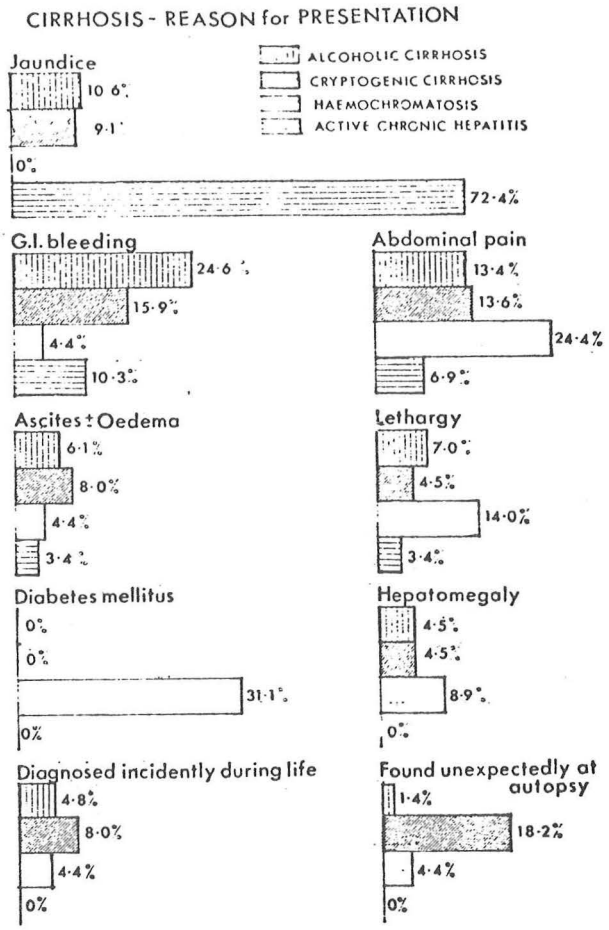
Distribution of Patients According to Ætiology and Sex

Type	Number of Patients	Men	Women	Incidence (Percentage)
Alcoholic	146	116	30	35·3
Cryptogenic	88	44	44	21·2
Hæmochromatosis	45	39	6	10·9
Active chronic hepatitis ..	32	8	24	7·7
Posticteric	25	9	16	6·0
Secondary biliary	24	10	14	5·8
Primary biliary	12	2	10	2·9
Cardiac	8	3	5	1·9
Wilson's disease	5	4	1	1·2
Fibrocystic disease	1	1	0	0·3
Unclassified[1]	28	23	5	6·8
Total	414	259	155	100

