ASPECTS OF IRON METABOLISM - SOME FACTORS AFFECTING IRON ABSORPTION

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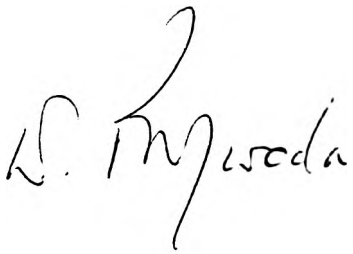
This thesis is my own work. No part of it has been presented at any other University. The information used in this thesis was obtained while I was employed by the University of the Witwatersrand and the Johannesburg Hospital.



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a) The Relationship between Marrow Iron Stores, Plasma Ferritin Concentration and Iron Absorption

b) Iron Absorption in Patients on Regular Dialysis Therapy

c) Patterns of Food Iron Absorption in Iron Deficient White and Indian Subjects and in Venesected Haemochromatotic Patients

d) The Importance of Gastric Hydrochloric Acid in the Absorption of Non-Haem Food Iron

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Abstract

The investigations which make up the body of this thesis touch on various aspects of iron absorption. Although many of the factors governing iron absorption have been elucidated specific problems remain to be defined in greater detail. The aim of these investigations was therefore to define more fully some of the quantitative and qualitative aspects of dietary iron absorption. To this end the relationship between iron absorption and body iron content was studied over a wide range of body iron status both in health and in various disease states. Food iron absorption from various dietary sources, including both non-haem and haem fractions, was also studied in detail and in addition studies were carried out of the manner in which luminal factors influence the availability of non-haem food iron. The specific studies include:-

a) The relationship between bone marrow iron content, plasma ferritin concentration and iron absorption This investigation addressed itself to the relationship between iron absorption and body storage iron content. Although the nature of the diet and the action of intestinal secretions determine the availability for absorption of iron, these factors do not appear to be the essential controlling mechanism for iron absorption. An inverse relationship between body iron content and iron absorption has been appreciated for some time. However, the early studies in which this relationship was first defined included patients

with only a fairly limited range of body iron content. The present study extended these observations to include a wide range of storage iron content, from iron depletion to gross iron overload. In order to obtain an accurate assessment of iron absorption, even in subjects replete with iron, the measurement of absorption was carried out using the whole body retention of radioiron by means of a shadow shield whole body counter. A small dose (3 mg) of ferrous ascorbate was used to provide a readily available source of iron and the iron status of the subjects was assessed by a number of measurements including the haemoglobin concentration, the plasma iron concentration, the total iron binding capacity and the concentration of non-haem iron in the bone marrow.

There were good inverse correlations between log percentage iron absorption and both the log marrow non-haem iron content ($r = -0,94$; $p < 0,001$) and the log plasma ferritin concentration ($r = -0,78$; $p < 0,001$). In addition there was a positive correlation between the marrow non-haem iron concentration and the plasma ferritin concentration ($r = + 0,84$; $p < 0,001$). These results suggest that the reticuloendothelial iron stores are the most important determinant of iron absorption at all levels of storage iron content. Furthermore, the close relationship between marrow non-haem iron content and plasma ferritin concentration provides additional evidence that plasma ferritin is a reliable index of storage iron level. Finally, the results

of this investigation helped to define the quantitative relationship between the plasma ferritin concentration and the reticuloendothelial iron content of the bone marrow over a wide range, giving some indication of the variability which can be expected from these measurements.

b) Iron absorption in patients on regular dialysis therapy The results of the first investigation suggested a further direction for study. This was to include, together with the measurement of iron status, an assessment of erythropoietic activity in order to define the contribution of this variable to the control of iron absorption. Although the body iron content had been shown to be the most significant factor influencing iron absorption, it is possible that erythropoietic demand might still modify this relationship. The evidence from previous studies in this regard is conflicting. The anaemia of renal failure is one of the classic examples of a hypoproliferative anaemia and the availability of a population of patients being treated for chronic renal failure by means of regular haemodialysis therapy presented an opportunity to study this question in more detail. Iron status was determined by means of a sensitive radioimmunoassay for plasma ferritin and the whole body counter was again used to measure the retention of radioiron; plasma iron turnover measurements were carried out concurrently, using a different isotope of iron. Iron absorption was measured at both physiologic as well as at pharmacologic doses of iron in order to assess whether the

pattern of absorption was similar at both dose levels and whether oral iron therapy would be efficacious for the treatment of iron deficiency developing in chronic dialysis patients.

The results obtained in this study did not differ significantly from those in the previous investigation. Iron absorption was still largely influenced by the level of storage iron in spite of the reduced marrow iron turnover. Although the addition of the erythrokinetic data as a covariate in the statistical analysis did increase the significance of some of the correlations, the degree of enhancement was slight. The major variable influencing iron aborption remains the body iron content. Pharmacologic doses of iron were absorbed in a manner comparable to that found in subjects with normal renal function, thus providing further evidence that the absorptive mechanism for iron in patients with renal failure responds to changes in body iron status rather than to the rate of erythropoiesis.

c) Patterns of food iron absorption in iron deficient White and Indian subjects and in venesected haemochromatotic patients In this investigation subjects from 2 different population groups and with various disturbances of iron balance were studied in order to ascertain differences in the ability to absorb different forms of food iron. The subjects included Indian women living near Chatsworth, Durban, who frequently suffer from iron deficiency anaemia, a group of patients treated for idiopathic haemochromatosis

and a group of hospitalised, white, iron deficient patients. Comparison of the data from these three groups was aimed at elucidating some of the factors which influence dietary iron absorption. Although the body iron content was depleted in all 3 groups of subjects major differences were found in their ability to absorb food iron. In particular the non-haem iron (in this instance wheat iron) was poorly absorbed by the majority of Indian females and by some of the iron deficient white patients. Although the haemochromatotic individuals absorbed all forms of iron efficiently they were not significantly different in this regard to the white iron deficient patients many of whom were able to achieve levels of absorption similar to those of the haemochromatotic individuals. No specific abnormality was thus identified in the haemochromatotic individuals. The reason for the poor absorption in the Indian women and in some of the white patients appeared to be a luminal defect, since both haemoglobin iron and ferrous ascorbate were absorbed adequately by these subjects. The further investigation of the nature of this defect led to the next study.

d) <u>The importance of gastric hydrochloric acid in the absorption of non-haem food iron</u> In this investigation the focus of interest was the influence of the intestinal secretions, particularly gastric juice, on the absorption of iron. That gastric acid serves to enhance the absorption of ferric iron is well established but disagreement still

existed about the role of other factors, in particular, the role of macromolecular components of gastric juice in the absorption of food iron. An in vitro method was developed whereby gastric juice was used to solubilize intrinsically labelled non-haem food iron. The degree of in vitro solubilisation was used to predict the in vivo absorption of wheat iron from the same bread fed to the gastric juice donors.

The degree of solubilisation of wheat iron was related to the pH of the gastric juice. Most of the solubilized iron was present in a low molecular weight form and there was a good correlation between the amount of low molecular weight iron in *in vitro* experiments and *in vivo* absorption of wheat iron. Similar quantities of iron were released by hydrochloric acid and the iron so released was also in a low molecular weight form.

Gastric hydrochloric acid was thus identified as being the only factor of importance in making the wheat iron available for absorption and hypochlorhydria was identified as the cause for the malabsorption of non-haem iron present in many of the Indian females.

e) The influence of dietary composition (haem versus non-haem iron content) on the absorption of iron from a meal

The results obtained in these two studies of dietary iron absorption showed marked differences between the absorption of non-haem iron and of haem iron. While the availability of non-haem food iron was markedly influenced by luminal

factors, these factors did not appear to affect significantly the absorption of haem iron. It was of some interest, therefore, to study the effect on total iron absorption, from a meal of fixed iron content, of varying the ratio of haem to non-haem iron. Two groups of Indian women were fed a series of 4 meals consisting of haemoglobin, given as a gravy, together with potato mash. The ratios of haem:non-haem iron in the meals varied from 1:2Ø to 3:1 and the iron content of each meal was adjusted so as to total 6 mg.

The results of this investigation emphasised the important contribution which haem iron can make to iron nutrition. The percentage absorption of non-haem iron decreased with increasing dose and the contribution of this component to the total amount of iron absorbed from the meal remained small. In contrast, the percentage absorption of haemoglobin iron remained constant at all doses from approximately Ø,25 - 4,5 mg of haem iron, resulting in a linear increase in the amount of iron absorbed from this constituent of the diet.

These results may explain some of the observed regional and socio-economic differences in iron nutrition, since the ratio of non-haem: haem iron rather than the total amount of iron in the diet may well be critical. Moreover, on the basis of these results it appears that haem iron could well be used to fortify the diet of selected population groups.

f) The effect of diet on the rate of iron accumulation in idiopathic haemochromatosis In both the studies where iron absorption was compared to storage iron content it was evident that the reciprocal relationship between iron stores and percentage absorption is present over an extremely wide range, including subjects with a marked excess of body iron. The relationship between body iron content and iron absorption in patients with idiopathic haemochromatosis has, however, been less well studied. In this condition body iron accumulates to excess and iron loading of parenchymal tissues occurs. The source of the excess iron is the diet. Previous studies in haemochromatotic subjects have shown that the percentage absorption of iron is low when these patients first present with increased iron stores and the fully developed disease. In spite of the low percentage absorption the amounts absorbed are inappropriate to iron needs. The absorption of iron by these subjects prior to the development of gross iron overload has not been studied thus far. However, patients who suffer from the disease but who have been rendered iron free by means of phlebotomy therapy, might reasonably be expected to have a similar absorptive capacity to that which was present prior to the development of significant iron overload. It was of some interest, therefore, to study certain aspects of iron absorption in a group of subjects with haemochromatosis who had been treated by means of venesection therapy and rendered free of excess iron. In this investigation the

absorption of iron from a meal supplemented with two different amounts of an available iron salt was assessed in such patients.

The results showed that the slope of the line relating the quantity of iron absorbed to the dose administered is similar in venesected haemochromatotic individuals to the line obtained for the same relationship in normal subjects. Although a parallel relationship between dose and amount absorbed was established in haemochromatotic and normal individuals the haemochromatotic subjects absorbed more iron at each of the doses administered. Iron absorption in these individuals is thus still subject to the same influences that are present in normal individuals, albeit that the control is being exerted at a somewhat higher level of absorption that normal. These results were considered in relation to a theoretic model of iron balance, which was developed to explain the equilibration of body iron content at the increased levels found in subjects genetically predisposed to develop iron overload. The predictions made according to the model were that the predominant effect of an increase in dietary iron content on genetically susceptible individuals would be a modest acceleration of the rate at which iron accumulates, without significantly affecting the resultant level of body iron content at which absorption and excretion equilibrate.

Final Conclusions

Taken together these investigations demonstrate the

remarkably close way in which iron absorption is controlled over a wide range of body iron content. Although the nature of the controlling mechanism has not been defined further insight has been gained into some of the quantitative aspects of iron absorption and body iron status. Any future hypothesis of the control of iron absorption must take into account the fact that iron absorption proceeds at levels related to storage iron content even when erythropoietic activity is depressed. Moreover, the fact that the absorptive capacity for non-haem food iron in individuals with idiopathic haemochromatosis cannot clearly be distinguished from that of normal individuals makes it extremely unlikely that luminal factors exert a controlling influence. Instead, the luminal factors provide the background, by making dietary iron available for absorption, against which the controlling mechanism exerts its effect. However, when defective, particularly in terms of gastric hydrochloric acid secretion, these luminal factors can become critical determinants of non-haem iron absorption and thereby could influence the iron status of individuals subsisting mainly on cereal based diets. Finally, the results of these studies, particularly those concerned with the composition of the diet and with iron absorption in haemochromatotic subjects, have relevance to iron fortification programmes; indicating on the one hand the importance of haem as a source of dietary iron, and on the other hand providing preliminary evidence that these

programmes are unlikely to be deleterious to individuals genetically predisposed to develop iron overload.

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CHAPTER 1

Introduction

1.1 Iron Needs

During intra-uterine life the foetus is in a relatively privileged position in regard to iron supply. Iron accumulation continues even in the face of maternal iron deficiency (Lanzkowski 1961; Shott et al 1972; Murray et al 1978; MacPhail et al 1980). By birth the human infant has accumulated, on average, about 80 mg of iron/kg body weight (Widdowson and Spray 1951; Josephs 1956). The major determinant of the newborn's iron content is birth weight (Widdowson and Spray 1951) and infants of normal birth weight have about 270 mg of iron. Most of the iron accumulated during gestation is present in the circulating red cell mass. Considerable amounts of iron, with which the infant would otherwise be endowed, can be lost if the cord is clamped before there is adequate transfer of blood from the separating placenta back to the infant (Yao et al 1969). In spite of the fact that most of the iron is incorporated into haemoglobin, the rate of foetal haemopoiesis does not appear to be the determining factor for foetal iron accumulation (Bothwell et al 1979). Considerable variation may occur in the cord blood haemoglobin concentrations of newborns (Guest and Brown 1956; American Academy of Paediatrics 1961) and in most cases the reason for this

variability is obscure (American Academy of Paediatrics 1969). Recent evidence suggests that there is an inverse relationship between the cord blood haemoglobin concentration and the cord blood ferritin concentration (MacPhail et al 198Ø). Since the plasma ferritin concentration reflects tissue iron stores in both adults, (Walters et al 1973; Lipschitz et al 1974; Bezwoda et al 1979) and in children and infants (Siimes et al 1974; Rios et al 1975) it seems reasonable to make the assumption that this is also the case in the newborn. The inverse relationship between cord blood haemoglobin and plasma ferritin implies that newborn infants with high haemoglobin levels have less iron in other body stores and vice versa and that the endowment with iron is relatively constant for all new born infants of similar birth weight.

During the first 6 weeks after birth major shifts in internal iron balance occur. Erythropoiesis virtually ceases during this period and the haemoglobin concentration rapidly falls from about 17 g/dl to about 11 g/dl (Lintzel et al 1944; Rios et al 1975). The iron thus liberated from the haemoglobin pool moves into stores for a short time. Erythropoietic activity resumes as the infant starts to grow, and the red cell mass expands in order to maintain the haemoglobin concentration at constant levels. Iron stores are rapidly re-utilised to provide for this increase in red cell mass. The underweight and/or premature infant starts at a disadvantage in regard to the amount of iron

available for erythropoiesis. A child with a normal birth weight will have 270 mg of iron available for haemopoiesis while the underweight infant may have as little as 160 mg for this purpose. Since growth rates are not constant during infancy and childhood the rate at which iron needs to be absorbed varies with the stage of development. The full-term infant, starting with a normal iron content of 270 mg, will need to absorb 100 mg of iron (0,3 mg/day) during the first year of life. To develop equally the smaller infant, with only 160 mg of iron, will need to absorb more than 200 mg (0,6-1,0 mg/day) of iron during that first year. Subsequent growth and development calls for the absorption of between 80 and as much as 580 mg of iron per year (0,3-1,6 mg/day) depending on age and stage of development. In summary, the mean requirements amount to 80 mg between age 1 - 2 years (0,3 mg/day), 200 mg per year between ages 2 - 12 years and 580 mg/year during the growth spurt at puberty.

The extra iron needed to support growth and development must be obtained from the diet together with a sufficient amount to replace the obligatory iron losses which take place from the skin, gastrointestinal and genitourinary tracts. These obligatory iron losses have been most intensively investigated in normal adult males (Green et al 1968), and, in these subjects, amount to 12 - 14 /ug/kg/day. While it has often been assumed that the losses in infancy and childhood are proportional to those in the adult, there is some evidence that during childhood they may be

considerably higher, reaching as much as 30 /ug/Kg/day (Garby et al 1964).

At puberty the iron requirements of girls begin to diverge from the amounts required by boys. While the adolescent male begins to accumulate a store of iron which ultimately becomes considerably larger than that found in the female the adolescent girl has a greater iron requirement than the male in order to compensate for the extra iron loss caused by menstruation. The late adolescent male requires approximately 1,2 mg of iron per day and the young female requires as much as 1,8 mg per day. To remain in iron balance during adult life males need to absorb an average of 1 mg of iron per day whereas females who are menstruating require nearly twice that. With each pregnancy a net extra amount of 740 mg of iron must be absorbed in order to ensure that the mother remains in normal iron balance, in spite of the fact that menstrual losses cease during this time.

The amount of iron that must be accumulated between birth and adulthood can be calculated from the difference between body iron content at birth and that of the normal adult, and amounts to about 3,5 g in males and about 2,5 g in females.

The various aspects of iron metabolism as they relate to these iron needs will be dealt with in more detail in the following sections.

1.2 Iron Stores

1.2.1 The nature and functions of storage iron

The need to conserve iron and to sequester it in a non toxic form has led to the developement of specialised proteins of iron storage. These proteins are present in a wide range of plant and animal tissues. In man, iron, which is in excess of immediate needs, is stored in the tissues in two related forms, ferritin and haemosiderin. Ferritin is the more soluble of the two compounds and consists of a protein (apoferritin) shell and a variable amount of iron. The protein shell encases the iron and prevents its precipitation. It is the presence of ferritin that gives the tissues the ability to store iron, which in the unbound state, would be toxic. As ferritin is never fully saturated with iron, free iron can be incorporated immediately on entry into the cell (Harrison et al 1974). In addition, as soon iron enters the tissues apoferritin synthesis is stimulated (Fineberg and Greenberg 1955) in a dose related fashion (Fineberg and Greenberg 1955a; Drysdale and Munro 1966). Ferritin is present in greatest concentration in liver, spleen, bone marrow and skeletal muscle. Although these organs constitute the major sites of iron storage, ferritin has also been found in virtually all the other body tissues (Granick 1943; Heilmeyer 1958; Shoden 1953; Richter 1957; Linder and Munro 1973), albeit in smaller quantities.

may be virtually iron-free (apoferritin), or it may contain up to 5000 atoms of iron (Fischbach 1965; Crichton 1973). The iron contained within the protein shell is in the form of ferric-oxide-phosphate micelles (Harrison 1977; Harrison 1977a), with the iron for the most part in the Fe^{3+} form. From various studies it appears that the iron within ferritin is probably laid down and removed in successive layers (Harrison et al 1974), with the most recently stored iron being the first to be removed and the first to be utilised.

The mechanism by which iron is incorporated into ferritin is still under investigation. It is known that the entry of iron into the cell stimulates ferritin synthesis. Initially iron-poor ferritin is synthesised but with time the iron content rises (Fineberg and Greenberg 1955; Drysdale and Munro 1966; Hoy and Harrison 1975). For iron storage to be effected the entire apoferritin molecule has to be synthesised. Subunits of apoferritin do not bind iron prior to assembly of the whole protein.

Storage iron is available for the formation of functional iron compounds when needed. The first evidence for this came from observations that haemosiderin was absent from the tissues of iron deficient individuals. Iron normally exchanges between the storage compartment and the active metabolic circuit although it may take a year or two for radioiron which has been introduced into the plasma to become evenly distributed and to label the entire storage

compartment (Green et al 1968).

Haemosiderin has certain similarities to ferritin but is a much less soluble iron complex than ferritin. Haemosiderin was first isolated by Cook (Cook 1929) who concluded that it consisted of organic granules impregnated with some form of ferric oxide. The iron content of haemosiderin may be as high as 41% (Ludewig 1957). Chemical studies on haemosiderin that has been treated to render it soluble and iron free indicate that it also contains apoferritin (Greenberg 1956; Ludewig 1959; Richter 1960; McKay 1964). Conversely, ferritin can be treated so as to give a product similar to haemosiderin (Matioli and Baker 1963). These studies appear to show that ferritin and haemosiderin are related compounds. However, there are components in haemosiderin which are not found in ferritin. These components include porphyrins, other pigments, (McKay and Fineberg 1964) sugars, (Ludewig 1957) sialic acid, (Ludewig and Glover 1966) lipids, (Ludewig and Franz 1970) and proteins which are not apoferritin (McKay and Fineberg 1964). It is not known if haemosiderin can be formed without prior formation of ferritin.

The two storage forms of iron are found in highest concentration in the same organs, namely in the liver, spleen, bone marrow and in skeletal muscle. Although the two compounds are found in the same sites there is a considerable difference in the localisation, within the tissues, of the two. In the liver the parenchymal cells

contain 95% of the ferritin (Van Wyk et al 1971; Cook et al 1972; Cook et al 1973) while most of the haemosiderin is present in the Kupffer cells. At physiologic concentrations of storage iron there is some preponderance of ferritin over haemosiderin (Shoden et al 1953; Kaldor 1958) with ferritin accounting for about 60 per cent of the iron store in both the liver and the reticuloendothelial cells of the bone marrow (Weinfeld 1956). As the iron stores increase in size so progressively larger amounts of haemosiderin are found and the ratio of ferritin to haemosiderin changes (Shoden et al 1953; Finch and Finch 1955; Morgan and Walters 1963). Under normal conditions the hepatic parenchymal cell receives most of the iron which it incorporates into ferritin from plasma transferrin (Hershko et al 1973). In pathological states, however, it may also derive considerable amounts of iron from haemoglobin which has been released into the plasma (Hershko et al 1972).

