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Influence of disease on iron status

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DISTRIBUTION OF IRON IN THE BODY

Most of the Fe in the body is found in the O_2-binding protein, haemoglobin, of the erythrocytes. However, Fe is also essential for O_2 utilization within the cell. Table 1 summarizes the main Fe-containing components within the body.

Fig. 1 illustrates the major pathways of Fe metabolism in man. The most important pathway in terms of Fe turnover is the delivery of Fe to the erythroid marrow for the synthesis of haemoglobin and the breakdown of erythrocytes at the end of their lifespan with Fe returning to the plasma. Fe excretion is limited to about 1 mg/d in men but menstruation increases total Fe losses to about 2 mg/d in women.

IMPORTANT COMPONENTS OF IRON METABOLISM

Plasma iron transport

The Fe-binding protein of the plasma is transferrin (for review, see De Jong *et al.* 1990). Transferrin is very similar to lactoferrin found in granulocytes and in milk. Both are monomeric glycoproteins with a molecular mass of about 80 kDa. Transferrin binds two atoms of Fe^{3+} and each Fe-binding site requires both Fe and an anion which is usually carbonate or bicarbonate. Transferrin has a very high affinity for Fe. Fe can be released by lowering the pH to less than 5·5 but there are specific mechanisms for delivering Fe to cells (see later).

The plasma concentration of transferrin in adults is normally about 2·5 g/l and transferrin can bind 1·4 μg Fe/mg. The protein is normally 20–40 % saturated with Fe.

Delivery of iron to cells

Delivery of transferrin-Fe to cells (particularly to immature erythrocytes for haemoglobin synthesis) takes place by interaction with specific receptors in the cell membrane (Huebers & Finch, 1987) followed by receptor-mediated endocytosis, removal of Fe and release of apotransferrin from the cell. The transferrin receptor is a transmembrane glycoprotein consisting of two identical subunits of molecular mass 95 kDa joined by a disulfide bond (Trowbridge & Schackelford, 1986).

Haem synthesis

Quantitatively, the major pathway of Fe metabolism involves haem synthesis and breakdown and this haem is largely present in haemoglobin. Haem synthesis and its control in the erythroid cell and the liver have been reviewed by Bottomley & Muller-Eberhard (1988).

Table 1. *Distribution of iron in the body (70 kg man)*

Protein	Location	Fe content (mg)
Haemoglobin	Erythrocytes	3000
Myoglobin	Muscle	400
Cytochromes, other haem and Fe, S proteins	All tissues	50
Transferrin	Plasma and extravascular fluid	5
Ferritin and haemosiderin	Liver, spleen and bone marrow	0–1000

Haem catabolism

This is an enzymic process mediated by the enzyme haem oxygenase which is located in the endoplasmic reticulum. The enzyme requires NADPH and molecular oxygen (Schacter, 1988). Enzyme activity (in the rat) is highest in the spleen and then in the bone marrow, liver, brain, kidney and lung. There is also activity in the intestinal mucosa. The immediate fate of the Fe released from haem is unknown but Fe is rapidly returned to the plasma as well as being incorporated into ferritin in the cells breaking down haem.

Iron storage proteins

Fe is stored in cells as ferritin which is a soluble, spherical protein enclosing a core of Fe. Particularly high concentrations are present in the liver, spleen and bone marrow. Ferritin is also found in low concentrations in plasma (Worwood, 1990). Human apoferritin (i.e. the molecule devoid of Fe) has a molecular mass of 480 kDa and is composed of twenty-four subunits of molecular mass about 19 kDa. The subunits form a nearly spherical shell that encloses a central core containing up to 4500 atoms of Fe in the form of ferric hydroxyphosphate.

Human ferritins are made up of two types of subunit in varying proportions. In liver and spleen, the L subunit dominates. In the most acidic isoferritins found in the heart and in erythrocytes, the H subunit predominates. The various isoferritins appear to have different functions. In Fe-loaded tissues it is the L-rich isoferritins which predominate, although H-rich isoferritins have the highest rates of Fe uptake *in vitro*.

Haemosiderin is a degraded form of ferritin in which the protein shells have partly disintegrated allowing the Fe cores to aggregate (Richter, 1984). It is usually found in lysosomes and may be seen under the light microscope after tissue sections have been stained with potassium ferrocyanide in the presence of HCl (Prussian blue or Perl's reaction).