from Powell et al, Med. J. Aust. 1971

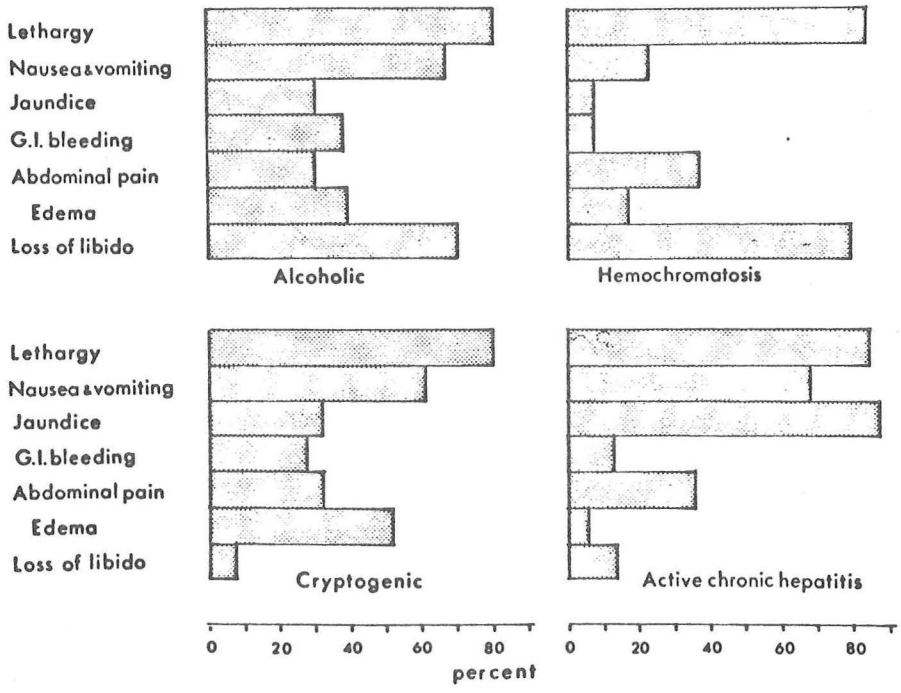
Figure 14

The presenting manifestation of patients with the 4 most common etiologies is depicted in Figure 15. Diabetes, abdominal pain and lethargy were common reasons for patients with hemochromatosis to seek medical attention but more specific hepatic manifestations such as jaundice, G.I. hemorrhage, ascites and edema were quite uncommon.



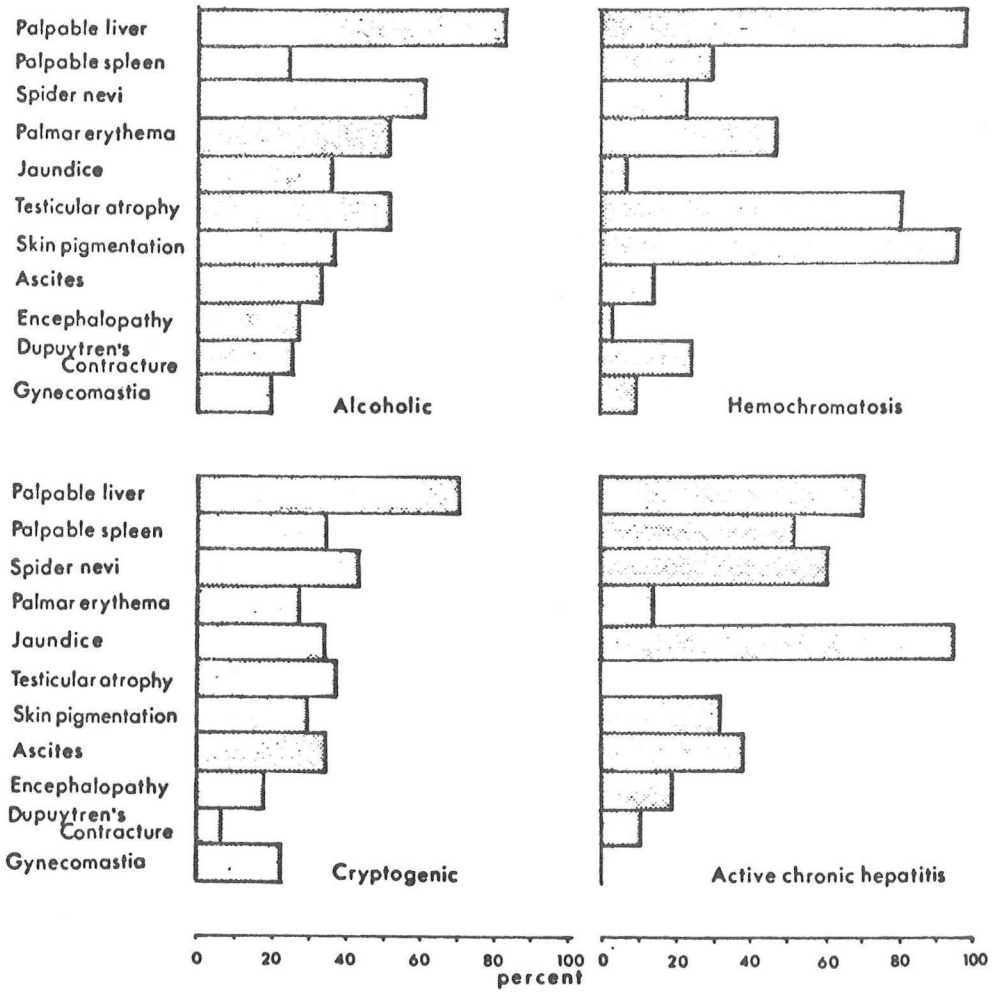
from Powell et al, Med. J. Aust. 1971
Figure 15