The transfer of iron from the storage compartment to the active metabolic circuit is as yet poorly understood. Although there is morphologic evidence that lysozymes are involved in the breakdown of rat liver ferritin (Trump et al 1975) it is probable that this process is more related to the formation of haemosiderin than to the mobilisation of storage iron. Mobilisation of the iron stored in ferritin can be achieved in vitro by the use of a number of chelating or reducing agents (Granick and Michaelis 1943; Bielig and Bayer 1955; Pape et al 1968) without disruption of the

protein envelope (Hoy et al 1974). The in vivo release of iron from ferritin in the liver probably involves the action of reduced riboflavins (Osaki and Sirivech 1971; Sirivech et al 1974; Crichton et al 1975; Jones et al 1978). These molecules are probably just small enough ($FMNH_2$ = 1.3 nm) to negotiate the channels in the protein shell and to react directly with the entrapped iron (Harrison et al 1975). The mechanism for iron release from reticuloendothelial cells may however be different since flavoprotein enzyme activity cannot be demonstrated in splenic tissue. In addition, the possible role of ascorbic acid in the process of iron release from storage sites has also to be considered. Ascorbic acid is one of the reducing agents which can cause the release of ferritin iron (Bielig and Bayer 1955). Defective release of iron from reticuloendothelial cells has been demonstrated in scorbutic guinea-pigs (Lipschitz et al 1971) and the sudden rise in plasma iron concentration when human subjects with scurvy are given ascorbic acid (Wapnick et al 197Ø) is consistent with a similar situation in man. However, the rate at which ascorbic acid causes iron release from ferritin is slower than the rate at which this process occurs physiologically (Crichton 1973; Sirivech et al 1974) and the role of ascorbic acid may well be to cause solubilisation of haemosiderin iron rather than the release of ferritin iron.

When iron is mobilised from tissue stores it is derived from both the ferritin and the haemosiderin fraction (Shoden

et al 1953; Hampton 1954). However, it is clear that these storage fractions are not homogenous. Isotopic studies have shown that recently deposited iron is mobilised more actively than the iron in older stores (Hampton 1954). In addition, iron derived from broken down red cells is preferentially used for the production of new erythrocytes (West et al 1952; Noyes et al 196Ø) which indicates that there is little mixing in reticuloendothelial cells between the iron released from effete red cells and that derived from other sources or present in pre-existing stores in these cells.

The entry of iron into the active metabolic circuit involves its transfer from the storage sites, via plasma transferrin, to the erythroid marrow and to other tissues. It is not known whether ferritin is transported to the cell membrane so that the iron can be passed directly to the transferrin molecule, or whether transferrin enters the cell to reach the iron, or whether there is some intermediate transport system. The last possibility is suggested by the observation that in some experimental animals copper deficiency causes iron deficient erythropoiesis with accumulation of iron at storage sites. This defect can be corrected by giving copper but the correction is even more rapid when caeruloplasmin is administered (Lee et al 1968; Ragan et al 1969). However, caeruloplasmin is not required for iron release from ferritin when an isolated perfused rat liver experimental system is used (Baker et al 1975).

Moreover, storage iron release does not seem to be impaired in human subjects with Wilson's disease or copper deficiency although haem synthesis may be impaired in the latter condition (Karpel et al 1972; Dunlap et al 1974). The mobilisation of ferritin iron is thought to require reduction to Fe^{2+} whereas transferrin iron is in the Fe^{3+} form. The iron thus has to be re-oxidised to Fe^{3+} prior to transferrin binding and since caeruloplasmin has been shown to have ferroxidase activity (Osaki et al 1966) a possible role in mediating the release and transferrin binding of reticuloendothelial iron has been ascribed to this capacity of ceruloplasmin. However, there is a second copper-containing plasma protein which also has ferroxidase activity (Topham and Frieden 1970), and, in addition, the oxidation of Fe^{2+} to Fe^{3+} is accelerated by transferrin itself (Bates and Schlabach 1976) and even by citrate and phosphate (Lee et al 1969; Aisen 1977). The role of caeruloplasmin as an intermediate in the process of iron release from ferritin and passage to transferrin has, therefore, still to be elucidated. Direct passage of iron between cells may occur to a limited extent by ropheocytosis between contiguous cells, particularly those of bone marrow (Bessis and Breton-Gorius 1962; Tanaka et al 1966), but the importance of this mechanism is probably limited. It is also unlikely that plasma ferritin plays any major role in iron transfer. The ferritin found in the plasma is probably in transit between different tissues but it appears

to contain little iron (Worwood et al 1975; Drysdale and Kirby 1976). Current evidence indicates that all significant internal exchange of mobilised storage iron is mediated via transferrin.

The rate at which iron can be mobilised can be estimated by the haemopoietic response to phlebotomy. A normal adult male can usually mobilise up to 60 mg of iron per day, enough to treble erythropoiesis, when the haematocrit is reduced to between 32 and 37 per cent. At this level of haematocrit the rate of mobilisation is similar in subjects with normal and those with increased iron stores. When the haematocrit is reduced further to between 25 and 30 percent patients with excess iron in parenchymal cells are able to mobilise iron more rapidly than normal subjects and may achieve rates of erythropoiesis five or six times higher than normal (Crosby 1958; Hillman and Henderson 1969).

Tissue iron stores serve two main functions. Firstly, the availability of storage iron serves to damp the effect of a variation in iron supply, via transferrin, particularly in those tissues with a large iron requirement such as the erythroid marrow. The second major function of ferritin synthesis is to deal with any large local increase in iron concentration, which may result from either haem catabolism or from iron absorption.

Storage iron accumulates gradually during growth and development. New born infants and pre-pubertal children

have virtually no storage iron. There is a brief period during the first six weeks of life, due to the physiologic decrease of the haemoglobin concentration, when iron enters stores. This iron is, however, rapidly re-utilised in order to maintain a normal haemoglobin concentration in the face of the rapid expansion of the red cell mass which occurs in growing children (Smith et al 1955; Rios et al 1975). From these small beginnings a reserve store of iron is eventually built up. In the young female this reserve amounts to about 5mg/kg at the time of the menarche and is thereafter maintained at this level (Cook et al 1976). In the male storage iron levels at the time of puberty are approximately the same as those in the female but between the ages of 15 and 30 years the young male continues to accumulate iron, eventually achieving a storage iron level of 12-15 mg/kg body weight. The total iron content of the male is approximately 50 mg/kg, with a reserve store of about 1000 mg (Bothwell et al 1979). The iron content of the female during the reproductive period of life is considerably smaller, averaging from 14 - 42 mg/kg, with a reserve store of approximately 300 mg. Iron balance in the female is therefore considerably more precarious than it is in the male. Part of the reason for the different iron content of males and females is the smaller muscle mass and smaller blood volume of the female, but iron losses are also greater in women. In older women when the demands made on iron nutrition by menstrual losses and by childbearing are no

longer present there is a significant, and progressive, increase in body iron content (Cook et al 1975; MacPhail et al 1981).

The size of the body iron store is a complex function of the amount of available iron present in the diet as well as internal and external iron exchange. Increased blood losses, either physiological or pathological, are often sufficient to create a negative iron balance and hence storage iron depletion. In addition, iron stores will be smaller than normal if malabsorption is present, such as the malabsorption which occurs after partial gastrectomy or with gluten-induced enteropathy (Bothwell and Finch 1962). Although iron depletion is the most frequently encountered abnormality of iron balance, particularly in certain population groups, there are situations in which iron stores are found to be increased. Iron stores rise in any anaemia which is not due to blood loss. In these circumstances the total body iron content does not increase but there is a relocation of the iron, with a decrease in the amount circulating in haemoglobin and a reciprocal increase in the quantity in stores. The anaemia in these individuals is characterised by a moderate shortening of the red cell life span, together with a partial block in the release of iron from reticuloendothelial cells (Cartwright and Lee 1971). A moderate increase in iron stores, not exceeding 1 - 2 g, is thus a feature of the mild anaemia that accompanies a variety of chronic disorders including inflammation and

neoplasia (Cartwright and Lee 1971). Although this mechanism is responsible for the majority of instances in which iron stores are increased there are also a number of conditions in which total body iron content is increased. These conditions are dealt with later in section 1.9.

1.2.2 The quantitative assessment of body iron stores

The most direct way of measuring body iron stores is to assess the amount of iron which can be mobilised by repeated phlebotomy. If this is done, storage iron will be mobilised and normal erythropoiesis will continue until the iron supply is exhausted (Haskins et al 1952). Due allowance has to be made for the fact that increased amounts of iron are absorbed during this period but it is known that the maximum amount which can be absorbed from the diet is about 3 mg per day. By determining the total amount of haemoglobin iron removed and the induced deficit in circulating haemoglobin iron, the iron stores originally present can be calculated by subtraction.

Alternative approaches to the assessment of body iron stores, most of which have been developed for research purposes, include isotope dilution (Finch 1959; Green et al 1968) and the determination of the non-haem iron content of various tissues by biochemical methods (Bothwell and Bradlow 196Ø; Bothwell and Isaacson 1962; MacDonald and Pechet 1964; Pechet et al 1965; MacDonald and Pechet 1965; Charlton and Bothwell 1966; Charlton et al 197Ø; Charlton et al 1971;

Sturgeon and Shoden 1971; Disler et al 1973). Measurements using the isotope dilution method have generally tended to give lower estimates of the body iron content than those arrived at by using the mobilisation of iron by phlebotomy (Haskins et al 1952; Green et al 1968). These findings suggest that there is a portion of the storage iron, presumably haemosiderin, which exchanges very slowly (if at all) with functional iron, but which is nevertheless available for erythropoiesis if a state of negative iron balance develops. The methods based on the chemical estimation of non-haem iron have usually concentrated on the iron content of particular organs and of these the liver iron store has been most intensively investigated. Using this method one recent study found that the mean value of hepatic iron content for Western men was Ø,27 mg/g and for women it was Ø,1 mg/g (Charlton et al 197Ø). The different values obtained for males and for females agree with other evidence indicating a smaller iron store in women.

Histologic assessment of liver biopsy specimens can also be of value in determining the amount of storage iron. Both the microscopic appearance of the iron deposits as well as their extent give an idea of the hepatic iron content. Normally only scanty deposits of golden brown haemosiderin granules are visible. With increasing amounts of hepatic iron the haemosiderin deposits are seen as larger and coarser granules present throughout the liver lobule. Liver biopsy is especially useful in the patient with idiopathic

haemochromatosis. One of the characteristic features of the fully developed disease is that the iron deposits are found mainly in parenchymal cells rather than in reticuloendothelial cells and the liver biopsy can help with the assessment of the degree of parenchymal cell iron overload as well as the extent of liver damage (Bothwell et al 1965). However, it must be remembered that liver biopsy is more hazardous than other investigations and that if it is done to assess iron content then chemical determination of the amount of non-haem iron in the liver should also be performed, so that as much information as possible can be obtained.

The iron content of other tissues, such as bone marrow (Gale et al 1963), spleen (Bothwell and Bradlow 196Ø) and skeletal muscle (Hallgren 1954; Torrance et al 1968), has also been studied. Roughly equal amounts of iron are found in each of these tissues. When body iron stores are increased, as in dietary iron overload, the marrow iron store rises in parallel with that in the liver (Gale et al 1963; Brink et al 1977). The iron storage disease, idiopathic haemochromatosis, is, however,an exception to this rule as in this condition the reticuloendothelial system appears to be unable to hold much iron (Fillet and Marsaglia 1975) and the quantity of iron in the bone marrow may be normal even when massive hepatic iron overload is evident (Brink et al 1977). Although muscle iron stores appear also to rise when iron overload is present the

increase is of much smaller degree than the rise in bone marrow and hepatic iron store.

Another method which can be used to assess the amount of storage iron is by the use of iron chelating agents. The amount of iron excreted in response to the administration of an iron chelator can be measured and related to dose and time. Parenteral administration of the iron specific chelator desferrioxamine results in the excretion of iron in the urine in amounts proportional to the quantity of parenchymal iron (Balcerzak et al 1963; Smith et al 1969; Walker et al 1971). After administration of the drug urine is collected for either 6 or for 24 hours. Excretion of less than Ø,6 mg/24 hours indicates diminished iron stores and more than 2,2 mg/24 hours is associated with abnormally large stores (Balcerzak et al 1968). The excretion of more than 8 mg of iron after a dose of 5ØØ mg desferrioxamine is virtually diagnostic of idiopathic haemochromatosis (Bothwell et al 1979). The fraction of the body store from which iron is mobilised by desferrioxamine is still under debate. In the rat there is evidence that desferrioxamine can mobilise iron from two compartments; the first compartment being the hepatic ferritin pool (Hershko et al 1973), and the other, another chelatable pool (Hershko et al 1978; Hershko et al 1978a) separate from the hepatic ferritin pool. The second pool appears to be located in (or on) reticuloendothelial cells. Studies using another chelating agent, DTPA, support the concept of

a second chelatable iron pool separate from ferritin, since DTPA, although able to chelate iron in proportion to the body store, has been shown to be unable to remove iron from ferritin. DTPA rapidly loses its effectiveness, presumably because of the small size of the iron pool which is accessable to this chelator. It appears likely that DTPA removes iron from the reticuloendothelial pool which is one of the two pools acted on by desferrioxamine. This reticuloendothelial pool probably consists of iron present on reticuloendothelial cell membranes in excess of that which can be removed by transferrin. With the occurrence of iron overload both pools increase in size thus accounting for the larger amounts of iron excreted in response to these chelating agents when iron overload is present. The ferritin pool is, however, quantitatively the most important and currently desferrioxamine is the only clinically useful chelating agent when iron overload is present. The factors which affect the amount of iron excreted in response to desferrioxamine, apart from the size of the two iron pools, include the dose administered and the ascorbic acid status of the subject (Wapnick et al 1969). Ascorbic acid deficiency limits the amount of iron that can be excreted in response to desferrioxamine. Although the administration of ascorbic acid can overcome this effect, and therefore this finding could have therapeutic implications, there is reason to believe that the administration of ascorbic acid to iron overloaded subjects may be deleterious (Henry and Nienhuis

1977). Care should be exercised when embarking on a programme of iron chelation in iron overloaded individuals.

Although the measurement of plasma iron and transferrin saturation, marrow sideroblast count and free erythrocyte protoporphyrin content may each provide information from which depletion of iron reserves can be inferred in the correct clinical context the major application of these investigations lies in the diagnosis of iron deficiency anaemia rather than in the quantitation of body iron stores. These investigations will be discussed in more detail in section 1.6.

The percentage absorption of iron following the administration of a small oral dose of a readily available iron salt (usually 3 mg iron as ferrous sulphate + 30 mg ascorbic acid) can also be used as a measure of iron nutrition, an increase in the percentage absorption being a well recognised index of increased iron requirement (Bothwell et al 1958; Pirzio-Biroli and Finch 1960; Kuhn et al 1968). In addition, Heinrich (1970) has shown that there is an inverse relationship between iron absorption and the amount of stainable iron in bone marrow particles, even in healthy individuals with normal haematologic indices. Although these studies have provided important evidence linking iron absorption to body iron status the assessment of iron stores by means of the Prussian Blue reaction on bone marrow aspirates and its correlation with iron absorption is only semi-quantitative and is subject to

observer error (Bentley and Williams 1974). Using newer techniques it has been possible, however, to demonstrate a remarkably close reciprocal relationship between marrow storage iron content and iron absorption. The estimation of bone marrow iron content was carried out by means of chemical analysis of bone marrow trephine biopsies thus providing a more objective measure of storage iron content. This investigation is described in more detail in Chapter 4.

The whole subject of the assessment of iron stores has recently been given new impetus by the development of techniques for measuring the ferritin content of the blood. The small amounts of ferritin present in the circulation can now be measured by sensitive radioimmunometric (Addison et al 1972; Miles et al 1974; Miles et al 1974a; Halliday et al 1975; Saab et al 1978), radioimmunoassay (Marcus and Zinberg 1975; Niitsu et al 1975; Laxton et al 1977; Barnett et al 1978; Deppe et al 1978; Goldie et al 1978) and enzyme linked immunoassay (Zuyderhout et al 1978) methods. There is considerable evidence that the plasma ferritin concentration reflects the size of the body iron store (Walters et al 1973; Jacobs 1977). Under normal conditions the plasma ferritin concentration varies with age and with sex. Immediately after birth there is a rise in the plasma ferritin concentration which is consistent with the known shift of iron from red cells into stores at this time (Rios et al 1975; MacPhail et al 1981). Thereafter the ferritin concentration falls rapidly, and remains extremely low

during the first year of life, reflecting the low iron content at this time. During childhood the values are similar in males and in females but they begin to diverge in adolescence as males accumulate iron. In males a major rise in the plasma ferritin concentration occurs between the ages of 15 and 30 years, during which time there is a trebling of the mean plasma ferritin concentration from about 30 /ug/l to 90 /ug/l. Thereafter the rise is more gradual, averaging 1 - 2 /ug/l/year (Cook et al 1976). In females the plasma ferritin concentration remains low during the entire childbearing period, with levels similar to those found at puberty. However, after the menopause there is a significant increase in the ferritin concentration. The levels in postmenopausal women after the sixth decade of life approach those found in the male (Cook et al 1976; MacPhail et al 1981). Studies in which iron stores have been measured quantitatively by phlebotomy have provided the most convincing evidence of the close relationship between plasma ferritin and iron stores (Jacobs et al 1972; Walters et al 1973; Charlton et al 1977). Results from these studies indicate that within the range of 20 - 100 /ug/l, 1 /ug/l of plasma ferritin is equivalent to 10 mg storage iron. Geometric mean values for plasma ferritin in a nutritionally normal population, between the ages of 18 and 45 years, are 94 /ug/l for males and 25 /ug/l for females (Cook et al 1976). In iron deficiency values above 12 /ug/l are uncommon (Jacobs et al 1972; Cook et al 1976; Lipschitz

et al 1974). With iron overload greatly elevated plasma ferritin concentrations are found (Jacobs et al 1972; Halliday et al 1977).

The relationship between plasma ferritin concentration and iron absorption has been investigated in a number of studies (Cook et al 1974; Disler et al 1975; Walters et al 1975; Charlton et al 1975; Heinrich et al 1975). Although a significant inverse relationship between plasma ferritin and iron absorption is evident from these investigations the degree of correlation was not particularly high nor did the investigations include any independant measure of body iron content. Further insight into the relationship between marrow storage iron content, plasma ferritin concentration and percentage iron absorption was gained from the studies described in Chapters 4 and 5. These investigations provided quantitative information regarding the relationship between plasma ferritin levels and bone marrow iron content on the one hand, and between plasma ferritin levels and iron absorption on the other. Although a close correlation between plasma ferritin and marrow iron content was evident, and although a major portion of the variation in plasma ferritin concentration could be explained by variation in storage iron level, the normal range of plasma ferritin concentration accommodates a considerable variation of marrow reticuloendothelial iron stores. These relationships are discussed in more detail in Chapter 4. It is clear, however, that plasma ferritin does provide a valid index of

iron status and that the relationship between plasma ferritin and iron absorption holds true even when the erythropoietic state of the bone marrow is depressed, as it is in patients with chronic renal failure being treated by means of haemodialysis therapy (Eschbach et al 1977; see also Chapter 5).

The ferritin concentration is not, however, an infallible index of iron stores. There are conditions where the plasma ferritin concentration is raised out of proportion to the amount of iron. These conditions include inflammatory diseases (Lipschitz et al 1974), acute and chronic liver disease (Reissman and Dietrich 1956; Aungst 1968; Jones et al 1974; Lipschitz et al 1974; Prieto et al 1975), surgical procedures (Elin et al 1977), rheumatoid arthritis (Smith et al 1977) and various neoplastic conditions (Reissman and Dietrich 1956; Jones et al 1973; Oertel et al 1977). In breast carcinoma a raised plasma ferritin concentration is particularly encountered in those with hepatic metastases (Bezwoda et al 1981). In some of the neoplastic diseases, including leukaemia (Worwood et al 1974) and hepatoma (Powell et al 1975; Prieto et al 1975; Hazard and Drysdale 1977; Hazard et al 1977; Kew et al 1978), there is evidence that ferritin may be produced in the tumour cells themselves. The occurrence of these clinical conditions needs to be taken into account when interpreting the meaning of a raised plasma ferritin concentration.

1.3 Internal Iron Exchange

The physiologic handling of iron is organised around the acquisition of new iron and the re-use, storage and conservation of all the iron which has been accumulated by the body. Iron containing compounds are required for a wide variety of metabolic functions. The most important processes in which iron plays a role are those concerned with oxidation and energy transfer via electrons in which advantage is taken of the physical peculiarities of the iron atom and its highly reactive nature. Although these properties of iron serve a useful purpose the propensity of iron to interact with other compounds has required the development in the body of specialised methods for handling iron as well as specialised iron transport and storage proteins.

Adult man has a complement of 35 - 40 mg/kg of functional iron and a variable additional amount (under normal circumstances another 10 - 15 mg/kg) of storage iron. The major portion of the functional iron (60%) is found in haemoglobin. The iron in myoglobin accounts for 8 percent of the body iron content. In these two compounds iron is found as an iron-porphyrin complex which is especially adapted for the reversible interaction of iron and oxygen. Other iron containing enzymes, in which the iron is bound to a variety of non-porphyrin compounds, account for less than 5 percent of the body iron. There is a continuous process

of renewal of the metabolically active iron containing compounds. Iron which is liberated from the breakdown of these compounds is conserved and re-used over and over again. To effect this conservation and the internal exchange of iron between various sites of metabolic need a specialised transport system has evolved.

At any one time the plasma contains only about 3 mg of non-haemoglobin iron. During the course of each day however, about 35 mg of iron passes through the plasma (Cook et al 1970). This implies that there is both rapid ingress and egress of iron from the plasma compartment. Both the entry of iron into the plasma and its removal from it need to be taken into account in any analysis of internal iron exchange.