Regulation of synthesis of transport and storage proteins

Studies in animals and cultured cells show that apoferritin is synthesized in response to Fe administration. This mechanism is well understood and is exercised at the level of translation (Klausner *et al.* 1993). The 5′ untranslated region of the ferritin mRNA contains a sequence which forms a 'stem loop'. This has been termed an Fe response element. Cytoplasmic proteins which bind to this sequence and prevent translation have been identified. In the presence of Fe this repressor protein (Fe-regulatory protein; IRP) is unable

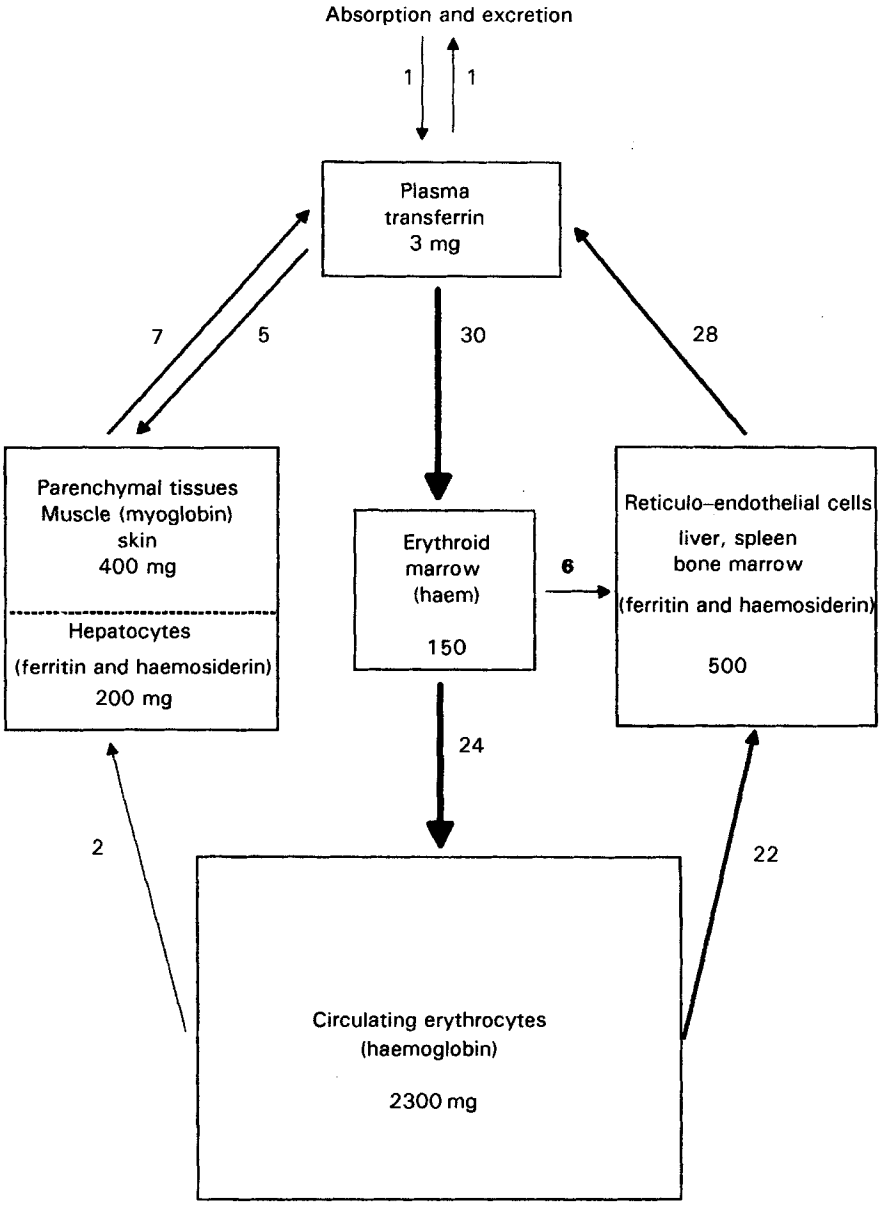

Fig. 1. The major pathways of iron metabolism. Values shown are mg Fe/d. The Fe in muscle is largely haem and in hepatic parenchymal cells ferritin and haemosiderin. (From British Nutrition Foundation, 1995; with permission.)

to bind the mRNA, polysomes form and translation proceeds. The IRP is soluble aconitase (*EC* 4.2.1.3) and in its Fe-replete state it may function as aconitase. A related mechanism operates in reverse for the transferrin receptor. There are 'stem loop' sequences in the 3′ untranslated region and protein binding prevents degradation of mRNA, hence Fe deficiency enhances transferrin-receptor synthesis. This translational regulation also applies to erythroid 5-aminolevulinate synthase (*EC* 2.3.1.37) and aconitase.