This pattern of mild clinical liver disease is also apparent in the symptoms that were present during the first month of illness (Figure 16) and the clinical signs that were present on first examination (Figure 17), where hepatogemally, testicular atrophy and pigmentation were the common findings.



from Powell et al, Med. J. Aust. 1971

Figure 16



from Powell et al, Med. J. Aust. 1971

Figure 17

Hemochromatotic liver disease presents with mild biochemical abnormalities as well. As can be seen in Figure 18, the mean values for routine liver function tests were the least disturbed and the mean serum albumin was the most well preserved in patients with iron storage disease.

LIVER TESTS ON PRESENTATION IN PATIENTS WITH CIRRHOSIS

Test	Alcoholic	Cryptogenic	CAH	Hemochromatosis
S. Bilirubin (mg/dl)	3.9	4.9	8.6	1.0
SGOT (Karmen U)	83	148	330	51
SAP (K.A. units)	20	37	26	12
S. Albumin (g/dl)	3.34	2.97	3.24	3.64

from Powell et al, Med. J. Aust. 1971
Figure 18

Complications of liver disease do develop in these patients and Figure 19 lists the frequency of the more common complications in the 4 groups. Liver failure, ascites, varices and G.I. hemorrhage all occurred least frequently in patients with hemochromatosis but this group had the highest incidence of hepatocellular carcinoma.

COMPLICATIONS IN PATIENTS WITH CIRRHOSIS

Complication	Alcoholic	Cryptogenic	CAH	Hemochromatosis
Liver failure (%)	35	32	77	10
Portal hypertension (%)	52	54	45	31
G.I. Hemorrhage (%)	32	34	30	9
Hepatoma (%)	5	10	0	20

from Powell et al, Med. J. Aust. 1971
Figure 19

This high frequency of hepatoma in patients with hemochromatosis has been echoed by every author who writes on the subject. The carcinogenic stimulus is not known although it is questionable whether iron itself plays this role because the incidence of hepatoma does not diminish in these patients despite effective iron depletion by venesection. All such patients have, however, progressed to the point of macronodular cirrhosis. It is possible, therefore, that the high incidence of primary liver cell tumor reflects prolonged survival with cirrhosis and liver cell regeneration serving as the carcinogenic stimulus. Hepatoma has now become the single most common cause of death in patients with hemochromatosis since effective therapy has reduced the contributions made by cardiac and hepatic failure. This is well documented in Figure 20 taken from Powell et al's comparative analysis of patients with cirrhosis.

CAUSES OF DEATH IN PATIENTS WITH CIRRHOSIS

Cause	Alcoholic	Cryptogenic	CAH	Hemochromatosis
Liver failure (%)	35	32	77	9
G.I. hemorrhage (%)	26	18	15	9
Infection (%)	8	8	8	14
Vascular (%)	1.5	17	0	9
Hepatoma (%)	9	11	0	37

from Powell et al, Med. J. Aust. 1971

Figure 20

There is no question that iron depletion with repeated phlebotomies will prevent the progression of liver disease and if initiated early enough, will prevent its development. There is even evidence that in some patients the amount of fibrous tissue will regress and an occasional well documented report of the complete reversal of cirrhosis and the return of the liver to an entirely normal architecture. The risk of developing hepatoma does not seem to be reduced significantly, however, by phlebotomy. A number of patients have been reported who have developed hepatoma years after being effectively treated with repeated venesection.

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

Powell, L. W., Mortimer, R. and Harris, O. D. Cirrhosis of the liver. A comparative study of the four major aetiological groups. Med. J. Aust. 1:941, 1971.

Bomford, A. and Williams, R. Long term results of venesection therapy in idiopathic hemochromatosis. Quart. J. Med. 45:611, 1976.

Williams, R., Smith, P. M., Spicer, E. J. F. Venesection therapy in idiopathic hemochromatosis. Quart. J. Med. 38:1, 1969.