Within the plasma compartment the non-haemoglobin iron is bound to a specialised protein, transferrin. This molecule functions as a true transport vehicle in that it is not destroyed during the process of distribution of iron. Once the iron has entered the plasma the flow is essentially unidirectional. Transferrin acquires most of its iron from reticuloendothelial cells with a smaller contribution from iron stored in hepatocytes and from newly acquired iron absorbed via the intestine. Transferrin delivers its iron mainly to developing cells of the erythroid series which together constitute the erythron (van Dyk and Anger 1965; van Dyk et al 1967; van Dyk et al 1968). Transferrin cannot acquire iron from these red cell precursors nor can it give

up iron again to the reticuloendothelial cells (Finch et al 1970). On average 22 mg of iron are released from the reticuloendothelial system (RES) into the plasma each day. This iron is derived from the catabolism of haemoglobin. About 15 mg of the daily reticuloendothelial iron turnover comes from the breakdown of senescent red cells which are engulfed by reticuloendothelial cells. The remainder (approximately 7 mg/day) comes from cellular wastage during erythropoiesis, that is, from red cell precursors which never reach the circulation and which are taken up by reticuloendothelial cells in the marrow, and from non-haem iron removed from viable red cells (Cook et al 1970). Other sources of entry of iron into the plasma include that which is derived from iron turnover in liver (7 mg/day),as well as that released by turnover in muscle, skin and other parenchymal sites, and the 1 to 1,5 mg of iron which is absorbed from the diet each day. In addition, about 3 mg of iron per day moves between extravascular and intravascular compartments (Finch et al 1970) in the form of transferrin bound iron recirculating between blood and lymph.

As has already been mentioned, the major site of removal of transferrin bound iron is the erythron. In man only immature red cells take up iron. Although some transferrin iron can be removed from the plasma by circulating reticulocytes, the quantity involved usually accounts for less than 1% of the daily turnover. All other labelled, transferrin bound iron which appears in the red

cells does so only after a delay denoting the intramedullary maturation and haemoglobinisation time. Within the marrow the more mature developing red cells are responsible for the greatest iron uptake (Labardini et al 1973; Papayannopoulou and Finch 1975). These cells form the greatest proportion of the erythroid cells present in the bone marrow and, indeed, marrow reticulocyte uptake accounts for about 1/3 of the iron uptake by the erythron. Most of this iron is subsequently discharged into the circulation incorporated into haemoglobin. Normally only the amount of iron required to meet the need for haemoglobin formation is taken up by the developing red cell. However, under certain circumstances iron in excess of that which can be utilised for haemoglobin formation is taken up by developing red cells, mainly by normoblasts (Yamada and Gabuzda 1974). These cells can incorporate the excess iron into ferritin. This phenomenon seems to be a function of the plasma iron concentration rather than of the iron content of reticuloendothelial cells (Bainton and Finch 1964) and the excess iron is probably extruded from the cell during maturation, possibly by being passed to monocytes (Deiss and Cartwright 1970). The excess iron can again be recirculated after reticuloendothelial processing. There is no evidence that the reverse process, namely, the passage of iron from reticuloendothelial cell to erythroid cell, occurs.

The other major site of uptake of iron from the plasma pool is the hepatocyte (Wada et al 1970; Hershko et al

1972). Erythroid iron uptake and hepatocyte iron uptake are inversely related. However, both are increased when the transferrin saturation rises. When the transferrin saturation rises the wastage iron of erythropoiesis increases and a more rapid circuit of iron, from intramedullary erythroid precursors to RE cell and back into the plasma, is established. If erythropoiesis is inhibited excess iron enters the hepatocyte. Apart from the iron which they take up from transferrin hepatocytes also normally take up about 2 mg of iron per day in the form of haemoglobin released into the circulation by the intravascular disintegration of red cells. This haemoglobin is bound to a specific carrier protein, haptoglobin, and the haemoglobin-haptoglobin complex is taken up by the hepatocyte and catabolised (Hershko et al 1972). About 2/3 of this iron is rapidly re-released into the plasma, the rest is stored in the liver (Dresch and Najean 1972). In quantitative terms the amount of transferrin bound iron which is removed from the plasma by the hepatocyte is very much smaller than that removed by the erythron (Worwood et al 1975). The daily interchange of iron which occurs in muscle accounts for an even smaller proportion of the plasma iron turnover. In small animals it amounts to no more than 5% of the transferrin bound iron (Cheney et al 1967).

1.4 Plasma Iron, Transferrin and Transferrin Saturation

The passage of iron through the plasma has already been

described. Under normal circumstances freely reactive iron is not found in any significant quantity in the plasma, although such iron may be found in the plasma in acute iron poisoning (Reissman et al 1955; Awai 1958). When massive iron overload occurs (e.g. in thalassaemia major or in haemochromatosis) small quantities of iron, loosely bound to albumen and other plasma proteins, may be present (Hershko et al 1978). Although this small amount of unbound iron is not of great quantitative importance in iron metabolism it may contribute significantly to the cell damage produced by iron overload (Hershko et al 1978). Normally 95% of the plasma iron is bound to a specific carrier protein called transferrin. It is transferrin which is responsible for the safe passage of iron through the plasma and for the delivery of iron to various sites of need. The requirement for a specialised mechanism for iron transport and apportionment becomes obvious when one considers tnat the erythroid bone marrow has a need for iron supply of about 2 orders of magnitude greater than the other body tissues. The importance of transferrin is further illustrated by the occurrence of the genetic defect of atransferrinemia which is characterised by a marked hypochromic anaemia which does not respond to iron therapy and which is associated with excess iron loading of other tissues (Heilmeyer et al 1961). In this condition the intravenous administration of transferrin restores iron kinetics to normal (Goya et al 1972).

Human transferrin is a single chain polypeptide with two active binding sites (Green and Feeney 1968; Mann et al 1970) and a molecular weight estimated at around 76,000 daltons (van Kreel et al 1972). The stability of the bond between iron and transferrin is dependant on the presence of carbonate ion which also appears to be necessary for the release of iron from transferrin (Bates and Schlabach 1973; Harris et al 1974). Although differential loading of the two binding sites has been demonstrated in vitro (Brody et al 1962; Loh et al 1971; Harris 1977; Van Eijk et al 1978) transferrin molecules with no iron atoms and others with either one or two iron atoms exist simultaneously in solution (Warner and Weber 1951; Stratil 1961; Van Eijk et al 1969; Wapnick et al 1970; Williams et al 1970; Aisen et al 1978). These findings imply that once the bond between iron and transferrin has formed it is an extremely tight one and that there is little or no exchange of iron between transferrin molecules. The in vivo saturation of transferrin thus represents a mixture of these various forms although as the plasma iron concentration rises the proportion of diferric molecules also increases.

Iron bound to transferrin is taken up predominantly by immature erythroid cells (Jeppsson 1967; Hemmaplardh et al 1974), which, taken together, form the erythron. Although iron release from transferrin can be brought about by various in vitro manipulations (Cleton et al 1963; Aisen and Liebman 1968; Pollack et al 1976; Morgan 1977; Carver and

Frieden 1978) it is not certain how this process occurs in vivo. It is known that the delivery of iron to the developing red cell takes place in 3 phases (Morgan 1974). The first phase consists of a non-specific adsorption of transferrin onto the cell membrane. This phase is rapid and is independant of the presence of iron on the transferrin molecule. It is probably predominantly due to electrostatic forces (Kornfeld 1968; Morgan 1974). The second phase is one of fixation and ingestion. Here the transferrin becomes tightly bound to a membrane receptor and the iron is rapidly translocated across the cell membrane to a cytosol binding protein (Nunes et al 198Ø). This phase of iron uptake is energy dependant and is proportional to the ATP level in the recipient cell. Iron is released to the cell during this phase, probably with the removal of carbonate ion (Schulman et al 1974). In spite of previous reports of preferential, or differential, removal of one of the atoms of iron from one of the binding sites on transferrin by erythroid cells, and removal of the other atom on the other binding site by non erythroid tissues (Fletcher and Huehns 1967; Fletcher and Huehns 1968; Chernelch and Brown 197Ø; Ganzoni et al 1972; Lane 1973; Zapolski et al 1974) recent studies suggest that both iron atoms are removed simultaneously (Huebers et al 1978) and that the iron uptake from the two sites by reticulocytes in vitro, or by body tissues in vivo, is similar (Harris 1977; Pootrakul et al 1977). The last phase in the process of iron release is also energy dependant and

results in the return of intact transferrin to the plasma. At present it is not clear how iron finds its way to the mitochondria. It is possible that it is transferred directly from transferrin implying pinocytosis of the transferrin molecule into the cell (Hemmaplardh et al 1974; Ulvik et al 1976). However, chelators which enter the cytoplasm can prevent mitochondrial iron uptake (Morgan 1971) which suggests that the iron is in a relatively available form and that it has been released from transferrin. The delivery of iron by transferrin is affected by the degree of saturation of the transferrin. Diferric transferrin delivers twice as much iron per cell as monoferric transferrin (Christensen 1978; Huebers 1978; Skarberg et al 1978) so that for the same plasma iron concentration variations in transferrin level, and consequently of saturation, can markedly affect iron supply to the recipient cell.

Although the transferrin concentration can be measured specifically by means of immunoassay it is often expressed in terms of its iron binding capacity since it is the major iron binding protein present in blood. Indeed, for the better understanding of iron metabolism it is preferable to estimate the functional capacity of the protein rather than to measure its concentration in absolute terms. There is good agreement between the measurement of transferrin by immunologic means and the estimation of total iron binding capacity if it is assumed that the molecular weight of

transferrin is 76,ØØØ daltons. The average total plasma iron binding capacity is about 33Ø ug/dl (Laurell 1947; Rath and Finch 1949; Brendstrup 1953; Bothwell et al 1959) and is inversely related to the size of the body iron stores and to the plasma ferritin concentration (Lipschitz et al 1974). The total iron binding capacity is not, however, a dependable index of iron nutrition as it is affected by factors other than body iron content. Among those factors, unrelated to body iron content, which influence the total binding capacity are protein malnutrition (McFarlane et al 1965; McFarlane et al 1969; Reeds and Laditan 1976), protein losses as such occur in nephrotic syndrome (Gitlin et al 1956; Ramsay 1958; Hancock et al 1976), and inflammatory and neoplastic conditions (Cartwright and Wintrobe 1949; Brendstrup 1953; Hallgren 1954), which all lower the iron binding capacity of the plasma. Although the major cause of a raised iron-binding capacity is iron deficiency (Laurell 1952; Ramsay 1958), pregnancy (Verloop et al 1958; Sturgeon 1959; Morgan 1961) and the taking of some oral contraceptive pills (Burton 1967) can also increase the plasma level of transferrin. There is no diurnal variation in the plasma iron-binding capacity.

In contrast to the iron binding capacity the plasma iron concentration exhibits a marked diurnal variation with a morning peak and an evening nadir (Hemmeler 1944; Hamilton 195Ø; Batey and Gallagher 1977). This variation is due mainly to fluctuations in reticuloendothelial iron release

(Laurell 1953; Lynch et al 1973; Fillet et al 1974). There may also be a considerable day by day variation (Heilmeyer 1937; Hoyer 1944; Johnston 1947; Ramsay 1958) in the level of plasma iron. Although there is a considerable difference between the iron stores of males and those of females the plasma iron concentration is remarkably similar in both sexes. In one study the mean level in males was 115 $\pm$ 31 /ug/dl and in females it was 115 $\pm$ 40 /ug/dl (Bothwell et al 1979). Considerable variation in erythropoietic demand can be accommodated within the normal range of iron level (Faura et al 1969). Very large changes in the rate of erythropoiesis or of red cell destruction (Schade and Stengel 1959) are required before the plasma iron concentation deviates from the normal range. An abnormally low plasma iron concentration is defined as a level of less than 40 /ug/dl but the level must be considered in relation to erythropoietic demand. The major cause of hypoferraemia is depletion of body iron stores (Hallgren 1954; Coleman et al 1955). Hypoferraemia may, however, result from inflammation, ascorbic acid depletion, a sudden very marked increase in erythropoietic demand, or from a diminished transferrin concentration due to nephrotic syndrome or kwashiorkor. In the protein depletion states the fall in plasma iron content is due to a reduction in transferrin levels but the degree of saturation of the transferrin with iron usually remains normal and normochromic erythropoiesis can be maintained. Hyperferraemia occurs more rarely than

hypoferraemia but is seen in liver disease, in certain anaemias and in iron overload when it involves parenchymal cells.

Transferrin saturation is determined by both the plasma iron concentration and by the level of transferrin. Transferrin saturation thus exhibits the same circadian fluctuation as the plasma iron, with a mean morning saturation of around 35 percent. In iron depletion the changes in plasma iron concentration and transferrin level are in the opposite direction. Transferrin content rises and the plasma iron level falls so that the fall in transferrin saturation is amplified. In states of iron depletion the transferrin saturation is characteristically between 5 and 15 percent (Bainton and Finch 1964). Where the fall in plasma iron is due to some inflammatory condition there is usually a corresponding, though lesser, fall in transferrin level and the decrement in percentage saturation of the iron binding capacity is not as striking. When the transferrin saturation drops below about 16 percent the iron supply to the erythron becomes inadequate to meet basal erythropoietic needs. Transferrin saturation is often a better predictor of iron deficient erythropoiesis than is the plasma iron concentration. Since diferric transferrin delivers twice as much iron to the cell as does monoferric transferrin an adequate iron supply can be maintained even at a plasma iron concentration of 40 ug/dl if the transferrin saturation is 50 percent. However, the same

level of plasma iron with a transferrin saturation of only 1Ø percent is inadequate to sustain erythropoiesis. It is evident, therefore, that the levels of plasma iron, transferrin and transferrin saturation cannot be taken in isolation and that the assessment of iron status from plasma measurements requires consideration of all the variables which can affect these values.

1.5 Ferrokinetics

The detailed study of the behaviour of radioactive iron introduced into the plasma has given insight into disorders of erythropoiesis and a fuller understanding of certain aspects of iron metabolism. The aspects of ferrokinetic measurements which are relevant to iron metabolism relate mainly to internal iron exchange. The plasma iron pool can be labelled by the introduction of a tracer amount of radioactive iron bound to transferrin. From a practical point of view this may be done by direct injection of between 5 and 1Ø μc of ^{59}Fe citrate or $^{59}Fe\ SO_4$ provided that the subjects' transferrin is not saturated and if only simple measurements, such as plasma iron turnover, are required. Where the plasma iron concentration is high and the transferrin almost saturated, or when more complex investigations are required, the better procedure is to bind the radioiron onto unsaturated transferrin, in vitro, prior to its injection (Bothwell et al 1979).

After being introduced into the plasma compartment the

labelled iron leaves the plasma in an exponential fashion with a half time that is normally between 70 and 100 minutes. Surface monitoring shows that after exit from the plasma the most significant site of radioiron localisation is, under normal circumstances, the bone marrow (Elmlinger et al 1953; Alfrey et al 1969). Only if the erythroid marrow is ablated does hepatocyte uptake become a quantitatively significant component in iron flow. Most of the iron which is taken up by the marrow subsequently appears in the circulating red cell mass after a delay which may be designated as the marrow transit time. The radioiron then remains in the red cell for its life span after which it is taken up by reticuloendothelial cells and then recycled for further erythropoiesis. Although the circuit just described accounts for most of the radioiron there is a fraction of the injected dose which enters the reticuloendothelial system almost immediately. This fraction is derived from extruded components of erythroid cells or from defective cells which do not enter the circulation, or which are phagocytosed as soon as they do. The proportion of iron which enters this circuit may be termed the "wastage iron" of erythropoiesis. Another pathway by which iron exits from the plasma is into the extravascular space from which it returns via the lymphatic system with a half mixing time of between 10 and 20 hours (Jarnum and Lassen 1961; Katz 1961; Awai and Brown 1963). Studies carried out in human subjects, using ^{59}Fe labelled

transferrin which had been partially desialated so that it could be separated from the recipients' transferrin by electrophoretic techniques, have shown that about 8% of the labelled transferrin recirculates through the extravascular compartment, returning to the blood stream with its radioiron still attached (Morgan et al 1967). Thus the initial model of the radioiron disappearance curve, which assumed that the transferrin bound plasma iron behaved as a single pool (Huff et al 1950; Huff et al 1951),has, on closer analysis, been shown to be too simplistic. The multicomponent nature of the radioiron disappearance curve is now generally accepted (Hosain and Finch 1964; Morgan et al 1967). At least three components to the curve can be recognised. In order to analyse the minor components of the radioiron disappearance curve it is necessary to continue measurements of the plasma activity into the second week.

The initial, linear, portion of the radioiron disappearance curve represents that proportion of the injected radioiron which will not re-appear in the plasma during the first two weeks of observation while the latter portion of the entire disappearance curve represents that portion of the injected radioiron which will reflux back into the plasma before reaching its final intracellular destination. The reflux portion of the plasma iron disappearance curve has been separated into two components. The earlier reflux component is found to be related to the plasma iron concentration and probably represents the return

of transferrin bound iron from the extravascular compartment. The second, slower, and also larger, reflux component is felt to represent largely the wastage iron of erythropoiesis, processed by the marrow reticuloendothelial cells and returned to the plasma (Cook et al 1970). Although most groups of investigators agree on the coefficients for the first component (Pollycove 1966; Hosain et al 1967; Najean et al 1967) there is much less agreement about values for the other two components.

For clinical purposes the following calculations, based on the analysis of ferrokinetic data are useful. Firstly the amount of iron flowing through the plasma per unit time or plasma iron turnover (PIT) can be calculated from the product of the fractional clearance rate and the plasma iron concentration. The fractional clearance rate is calculated by dividing the natural logarithm of 2 (0,693) by the half time for radioactivity in the plasma.

Fraction cleared per day thus =

$$\frac{0{,}693 \times 24 \times 60}{T\ 1/2\ (min)} = \frac{1000}{T\ 1/2\ (min)}$$

while the plasma iron concentration in mg/dl =

$$\frac{\text{Plasma iron (}\mu\text{g/dl)}}{1000}$$

Thus PIT (mg/dl plasma/day) =

$$\frac{\text{Plasma iron concentration (}\mu\text{g/dl)}}{\text{T 1/2 (min)}}$$

The plasma iron turnover, however, includes both early reflux iron as well as iron taken up by non erythroid tissues. These factors, together with the fact that there may be considerable fluctuations in plasma iron concentration and in rates of erythropoieis, fluctuations which may occur in either circadian or random fashion, make the application of appropriate correction factors necessary (Hosain et al 1967; Cook et al 1970; Najean et al 1977; Skarberg et al 1978). In addition, it is also important to take into account the relative proportions of labelled mono and diferric transferrin since the ratio influences the amount of iron taken up by the erythron (Skarberg et al 1978). The application of a suitable correction factor allows the derivation of the standard erythron iron turnover (SEIT) (Cook et al 1970), as follows : -

SEIT = PIT - (plasma iron concentration (µg/dl) x 100 - haematocrit) x 0,0038.

This expression standardises the erythroid iron turnover to a constant plasma iron concentration and permits comparison between individuals. The SEIT includes the

wastage iron of erythropoiesis, which in normal subjects amounts to about 34% of the erythron iron turnover. Standard erythron iron turnover is normally about Ø,54 mg/dl whole blood/day, with Ø,35 mg iron/dl whole blood/day being involved in effective erythropoiesis (Cook et al 197Ø).

Other ferrokinetic measurements which may be used include the marrow transit time, which normally amounts to about 3 - 4 days (Finch et al 197Ø; Labardini et al 1973), and the red cell utilization of iron, which is the amount of radioiron in the red cell mass 1Ø - 14 days after injection and which is normally about 8Ø percent. It must be remembered, however, that the red cell utilisation figure cannot accurately separate the iron which has entered the erythroid pathway the first time around from that which has refluxed from the reticuloendothelial cell and re-entered the erythroid pathway. The percentage utilization, nevertheless, does give some indication of the proportion of newly formed red cells which can maintain themselves in the circulation. The figure is, however, inflated by at least one third since this is the usual amount of reflux iron and for clinical purposes the more accurate index of effective erythropoiesis is the reticulocyte count.

The major application of ferrokinetic measurements has been in clarifying the pathophysiology of a number of red cell disorders. While the ferrokinetic profiles of the different disease entities are often not unique insight has been gained into the nature of the disturbance of red cell

production and destruction in different disease states. With bone marrow damage and hypoplasia, such as that caused by radiation or chemotherapeutic drugs, the plasma iron level rises until the transferrin is almost fully saturated and the plasma radioiron disappearance half time increases to between 3 - 6 hours. There is a moderate reduction in plasma iron turnover but a marked change to non-erythron iron uptake with increased deposition of iron in the liver (Alfrey et al 1966). Similar, but more marked, changes occur in aplastic anaemia or red cell aplasia with the non-erythron turnover accounting for all the plasma iron turnover and with virtually no red cell utilization of iron. Since there is no erythropoiesis, and hence no wastage iron of erythropoiesis, the late reflux of radioiron back into the plasma is absent. In these subjects the main destination of the radioiron is the liver (Hosain and Finch 1966). Less severe degrees of marrow damage produce less marked kinetic changes. A decrease in the erythroid marrow cellularity to one half to one third of normal may not elevate the plasma iron concentration although the plasma iron disappearance rate lengthens somewhat and the standard erythron iron turnover and red cell utilization both decrease. Similar changes are also found when there is impaired erythropoietin stimulation of the bone marrow but the marrow transit times can be used to distinguish between the two types of hypoproliferation if there is sufficient red cell activity to measure it. The patient with marrow

damage will have an increased erythropoietin level and the marrow transit time will therefore be shortened in proportion to the degree of anaemia present. In contrast, if the anaemia is due to decreased erythropoietin stimulation the marrow transit time is normal.