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Regulation of iron absorption

The body Fe content is normally maintained at a constant level by variation in the amount of Fe absorbed. It is clear that this depends on the availability of Fe in the diet and is also influenced both by the storage Fe content of the body and by the rate of erythropoiesis. However, the way in which Fe absorption is regulated in the intestinal epithelial cell remains largely a mystery (Peters *et al.* 1988).

IRON STATUS

Normal Fe status implies both the presence of erythropoiesis which is not limited by Fe and a small reserve of 'storage Fe' to cope with normal physiological functions and to permit a rapid response to acute loss of blood (Fe). The limits of normality are difficult to define and some argue that physiological normality is an absence of storage Fe (Sullivan, 1992). However, the extremes of Fe-deficiency anaemia and haemochromatosis are well understood. There is also the possibility of maldistribution of Fe within the body. In the anaemia associated with inflammation or infection, where there is a partial failure of erythropoiesis and of Fe release from phagocytic cells in liver, spleen and bone marrow, there is an accumulation of Fe as ferritin and haemosiderin in these cells. Thus, determination of Fe status requires an estimate of the amount of haemoglobin-Fe in the body (usually by measuring the haemoglobin concentration of the blood) and the level of storage Fe. Occasionally further investigations into Fe loss, Fe absorption and flow-rates within the body are also required. Fig. 2 illustrates the situation for normal Fe status, Fe deficiency, Fe overload and the anaemia of chronic disease. Fig. 3 shows the various indicators of Fe status which are in clinical use.

Tissue iron concentrations (iron stores)

The liver and bone marrow are important and relatively accessible storage sites and the amount of Fe present can be estimated either visually using the Prussian blue reaction on tissue sections or by a chemical determination. Methods for chemical and histological assessment of tissue Fe concentrations have been described by Torrance & Bothwell (1980). Chemical determination of liver Fe concentration is most widely applied for the demonstration of Fe overload and allows the important distinction to be made between the relatively minor increases in liver non-haem-Fe sometimes found in patients with cirrhosis of the liver and Fe overload associated with inherited haemochromatosis (Bassett *et al.* 1986). Estimation of Fe concentration in the bone marrow is, in contrast, usually carried out by the histochemical method and is used to detect the absence of storage Fe. In particular, assessing marrow Fe histologically distinguishes between true Fe deficiency and other chronic disorders in which there is impaired release of Fe from reticulo-endothelial cells.

Serum ferritin concentrations. Small amounts of ferritin circulate within the plasma. Serum ferritin concentrations are normally within the range 15–300 μg/l; values are lower in children than in adults and from puberty to middle age mean concentrations are higher in men than in women (Worwood, 1982). A concentration of less than approximately 15 μg/l is usually found in the absence of storage Fe. Ferritin concentrations are determined by sensitive immunological assays, usually ELISA.