Powell, L. W. and Kerr, J. F. R. Reversal of "Cirrhosis" in idiopathic haemochromatosis following long-term intensive venesection therapy. Aust. Ann. Med. 1:54, 1970.

Knauer, C. M., Gamble, C. N. and Monroe, L. S. The reversal of hemochromatotic cirrhosis by multiple phlebotomies. Gastroenterology 49:667, 1965.

DIABETES

Incidence: Diabetes is considered to be one of the cardinal clinical manifestations of hemochromatosis. It is far from a universal finding in such patients however. The prevalence of diabetes in the patients reported in the literature has varied widely from as few as 29% to as many as 80%. Most series report an incidence of greater than 50% but it is not always clear what criteria are being used to establish the diagnosis of either entity.

Pathogenesis: It was presumed initially that the diabetes of hemochromatosis was the direct result of the deposition of iron and consequent fibrosis of the pancreas. A number of pieces of evidence have challenged this view.

(i) There is a high incidence (25% to 47%) of diabetes in first degree relatives of patients with hemochromatosis. In a study reported by Dymock et al, this high incidence was limited to patients who had diabetes themselves (25%) and was not seen in the relatives of patients with hemochromatosis who did not have diabetes (4%).

(ii) Evidence of pancreatic exocrine failure is very unusual in patients with hemochromatosis and almost always precedes endocrine failure (diabetes) in patients with other diseases affecting the pancreas.

(iii) Diabetic complications such as neuropathy, retinopathy and nephropathy do occur in patients with hemochromatosis with some frequency but are rarely associated with the carbohydrate intolerance of cirrhosis or diabetes associated with other pancreatic disorders. It is noteworthy, however, that these complications correlate with the duration of diabetes in patients with hemochromatosis and are unusual in patients whose diabetes has not been present for 10 years. It is less common for patients with pancreatic insufficiency to survive this long, although occasional patients are described who have developed neuropathy and retinopathy many years after pancreatectomy or the development of diabetes from chronic pancreatitis. These arguments strongly suggest that a major factor in the diabetes of this disorder is the inheritence of genetic diabetes. Dymock et al studied the insulin response to I.V. glucose loads in patients with hemochromatosis and diabetes and described a pattern in some patients which reflected a decreased peripheral sensitivity to insulin. These patients had a normal early phase of insulin release but maintained high levels of insulin late in the test. This pattern is commonly seen in patients with cirrhosis from any cause.

Current wisdom would suggest that the carbohydrate intolerance and diabetes associated with hemochromatosis is multifactorial in origin. Genetic diabetes is a major influence but there are also contributions from the insulin insensitivity common to cirrhosis and probably also from iron-induced damage to the islets of Langerham.

Clinical manifestations and therapy: Most reports comment on the relative ease of diabetes control in patients with hemochromatosis. The majority of patients described have been insulin-dependent, however. Complications when they develop also tend to be relatively mild. The retinopathy, for example, tends to be a fundoscopic phenomenon and there is usually no loss of visual acuity.

While there is an occasional patient whose insulin requirements are dramatically reduced by effectively removing the iron load with phlebotomy, the diabetes in most patients remains unaltered by repeated venosections.

Grace, N. D., Powell, L. W. Iron storage disorders of the liver. Gastroenterology 64:1257, 1974.

Galton, D. J. Diabetic retinopathy and hemochromatosis. B. M. J. 1:1169, 1965.

Dymock, I. W., Cassar, J., Pyke, D. A. et al. Observations on the pathogenesis, complications and treatment of diabetes in 115 cases of hemochromatosis. Am. J. Med. 52:203, 1972.

Stocks, A. E. and Powell, L. W. Carbohydrate intolerance in Idiopathic hemochromatosis and cirrhosis of the liver. Quart. J. Med. 42:733, 1973.

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

Becker, D. and Miller, M. Presence of diabetic glenerulosclerosis in patients with hemochromatosis. N. Engl. J. Med. 263:371, 1960.