In iron deficiency anaemia the plasma iron concentration is low, the half clearance time of radioiron is markedly shortened, and, on surface monitoring, the iron is taken up only over marrow bearing sites (Hosain and Finch 1966). The restriction in iron supply, however, results in only a modest increase in plasma iron turnover in spite of the rapid clearance of iron from the plasma. The fact that the standard erythron iron turnover is increased to only 1,5 times the basal level, at a time when the circulating red cell mass is reduced to about one half, indicates that the efficiency of erythropoiesis is markedly impaired. At these levels some three times more iron than normal is being used to produce each viable red cell. This is so in spite of an apparent increase in the percentage utilisation of iron to nearly 100 percent. The increased utilization is not, however, due to a more efficient utilization of a scarce resource; instead there is an increase in the wastage iron of erythropoeisis but the recircuiting of this iron back into the erythropoietic pathway eventually results in the appearance of all the radioiron in the circulating red cell mass. Both Pollycove and Mortimer (1961) and Cook and co-workers (1970) have identified an increase in the reflux of

iron from the erythroid marrow to the plasma in iron deficiency and the major kinetic defect, therefore, appears to be ineffective erythropoiesis. The recircuiting of this iron results in a prolongation of the marrow transit time as the iron retraces its path (Labardini et al 1973).

The restricted iron supply in inflammation is due to a block in iron release from reticuloendothelial cells rather than to a deficit in total body iron (Freireich et al 1957; Fillet et al 1974; Hershko et al 1974). In addition, the re-utilization of the iron recovered from haemoglobin taken up from the plasma by the hepatocytes is also defective (Hershko 1974a). At the same time the marrow iron requirement is doubled because the red cell life span is characteristically reduced to about half normal (Cartwright 1966). The plasma iron concentration falls to between 35 - 50 ug/dl and the kinetic data in this type of anaemia are thus similar to that found in true iron deficiency.

In conditions where there is ineffective erythropoiesis, such as in the megaloblastic anaemias where there is defective nuclear maturation, or in patients with haemoglobin synthesis defects, such as thalassaemia, or in stromal diseases of the marrow such as myelofibrosis, the kinetic pattern is essentially similar. The plasma iron concentration is raised and both plasma iron turnover and standard erythron iron turnover are increased, the most marked increase in both these values occuring in thalassaemia major. The efficiency of erythropoiesis is,

however, markedly impaired and the ineffective nature of the erythropoiesis is revealed by the unexpectedly low red cell utilization of radioiron. The marrow radioiron transit time is also prolonged because of recirculation of radioiron from red cell precursors which are destroyed rapidly without gaining acess to the circulation.

Although there may be considerable differences between the kinetic patterns encountered in different individuals with haemolytic anaemia, haemolytic states in general are characterised by a reduction of the plasma half clearance time of radioiron and by plasma iron and standard erythron iron turnovers that are raised approximately two to six fold. The rapid clearance of radioiron indicates a high degree of iron extraction by the erythroid marrow but the limiting factor in plasma iron turnover is the plasma iron concentration which generally remains within normal limits. The marrow transit time is reduced and the red cell utilization of radioiron is normal or slightly decreased.

The investigation of iron absorption in patients with chronic renal failure, described in Chapter 5, included some of the ferrokinetic measurements described in this section. Previous studies in patients with chronic renal failure have revealed that two different patterns of radioiron turnover may be present. In those patients with chronic renal failure who require repeated transfusion the plasma radioiron clearance is prolonged and the standard erythron iron turnover is reduced to about half normal with a

diminished red cell utilization of radioiron. In contrast, those patients who require little or no transfusion have a normal plasma iron concentration, an intermediate to normal erythron iron turnover and a nearly normal red cell utilization of radioiron. The anaemia in these individuals is due to increased destruction which is not being compensated for by an appropriate increase in production (Eschbach 1970). The results of the radioiron studies described in Chapter 5 are in keeping with these ferrokinetic patterns, although the majority of the patients encountered in that particular study showed a normal or near normal SEIT.

1.6 Iron Absorption

Since the body is capable of only very limited changes in iron excretion absorption and the control thereof plays the major role in maintaining iron homeostasis. In order to remain in iron balance the normal male, with his 1000 mg store, must absorb roughly 6 percent of the dietary iron each day while the female who has greater needs must absorb as much as 15 percent of the dietary iron each day. This high percentage can only be achieved if the composition of the diet permits. The upper limit of absorption by the non iron deficient individual on a good diet appears to be about 3,5 mg per day.

Absorption may strictly be defined as the transfer of iron from the lumen of the bowel to the blood stream.

However the quantity of dietary iron which is absorbed is the end result of a complex series of changes and interactions between the various constituents of food, the intestinal secretions, the chemical and physical process of iron uptake by the mucosal cells and, finally, of the process of transfer from mucosal cell to bloodstream. The acquisition of iron can therefore be considered as taking place in two phases. The first or luminal phase is dependant on the nature of the iron presented to the gastrointestinal tract and the effect of gastrointestinal secretions on the availability of this iron for uptake. The second phase, or mucosal phase, includes the entry of iron into the mucosal cell, where it is further processed, and its final discharge into the plasma iron pool.

1.6.1 The luminal phase

Since all the iron absorbed by the body comes from the diet it would appear self evident that the nature of the diet can influence iron absorption. However, it was not until 1951 that the first studies of the process of food iron absorption were carried out by Moore and Dubach (Moore and Dubach 1951). The principal finding of this, and of a number of subsequent studies (Layrisse and Martinez-Torres 1971; Martinez-Torres and Layrisse 1973), was that food iron of animal origin is generally better absorbed than is vegetable iron. Dietary iron of animal origin is present mainly as the haem containing compounds haemoglobin and myoglobin. Normal subjects absorb 10-20 percent of iron

present in meat (Heinrich et al 1969; Heinrich et al 1971; Martinez-Torres and Layrisse 1973) whereas the percentage absorption from vegetable sources is generally below 10 percent, with absorption from many vegetable staples being considerably lower than this (Layrisse and Martinez-Torres 1971; Martinez-Torres and Layrisse 1973). There is however considerable variation in the absorption from different foodstuffs. Western diets generally have an iron content of around 6 mg per 1000 calories (Wretling 1968; United States Department of Health 1972) and approximately 50 percent of the dietary iron is in the form of haem iron. Nutritional surveys in various countries have shown that similar, and sometimes even greater, amounts of iron are consumed in countries where iron deficiency is prevalent (United States Department of Defence Nutrition Surveys 1956-1963; Ramsay 1957; Beaton et al 1970). However, the nature of the dietary iron may vary considerably from country to country. Among the factors which determine differences in the composition of the diet are socio-economic status (Cook et al 1976; Finch et al 1977), ethnic custom (Davis et al 1960; Mayet 1976), the handling of foodstuffs (Hofvander 1963) and the cooking utensils employed. Where meals are cooked in iron pots considerable amounts of iron can be added during the process of preparation (Bothwell et al 1964; Moore 1965).

Essential to the understanding of the influence of diet on iron absorption has been the development of the common pool hypothesis (Cook et al 1972; Cook 1977). This

hypothesis postulates that when several foodstuffs are consumed together all the non-haem iron contained in the meal forms a common pool within the lumen of the gastrointestinal tract, and that this pool is then acted on in common by the various luminal factors which affect the availability of iron for absorption. Furthermore it is postulated that this pool can easily be labelled by the addition of an isotope of iron added as a simple iron salt just prior to cooking (Cook et al 1972; Thein-Thau et al 1977). While studies using this technique have yielded much valuable information on the availability for absorption of food iron it has become obvious that there are exceptions to the common pool theory. Instances where the results of absorption studies are discrepant with those predicted by the common pool theory are; iron absorption from unmilled, unpolished rice (Bjorn-Rasmussen et al 1973),absorption from ferritin (Martinez-Torres et al 197Ø) and from the insoluble iron compounds ferric-hydroxide (Cook et al 1973; Disler et al 1975) and ferric-oxide (Derman et al 1977) which are poorly or not at all absorbed, whereas the iron chelate complex iron-lll-EDTA is better absorbed than is the intrinsic food iron in cereal and curry meals (MacPhail et al 1981).

Recent studies on single foods have shown that the milling of cereals greatly influences the bioavailability of the iron contained in them. Iron in milled polished rice, for instance, is about four times better absorbed than is

the iron in unmilled rice (Bjorn-Rasmussen et al 1973). Similarly, the bioavailability of wheat iron in bread baked with flour of different extraction varies in proportion with the bran content (Bjorn-Rasmussen 1974). In addition, vegetable foodstuffs also contain substances which can have important effects on the availability of iron. These include polyphenols, phytates, carbonates, and phosphates (Heilmeyer 1931; Hegsted et al 1949; Sharpe et al 1950; Conrad 1970; Disler et al 1975; Disler et al 1975a). All these substances act as inhibitors of iron absorption, generally by the formation of large polymers with iron (Benjamin et al 1967). There are also dietary constituents which tend to make iron more available for absorption. Of major importance in this regard is ascorbic acid (Sayers et al 1973) but other organic acids, amines, sugars and tricarboxylic acids may also have an effect (Hussain et al 1965; Conrad and Schade 1968; Layrisse et al 1968; Schade et al 1968; Jacobs and Miles 1969; Martinez-Torres and Layrisse 1970).

The concept of a common intraluminal pool has also been found to be valid for the haem iron in food. In other words if a small amount of radiolabelled haemoglobin is added to a meal in which there are haem containing items such as meat the absorption of the tag reflects the absorption of the haem iron from the whole meal (Martinez-Torres and Layrisse 1971).

Since the presence of one type of foodstuff can influence the absorption of iron from another food (Layrisse

et al 1968) iron absorption from a single constituent of a mixed meal does not provide a valid estimate of the total iron absorption from that meal. However, the development of techniques which allow the haem and the non-haem iron pools to be labelled seperately has made it possible to study the interaction of the two dietary constituents (Bjorn-Rasmussen et al 1974; Layrisse et al 1974). One example of such an interaction is the finding that the presence of meat in a meal appears to make the non-haem iron more available for absorption (Cook and Monsen 1976).

In spite of the large number of food iron absorption studies which have been carried out in the past the absorption of iron from mixed meals in subjects who suffer from conditions associated with abnormalities of iron metabolism has not received much attention. The investigation described in Chapter 6 addresses itself to this problem. Iron absorption from both the haem iron and the non-haem iron pool was assessed in patients suffering from idiopathic haemochromatosis, in a group of hospital patients suffering from iron deficiency anaemia and in a group of Indian housewives in whom iron deficiency, thought to be related to dietary factors, is common. In addition to demonstrating that the absorptive capacities of iron deficient and haemochromatotic subjects overlap and that the two groups cannot be distinguished quantitatively there were also considerable discrepancies between the absorption of the two types of dietary iron, particularly amongst the

Indian women, many of whom appeared to absorb non-haem iron extremely poorly. This malabsorption of non-haem iron may be a cause of nutritional iron deficiency, at least in selected population groups. These results are discussed in more detail in Chapter 6.

Apart from the variation in iron absorption caused by inherent differences between different foodstuffs the luminal secretions also influence the physical nature of the iron ingested. Gastric secretions, especially, have been studied intensively over the past decade. The major importance of gastric hydrochloric acid in the process of iron absorption was first noted in achlorhydric subjects (Goldberg et al 1963; Cook et al 1964; Jacobs et al 1964; Schade et al 1968) who absorb ferric salts poorly, even when they are deficient in iron. Iron absorption in achlorhydric patients can be increased by the simultaneous administration of hydrochloric acid (Jacobs et al 1964) or gastric juice and neutralization has been shown to diminish or abolish the enhancement (Cook et al 1964). In addition, a relationship has been demonstrated in anaemic subjects between the maximal acid output and the amount of iron absorbed from a test meal (Jacobs et al 1967).

Whether other components of the gastric juice facilitate iron absorption is less clear. Binding of inorganic iron by macromolecular constituents of gastric juice such as glycoproteins has been demonstrated in vitro; although the binding is weak and probably ionic in nature

(Winter and Williams 1968; Swan and Glass 1973) it is agreed that the complexes are not precipitated when the pH is raised (Swan and Glass 1973; Beutler et al 1963; Davis et al 1966; Jacobs and Miles 1969). This led to the suggestion that the loose binding preserves the availability for absorption in the upper small intestine of the ingested iron (Jacobs and Miles 1969). It was postulated that the iron could be transferred from the large molecules to absorbable low-molecular-weight ligands such as ascorbic acid and evidence compatible with this hypothesis was obtained by sampling the contents of the jejunal lumen after a mixed meal (Glover and Jacobs 1971). The non-specific nature of the binding has, however, created the suspicion that ligands in the ingested food may be of much greater significance in this regard than endogenous ones (Swan and Glass 1973). The evidence from absorption studies is conflicting. Jacobs and co-workers (1968) found no difference between the effect of gastric juice and that of HCl of similar molarity on iron absorption in achlorhydric subjects; but Jacobs and Owen (1969) reported that iron absorption was potentiated significantly by gastric juice even when the effect of the acid was eliminated.

The influence of gastric factors on the bioavailability of non-haem food iron was studied in greater detail in the investigation described in Chapter 7. A previous investigation of food iron absorption in various groups of subjects (see Chapter 6) had suggested that the

malabsorption of non-haem iron which was present in some individuals in the study might be due to luminal factors since the capacity of the mucosa to absorb iron presented in an available form, either as ferrous ascorbate or as haem iron, appeared to be intact. Using a system where the solubilisation of iron by gastric juice and hydrochloric acid solutions was measured in vitro it was found that both hydrochloric acid and gastric juice liberated similar quantities of iron in a low molecular weight form from intrinsically labelled wheat. Absorption studies carried out in gastric juice donors showed that there was a close correlation between in vitro solubilisation, particularly in the form of the low molecular weight fraction, and wheat iron absorption in vivo. It thus seems clear that hydrochloric acid is the only component of gastric juice that is of importance in modifying the absorption of non-haem food iron.

Other intestinal secretions have also been investigated. It is probable that the bicarbonate in pancreatic secretions promotes the formation of unavailable iron hydroxide polymers (Benjamin et al 1967) and the net effect of the formation of these would be to limit iron absorption. Bile has been shown to enhance the absorption of ferrous salts in iron deficient dogs (Wheby et al 1962), and ligation of the bile duct reduces ferric iron absorption in rats (Webling and Holdsworth 1966; Conrad and Schade 1968). Although it has been suggested that it is ascorbic

acid in bile which is responsible for this effect (Conrad and Schade 1968) *in vitro* studies suggest that iron and bile interact to form high molecular weight complexes (Jacobs and Miles 197Ø). Mucopolysaccharides or polymerised bile salts are probably responsible for the formation of these complexes and it appears that in man bile salts have no effect on iron absorption (Brise 1962).

The absorption of haem iron is influenced to a much lesser degree by intraluminal factors when compared to vegetable iron absorption. In particular, haem iron absorption is uninfluenced by the presence or absence of hydrochloric acid or ascorbic acid (Turnbull et al 1962), or by the presence of inhibitors of iron absorption such as desferrioxamine (Turnbull et al 1962; Moss 1964). This is because haem iron is absorbed as the metalloporphyrin ring and the valence of the iron is no longer of critical importance. However, when haem is administered without protein it is poorly absorbed, probably because of the formation of macromolecular haem polymers (Turnbull et al 1962; Conrad et al 1967; Conrad et al 1967), but when haem is given with meat its iron is well absorbed (Turnbull et al 1962; Martinez-Torres and Layrisse 1971), possibly because the primary amines formed by the enzymatic breakdown of protein tend to stabilise the haem in a monomeric form (Conrad 1963).

The important contribution which haem iron can make to overall iron nutrition is emphasised by the studies of

Layrisse and co-workers (1974) and Bjorn-Rasmussen and co-workers (1974) who demonstrated that the relatively small amounts of haem iron present in the diet can account for a major proportion of the total amount of iron absorbed each day. The relative contribution of haem to total iron absorption from a mixed meal was studied in more detail in the investigation described in Chapter 8. In this investigation the effect on total iron absorption from a meal of fixed iron content of varying the ratio of haem to non-haem iron was studied. The results of this investigation showed that the percentage absorption of haem iron remains relatively constant within the physiologic dose range, resulting in a linear rise in the amount of haem iron absorbed with increasing dose.

Apart from the importance of meat as a source of dietary haem, Layrisse and co-workers (1973) have shown that the presence of meat enhances the absorption of the non-haem iron present in a meal. This effect is so striking that a vegetable meal would have to be fortified with as much as 70 mg of iron in order to achieve the same nutritive value in terms of iron as a meal with only 5 mg of additional iron as long as meat is present. These findings have obvious implications for iron fortification programmes.

The results of these various studies, taking into account both the composition of the diet as well as the interactions between the various constituents, allow diets to be characterised according to the bio-availability of the

constituent iron (Monsen et al 1978). The most important factors which determine the bio-availability of food iron are the amounts of meat, poultry or fish and/or of ascorbic acid, contained in the meal. A diet of low availability is one containing less than 30 g meat, poultry or fish or less than 25 mg of ascorbic acid. The comparable figures for a diet of medium availability are 30 to 90 g meat, poultry or fish or 25 to 75 mg ascorbic acid, while a diet of high availability is one containing more than 90 g meat, poultry or fish or more than 75 mg ascorbic acid; alternatively it is one containing 30 to 90 g meat, poultry or fish plus 25 to 75 mg of ascorbic acid. Recently Hallberg (1982) has proposed the expression of "bio-available nutrient density" where food iron availability is expressed in terms of energy content. A diet of good bio-available nutrient density would be one in which the diet alone covers 90 percent of the iron requirement of menstruating women, the group most at risk of developing iron deficiency.

The bio-availability of food iron is, however, only one of the factors which influences iron absorption. The variability of iron stores among different individuals has a major influence on the amount of iron absorbed from the non-haem pool. This variability makes it necessary, when considering the results of food iron absorption studies, to obtain an independant measure of absorptive capacity. The concept of a reference dose of radioiron was introduced by Layrisse and co-workers (1969). Comparison of iron

absorption from the test meal and the reference salt is an index of the bio-availability of the non-haem food iron in the meal (Bjorn-Rasmussen et al 1976). Although it is generally accepted that the absorption of iron from a readily available iron salt is an index of body iron needs and that a similar relationship should pertain to dietary iron absorption the presence of significant numbers of individuals with malabsorption of non-haem food iron can disturb this relationship (see also Chapter 6). The absorption of haem iron in physiologic quantities appears to be independant of the "reference" iron absorption (see Chapter 8).

1.6.2 The mucosal phase

Although iron can pass across the intestinal mucosa at a number of sites in the gastrointestinal tract the most active site of iron absorption in vivo is the duodenum and upper jejunum (Wheby 1970). Studies with everted gut pouches suggest that the properties of the mucosa determine this finding (Dowdle et al 1960; Manis and Schachter 1962; Howard and Jacobs 1972). The metabolic changes which occur in mucosal cells during iron absorption are still obscure. There is a direct relationship between the concentration of iron in the lumen and the amount entering the mucosa (Thomson and Valberg 1971). Electronmicroscopic studies suggest that the iron becomes attached to the glycocalix of the brush border of the mucosal cells (Kimber et al 1973).

Active metabolic processes appear to be involved in the transfer of iron across the cell membrane (Dowdle et al 196Ø; Manis and Schachter 1962; Pearson and Reich 1965), since metabolic inhibitors will reduce iron uptake by the mucosa of everted gut pouches. In addition, enzyme inducers such as phenobarbitone increase the amount of iron taken up by the mucosa of rats (Thomas et al 1972).

Once iron has entered the mucosal cell it must be transferred to the plasma. Transfer occurs in two phases. The first component is a rapid one (Wheby et al 1964) and during this phase 6Ø - 8Ø percent of the eventual total amount of iron which will be absorbed is transferred. This rapid phase is followed by a second, slower, component which may last from 12 - 24 hours. Some or all the iron transferred during the slower phase has been temporarily stored within the cell as ferritin (Conrad and Crosby 1963; Charlton et al 1963; Charlton et al 1965; Worwood and Jacobs 1971; Forth and Rummel 1973). A variable proportion of the iron that enters the cell is eventually discarded when the cells exfoliate (Charlton et al 1963; Charlton et al 1965; Forth and Rummel 1973). In one study (Powell 197Ø) a mean mucosal uptake of 12 percent was observed in normal subjects but with eventual transfer to the plasma of only 3,6 percent. In iron deficiency the figures were 33,5 percent uptake and 29,8 percent transfer. Although there is a relationship between the amount of absorbable iron within the lumen of the bowel and the amount transferred the

relationship is not a constant one as is the case with mucosal uptake. It seems likely that there is an active, carrier mediated, transfer system which results in a smaller percentage being transferred at higher concentrations of luminal iron (Bothwell et al 1958; Bannerman et al 1962; Gitlin and Cruchaud 1962; Lindell et al 1963; Moore 1964; Thomson and Valberg 1971). In iron deficiency the transfer system may be set at a higher level, and in iron overload at a lower level.