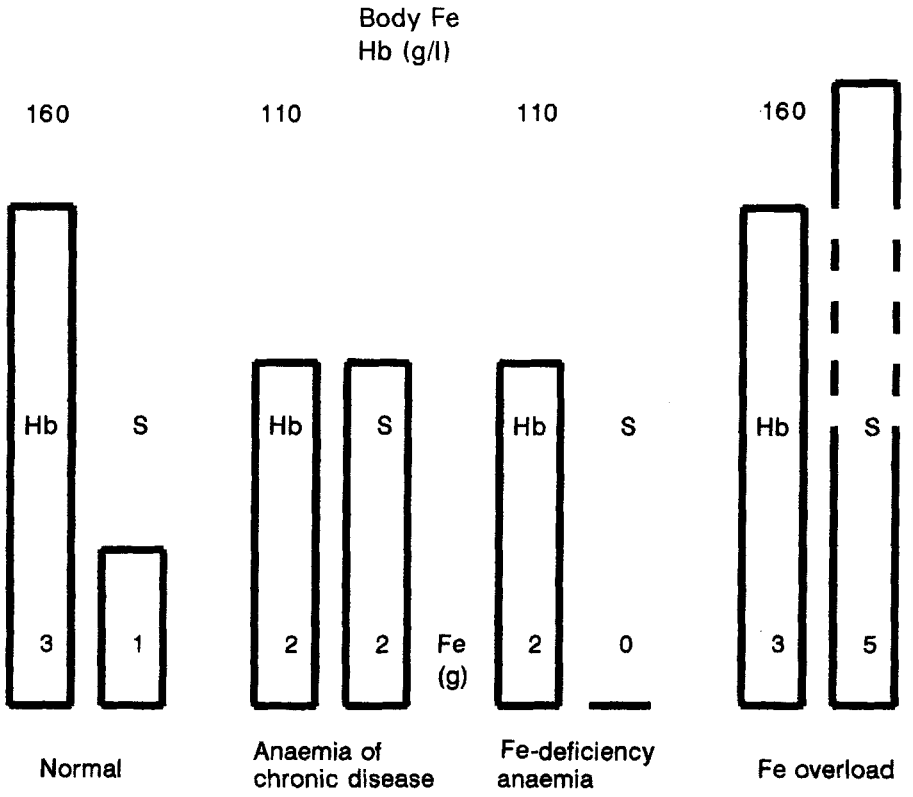

Fig. 2. Body iron: total haemoglobin (Hb) and storage Fe (S) levels in various physiological conditions. Total haemoglobin and storage Fe levels (g) are shown. The normal subject is a 70 kg man.

Transport iron

Serum Fe concentrations can be measured by colorimetric methods (International Committee for Standardization in Haematology, 1990) which are relatively specific for transferrin Fe and do not measure haem-Fe. Measurement of serum Fe concentrations alone provides little useful clinical information because of the considerable variation from hour-to-hour and day-to-day in normal individuals (see p. 415). More information can be obtained by measuring both the serum Fe concentration and the total Fe-binding capacity (TIBC) from which the percentage of transferrin saturation with Fe may be calculated. The TIBC is a measurement of transferrin concentration and may be estimated by saturating the transferrin Fe-binding capacity with excess Fe and removing the excess by adding soluble $MgCO_3$ or some other absorbent. This is followed by determination of the Fe content of the saturated serum. As an alternative transferrin concentrations may be measured directly by immunological assay. Normally, in adults, the median Fe concentration is about 17 μmol/l, the TIBC is approximately 65 mol Fe/l and the transferrin saturation approximately 25 %. A transferrin saturation of 16 % or less is usually considered to indicate an inadequate Fe supply for erythropoiesis (Bainton & Finch, 1964). A raised TIBC (greater than 70 μmol/l) is characteristic of a deficiency of storage Fe.

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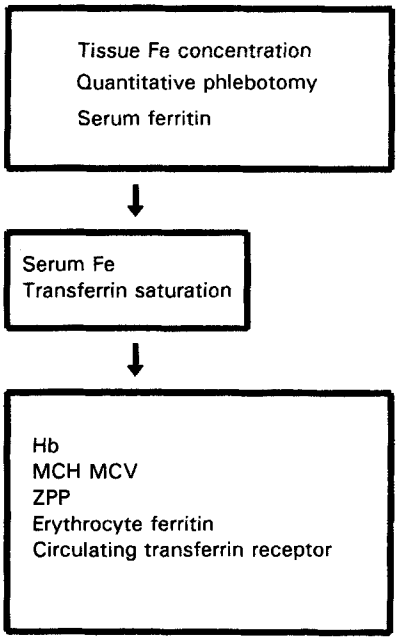

Fig. 3. Indicators of iron status, relating to stores → transport → erythropoiesis. Hb, haemoglobin; MCH, mean cell haemoglobin; MCV, mean cell volume, ZPP, zinc protoporphyrin.

Functional iron status (detection of iron-deficient erythropoiesis)

The primary measurement here is the determination of haemoglobin concentration (Dacie & Lewis, 1995). Fe deficiency is indicated by a low mean cell volume (MCV) and a low mean cell haemoglobin concentration (MCH). Thus, blood cell analysers can indicate the presence of microcytic anaemia which can be due to a reduced supply of Fe to the bone marrow or to a deficit in haemoglobin synthesis such as in thalassaemia. Further tests are usually necessary to distinguish between simple Fe deficiency (absence of storage Fe) and a supply deficiency that is secondary to another disease process (see p. 416).

Erythrocyte protoporphyrin (zinc protoporphyrin). This assay has been performed for many years as a test for Pb poisoning. More recently, there has been much interest in its use in evaluating the Fe supply to the bone marrow. The 'free' protoporphyrin concentration of erythrocytes increases in Fe deficiency. A widely-used technique directly measures the fluorescence of zinc protoporphyrin (μmol/mol haem) in a haematofluorometer (Labbe & Rettmer, 1989). The normal range in adults is less than 80 μmol/mol haem.