PIGMENTATION

Increased skin pigmentation is very common in patients who have symptomatic hemochromatosis. The pigmentation is most marked in sun-exposed areas but is also heavy in scars and in the perineal region. Increased pigment affects the buccal mucosa in 10-15% of patients and has been described in the conjunctive and lid martins. The pigmentation is caused mainly by an increase in cutaneous melanin. This melanosis is of uncertain cause but may be a direct consequence of iron deposition. It has been suggested that iron in the cutaneous tissues may favor melanin deposition by increasing oxidation of tyrosine, a feature of normal melanogenesis. In untreated patients, there is an increase in cutaneous iron and if this is very heavy, a particular "slate" color is added to the "bronze" produced by melanin. By and large, the increase in skin pigmentation is diminished by effective venesection therapy. Some slight increase in color, particularly in scars, may persist after iron depletion.

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

Williams, R., Smith, PM, Spicer, E. J. F. et al. Venesection therapy in idiopathic hemochromatosis. Quart. J. Med. 38:1, 1969.

Davies, G., Dymock, I., Harry, J. et al. Deposition of melanin and iron in occular structures in haemochromatosis. Brit. J. Opthal. 56:338, 1972.

CARDIOMYOPATHY

In 1955, Finch and Finch emphasized the importance of the myocardial involvement in hemochromatosis by pointing out that heart disease was the single most common cause of death in this disorder. Symptomatic myocardial disease was present at presentation in 15% of patients reported at that time and was noted to be an especially common feature in younger patients. This has been underscored by a recent paper from our institution which reviewed the presentation and course of idiopathic hemochromatosis in patients less than 30 years of age. Cardiomyopathy was described in almost 60% of such patients.

The manifestations of heart involvement are those of congestive heart failure and/or arrhythmias. Symptoms and signs of right sided heart failure may predominate but there is usually biventricular dilatation. The disorder is usually categorized as a restrictive cardiomyopathy. Hemodynamic evaluation demonstrates a marked decrease in myocardial contractility. The common arrhythmias (which may be extremely difficult to control with conventional therapy) include supraventricular tachyarrhythmias, ventricular ectopics and episodes of ventricular tachycardia. Varying degrees of AV block are also common. EKG's otherwise are characterized by low voltage and nonspecific ST + T wave changes. Microscopically, the iron deposition begins in and is more extensive in ventricular rather than atrial muscle. The iron deposits are always more extensive in working than in conducting myocardium. Iron deposits are found in both interstitial cells and in myocardial fibres. The heart disease of hemochromatosis is entirely reversible. With effective removal of the iron load, all of the symptoms and signs of the cardiomyopathy will remit and patients are able to resume normal and active lives.

Finch, S. C. and Finch, C. A. Idiopathic hemochromatosis, an iron storage disease. Medicine 30:381, 1955.

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

Engle, M. A., Erlandson, M., Smith, C. H. Late cardiac complications of chronic, severe, refractory anemia with hemochromatosis. Circulation 30:698, 1964.

Buja, L. M., Roberts, W. C. Iron in the heart. Etiology and significance. Am. J. Med. 51:209, 1971.

Lamon, J. M., Marynick, S. P., Rosenblatt, R. Idiopathic hemochromatosis in a young female. Gastroenterology 76:178, 1980.

ENDOCRINE

At autopsy, patients with untreated hemochromatosis frequently have excessive hemosiderin deposits in the pituitary, the gonads, the adrenals, the thyroid and the parathyroids. None of these organs demonstrate an increase in fibrous tissue, however. Functional impairment of these endocrine organs is essentially confined to the pituitary and the gonads and is very common. Of 14 patients studied extensively by Stocks and Martin, only 6 had completely normal pitui-

tary function and 4 showed severe depression. There is a predominant impairment of gonadatrophic function in these patients. Prominent findings among male patients are loss of libido with failure to maintain an erection and marked testicular atrophy. Women develop secondary amenorrhaea and also lose libido. Gynecomastia is very uncommon in patients with hemochromatosis who do not have a major component of superimposed alcohol-related liver disease. The gondal failure is secondary to pituitary failure and is not considered to be the result of direct iron-deposition in the testes. While one can detect other evidence of pituitary insufficiency by stimulating growth hormone response by hypoglycemia or the TSH response to TRF, it is very unusual for these other features of hypopituitarism to achieve clinical expression. Unfortunately, the pituitary dysfunction is permanent and there is no improvement in the clinical manifestations following effective phlebotomy therapy.