Considerable investigation into the nature of the transport carrier has taken place over the last decade. It has been suggested that transferrin itself is responsible (Levine et al 1972). More probably the carrier is a separate protein which is similar to , but distinct from, transferrin (Wheby and Jones 1963; Huebers et al 1976; El-Shobaki and Rummel 1978). The carrier mediated mechanism exhibits considerable specificity. In iron deficiency the absorption of magnesium, calcium, copper, mercury and cesium is not increased (Pollack et al 1965) but the absorption of metals close to iron in the periodic table of elements is enhanced.

1.6.3 The regulation of iron absorption

The most important factor in the physiological regulation of iron absorption is clearly the body iron content. Iron absorption, although influenced by the amount of iron available for absorption, is a function of total

body iron stores (Bothwell et al 1958; Pirzio-Biroli and Finch 1960; Kuhn et al 1968). As body iron stores are depleted a higher percentage of the available iron in the diet is absorbed, even in the absence of anaemia. The relationship between body iron stores and iron absorption is dealt with in more detail in Chapter 4. Increased erythropoiesis, such as follows bleeding or acute haemolysis, stimulates iron absorption while inhibition of erythropoiesis by hypertransfusion results in decreased absorption (Bothwell et al 1958; Erlandson et al 1962; Conrad et al 1967). However, not all haemolytic states are associated with comparable increases in iron absorption and in renal failure, which is characterised by a hypoproliferative anaemia, iron absorption still appears to be related to body iron stores despite decreased erythropoiesis (Eschbach et al 1970; Eschbach et al 1977). The relationship between iron absorption and iron stores in renal failure is described in more detail in Chapter 5. In this investigation simultaneous ferrokinetic and iron absorption measurements were performed in patients with chronic renal failure on regular dialysis therapy. Although no correlation was noted between total erythropoietic activity, as measured by the standard erythron iron turnover, and absorption the degree of iron absorption was still governed by body iron content.

The mechanism by which iron absorption is regulated in response to distant events in the body is as yet not

understood. The local regulation of iron absorption by variation of the ratio of the amount incorporated into ferritin and the amount available for transfer across the mucosal cell has been postulated as a controlling mechanism. It seems more likely however that these variations occur in response to some controlling mechanism rather than being the regulating factor. The size of the plasma iron pool per se also seems an unlikely method of control since large changes of body iron store can occur whilst a normal plasma iron concentration is maintained. Transferrin saturation has also been shown not to be the determining factor. The creation of additional unsaturated binding sites, by injection of unsaturated transferrin, does not result in increased absorption from the intestinal tract (Schade et al 1969; Levine et al 1972). Furthermore, in disease states such as thalassaemia and idiopathic haemochromatosis inappropriately high absorption can occur, even with a high degree of transferrin saturation (Bothwell and Charlton 1970). Other humoral factors, including erythropoietin and ferritin, have also been investigated for a possible role as the controlling influence on iron absorption but the experimental data do not support the candidacy of any of them. In an attempt to explain the regulation of iron absorption without invoking the existence of a humoral mechanism Cavill and co-workers (Cavill et al 1975) have postulated the presence of a "labile iron pool" which is maintained by the balance between egress and ingress of iron

from various sources including the reticuloendothelial cell, parenchymal stores and the gastrointestinal tract. According to this hypothesis internal iron exchange is automatically regulated by the iron needs of the dominant site of uptake, normally the erythroid marrow. An increase in erythropoietic demand, particularly when coupled to storage iron depletion, would therefore automatically increase the inflow of iron from the gut, one of the important supplier tissues, by creating additional binding sites on the transferrin molecule. This ingenious concept could theoretically explain how changes in iron absorption occur, and in particular how they occur without having to postulate any humoral regulatory mechanism. However, recent experimental studies in rats, in whom acute changes in iron requirement were induced by exchange transfusion with reticulocyte rich blood (Finch et al 1982), do not wholy bear out this concept. Although the increased iron requirement was accompanied by a significant increase in iron turnover and iron absorption the changes observed were not mediated by a decrease in plasma iron concentration or by an increase in unsaturated iron binding capacity. Indeed, a higher plasma transferrin saturation augmented the movement of iron into the plasma from iron-donating tissues. These findings are in accord with earlier reports in which the production of hypertransferrinaemia failed to increase iron absorption (Schade et al 1969; Levine et al 1972). Further work remains to be done in order to elucidate fully

the mechanism by which iron absorption is regulated, both in normal subjects and in patients with various disease states.

1.7 Iron Loss

Under physiologic circumstances iron leaves the body mainly as intracellular iron compounds lost during the turnover of cells from epithelial surfaces. In addition, small quantities of red cells are lost from the gastrointestinal tract even under normal circumstances. Larger quantities of blood are lost during menstruation. The losses in males, as estimated over prolonged periods of time by whole body excretion of ^{55}Fe, amount to between Ø,9 to 1,2 mg per day (Green et al 1968). The radioisotopic method for calculating iron losses has been compared to the results obtained by adding together the quantities of iron lost individually from the skin, the gastrointestinal tract and the urinary tract, with good agreement between the estimates obtained by these two approaches. Approximately one third of the iron losses are due to loss of red cells from the bowel, about 2Ø% of the iron loss is due to turnover of epithelial cells of the skin and approximately the same amount is lost through exfoliation of mucosal cells. In addition, small amounts of iron are lost in sweat, bile and urine (Finch 1969). The iron losses are influenced by iron status so that the total losses may vary from 8 /ug/kg/day in iron deficiency to 2Ø /ug/kg/day in iron overload (Dubach et al 1955; Green et al 1968).

Although not physiologic, an important factor in gastrointestinal iron loss, which has to be recognised, is the ingestion of analgesic compounds and in particular the use of aspirin. Even in the absence of any symptoms of gastrointestinal irritation the rate of blood loss from the bowel increases four fold, from an average of Ø,4 ml blood per day to more than 2 ml of blood per day (Beeken 1968; Baird et al 197Ø), in regular aspirin takers. At higher doses of aspirin even more blood than this may be lost.

In the female menstrual losses and the iron loss associated with pregnancy are considerable. The average monthly loss due to menstruation is between 26 and 3Ø ml (Hallberg et al 1966; Cole et al 1971). In terms of iron loss this amounts to Ø,4 to Ø,5 mg/day. In both the studies from which these figures are derived more than 1Ø percent of the women had losses of more than 8Ø ml per month which represents an iron loss of more than 1 mg per day. To these losses must be added the other unavoidable losses and the total iron loss in premenopausal women is thus about 1,4 to 1,5 mg per day compared to Ø,9 to 1 mg per day in men. Pregnancy also has its price in terms of iron. Although iron losses due to menstruation cease, the foetus, cord and placenta require about 36Ø mg of iron (Widdowson and Spray 1951; McCoy et al 1961) while maternal blood loss at parturition may account for a further 15Ø mg of iron (McCoy 1961). To these quantities must be added the 23Ø mg of obligatory iron loss occurring during the 9 months of

pregnancy. The iron cost of pregnancy is thus some 740 mg of iron or 2,6 mg per day. Breast feeding may result in further losses of up to 1 mg of iron per day (Bothwell et al 1979).

Thus, although there is no mechanism in the body for iron excretion per se, iron loss is a continuing process which must be compensated for by absorption of dietary iron. The greater losses in women play a major role in determining the difference in body iron content between males and females, particularly in the premenopausal female.

1.8 Iron Deficiency

1.8.1 The causes of iron deficiency

Iron deficiency may develop suddenly or gradually. Since each g of haemoglobin contains about 3,5 mg of iron acute haemorrhage leads to a rapid fall in total body iron and mobilisation of storage iron in order to replace the lost haemoglobin. A more gradual depletion of body iron may result from persistent negative balance when the demands of growth, menstruation or pregnancy or even obligatory iron loss cannot be met by absorption. Iron deficiency is particularly prone to occur during the period of rapid growth between 6 months and 2 years of age, during the adolescent growth spurt, in females when the added burden of menstruation plays a part and in multiparous premenopausal women. The term "nutritional iron deficiency" should be restricted to those instances where physiologic demand

outstrips iron supply.

A monthly menstrual loss of more than 80 ml, equivalent to 1,2 mg of iron per day, probably occurs in as many as 10 percent of premenopausal women and is capable of causing iron deficiency anaemia (Hallberg et al 1966; Cole et al 1971). Blood loss of this order may be difficult to detect clinically since up to half the women who lost this amount of blood described their menstruation as moderate or even scanty (Hallberg et al 1966). The factors which contribute to excessive menstrual loss include heredity (Rybo and Hallberg 1966), parity and body mass (Cole et al 1971), the use of intrauterine contraceptive devices (Guillebaud et al 1976) and uterine fibroids (Rybo 1973). Uterine fibroids, however, are likely to be present only if menstrual blood loss exceeds 200 ml per month and specific gynaecologic abnormalities are uncommon with menstrual blood losses of less than this amount.

In adult males and in post menopausal females the prime cause of iron deficiency is gastrointestinal blood loss. The daily blood loss which can produce iron deficiency need only to be of the order of 7 - 10 ml per day in an adult male eating a Western type diet (Bothwell et al 1979). Such amounts usually go unnoticed by the individual. In iron deficient subjects who are bleeding from the bowel every effort should be made to find the specific cause of the blood loss. A large number of structural, inflammatory or neoplastic conditions of the gastrointestinal tract may be

responsible. In many instances, however, it is impossible to find a specific cause for the gastrointestinal bleeding (Bothwell et al 1965). Hookworm infestation is an important cause of gastrointestinal blood loss in the less developed parts of the world (Farid et al 1969) and each worm of the Necator americanus species is capable of removing up to Ø,1 ml of blood per day (Roche and Layrisse 1966) while Ancylostoma duodenale may remove as much as Ø,25 ml per day (Roche et al 1957; Farid et al 1965). Blood loss from the urinary tract due to heavy infestation with Schistosoma hematobium may also be sufficient to lead to iron depletion in countries where such infestations are endemic (Farid et al 1968). A less common cause of iron deficiency is intravascular haemolysis with consequent haemoglobinuria and haemosidirinuria. In paroxysmal nocturnal haemoglobinuria the iron loss may be as much as 15 mg per day (Hartman et al 1966) and similar losses have been observed in patients with traumatic haemolysis of red cells due to artificial heart valves (Hartman et al 1966). Blood donors are also at risk of becoming iron depleted (Finch et al 1977) or even frankly anaemic (Mehta 1968).

True malabsorption of iron is less frequently encountered as a cause of iron deficiency than is blood loss. However in countries such as India, Pakistan, Haiti and Bangladesh there is a high incidence of bowel disease with histologic abnormalities of the mucosa. These diseases may cause iron malabsorption and contribute to the high

incidence of iron deficiency found in these countries (Baker et al 1962; Klipstein et al 1966; Cowan et al 1968). Defective iron absorption has clearly been shown in idiopathic steatorrheoa (Badenoch and Callender 1954; Badenoch and Callender 1960; Anad et al 1977). Chronic gastritis with mucosal atrophy and achlorhydria as a cause of iron deficiency has been the subject of some controversy over the years. This finding is present in a significant number of patients with iron deficiency anaemia (Witts 1966; Witts 1969) but it has been suggested that the mucosal atrophy is the result rather than the cause of the iron deficiency (Jacobs et al 1966; Witts 1969). While there is some experimental evidence to support such a view (Murray and Stein 1968; Witts 1969) the importance of gastric hydrochloric acid in making non-haem food iron available for absorption means that in at least a proportion of patients hypochlorhydria must contribute to the iron deficiency (see also Chapters 6 and 7).

In Western countries gastric surgery, often accompanied by bleeding, may lead to iron deficiency due to malabsorption caused by hypochlorhydria and the loss of the normal mixing and retentive function of the stomach (Kelly et al 1967). In recent years peptic ulceration has been treated by less destructive operations than gastrectomy. While the haematologic effects of vagotomy and pyloroplasty appear to be less than those of gastrectomy (Cox et al 1968), long term information is not yet available.

Finally, the practice of pica or ingestion of non food materials has come under some scrutiny. While some types of clay and alkaline earths are capable of markedly inhibiting iron absorption (Minnich et al 1968) both the nature and quantity of the substances ingested are the determining factors. The practice appears to be common in South African black women but iron deficiency is not prominent among these women (Sayers et al 1974).

1.8.2 The effects of iron deficiency

The most clearly demonstrated impact of depletion of body iron stores is the inability to replace rapidly red cell losses due to haemorrhage or to adjust fully to the increased haemopoietic demands of pregnancy and growth. If depletion is severe enough iron deficiency anaemia is the result. There is clear evidence that severe nutritional iron deficiency anaemia is detrimental to health. In pregnant Malayan women with haemoglobin concentrations of below 6,5 g/dl the maternal mortality was three times as high, at 15,5/1000, compared to non anaemic controls (Llewellin-Jones 1965). The prenatal and perinatal infant loss and the prematurity rate were also significantly higher in the infants of anaemic mothers. Iron deficiency anaemia has also been shown in several physiologic studies to have an effect on effort tolerance and work capacity. Myocardial function is not impaired (Levine et al 1977) but there is a greater than normal increment in cardiac output per unit

increase in oxygen consumption in response to exercise (Varat et al 1972). Since the maximum cardiac output and respiratory minute volume remain unaltered (Sproule et al 196Ø; Cotes et al 1972; Davies et al 1972) maximum work capacity must be reduced. A direct relationship between Harvard Step Test scores and haemoglobin concentration has been found in a number of studies (Viteri and Torun 1974; Garby and Areekul 1974) where this has been examined in subjects with haemoglobin concentrations within the range of 4 to 16 g/dl. The relevance of these physiologic findings to clinical defects is not clear, because of the many variables involved, but there are at least two studies which suggest that manual labourers suffering from iron deficiency anaemia are less efficient workers than are subjects with normal haemoglobin concentrations (Basta et al 1979; Edgerton et al 1979). There is, however, some controversy regarding the level of haemoglobin above which exercise capacity shows no further increase. In some studies there was no further increase in work capacity with haemoglobin levels of above 1Ø - 12 g/dl (Davies et al 1972; Finch et al 1976). Since there are other compensatory mechanisms that are able to adjust oxygen delivery to tissue demands (Finch and Lenfant 1972) the change in oxygen carrying capacity caused by a small deficit in haemoglobin concentration is hardly likely to be a critical determinant of work capacity.

Apart from causing anaemia iron depletion is associated with structural changes in skin, mucuous membranes and in

the gastrointestinal tract. These changes include angular stomatitis, glossitis, webbing of the oesophagus at the level of the cricoid plane, gastric atrophy and koilonychia. Deficiencies of other nutrients such as zinc and pyridoxine (Jacobs and Cavell 1968) may play a role in the pathogenesis of these changes.

The structural changes which have been known about for a long time may be less important than the effects of iron deficiency produced by depletion of certain tissue enzyme systems. There is good evidence that a number of functional iron containing tissue compounds are depleted in the iron deficient state (Adamson et al 1968; Jacobs 1969; Fairbanks et al 1971; Siimes et al 198Ø). These compounds include myoglobin, the cytochromes, catalase and peroxidase and the metalloflavoprotein enzymes such as NADH, as well as succinic, xanthine, aldehyde and alphaglycerophosphate dehydrogenases. Depletion of these enzymes occurs more readily in skin than in skeletal muscle, and skeletal muscle depletion occurs before myocardial enzyme levels fall. Experiments in rats have linked the depletion of alpha glycerophosphate dehydrogenase to muscle dysfunction, resulting in an over production of lactic acid (Finch et al 1976) and muscle weakness.

Other biochemical defects have also been described in iron deficiency and these include depletion of cerebral iron in iron deficient young rats (Dallman et al 1975), reduced levels of aldehyde and mono-amine oxidase (Dallman et al

1968; Mackler et al 1978) and increased urinary catecholamine excretion (Voorhess et al 1975). The maintenance of a normal body temperature in rats has recently been shown to be iron dependant. The inability of iron deficient rats to maintain their body temperature at normal levels on cold exposure is due to impaired conversion of triiodothyronine to thyroxine (Dillman et al 1979; Dillman et al 198Ø). Again, the clinical relevance of these studies is unclear. However, there is suggestive evidence that infants suffering from iron deficiency, particularly during the first 2 years of life, show developmental and behavioural abnormalities (Oski and Honig 1978).

Iron has also been thought for centuries to play a role in man's defence against infection. Galen, although unaware of the iron content of haemoglobin, thought that the "pouring forth of the superfluity of the blood" in menstruation protected women against disease. Indeed, some recent studies have tended to suggest that iron treatment is associated with an increased propensity to infection (Masawe et al 1974; Murray et al 1978; Brain 1979). There is some theoretic backing for this idea since the growth of some micro-organisms has been shown to be iron dependant (Sussman 1974). In contrast to these findings there is evidence that iron deficiency results in decreased resistance to infection. Impairment of cellular immunity has been reported in iron deficient children (Joynson et al 1972; MacDougall et al 1975). Other studies have shown

abnormalities in granulocytic function (Yetgin et al 1979) in iron deficiency which can be reversed by giving iron (Chandra 1973). The relevance of these findings to clinical infective disease is still unclear.

Thus, while the most obvious effect of iron deficiency is anaemia it seems clear that iron depletion has widespread tissue effects the importance of which will only become clear in the future. Tissue depletion of iron usually develops in concert with anaemia but it is possible that both anaemia without tissue depletion and tissue depletion without anaemia may occur (Henderson 1954; Verloop 1970).

1.8.3 The diagnosis of iron deficiency

The methods available for the measurement of the total amount of storage iron have been dealt with previously. Many of these techniques, however, are suited mainly to experimental studies but it is the experimental studies which have helped to define the meaning of tests which can be used in the clinical investigation of patients with anaemia. In particular, these studies have helped to define the meaning of the plasma ferritin concentration and of the plasma iron and transferrin levels. Apart from being helpful in the diagnosis of iron deficiency the plama iron level and the degree of transferrin saturation can give valuable information about the state of erythropoiesis. When assessing the meaning of plasma iron and transferrin values a number of factors need to be taken into account.

Although an increase in the total iron-binding capacity does occur with storage iron depletion there are other factors which make this investigation less reliable as a test for iron deficiency. However, a fall in the plasma iron concentration to below normal vales together with a raised total iron binding capacity and a transferrin saturation of below 15 percent is good evidence of iron deficiency (Bainton and Finch 1964) and is usually associated with evidence of iron deficient erythropoiesis.

From the clinical point of view one of the most common methods used to assess iron stores is the histologic examination of bone marrow. This investigation can give a good idea of the quantity of reticuloendothelial storage iron. Visual estimations of the quantity of haemosiderin correlate reasonably well with the concentration of non-haem iron in the bone marrow as determined by chemical methods (Kerr 1957; Gale et al 1963; Torrance et al 1963; Pritchard and Scott 197Ø). Good marrow specimens are essential and a portion of the marrow must be stained specifically for iron in order to obtain an accurate estimate. For clinical purposes the best system of grading the marrow iron content is into the simple categories of absent, reduced, normal or increased. In most healthy individuals there is a moderate amount of visible iron, with men tending to have more than women (Stevens et al 1953). However, up to one third of healthy and otherwise normal normal women in Western countries have little or no stainable iron in the bone

marrow. This is also the finding in frank iron deficiency, where there is no stainable iron, since the reticuloendothelial stores are used up before erythropoiesis becomes compromised. The visual estimation of iron stores thus has limitations and the results must be evaluated in conjunction with other evidence of iron depletion. The development of sensitive methods for the determination of the plasma ferritin concentration has provided a simple means of diagnosing storage iron depletion (Walters et al 1973; Lipschitz et al 1974). The use of this assay in the study of body iron content has already been discussed. From the clinical point of view the major value of the plasma ferritin estimation in an anaemic patient is the fact that a level below 12 /ug/l is diagnostic of iron deficiency. Among a total of 154 cases of uncomplicated iron deficiency, values above the cut off level of 12 /ug/l were seen in only 2,6 percent of cases (Addison et al 1972; Jacobs et al 1972; Siimes et al 1974; Lipschitz et al 1974; Leyland et al 1975; Heinrich et al 1977). Unfortunately, a plasma ferritin value in the normal range does not exclude iron deficiency anaemia because several disorders, such as infection, chronic disease and liver disease, elevate the plasma ferritin independantly. An advantage of the plasma ferritin estimation over the plasma iron level is its relative stability with repeated measurements in the same subject which contrasts with the relatively poor reproducibility of plasma iron measurements (Cook et al 1974; Pilon et al

1981).

The excretion of iron in response to parenteral administration of a chelating agent while being of value in the diagnosis of iron overload is less useful in helping to diagnose iron deficiency, since values in subjects with depleted or absent iron stores show considerable overlap with those obtained in normal individuals (Weinfeld 1956; Hedenberg 1969).

Once storage iron depletion is well advanced evidence of iron deficient erythropoiesis can be found and is identified by a number of features. These features include a plasma ferritin concentration of below 12 /ug/l, a plasma iron concentration of below 40 /ug/dl togetner with a percentage saturation of below 15 percent, reduction or absence of marrow sideroblasts, rapid disappearance of injected radioiron from the plasma and an increase in the red cell protoporphyrin concentration. The estimation of free red cell protoporphyrin concentration will come to assume an increasingly important place in the diagnosis of iron deficient erythropoiesis since it can be carried out fairly rapidly and relatively simply. Increased free erythrocyte protoporphyrin concentrations result from an imbalance between porphyrin synthesis and iron uptake by red cell precursors (Dagg et al 1966; Langer et al 1972; Koller et al 1978). Reduction in the supply of iron, whether due to iron deficiency or due to a sudden increase in erythropoiesis, results in an accumulation in the

erythrocyte of free protoporphyrin, even before there is a noticable reduction of the haemoglobin concentration. The concentration of free erythrocyte protoporphyrin is usually expressed as /ug/dl red blood cells, or perhaps even better as the protoporphyrin (pp) : haem (hm) ratio (Labbe et al 1979). Normal values for red cell protoporphyrin are about 35 $\pm$ 15 /ug/dl red cells or a pp:hm ratio of 16. With transferrin saturations of less than 16 percent the protoporphyrin level rises to more than 70 /ug/dl red cells and the pp:hm ratio increases to over 100. The protoporphyrin levels in normal cells appear to be stable for the life span of those cells although in iron deficient cells there may be a gradual elution of the protoporphyrin (Langer et al 1972). Since the red cell mass turns over at only about 1 percent per day several weeks of iron deficient erythropoiesis are required before the free erythrocyte protoprophyrin increase is recognisable.