Serum transferrin receptor. Another indicator of Fe-deficient erythropoiesis is the serum transferrin receptor level. Soluble transferrin receptors are detectable in the circulation by immunoassay and appear to reflect the number of transferrin receptors on immature erythrocytes and, thus, the level of bone marrow erythropoiesis (Cazzola & Beguin, 1992). A mean level of 5·6 mg/l has been reported by Flowers *et al.* (1989). The assay is potentially of considerable value as it provides an alternative to the very cumbersome ferrokinetic studies which were previously necessary. In normal subjects the serum transferrin receptor level also provides a sensitive indicator of functional Fe

deficiency in subjects without Fe stores who have not yet developed Fe-deficiency anaemia (Skikne *et al.* 1990).

METHODOLOGICAL AND BIOLOGICAL VARIATION OF ASSAYS

The blood assays vary greatly in both methodological and biological stability (British Nutrition Foundation, 1995). Haemoglobin concentrations are stable and a simple and well-standardized method of determination (Dacie & Lewis, 1995) ensures relatively low day-to-day variation in individuals. Automated cell counters analyse at least 10 000 cells and, thus, reduce errors. The more complicated procedures involved in immunoassays mean higher methodological variation for ferritin assays and overall CV of approximately 15 %. The serum Fe determination is an example of extremes with reasonably low methodological variation coupled with extreme physiological variability giving an overall 'within subject' CV of approximately 30 % when venous samples are taken at the same time of the day.

IRON STATUS AND DISEASE

Iron deficiency

Table 2 shows the changes that take place for the various blood measurements for assessing Fe status in Fe-deficiency anaemia. There is little difficulty in making such a diagnosis. Fe-deficiency anaemia has been estimated to affect 500 million people worldwide. It is considered that Fe deficiency even in the absence of anaemia has serious consequences in terms of both mental and physical development and in work performance (Cook *et al.* 1994).

Recently there has been some interest in what has been called 'functional Fe deficiency'. This situation arises in the treatment of patients undergoing renal dialysis with erythropoietin in order to cure their anaemia. Sometimes a failure to respond is due to the fact that although there is apparently sufficient storage Fe available, this Fe cannot be mobilized rapidly enough to regenerate haemoglobin (Macdougal *et al.* 1989). A more rapid response is assured by injecting Fe. Functional Fe deficiency can be detected by the presence of a raised percentage of hypochromic erythrocytes (greater than 8 %).

Iron overload

Genetic haemochromatosis is one of the most common inherited conditions in Northern European populations (Worwood, 1994). The prevalence varies from 1 in 2 000 to 1 in 200. A prevalence of 1 in 400 means that approximately 10 % of the population may carry one copy of the gene. Diagnosis of haemochromatosis and Fe overload requires the demonstration of an elevated transferrin saturation (greater than 60 % in men, 50 % in women) and a raised serum ferritin concentration (greater than 300 μg/l in men and 200 μg/l in women). Both transferrin saturation and ferritin concentration must be abnormal in a second sample and the diagnosis usually requires demonstration of Fe overload by measurement of the liver Fe concentration. Early detection allows successful treatment by phlebotomy and the identification of other family members at risk from Fe overload. Once cirrhosis of the liver has developed, this cannot be reversed and in many cases hepatoma develops. The recent identification of a candidate, causative mutation for haemochroma-

Table 2. *Cut-off points for defining iron status in adults*
(From British Nutrition Foundation, 1995; with permission)

Factor	Overload	Normal	Fe-depleted non-anaemic	Fe-deficient erythropoiesis	Anaemia
Haemoglobin (g/l)	–	≥ 130*	≥ 130*	≥ 130*	< 130*
		≥ 120†	≥ 120†	≥ 120†	< 120†
Ferritin (μg/l)	> 300*	≥ 13	< 13	< 13	< 13
	> 200†				
Transferrin saturation (%)	> 60*	> 16	> 16	< 16	< 16
	> 50†				
Zinc protoporphyrin (μmol/mol haem)	–	< 80	< 80	≥ 80	≥ 80
Serum transferrin receptor (mg/l)	–	< 8·5	< 8·5	≥ 8·5	≥ 8·5

*Values for men.
†Values for women.

tosis in a novel MHC class I-like gene (Feder *et al.* 1996) provides the opportunity to develop effective genetic testing.