Stocks, A. E. and Martin, F. I. R. Pituitary function in haemochromatosis. Am. J. Med. 45:839, 1968.

Stocks, A. E. and Powell, L. W. Pituitary function in idiopathic haemochromatosis and cirrhosis of the liver. Lancet (ii):298, 1972.

ARTHROPATHY

It was not apparent until Schumaker's paper in 1964 that a particular arthropathy was associated with hemochromatosis. In retrospect, a number of patients had complained of mild symptoms of arthritis and had been presumed to have had degenerative joint disease. Once attention was drawn to the association, a rather characteristic arthropathy was delineated. When looked for a progressive polyarthritis is found in more than half the patients with idiopathic hemochromatosis. The disease often starts in, and is usually most manifest in, the metacarpo-phalangeal and proximal interphalangeal joints of the hands. It spreads to involve larger joints particularly the wrists, hips, knees, ankles and elbows. The small joints of the feet are seldom involved, and then only mildly.

X-ray findings: In the small joints, the major radiological findings consist of subarticular cysts which erode into the joint cavity and lead to a loss of joint space with erosion of the articular surface and osteophyte formation. In the larger joints, there is, characteristically, chondrocalcinosis with calcification of articular cartilage as well as the fibrocartilage of the menisci in the knees and the articular labrum of the hips. Subarticular cysts with loss of articular cartilage and narrowed joint spaces may also be seen in the large joints. Calcification may also be observed in the symphisis pubis, the tendo Achilles, the plantar fascia and the intervertebral discs in occasional patients.

Clinical features: Symptoms for the most part are mild and consistent stiffness of fingers and occasional pain after excessive use of the hands. This is associated with bony swelling of the involved joints and some limitation of finger flexion. With time, the symptoms and bony swelling increase. Pain and limitation of joint movement also may signal the presence of disease in large joints and in some patients, this has progressed to the point where hip replacements have been deemed justified. Superimposed on this chronic progressive joint disease, there may occur acute episodes of inflammatory arthritis.

The affected joint (often a knee) becomes swollen, red, warm and tender. Aspiration of such a joint reveals an inflammatory fluid and the positive birefringent rhomboid-shaped crystals of calcium pyrophosphate dihydrate may be found. Thus, hemochromatosis becomes one of the associations (along with diabetes, hypertension, hyperuricemia, hyperparathyroidism and possibly Wilson's disease, ochronosis and hypophosphatasia) of the "pseudegout" syndrome.

Pathogenesis: In untreated patients, there is often a heavy deposition of iron granules in the synovial cells of involved and uninvolved joints and it is presumed that iron is the initiating event in the arthropathy. A similar arthropathy occurs in rural Asia among peasants exposed to very heavy iron ingestion through drinking water. This disease, called Kaschin-Beck's disease, affects youths (but not adults) in endemic areas and is worse in the spring, when apparently the iron content of the water is at its peak. Children removed from this environment are not subsequently affected. In general, patients with hemochromatosis who develop arthritis are older and have had symptoms of the disease for longer than those without joint involvement. In some patients, however, arthritis precedes other symptoms of the disease and joint destruction has been documented before the onset of liver damage at a time when a biopsy showed only excess parenchymal iron deposition. If iron deposition serves as a trigger for the onset of arthropathy, its presence is certainly not required for the perpetuation and progression of joint disease. Phlebotomy has no ameliorative effect on the course of the joint manifestations and, indeed, joint symptoms may first occur during or even after venesection therapy. It has been shown *in vitro* that iron interferes with cartilagenous pyrophosphatase activity. This may initiate a sequence of events which leads to pyrophosphate crystal formation and calcification of the cartilage. The process then is apparently self-perpetuative.

Schumaker, H. R. Hemochromatosis and arthritis. Arth. Rheum. 7:41, 1964.

Wardle, E. N. and Patton, J. T. Bone and joint changes in hemochromatosis. Ann. Rheum. Dis. 28:15, 1969.