The classic hypochromic microcytic anaemia of iron deficiency is the end stage of the process of iron depletion, which may have developed over a prolonged period of time. Morphologic changes are first evident in the reticulocyte but eventually all the erythrocytes reflect the diminished haemoglobin synthesis by becoming hypochromic and microcytic. The rise in the proportion of microcytes is a sensitive indicator of inadequate transferrin saturation. In established iron deficiency anaemia the mean cell volume falls below 84 fl, the mean cell haemoglobin (MCH) below 27

pg and the mean corpuscular haemoglobin concentration (MCHC) below 32 g/dl (RBC). The drop in these red cell indices tends to parallel the drop in haemoglobin concentration.

The haemoglobin concentration below which an individual can be considered to be anaemic is still the subject of debate. The World Health Organisation has set a series of levels below which anaemia is likely to be present (Table 1) but the true level should ideally be assessed for each population (WHO 1968; WHO 1972).

Table 1.

Haemoglobin concentrations below which anaemia is likely to be present

(sea level)*

Age (yrs)		Haemoglobin (g/dl)
Ø,5 - 6		11
6 - 12		12
12 - 15		12
> 15	- male	13
	- non pregnant female	12
	- pregnant female	11

*Modification for altitude : Ø,3 g/dl increase for each percent decrease in oxygen saturation (Hurtado 196Ø).

In one study of the distribution of haemoglobin levels

(Cook et al 1971) the cumulative frequency of distribution in males was shown to follow a single Gaussian curve with minimal deviation at the lower end. However, a similar plot in females showed considerable deviation from a normal distribution. The haemoglobin concentration at which the plot deviated from the pattern typical of a single Gaussian distribution was taken as the cut off limit for that population. Because of considerable overlap of distributions it is, however, not possible to fix a figure dividing normal from abnormal and the accuracy with which any level will identify an anaemic individual is a matter of probability. The lower the level of haemoglobin the lower the probability that the person is within his or her physiologic norm.

Definitive proof that iron deficiency is the reason for iron deficient erythropoiesis in any particular patient depends on its correction with iron therapy. However, due care must be exercised before giving or interpreting a trial of iron therapy. On the one hand the injudicious use of iron in conditions associated with globin or haem synthetic abnormalities, such as thalassaemia or other iron loading anaemias, may be of detriment to the patient. On the other hand blood loss may be so heavy that iron therapy cannot keep pace, or causes of anaemia such as haemolysis may resolve spontaneously, making the interpretation of a trial of therapy difficult. Thus the investigation of anaemia in any particular patient remains a complex exercise in which

many factors have to be taken into account.

1.8.4 The treatment of iron deficiency Iron deficiency anaemia is not a complete diagnosis. In each instance the cause for the deficit must be established and specific treatment for any underlying condition must be given, wherever necessary.

The aim of iron therapy is to repair the anaemia and to restore the body iron reserve. This is accomplished most simply, and with least expense, by the use of oral iron therapy. Parenteral iron therapy is indicated only if oral treatment is unsuitable and transfusion is indicated only if the severity of the anaemia threatens life.

A therapeutic trial with oral iron usually consists of doses of between 37 and 74 mg of iron as ferrous salt given three times daily. This form of oral iron supplementation is the cheapest available. In general it is preferable to administer the iron fasting, or at least away from meal times, as foods and particularly vegetable foods, can significantly reduce iron absorption (Layrisse et al 1973; Ekenved 1976). However, some of the newer controlled release preparations appear to be better absorbed when given together with food than ferrous sulphate given in the fasting state. In addition, fewer side effects are produced by these preparations (Rybo and Solvell 1971; Hallberg et al 1978) but their cost is also greater. A positive response to iron therapy is defined as a daily increase in haemoglobin concentration of more than Ø,1 g/dl from the

fourth day onwards. Generally, if the haemoglobin rise is less than 2 g in a 3 week period this is considered as a therapeutic failure. Normal subjects with adequate iron stores will show no rise in the haemoglobin concentration when given supplemental iron. In order to achieve the desired rate of increase in iron deficient subjects some 40 - 60 mg must be absorbed each day. This amount will allow for a red cell production rate of three times normal. Absorption of this quantity of iron will lead to an appropriate increment in the plasma iron concentration and hence of iron delivery to the bone marrow. Although the percentage absorption of the iron salt falls as the dose increases the absolute amounts retained in the body rise with increasing dosage. However, gastrointestinal side effects of oral iron are also dose related and at higher dosages patient intolerance of iron may limit the effectiveness of treatment. The percentage absorption also appears to change somewhat during the course of treatment (Norrby and Solvell 1974). Ferric salts are absorbed less well than ferrous salts and the recommendation for oral iron treatment must remain with a standard preparation of ferrous iron e.g. ferrous sulphate B.P. or ferrous gluconate.

A variety of parenteral iron preparations are available. These include saccharated iron oxide (Nissim 1947), iron dextrin (Andersson 1950), iron-sorbitol-citric-acid complex (Lindvall and Anderson 1961), iron poly-sorbitol-gluconic-acid complex and iron dextran (Evans and

Ramsay 1957). The use of parenteral iron compounds is associated with an increased risk of side effects, which include allergic reactions, cardio-vascular toxicity, fever and lymphadenopathy and possibly the development of sarcomatous lesions with the use of the intramuscular forms of iron.

The problem of the prevention of nutritional iron deficiency lies outside the scope of this review. Suffice it to say that, as with the treatment of individual patients with iron deficiency, the target population for a preventative programme must be analysed in order identify the underlying causes of iron deficiency. Factors which may be specifically treatable, either by medical means or by social manipulation, need attention. Examples include treatment of hookworm and other parasitic infestations, birth control programmes and improvement of general nutrition. Iron supplementation for specific groups at high risk of developing iron deficiency, such as pregnant females and growing children, is also required.

The fortification of certain staple food-stuffs with iron has a somewhat different emphasis, being aimed at the population as a whole. Systematic studies are going on in several countries in an attempt to find suitable iron compounds and vehicles for fortification and to define suitable levels of iron fortification. In several industrialised countries iron fortification of certain staple foods has been instituted for several years. In

Sweden, where foods have been fortified for many years, the level of fortification has been increased step wise from 3 mg iron /100 g white wheat flour in 1944 to 6,5 mg/100 g of all sifted flour in 1970. During this time the prevalence of iron deficiency in Swedish women in the fertile age period has decreased from approximately 25 - 30% to between 5 - 10% (Garby et al 1967; Garby et al 1969; Bengtsson et al 1973; Hallberg et al 1979). Although other social, economic, dietary and medical factors may also have contributed to this change the decrease in the prevalence of iron deficiency is impressive. Although the ideal iron compound and the ideal dietary vehicle for iron fortification has not yet been defined there are indications that fortification may in future make a significant impact on the global problem of iron deficiency.

An aspect of iron fortification which has to be considered before it is generally adopted is whether the increase in dietary iron content would have a deleterious effect on individuals predisposed to develop iron overload. Although less frequent than iron deficiency, genetic conditions in which there is a predisposition to accumulate iron to excess do occur in the general population. The clinical expression of these conditions might be exacerbated or occur at a younger age if greater amounts of iron were present in the diet. A proposal to increase the level of iron fortification of flour in the U.S.A. from its current figure of 13,0 - 16,5 mg per pound to 40 mg per pound

(Waddell et al 1972) engendered a heated debate that extended over several years. This fortification level was expected to increase the average intake of iron in adult males from 17,9 mg to 21,5 mg. Eventually the proposal was rejected on the grounds of unproven efficacy and doubts about its safety. In terms of this second point it was felt that individuals suffering from several "iron loading" states, such as idiopathic haemochromatosis and thalassaemia major, would be at particular risk (Crosby 1973). Although the number of subjects at risk is not accurately known it has been estimated that there are about 20,000 individuals with idiopathic haemochromatosis and 5,000 with thalassaemia major in the United States. In 1978 Bothwell and co-workers proposed a mathematical model of iron balance, which took into account dietary iron intake as well as iron excretion, to explain iron accumulation in subjects genetically predisposed to develop iron overload. This model was based on data available from the literature and required testing. However, according to these calculations, the proposed increase in iron fortification in the U.S.A. would merely lead to somewhat earlier clinical presentation of haemochromatotic individuals, leading in turn to earlier diagnosis and treatment (Bothwell et al 1978). Accordingly, it appeared unlikely that the prevalence of clinically significant iron overload would increase markedly as a result of iron fortification. There are, however, few studies which have concerned themselves with iron

absorption, from fortified diets, amongst individuals suffering from haemochromatosis. The opportunity to confirm some of the predictions in the mathematical model came with the investigation described in Chapter 9, where the absorption of iron from maize meals fortified with different quantities of ferrous sulphate was studied in fully treated haemochromatotic subjects. The results confirmed some of the predictions of the model, namely that the relationship between dose administered and amount absorbed is similar in haemochromatotic individuals and normals, albeit that this relationship is set at a somewhat higher level of absorption in the haemochromatotic subjects (Bezwoda et al 1981). Iron fortification programmes, therefore, do not appear to be associated with an excess risk of increasing the incidence of clinically significant iron overload.

1.9 Iron Overload

1.9.1 The causes of iron overload

Although iron overload occurs much less frequently than iron deficiency there are a number of specific circumstances where it may be found. Iron overload may be produced by inappropriately increased mucosal absorption of iron, as in idiopathic haemochromatosis and the iron loading anaemias, by parenteral administration of iron (either in the form of blood transfusions or as therapeutic injections of iron preparations), and by grossly excessive dietary intake over a prolonged period of time.

In the tissues iron is seen microscopically and histochemically as haemosiderin aggregates and the term haemosiderosis or siderosis may be applied to any visible increase in tissue iron. The excess iron (> 2000 mg) may be deposited either in reticuloendothelial or parenchymal cells. Important considerations when dealing with a generalised increase in tissue iron include the quantity of excess iron and the presence of tissue damage. In idiopathic haemochromatosis the source of the excess iron is the diet and there appears to be an error of metabolism in which iron absorption, although still influenced by stores, is inappropriately high (Bothwell et al 1978). However, absorption studies in patients with haemochromatosis have failed to elucidate the nature of the defect. Absorption is probably not increased to any marked degree, if at all, in untreated subjects (Chodos et al 1957; Pirzio-Biroli et al 1958; Smith et al 1969; Powell et al 1970). Presumably absorption is depressed in such individuals by the massively increased stores since it has been shown that iron absorption is inversely proportional to the quantity of storage iron in idiopathic haemochromatosis (Walters et al 1975) as it is in normal subjects. The absorption of available iron salts and the absorption of haem food iron is similar in individuals with treated haemochromatosis and those with iron deficiency anaemia (Bezwoda et al 1976, see also Chapter 6). Although a number of studies show that non-haem food iron is well absorbed by haemochromatotic

patients who have been treated by means of phlebotomy iron deficient individuals may also absorb similar quantities of iron from this dietary source (Powell et al 1970; Bezwoda et al 1976). The investigation described in Chapter 9 provided the opportunity to study this question in more detail and over a larger range of dietary iron content. Maize meals fortified with different quantities of ferrous sulphate were fed to a group of fully treated haemochromatotic subjects. The results of this investigation indicate that the amount of non-haem iron absorbed by haemochromatotic individuals is influenced by dose in a manner comparable to that which occurs in normal subjects. Although the relationship is similar in some respects the regulatory mechanism may be set at a higher level in haemochromatotic individuals compared to normal subjects. Although it is unlikely that differences in absorptive capacity are the cause of haemochromatosis any future hypothesis of the control of iron absorption will have to take into account the findings in these individuals.

There is considerable evidence that idiopathic haemochromatosis has a genetic basis although the fully developed condition is rarely found in more than one member of a family (Dillingham 1960; Felts et al 1967). Recent studies have demonstrated a significant association of idiopathic haemochromatosis with histocompatibility antigens, especially HLA-A3 (Simon et al 1975; Simon et al 1977; Simon et al 1977), and have confirmed that this

condition is a genetic disorder with at least one susceptibility gene located on chromosome 6 in close proximity to the A locus (Simon et al 1978; Beaumont et al 1979; Cartwright et al 1979). Detailed analysis of families with idiopathic haemochromatosis has confirmed that in most, if not all, the disease is inherited as an autosomal recessive trait (Simon et al 1977; Cartwright et al 1979). On this basis the homozygote requires two abnormal genes for full expression of the disease. An autosomal recessive mode of inheritance is consistent with the long-standing observation that the disease occurs about five times more frequently in siblings than in the offspring of a haemochromatotic parent. The occurrence of vertical transmission in some families (Debre et al 1958) is probably due to a homozygous-heterozygous mating, with an autosomal recessive mode of inheritance; such matings are not unlikely since the gene frequency has been estimated to be as high as 1 in 20 in some populations (Simon et al 1975; Simon et al 1977; Cartwright et al 1979). The observation that in families where one of the children of the proband is affected other children are frequently found to be affected is also consistent with this interpretation. Close family members who are heterozygotes sometimes have raised iron stores (Debre et al 1958; Scheuer et al 1962; Ploem et al 1965) although in one study neither clinical evidence of iron overload, nor any continued increase in iron stores, could be demonstrated in a group of 44 relatives who had

been studied for up to 16 years (Basset et al 1981).

Diagnostic criteria in idiopathic haemochromatosis are limited to the phenotypic expression of the disorder. Although the rate of development of iron overload may be modified by many factors, including age, sex, intake of iron and blood loss, the fully developed condition is characterised by massive (> 15 g) iron overload and evidence of tissue damage, particularly in the liver. In haemochromatosis iron circulates to excess in the plasma and there is a high transferrin saturation with deposition of excess iron in parenchymal cells (Jandl and Katz 1963; Buchanan 1969). The exact nature of the defect has not been fully elucidated. Despite earlier reports to the contrary transferrin itself is not abnormal (Bothwell et al 1962; Wheby et al 1964) nor have any consistent abnormalities been found either in the nature or in the distribution of ferritin. Current evidence suggests that the reticuloendothelial cells are unable to hold iron in this disease, although there may be an additional mucosal abnormality which allows inappropriate amounts of iron to be absorbed.

In situations where there are normal physiologic mechanisms for the handling of iron, but where the amounts presented to the body are excessive, iron overload may also develop. Parenteral iron overload is nearly always due to some form of refractory, non-haemorrhagic, anaemia treated by repeated blood transfusions. Since the capacity to

excrete iron is limited the iron stores will be increased by the amount of iron contained in the transfused blood. In addition, in some of these conditions where anaemia occurs in spite of increased erythropoietic turnover oral iron absorption may also be excessive. In these conditions the excess iron is derived from the breakdown of effete red cells and cannot be re-utilised for effective erythropoiesis. The excess iron is deposited mainly in the reticuloendothelial system (Lynch et al 1974).

Absorption of excessive amounts of iron resulting from an increased dietary load has best been described in Southern African Blacks. The excessive reticuloendothelial deposits found in these individuals are due to the absorption of large amounts of available iron from traditional maize and sorghum beer (Derman et al 198Ø). Although these drinks do contain alcohol, which may be responsible for some of the liver damage seen in these subjects, the alcohol per se does not appear to be the major factor in the production of the iron overload (MacPhail et al 1979). In this situation the reticuloendothelial iron is still derived from the catabolism of haemoglobin but the need to supply the erythroid marrow from this source is lessened as a result of the larger inflow from the gut. As a consequence storage iron accumulates. The reticuloendothelial iron overload may be further aggravated by ascorbic acid deficiency. Depletion of vitamin C has been shown to cause impaired release of iron from

reticuloendothelial cells (Bothwell et al 1964; Lipschitz et al 1971) and iron overload itself may lead to ascorbic acid depletion (Lynch et al 1967b).

1.9.2 The effects of iron overload

Acute iron toxicity from massive iron poisoning has been known for some time. The clinical features include abdominal pain and vomiting, metabolic acidosis and cardiovascular collapse (Aldrich 1958; Fischer et al 1971). Chronic iron toxicity is a more prolonged process and is a function of both the concentration of the iron and its tissue distribution. The tissues which suffer most damage in iron overload are the liver, the heart and the endocrine system. Cirrhosis is rare in patients with aplastic anaemia and transfusional iron overload, since the excess iron is deposited mainly in reticuloendothelial cells, but is almost invariably present when the diagnosis of idiopathic haemochromatosis is made (Finch and Finch 1955). In idiopathic haemochromatosis the body store of iron is > 15 g and iron is deposited predominantly in hepatic and other parenchymal cells. In the dietary iron overload of Southern African Blacks a survey, done between 1959 - 1960, showed that there was a striking correlation between the degree of iron overload and the degree of portal fibrosis (Bothwell and Isaacson 1962). Since that time there has been a significant decrease in the degree of hepatic iron overload but no decrease, in fact even a rise, in the degree of

portal fibrosis and cirrhosis in this population. This change has been ascribed to differences in both the quantity and the nature of the alcohol consumed (MacPhail et al 1979).

Although the mechanism by which iron damages tissues is not well understood it is possible that reactive iron damages lysosomal membranes and that the subsequent release of acid hydrolases into the cell cytoplasm produces the tissue changes. This theory is supported by the findings in haemochromatosis of diffuse deposits of ferritin and haemosiderin in lysosome-like structures (Richter 1978). Some of the tissue damage can be reversed by removal of the iron by vigorous phlebotomy in subjects with idiopathic haemochromatosis (Williams et al 1969). Chronic iron overload also leads to defects in ascorbic acid metabolism and may result in subclinical and clinical scurvey (Seftel et al 1966; Lynch et al 1967). The osteoporosis seen in siderotic South African blacks may be caused, at least in part, by iron induced ascorbic acid depletion (Seftel et al 1966). Similar defects have been reproduced in iron overloaded guinea pigs (Wapnick et al 1971) and have also been described in other forms of iron overload, both in man (Dellbarre 196Ø; Du Lac et al 1967) and in animals (Hartley et al 1959).

1.9.3 The treatment of iron overload

Phlebotomy therapy is the only currently feasable

treatment for idiopathic haemochromatosis since one phelebotomy of 500 ml removes 200 mg of iron, in contrast to desferrioxamine which removes only 10 to 20 mg of iron per single dose (0,5 g) (Harker et al 1968; Modell 1979). Although no prospective controlled trial of phlebotomy therapy has been reported, either in patients with established disease or in asymptomatic relatives with increased iron stores, the evidence is highly suggestive that removal of iron from patients with idiopathic haemochromatosis is of benefit at all stages of the disease (Williams 1969; Powell 1970; Bomford and Williams 1976).

In patients with iron loading-anaemias it is usually not possible to remove excess iron by phlebotomy. Hence agents which increase the excretion of iron by chelation must be used. Up to the present time the most effective and least toxic chelating agent has been desferrioxamine. Early studies, in which the drug was given by intramuscular injection, showed that intermittent administration fails to induce sufficient iron excretion to cause a negative iron balance in patients with thalassaemia (Modell 1979). Iron excretion after desferrioxamine may be increased by increasing the dose, giving it daily, giving oral ascorbic acid together with desferrioxamine and by giving the desferrioxamine intravenously (Modell and Beck 1974). Subcutaneous infusion of 1500 mg of desferrioxamine, using a portable pump over a 12 hour period every day was reported by Nienhuis and co-workers (Nienhuis et al 1978) to result

in the excretion of between 15 and 55 mg of iron per day. Most patients older than 5 years achieved a net negative iron balance on this regimen. Larger doses, up to 4 g of desferrioxamine daily, have been used by other workers (Pippard et al 1978) with even more iron being removed. Although there is evidence that the administration of ascorbic acid together with desferrioxamine considerably enhances iron excretion (Wapnick et al 1970; Modell 1974; O'Brien 1974; Hussain et al 1977) some hazards of ascorbic acid treatment have been reported (Nienhuis et al 1979). In particular, cardiac damage may become overt at the start of a programme of iron chelation with additional ascorbic acid. Other approaches to the treatment of these patients include the use of neocyte transfusions together with chelation therapy and the possibility of bone marrow transplantation has been broached, but whatever mode of treatment is chosen, the costs of therapy in thalassaemia are high (Modell 1979). The development of orally effecive chelators is greatly to be desired but remains an area where considerable research still has to be done and it can be anticipated that primary and secondary iron overload will continue to be significant clinical problems for some years to come.

CHAPTER 2

The Aims of This Thesis

The physiologic handling of iron is organised around the accumulation of iron from the diet and its conservation and re-use by the body. Dietary iron comes from two main sources, haem iron which is present in meat and the non-haem or vegetable iron pool. Although considerable progress has been made over the last two decades in understanding the factors which influence iron absorption there are a number of areas where further clarification is necessary. Those areas requiring further elucidation include the relationship of iron absorption to body iron content, the interaction between the various dietary components and the contribution of luminal factors, and in particular the effect of gastric juice, in making iron available for absorption.