Anaemia associated with inflammation, infection and other chronic disorders

Inflammation, infection, surgery or malignancy are often associated with anaemia. As well as anaemia, there may be a low serum Fe concentration and evidence of increased levels of storage Fe in the bone marrow. It is now thought that inflammation etc. leads to the release of interleukin 1β (IL1β) and other cytokines. IL1β stimulates ferritin synthesis (Rogers, 1996) and also inhibits erythropoiesis. The initial effect seems to be the stimulus of apoferritin synthesis which traps Fe in phagocytic cells. This leads to a reduced supply of Fe to the bone marrow. Distinguishing between patients with the anaemia of chronic disease who have no storage Fe and those who have apparently adequate amounts of storage Fe has been a problem for many years. Many clinical studies have demonstrated that patients with the anaemia of chronic disease, with no stainable Fe in the bone marrow, may have serum ferritin concentrations considerably in excess of 15 μg/l and there has been much debate (Witte, 1991) about the practical application of the serum ferritin assay in this situation. Values of less than 15 μg/l indicate the absence of storage Fe and values of greater than 100 μg/l indicate the presence of storage Fe. It is the 'grey' area from 15 to 100 μg/l which is difficult to interpret. Although it would seem logical to combine the assay of serum ferritin with a measure of disease severity such as the erythrocyte sedimentation rate or C reactive protein, this approach does not appear to be significantly better than measuring serum ferritin concentration on its own (Coenen *et al.* 1991). Ferguson *et al.* (1992) have suggested that in patients with chronic disease, only those with an absence of storage Fe have raised levels of the serum transferrin receptor. However, later studies have not suggested that the assay of serum transferrin receptor adds much information to the assay of serum ferritin (see Kurer *et al.* 1995). Various algorithms involving measurement of serum ferritin concentration, transferrin saturation, MCV and MCH have been proposed. In general these appear to offer some improvement on a simple determination of serum ferritin concentration (see Mulherin *et al.* 1996).

Liver disease

Serum ferritin concentrations are particularly influenced by the presence of liver disease. Extremely high concentrations may be found in patients with hepatitis and cirrhosis of the

liver (Prieto *et al.* 1973). In the context of liver disease, the only simple interpretation is that a serum ferritin concentration in the normal range excludes Fe overload. In hospital practice, apparently unexplained elevations of serum ferritin concentration are found in patients with liver disease, in patients with human immunodeficiency virus infection and patients with malignant disorders (Lee & Means, 1995). The highest concentrations of serum ferritin are found in patients with transfusional Fe overload as well as liver disease.

Other factors affecting measurements of iron status

Starvation, or even fasting briefly, can cause elevation of the serum ferritin concentration (Lundberg *et al.* 1984) and vitamin C deficiency may reduce both ferritin and Fe concentrations (Chapman *et al.* 1982). This is perhaps the only known instance where serum ferritin concentrations may be depressed in the absence of tissue Fe deficiency. Haemolysis can increase serum ferritin concentrations and serum Fe concentrations. Very high concentrations of circulating ferritin and Fe are found in patients with active erythrophagocytosis (Esumi *et al.* 1988). One interesting cause of apparently unexplained ferritinaemia is that associated with inherited cataract formation. It has now been demonstrated that there is a mutation in the 'stem loop' structure of the ferritin L subunit which causes the very high concentrations of ferritin found in this condition apparently in the absence of high levels of storage Fe (Beaumont *et al.* 1995).

What is normal iron status?

There has been much interest in recent years in the proposition that even moderate increases in storage Fe may be associated with pathology, for example, in higher rates of myocardial infarction (Salonen *et al.* 1992). In general, it is difficult to demonstrate that elevations in serum ferritin concentration actually reflect enhanced levels of storage Fe rather than being a result of the chronic disease process described previously.

CONCLUSION

Most diagnostic problems in Fe metabolism can be resolved by a careful consideration of haemoglobin-Fe concentration, erythrocyte indices and serum ferritin concentration. However, serum ferritin concentration must be assessed in terms of other disease processes particularly infection, inflammation and liver disease. The determination of serum Fe and transferrin saturation is essential in the diagnosis of genetic haemochromatosis and this includes the screening of other family members. Although both zinc protoporphyrin and measurements of circulating transferrin receptor have particular value in epidemiological studies, they probably add relatively little to the diagnostic armoury at present.

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