Dymock, I. W., Hamilton, E. B. D., Laws, J. W., et al. Arthropathy of hemochromatosis. Clinical and radiological analysis of 63 patients with iron overload. Ann. Rheum. Dis. 29:469, 1970.

Webb, J., Corrigan, A. B. and Robinson, R. G. Hemochromatosis and "pseudogout". Med. J. Aust. 2:24, 1972.

GENETICS OF HEMOCHROMATOSIS

The familial tendency of hemochromatosis was recognized as early as 1935 when Sheldon in his classic review suggested that the disease was caused by an inherited inborn error of metabolism. Despite the continued reporting of occasional examples of familial co-occurrence of the disease, dispute raged through the 1960s as to whether or not the excess iron deposition in patients with hemochromatosis was entirely the consequence of acquired environmental factors (tranfusions, iron ingestion, alcoholism, cirrhosis). As first degree relatives were evaluated with respect to iron stores, it became clear that an abnormality in iron autobolism was common in family members of patients with hemochromatosis even if fully expressed clinical disease was not. The

modifying influence that environmental factors such as alcoholism, dietary iron, liver disease and diabetes have on iron absorption has not allowed a clear picture of the pattern of inheritance of hemochromatosis to emerge because definitive recognition of affected individuals who have not yet developed iron overload is not possible, given our continued ignorance of the metabolic basis of the disease.

Scheinberg proposed an attractive if simplistic hypothesis which argued that the disease was transmitted as an autosomal recessive. A patient homozygous for the disorder would develop clinically apparent disease usually at a young age. A patient heterozygous for the gene would have a very variable clinical expression. Such a patient would have an abnormality of iron absorption leading to the accumulation of from 2 to 5 grams of iron. This would be clinically inapparent. If one of a number of environmental influences (particularly alcohol-induced liver disease) were superimposed, the iron storage could increase to the 20 grams or more seen in homozygotes and the full-blown clinical disorder would result. Other authors argued in favor of an autosomal dominant inheritance with variable expressivity determined principally by age, sex and alcohol ingestion.

New impetus to the definition of the hereditary characteristics of hemochromatosis were provided by Simon et al who demonstrated an association between the histocompatibility antigens HLA-A_3 and HLA-B_{14} and the disease. The strong association between HLA-A_3 and symptomatic hemochromatosis and the weaker association between the disease and HLA-B_7 and B_{14} were quickly confirmed by other authors.

	Hemochromatosis			Controls		
Authors	A_3	B_7	B_{14}	A_3	B_7	B_{14}
Simon et al 1976	78.4%	49%	25%	27%	25%	3.4%
Bomford et al 1977	69%	34%	20%	31%	20%	6%
Simon et al 1977	75%		31%	27%		3.4%
Bassett et al 1979	60%			22%		

That these HLA antigens were not directly involved in transmitting the disease was made clear by the demonstration that as many as 40% of unaffected relatives also carried the HLA-A_3 antigen.

In 1977, Simon et al demonstrated that siblings with symptomatic disease very frequently shared two identical HLA haplotypes. Other members of the family might share one or other of the proband's haplotypes but such persons either had no disease or only minor degrees of iron overload. It seemed from these studies that there was a hemochromatosis gene located on chromosome 6 (the HLA chromosome) very close to A_3. Symptomatic disease (i.e. hemochromatosis) appeared to require a double dose of the hemochromatosis allele (i.e. was inherited as an autosomal recessive). A variable degree of expressivity accompanied the heterozygote state influenced by the usual environmental

factors. There were a number of different HLA haplotypes which carried the hemochromatosis allele but HLA-A_3 B_{14} was the most commonly associated combination.

In 1979, Cartwright et al defined 19 haplotypes bearing the hemochromatosis allele among 10 patients with evidence of iron overload (9 with hemochromatosis and 1 with minor iron overload and judged to be a heterozygote). The family members (261 persons) of these families were then typed and assigned a status determined by their haplotype. Persons with two "hemochromatosis" haplotypes were judged to be homozygous. Those with one haplotype were considered heterozygotes and those with entirely different haplotypes were considered normal. The iron storage status of all of these patients was then determined.