Although much evidence has accumulated indicating a close link between iron absorption and body iron content the delineation of this relationship over a wide range of storage iron concentration has awaited the availability of accurate and quantative methods for estimating the size of the body iron stores. The studies in Chapters 4 and 5 investigated some of these relationships. Similarly, the absorption of iron from the two dietary iron pools in subjects with different disorders of iron metabolism required further elucidation, which was made possible only

by the development of methods for labelling both dietary iron pools separately. Iron absorption from these dietary pools was investigated in the studies described in Chapters 6 and 8. In addition the influence of luminal factors on the availability of non-haem iron has been the subject of some controversy, particularly in regard to the effect of gastric juice. Chapter 7 describes a method which was developed whereby the in vitro solubilisation of food iron could be related to in vivo absorption. Finally, the existence of conditions in which there is an excessive accumulation of body iron has to be taken into account in any overall hypothesis of the control of iron absorption. How does the handling of iron in these subjects differ from that in normal individuals, and what would the effect be on them of increasing the amount of iron in the diet? The investigation described in chapter 9 studied the effect of food iron fortification in subjects with idiopathic haemochromatosis.

Although these studies touch on a number of aspects of iron metabolism the central theme has been the more accurate delineation of the factors which influence absorption and the effect of diet on iron nutrition.

CHAPTER 3

Methods

This section describes some of the experimental methods and patient data common to the investigations described in the following chapters. Additional methods, peculiar to specific studies, are described in the appropriate chapters.

3.1 Study Populations

All human studies were performed using volunteer subjects. The nature of the investigation was explained to each individual and informed consent was obtained. The Committee for Research on Human Subjects of the Faculty of Medicine of the University of the Witwatersrand approved all studies, which were carried out in conformity with the principles embodied in the Declaration of Helsinki. The doses of radioactive isotope administered, either as extrinsic or as intrinsic tags for the food iron absorption studies, or as labelled iron salts for the reference iron absorption studies, ranged from 2,5 - 3,0 /uCi of 59Fe and from 3 - 5 /uCi of 55Fe. It was calculated that if all the isotope administered on each occasion had been retained the total radiation dose averaged over a period of 13 weeks would have been approximately 20% of the permissible whole body burden for continuous exposure in the case of 59Fe and 0,2% in the case of 55Fe (International Committee for

Radiological Protection 1960).

3.2 Haematologic and Chemical Methods

Haemoglobin estimations were performed by the cyanmethaemoglobin method.

Plasma iron concentrations were measured either by a modification (Bothwell and Finch 1962) of the method of Bothwell and Mallett (1955) or according to the method of the International Society for Haematology Iron Panel (I.C.S.H Iron Panel 1978). The method of Bothwell and Mallett is based on the stochiometric reaction of iron, dissociated from transferrin, and a suitable chromogen. The reaction is read by the intensity of the colour produced. Plasma is acidified to dissociate the iron; plasma proteins are precipitated with trichloroacetic acid and the precipitate is removed by centrifugation; aliquots of the supernates are added to a solution of sulphonated bathopenanthroline, as the chromogen, in the presence of saturated sodium acetate as buffer; the optical density of the sample is read at 535 nm and compared to a standard curve. The I.C.S.H. panel method has also used these three basic steps namely : (i) plasma or serum is acidified to dissociate the iron-transferrin complex; (ii) plasma proteins are then precipitated with a protein precipitant solution (10% trichloroacetic acid, 1M hydrochloric acid and 3% thioglycollic acid) and removed by centrifugation; (iii) finally a sensitive iron chromogen is added to the

supernatant to determine the iron concentration colorimetrically. The modifications introduced into this method, compared to the method of Bothwell and Mallett, include the use of thioglycollic acid which acts as a reducing agent and which prevents the co-precipitation of ferric iron together with the proteins. Such precipitation would otherwise occur in stored plasma if trichloroacetic acid alone were used. In addition, the reducing agent is added after acidification in order to avoid the release of iron from haemoglobin present in the plasma. The chromogen solution used consists of 1,5 M sodium acetate containing Ø,Ø25% bathophenanthroline sulphonate and the optical density of the solution is read in a spectrophotometer with the wavelength set at 535 nm against a blank of distilled water and compared to an iron standard. The standard is prepared from polished, certified, electrolytic iron wire dissolved in hydrochloric acid. All glassware and water used are iron free (< 1 /ug/dl) and all chemical agents are reagent grade with the lowest possible iron content. In this laboratory there is good agreement between the 2 methods so that the change from one method to the other was not felt to affect the interpretation of the results.

Unsaturated iron binding capacity (UIBC) was measured according to the method of Herbert and coworkers (1966). In this method radioactive ferrous ammonium sulphate of known specific activity is used to saturate the transferrin fully and, indeed, to exceed the transferrin binding capacity.

The unbound excess iron is removed by adding haemoglobin-coated charcoal particles which adsorb free iron, followed by centrifugation. The UIBC is determined by the residual amount of radioactivity in the supernate. The use of radioactive iron to saturate the transferrin is mandatory in this method because of iron contamination from the haemoglobin coated charcoal. The validity of this adsorbent method depends on at least three assumptions. Firstly it is assumed that the added iron binds quantitatively with any unsaturated transferrin, secondly, that the subsequent addition of adsorbent does not result in any iron being removed form the transferrin, and thirdly, that the adsorbent quantitatvely removes unbound iron. Some of these assumptions have been tested (Cook 197Ø) and both complete saturation of transferrin by radioactive iron as well as continued binding of the radioiron after addition of the adsorbent occur to a sufficient degree to make the method valid. It appears, however, that there may be isotopic exchange between the excess of unbound radioiron and native transferrin iron with increasing incubation time resulting in a gradual rise of the UIBC over a number of hours. The magnitude of the error has, under normal laboratory conditions, been estimated at about 3,4 percent (Cook 197Ø).

Measurement of desferrioxamine-induced urinary iron excretion was performed after the method of Lundvall and Weinfeld (1969) after desferrioxamine, 1Ø mg/Kg body weight, was injected intramuscularly.

The iron content of digested food samples was estimated by a modification (Bothwell and Finch 1962) of the method of Lorber (1927). The procedure involves wet ashing the test materials by heating in a mixture of concentrated nitric and sulphuric acid followed by decolorisation with $H_2Ø_2$. After transfer of the clear solution to a volumetric flask the pH of the sample is adjusted to above 4,Ø. Chromogen solution (disodium-4, 7 diphenyl-1, 1Ø phenanthroline disulphonate) is added to an aliquot of the sample and the absorbance is read in a spectrophotometer at 535 nm and compared to an iron standard. Both acid blanks and reagent blanks are used to enhance the accuracy of the method.

The ascorbic acid content of food samples was measured in metaphosphoric acid extracts of the relevant food by a method (Roe 1954) based upon the reduction of 2, 6 dichlorophenol indophenol (B.D.H. Chemicals Ltd., Poole, England).

Bone marrow biopsies were obtained using a Westerman Jensen needle and the specimens were stored in buffered formol saline prior to further processing. The wet weight of each specimen was obtained after all the excess liquid had been removed by careful blotting. The non-haem iron concentration was measured by the bathophenanthroline method of Torrance and Bothwell (1968). This method is based on acid extraction of the non-haem iron in the sample by incubating a small, weighed, piece of tissue for 2Ø hours at 65°C in a mixture of 3 N hydrochloric acid and Ø,61 M

trichloroacetic acid. During the incubation the tissue disintigrates leaving a small residue and a clear extract. An aliquot of the extract is used for colorimetric determination of the iron content using a mixture of disodium 4, 7 diphenyl-1, 10 bathophenanthroline (0,1 percent solution) and thioglycollic (mercaptoacetic) acid (1 percent solution). The absorbance of the sample is read in a spectrophotometer at a wavelength of 535 nm against a distilled water blank and compared to a reagent blank and an iron standard. For the size of the trephine biopsy samples that were generally obtained the volume of the acid mixture used to obtain the extract was 0,5 ml. A ratio of 0,4 ml of the extract together with 0,8 ml of the chromogen solution was found to give optimum sensitivity. Although there have been various suggestions regarding the expression of results (Weinfeld 1965) the weight of the entire needle biopsy specimen was used for the calculation of iron content. The results of a previous study from this laboratory (Brink et al 1977), in which good agreement was found between the iron content of trephine needle biopsy specimens from the right and from the left illiac crests in patients who had both areas biopsied, suggested that the marrow forms a fairly constant fraction of the total weight of the needle biopsy specimens and that meaningful measurements of the reticuloendothelial iron stores can be obtained in this way.

3.3 Plasma Ferritin Estimation

Two immunoassays for measuring ferritin were used to obtain the results presented in this thesis. The two-site radioimmunometric assay (IRMA) described by Miles and co-workers (1974) was used to measure plasma ferritin concentration in the studies described in Chapters 7 and 8 whereas in the other studies a radioimmunoassay was used.

In summary, the method used in the immunoradiometric assay (IRMA) includes the following steps: antiserum is raised in rabbits against human recrystallised ferritin; ferritin antibody is isolated by mixing immune serum with ferritin that has been previously rendered insoluble by allowing it to react with cyanogen bromide activated Sepharose 4B; after elution from the immunoadsorbent some of the specific IgG is labelled with ^{125}I by reaction with chloramine T; both labelled and unlabelled antibody are used in a "sandwich" technique or 2-site-IRMA in which the unknown antigen is sandwiched between solid phase unlabelled antibody and ^{125}I-labelled IgG. The solid phase is prepared by placing rabbit antiferritin serum in disposable polystyrene tubes. The labelled antibody becomes adsorbed to the surface and after rinsing the coated tube 200 /ul of ferritin standard or the unknown serum, diluted 1:20, is placed in the bottom of the tube and allowed to stand for 24 hours (reaction 1). During this phase the antigen becomes attached to the tube by binding with the solid phase antibody. After washing, ^{125}I-labelled antiferritin antibody is added to the tube for a further 24 to 28 hours,

(reaction 2), during which time the labelled IgG binds in proportionate amounts to the previously insolubilised antigen. After rinsing away any ^{125}I-labelled antiferritin antibody that has not reacted with the ferritin the radioactivity retained within the tube is estimated and is directly proportional to the amount of antigen present.

In the studies described in Chapters 4,5,6 and 9 the IRMA was replaced by a simpler, less time-consuming,radioimmunoassay. The method used was based largely on that of Deppe and co-workers (1978) and incorporated some of the steps discussed by Barnett and co-workers (1978). A detailed account of this radioimmunoassay has been published previously (Bothwell et al 1979). Briefly, the radioimmunoassay uses labelled antigen rather than labelled antibody and ^{125}I-labelled ferritin is mixed with 50 to 100 μl ferritin standard or the unknown plasma. Specific rabbit IgG antiferritin antibody is then added in amounts insufficient to bind fully the labelled ferritin present in the unknown plasma. In this way a competition for binding to the ferritin antibody is established between labelled ferritin and the ferritin in the test plasma. After incubation additional, but unlabelled, rabbit antiferritin antibody is added as carrier followed by an antiserum raised in sheep against rabbit immunoglobulin. During a second incubation the soluble ferritin-antiferritin complexes are precipitated by the sheep antiserum and are then removed by centrifugation. Since the rabbit

antiferritin has the same affinity for the labelled ferritin as it does for the unlabelled ferritin in the unknown plasma the resulting ferritin-antiferritin complex reflects the relative proportions of the two ferritins present in the original mixture.

There was good agreement between the IRMA and the RIA with a co-efficient of correlation of + Ø,98, r^2 = Ø,96, (MacPhail 1981). Regression analysis gave the relationship: RIA = Ø,81 x IRMA + 23,8, so that the results of plasma ferritin assays in the various studies were felt to be comparable.

3.4 Radioiron Absorption Methods

Iron absorption was determined either by means of an indirect method based on the incorporation of radioiron into the red cell mass or by means of whole body retention of ^{59}Fe using a whole body shadow shield counter.

When two isotopes were used to label separate dietary iron pools the indirect method was used. Duplicate 1Ø ml blood samples were collected prior to the administration of isotope and again 2 weeks later. Blood samples and aliquots of the radioactive foods were prepared for differential radioactive counting by the method of Katz and coworkers (1974). In this method, wet ashing of the food or blood sample is first carried out in a mixture of concentrated sulphuric and nitric acid. The solubilized iron is then precipitated with ammonium hydroxide, redissolved in acid,

and transferred to counting vials. This iron is then precipitated and redissolved again before scintillant is added to the counting vials. The quantities of ^{55}Fe and ^{59}Fe in the processed samples were determined by means of a liquid scintillation system (Insta-Gel, Packard Instrument Company, Downers Grove, Illinois) and a Packard Tri-Carb AAA spectrometer (model 3375) which automatically adjusts for quenching. The counting efficiency was 24% for ^{55}Fe and 42% for ^{59}Fe at optimal gain and window settings.

In those studies which involved the administration of ^{59}Fe only the radioactivity in duplicate 4 ml blood samples, collected immediately before and 2 weeks after the administration of radioactive tracer and carrier iron, was measured in a Packard Autogamma Tri-Carb (model 3001) spectrometer. Because of the characteristics of ^{59}Fe no sample preparation is required when only this isotope is being measured and radioactivity can be conveniently determined using a well type NaI scintillation crystal.

Percentage radioactive absorption of both 55 and 59 Fe labelled iron was calculated on the assumption that 100% of the absorbed radioactivity was present in the haemoglobin of circulating red cells and that the blood volume of each subject was 65 ml/Kg. The assumption of 100% utilization is valid when absorption studies are carried out in iron-deficient subjects who are avid for iron.

In those studies where absorption of a readily available iron salt was related to bone marrow storage iron

content and to radioiron turnover (Chapters 4 and 5) and where there was a wider range of body iron content, or renal failure was present, the radioiron absorption was assessed by the whole body retention of ^{59}Fe 14 days after the oral administration of ferrous ascorbate labelled with ^{59}Fe. After an overnight fast the background radioactivity of each patient was measured in a New England Nuclear Enterprises Shadow Shield Whole Body Counter (NE 8108, New England Nuclear Enterprises Limited, Sighthill, Edinburgh, Scotland). This counter consists of opposing, partially shielded, collimated NaI crystal detectors placed entirely within the "shadow" of a lead shield and the subject moves relative to the detector during the counting procedure by means of a motorised carriage which slowly traverses the scanning field. After measurement of background radioactivity, due to naturally occuring 40 K, the radio labelled ^{59}Fe iron salt was administered orally. The test dose consisted of 3 mg iron as ferrous sulphate in 3 ml 0,01 N HCl to which 30 mg ascorbic acid, freshly dissolved in 50 ml distilled water, was added together with 2 - 5 /uCi ^{59}Fe in 0,1 N HCl. Two rinsings of the plastic beaker with distilled water were also drunk. Immediately afterwards the radioactivity present in the patient was measured again and 3 hours later it was measured for the third time. The variation between the latter 2 readings was found to be not more than 2,4 percent and for convenience the first of the two measurements was used. No food or fluid was allowed for

4 hours after the administration of iron. After 14 days the radioactivity in each patient was measured for the fourth time and the percentage absorption was calculated. Corrections for radioactive decay were made by counting on each occasion a suitable ^{59}Fe standard in a plastic vial. The whole body retention method has the advantage of not being dependant on iron utilisation. This method has been compared to the indirect methods by other authors notably by Heinrich (1970) and is felt to be the most accurate method currently available.

Chapter 4

The Relationship between Marrow Iron Stores, Plasma Ferritin Concentration and Iron Absorption

Some of the evidence which indicates that the level of iron store is a major determinant of iron absorption has already been reviewed in Chapter 1. Important early studies in this regard were those of Bothwell and co-workers (1958), Pirzio-Biroli and Finch (1960) and Kuhn and co-workers (1968). These studies provided convincing evidence that iron absorption is increased in states of iron depletion and diminished with storage iron excess. Further insight into this relationship has been obtained in recent years by using the plasma ferritin concentration as a measure of the quantity of iron stored in the body. In 5 such studies an inverse correlation has been noted between the plasma ferritin concentration and the percentage iron absorption (Cook et al 1974; Disler et al 1975; Walters et al 1975; Charlton et al 1977; Heinrich et al 1977). However, the degree of correlation obtained in these studies was not particularly close and since the study populations exhibited a comparatively narrow range of iron stores it was decided to try to define the relationship over a wider range using several indices of iron status. The indices studied included the plasma ferritin concentration and the concentration of non-haem iron in the marrow as well as

haematologic indices including haemoglobin concentration, plasma iron and percentage saturation.

4.1 Subjects and Methods

Sixty five patients (55 white and 10 black) attending the Haematology Clinic of the Johannesburg Hospital were studied. Appart from the Hb concentration, erythrocyte count and PCV, the following tests were carried out: bone marrow non-haem iron concentration, plasma iron concentration and iron binding capacity, and plasma ferritin concentration. There were 36 males and 29 females. The percentage absorption from a 3 mg dose of ferrous iron was measured in 50 patients (40 white and 10 black, 28 males and 22 females). A further 15 patients (8 males and 7 females) underwent the investigation of iron status but without having absorption studies performed.

Iron deficiency was diagnosed in 18 patients on the basis of a Hb concentration below 11 g/dl, morphological evidence of iron deficient erythropoiesis and absence of stainable iron on the bone marrow aspirate. The cause of the iron deficiency was peptic ulceration with gastrointestinal haemorrhage in 5 patients, chronic aspirin ingestion in 2 patients, menorrhagia in 4 patients and gastrointestinal blood loss of undertermined origin in another 4 patients. No cause for the iron deficiency was found in the remaining 3 patients.

The next largest group consisted of 13 patients with myeloproliferative disorders, all of whom were in a stable

phase of the disease at the time of study. There were 6 patients with polycythaemia vera, 3 of whom had undergone previous venesection therapy; 5 patients with myelofibrosis; and 2 patients with chronic myelogenous leukaemia who were in peripheral blood remission at the time of study. Four of the patients with myeloproliferative disorders were on maintenance therapy with busulphan.

The third group consisted of 1Ø patients who were suffering from various lymphomas. Therapy for the underlying disease had been completed at least 3 months previously and peripheral blood indices were normal. The study was performed in these two groups when routine marrow surveillance was due.

Six patients were suffering from rheumatoid arthritis and anaemia. In 5 the anaemia was due to iron deficiency and was presumed to be the result of gastrointestinal blood loss due to longterm ingestion of anti-inflammatory drugs, since no other cause could be found. The remaining patient showed the features of the so-called anaemia of chronic disease, with a low plasma iron concentration and iron-binding capacity but plentiful stainable iron on bone marrow aspiration. Another 2 patients also had the features of the anaemia of chronic disease and joint symptoms but the nature of the rheumatic complaint had not been defined despite extensive investigation.

There were 4 patients with idiopathic haemochromatosis. The diagnosis was based on the classical clinical and

laboratory findings, including severe parenchymal iron overload on liver biopsy and a 24 h urinary iron excretion of greater than 8 mg after the injection of desferrioxamine. Three of these patients had had at least 30 l blood removed prior to study, while only 10 l of blood had been removed from the fourth. Unlike the others he still had a raised plasma iron concentration, with almost complete saturation of the plasma transferrin. Venesections were delayed in all 4 patients for at least 3 weeks prior to study. Another 2 patients were relatives of one of the haemochromatotic subjects. Neither of them showed the phenotypic features of haemochromatosis, neither had been venesected or had any other treatment and desferrioxamine induced urinary iron excretion was only mildly elevated, amounting to 3,1 mg of iron in one subject and 3,4 mg in the other.

Six other patents with iron overload were studied. Five were males (3 black and 2 white) with a history of heavy consumption of home brewed alcoholic beverages which are known to contain large amounts of iron (Bothwell et al 1964), and 1 was a white female heterozygous for thalassaemia.

The final 4 patients had localised squamous carcinomas of the extremities. Metastatic carcinoma was excluded by radioisotopic scanning and bone marrow biopsies were performed as part of an assessment of the value of this procedure in the staging of solid tumours.

The methods used in this investigation are described in

detail in Chapter 3. Briefly the plasma iron concentration was measured according to ICSH panel method (1978) and the plasma ferritin concentration was estimated according to the method of Deppe et al (1978). Iron absorption was measured by the whole body retention of ^{59}Fe as determined by the use of a whole body Shadow-Shield Counter. Marrow non-haem iron content was measured by the method of Torrance and Bothwell (1968).

4.2 Results

As was to be expected from the patients chosen for study there was a wide range in the results of the various measurements. The Hb concentrations varied between 6,9 g/dl and 16,2 g/dl, the plasma iron concentrations between 21,1 /ug/dl and 241,6 /ug/dl, the transferrin saturations between 4 percent and 91,5 percent, the plasma ferritin concentrations between less than 1 /ug/l and 5000 /ug/l, the marrow non-haem iron concentrations bewteen 14,4 /ug/g and 2019 /ug/g, and iron absorptions between 3,2 percent and 93,7 percent. With the exception of the Hb concentrations the data did not follow normal distributions.

The theoretical model $y = \alpha\ x^b$ seemed to be the best expression of the relationship between these variables. Since the model is non-linear a log transformation was applied to the variables and the following linear equation was fitted.

$$\log (y) = \log \alpha + \beta \log (x)$$

Using this model significant negative correlations were found between iron absorption and each of the other measurements (Table 2), particularly noteworthy being those with the marrow non-haem iron concentration (Figure 1) and with the plasma ferritin concentration (Figure 2).