Iron Status in Normal Subjects.

Determination	Male Subjects			Female Subjects		
	No.	Mean	95% limits	No.	Mean	95% limits
Age (yr)	44	33	0–77	52	36	0–80
Serum iron (μg/100 ml)	44	106	50–162	52	101	42–160
Transferrin saturation (%)	44	32	14–50	52	31	10–50
Serum ferritin* (ng/ml)	41	93	16–542	50	48	7–330
Urinary iron† (mg/24 hr)	19	1.3	0.4–2.2	22	1.0	0.2–1.8
Parenchymal-cell iron (grade)‡	22	0.2	0–1	14	0	0
Hepatic iron (μg/100 mg wet liver)	22	12	0–29	14	7	0–19

from Cartwright et al, N. Engl. J. Med. 1979

Figure 23

None of the 96 subjects judged to be normal by haplotype analysis had a major iron load (transferrin saturation >79% and hepatic iron >400μg/100 mg wet liver), and only one a minor load (transferrin saturation >50%, hepatic Fe 30 to 400μg/100 mg wet liver).

Iron Status of Heterozygotes.

Determination	Male Subjects			Female Subjects		
	No.	Mean	95% limits	No.	Mean	95% limits
Age (yr)	67	33	0–75	78	34	9–77
Serum iron (μg/100 ml)	67	135	62–208	78	110	40–181
Transferrin saturation (%)	67	44	19–69	78	37	10–63
Serum ferritin* (ng/ml)	64	96	15–617	76	50	8–335
Urinary iron† (mg/24 hr)	38	1.6	0.2–3.0	27	1.3	0–2.7
Parenchymal-cell iron (grade)‡	22	1.1	0–3	8	1.1	0–2
Hepatic iron (μg/100 mg wet liver)	22	96	0–282	8	63	0–191

from Cartwright et al, N. Engl. J. Med. 1979

Figure 24

Of the 145 "heterozygotes", 100 had no abnormality of Fe storage and 45 showed evidence of a minor iron load. Serum iron and transferrin saturation were higher in male than female heterozygotes and increased progressively with age.

Iron Status in Homozygotes.

Determination	Male Subjects			Female Subjects		
	No.	Mean	Determined range	No.	Mean	Determined range
Age (yr)	13	48	25–70	7	37	7–64
Serum iron (μg/100 ml)	13	244	173–307	7	204	145–261
Transferrin saturation (%)	13	95	80–100	7	83	73–90
Serum ferritin* (ng/ml)	12	2099	565–11,560	6	301	106–1390
Urinary iron (mg/24 hr)†	12	11.2	4.4–26.5	7	2.4	0.8–4.6
Parenchymal-cell iron (grade)‡	13	3.9	3–4	5	3.4	2–4
Hepatic iron (μg/100 mg wet liver)	10	877	486–1417	5	478	59–1109

from Cartwright et al, N. Engl. J. Med. 1979

Figure 25

Twenty homozygotes were identified in this study. Seventeen of these patients had a major iron overload and three females had evidence of a minor iron load. Clinically manifest disease was present in 11 of the 13 males (the other two were only 25 and 28 years old). Only one of the 7 women had clinically manifest disease.

IRON STATUS OF FAMILY MEMBERS BY GENOTYPE

	Genotype		
Iron Status	Normal	Heterozygote	Homozygote
Normal	95	100	0
Minor load[1]	1	45	3
Major load[2]	0	0	17

[1] ≡ transferrin sat. >50%, hepatic iron conc. 30-400μg/100 mg wet wt.

[2] ≡ transferrin sat. >75%, hepatic iron conc. >400μg/100 mg wet wt.

Figure 26

These findings have been confirmed in a similar study of 111 members from 12 families reported from Canada. These data underscored the utility of HLA typing to identify the status of relatives in a family with an identified proband and further supported the notion that hemochromatosis is transmitted as an autosomal recessive with partial expressivity of the heterozygote state. This transmission pattern also helps to explain why symptomatic hemochromatosis is not unusual among siblings but is much less frequently observed in successive generations unless there is consanguinity.

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