Further statistical analysis revealed that 88 percent of the variation in log percentage iron absorption could be explained by variation in the amount of storage iron ($r = - Ø,94$, $r^2 = Ø,884$). Somewhat less of the variation (60%) was explained when plasma ferritin was considered as the variable ($r = -Ø,78$, $r^2 = ,6Ø8$).

In addition there was a significant correlation between marrow iron (y) and plasma ferritin (x) ($r = + Ø.84$, r^2, Ø,7Ø6, $p < Ø,ØØ1$) (Figure 3). This indicates that 7Ø% of the variation in bone marrow iron can be explained by the variation in plasma ferritin.

The fitted regression line is:

log (bone marrow iron) = 1,319 + Ø,442 log (ferritin)

4.3 Discussion

While the results of previous studies have established that there is an inverse relationship between the size of the body iron stores and iron absorption the nature of this relationship has not been precisely defined over a wide range of iron stores. In some studies the assessment of the size of the iron stores has been imprecise or indirect and other variables have been present. For example, in a previous study of Indian women living near Durban the

Table 2 Correlation between various measurements of iron nutrition using Pearson correlation coefficients

	Haemoglobin concentration (g/dl)	Log plasma iron concentration	Log % transferrin saturation	Log total iron binding capacity	Log plasma ferritin concentration	Log marrow non-haem iron concentration
Log % iron absorption	r - 0,30 P < 0,02	r - 0,56 P < 0,001	r - 0,65 P < 0,001	r + 0,40 P < 0,002	r - 0,78 P < 0,001	r - 0,94 P < 0,001
Hb concentration (g/dl)		r + 0,70 P < 0,001	r + 0,60 P < 0,001	r - 0,001 P < 0,4	r + 0,32 P < 0,01	r + 0,37 P < 0,005
Log plasma iron concentration			r - 0,91 P < 0,001	r + 0,15 P < 0,1	r + 0,64 P < 0,001	r + 0,62 P < 0,001
Log % transferrin saturation				r - 0,54 P < 0,001	r + 0,68 P < 0,001	r + 0,69 P < 0,001
Log total iron binding capacity					r - 0,38 P < 0,005	r - 0,40 P < 0,002
Log plasma ferritin concentration						r + 0,84 P < 0,001

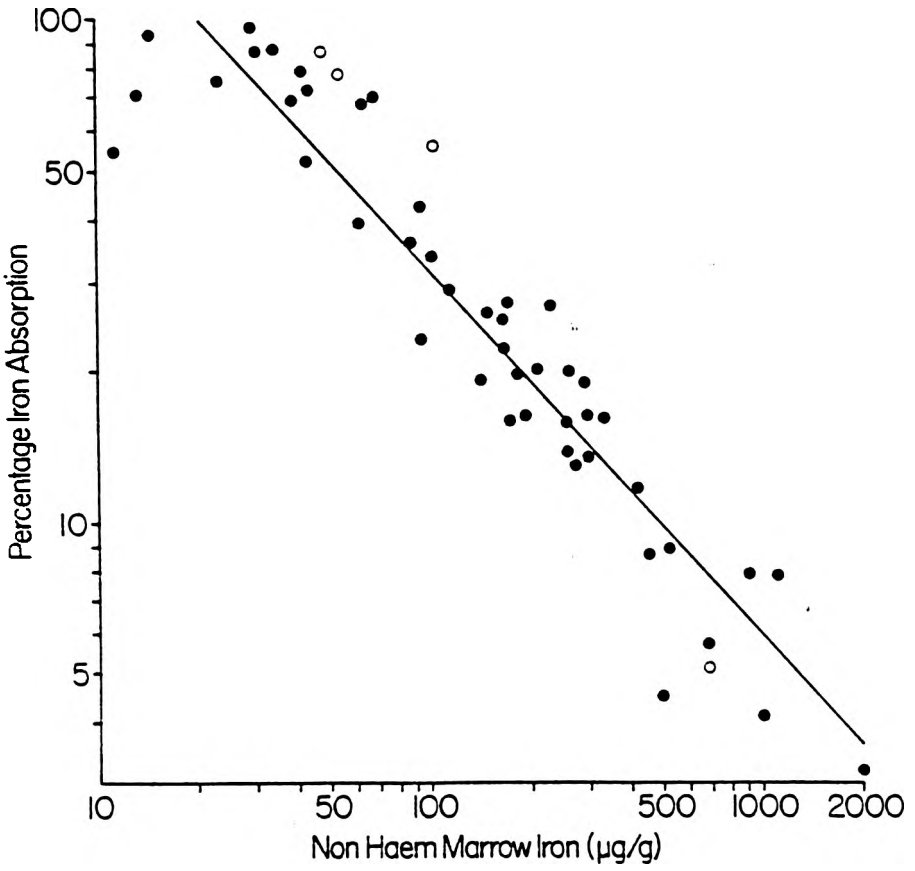

Figure 1. Relationship between the percentage absorption of a 3 mg dose of ferrous iron and the marrow non-haem iron concentration in a group of 50 subjects. (The open circles represent 4 patients with idiopathic haemochromatosis).

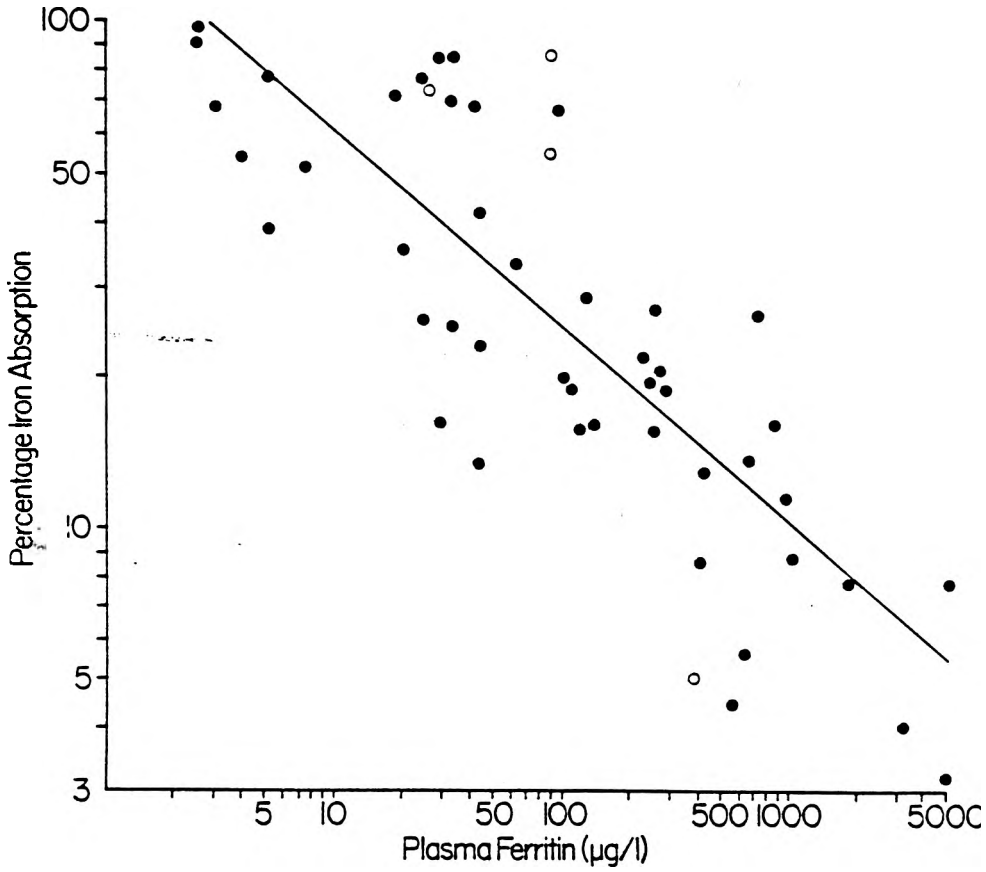

Figure 2. Relationship between the percentage absorption of a 3 mg dose of ferrous iron and the plasma ferritin concentration in a group of 50 subjects. (The open circles represent 4 patients with idiopathic haemochromatosis).

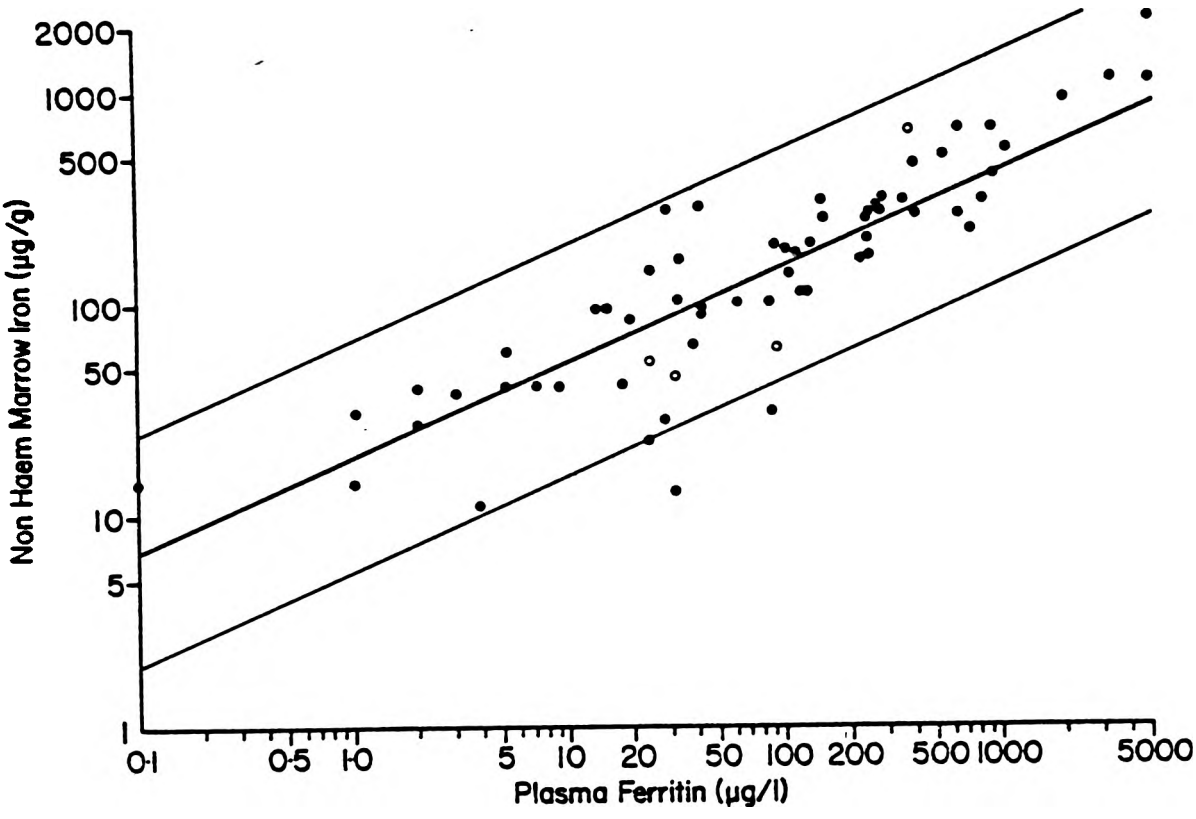

Figure 3. Relationship between plasma ferritin concentration and the marrow non-haem iron concentration in 50 subjects. (The open circles represent 4 patients with idiopathic haemochromatosis).

correlation coefficient between the log of the plasma ferritin concentration (which ranged between Ø and 35Ø /ug/l) and the percentage iron absorption was only Ø,43 ($p < Ø,ØØ1$) (Charlton et al 1977). The reason for the relatively poor though significant correlation could not be explained with certainty but malabsorption of iron could have played a part. In the present study the concentration of non-haem iron in trephine specimens of bone marrow provided a direct measure of the size of the reticuloendothelial iron stores. While malabsorption was not excluded the effects of intraluminal factors were minimised by administering 3 mg iron in the form of ferrous ascorbate while the subjects were fasting and any inaccuracies which might have been introduced into the previous study by variations in the red cell utilisation of the absorbed radioiron were eliminated by the use of a whole body counter.

A strong negative correlation was found between the log concentration of non-haem iron in the bone marrow and the log percentage iron absorption ($r = -Ø,94$; $p < Ø,ØØ1$). In previous studies the data have suggested that the relationship between iron stores and iron absorption is a log normal one but iron absorption continues, albeit at a very low rate, in the presence of the grossly increased stores found in some of the subjects in the present study. A log log relationship therefore seems more appropriate over a wide range of body iron stores, from grossly depleted to

haemochromatotic levels.

Although the present study confirms the direct relationship between plasma ferritin concentration and marrow non-haem iron content, the results indicate that considerable variation of the marrow iron concentration may be present within the normal range of plasma ferritin concentration.

For the equation:

$$\log (y) = \log \alpha + \beta \log (x)$$

the $1-\alpha$ confidence intervals for b's were calculated using the t-table at n-2 degrees of freedom, as follows:

$$(1)\quad b - t(1-\alpha/2;\ n-2)\ s(b) \leq \beta \leq t\ (1-\alpha/2;\ n-2)\ s(b) + b.$$

At a significance level of $\alpha = 0{,}05$ the confidence bounds for b_o can be calculated by substituting the values:

$b_o = 1{,}319$

$t\ (0{,}975;\ 63) = 1{,}998$

$s(b_o) = 0{,}07$

into equation (1) which gives

$$1{,}319 - (1{,}998)\ (0{,}007) \leq b_o \leq (1{,}998)\ (0{,}07) + 1{,}319$$

$$1{,}179 \leq b_o \leq 1{,}459$$

Then in the same way for b_1 substituting

$b_1 = 0{,}449$

$t(Ø,975;\ 63) = 1,998$

$s(b_1) = Ø,Ø45$

into equation (1) gives:

$$Ø,442 - (1,998)\ (Ø,Ø34) \leq b_1 \leq (1,998)\ (Ø,Ø34) + Ø,442$$

$$Ø,374 \leq b_1 \leq Ø,5Ø9$$

The resulting confidence bounds indicate that b_o can range from 1,179 - 1,459 and b_1 from Ø,374 - Ø,5Ø9.

Whenever a new prediction is made for a given value of ferritin, using this equation, the variation of bone marrow iron for that particular ferritin level has to be taken into consideration. Using the estimated variation, confidence limits can be calculated for the predicted value. The standard deviation of a predicted value is given by the formula:

$$(2) \quad S(y') = MSE\left\{1 + 1/n + \frac{(x' - x)^2}{\Sigma (x_i - x)^2}\right\}^{1/2}$$

where

MSE = mean square error in the regression analysis

n = sample size

x' = the new x value on which the prediction is based

x = the mean of the sample used in estimation

$\Sigma (x_i - x)^2$ = the sum of the squares of the x values used in the estimation.

The 1- α confidence limits for the new predicted value of y', are then given by:

$$(3)\quad \hat{y} - t\,(\alpha/2;\ n-2)\ s(y') \leq y' \leq t(\alpha/2;n-2)\ s(y') + \hat{y}$$

where $t(\alpha/2;\ n-2)$ is a value from the t table at n-2 degrees of freedom and $\alpha/2$ significance level and y is the predicted value from the regression equation.

For example, if the observed value of plasma ferritin is 10 µg/l then by substituting in equations (2) and (3) the upper and lower 95% confidence limits for the prediction of bone marrow non-haem iron content can be calculated to be 23 and 263 µg/g respectively. Similarly, if the observed ferritin value is 100 µg/l then the 95% confidence limits for bone marrow iron content are 47-550 µg/g.

These predictions were made on the assumption that the relationship between plasma ferritin and bone marrow iron content remained constant over the range studied. Although it is known that plasma ferritin is inappropriately raised in relation to body iron stores in certain conditions such as acute liver disease and disseminated neoplastic disorders, there is no evidence that this occurred in the present study, so that the values are believed to reflect storage iron content.

In view of the relationships between marrow iron content and plasma ferritin on the one hand and marrow iron content and absorption on the other hand, plasma ferritin is also a predictor of iron absorption, although statistical analysis of the data reveals that absorption can vary considerably among subjects with similar plasma ferritin

values. The addition of the other haematologic indices such as plasma iron, percentage saturation and haemoglobin level as covariates did not greatly improve the significance of the correlations.

The relatively poor though significant negative correlation between the log percentage iron absorption and the Hb concentration can be ascribed to the fact that the commonest cause of anaemia in the group studied was iron deficiency. This was reflected by the significant correlation between the Hb concentration and 4 of the 5 other measurements of iron status.

Early experimental studies suggested that the rate of erythropoiesis was the second major determinant of iron absorption. However, the weight of evidence obtained in human studies indicates that this is not so and iron absorption has been found to be normal in the great majority of chronic haemolytic states (Labardini et al 1973). The present results lend further support to the concept that the rate of erythropoiesis does not greatly influence iron absorption, since absorption levels were appropriate to the concentrations of storage iron in a variety of miscellaneous blood disorders. Even more persuasive evidence has recently been reported by Eschbach et al (1977) who found absorption levels in keeping with iron stores over a wide range in subjects with the markedly decreased red cell production that results from chronic renal failure. Further aspects of iron absorption in renal failure will be discussed in

Chapter 5.

There are two situations in which iron absorption has been shown to be inappropriately high in relation to the body store of iron. The first includes all those conditions characterised by excessive but ineffective erythropoietic activity, such as thalassaemia major and the other is the genetic disorder, idiopathic haemochromatosis. However, it should be stressed that no study has thus far been reported in which the relationship between stores and iron absorption has been systematically delineated in these conditions. Although there were 4 patients with idiopathic haemochromatosis in the present study, 3 of them had been fully treated by repeated venesections and were actually iron-deficient at the time of study. It has previously been reported that reticuloendothelial iron stores are inappropriately low in relation to the amount of iron deposited in parenchymal cells in this disorder (Brink et al 1976) and if the relationship between iron stores and absorption is to be defined in these patients in future studies it will be necessary to correlate absorption with the concentration of non-haem iron in a tissue such as liver instead of the bone marrow.

While the results of the present study emphasise the critical influence that the body iron stores exert on the absorption of iron they have not defined how this control is exercised. Significant negative correlations were noted between several factors operating in the plasma, including

the plasma iron concentration, the total iron-binding capacity, the percentage saturation of the transferrin and the ferritin concentration, but there is currently no convincing evidence to suggest that any of these factors directly modify the absorptive behaviour of the intestinal mucosal cells.

4.4 Summary

The percentage absorption from a 3 mg dose of ferrous iron was measured in subjects with iron stores that varied over a wide range. Iron status was assessed by a number of measurements, including the haemoglobin concentration, the plasma iron concentration, the total iron-binding capacity, the plasma ferritin concentration and the concentration of non-haem iron in the bone marrow. There were good inverse correlations between the log percentage iron absorption and both the log marrow non-haem iron concentration ($r = 0{,}94$; $p < 0{,}001$) and the log plasma ferritin concentration ($r = 0{,}78$; $p < 0{,}001$). In addition, there was a positive correlation between the marrow non-haem iron concentration and the plasma ferritin concentration ($r + 0{,}84$; $p < 0{,}001$). These results suggest that reticuloendothelial iron stores represent an important determinant of iron absorption and that their size can be gauged from the plasma ferritin concentration.

Chapter 5

Iron Absorption in Patients on Regular Dialysis Therapy

Although the previous investigation confirmed the major role of iron stores in determining iron absorption, the effect of erythropoietic rate on iron absorption still required some elucidation. After all, one of the major functions of iron is its activity in haemoglobin formation and oxygen transport. There is experimental evidence in rats that the rate of erythropoiesis does influence iron absorption (Bothwell et al 1958).

The anaemia of chronic renal failure is a hypoproliferative one (Eschbach et al 1967; Adamson et al 1968) and it did not, therefore, appear surprising when the results of several studies in man indicated that iron absorption was depressed in the presence of renal failure (Boddy et al 1970; Fam et al 1976; Blumberg et al 1971; Lawson et al 1971). These findings have not, however, been supported by more recent reports in which it has been shown that the rate of iron absorption is not affected by erythropoietic activity but is inversely related to the size of the body iron stores, both in normal subjects (see also Chapter 1 & Chapter 4) and in patients with chronic renal failure (Comty et al 1968; Eschbach et al 1970; Brozovitch et al 1971; Koch et al 1975; Eschbach et al 1977; Milman and Larsen 1976; Gokal et al 1979). These findings have

practical implications, since they suggest that the iron deficiency that can occur in patients on regular maintenance dialysis as a result of blood loss into the dialyser can be treated effectively using oral iron supplements.

In the present study more insight was obtained into the problem by assessing and correlating a number of variables in patients with chronic renal failure. These included: (a) erythropoietic activity using ferrokinetic measurements; (b) iron stores using plasma ferritin measurements, and (c) iron absorption at physiological and at therapeutic quantities of iron.

5.1 Subjects and methods

Fifteen patients (10 males and 5 females), who were undergoing maintenance haemodialysis therapy for chronic renal failure, were studied. All were being dialysed for 5 h, 2 - 3 times weekly,using either an automatic delivery system (Drake Willock or Cobe monitors) or a recirculating single pass delivery system (Travenol). The dialysers that were used were either flat plate (Gambro or Vivacell Ultraflow) or coil (Travenol). The patients had been on this therapy for between 9 and 22 months prior to study. Control was good in terms of blood pressure, fluid balance and biochemical stability and 12 were being dialysed at home. They were being maintained on a daily diet containing 1-1,2 g/kg protein and all but 1 had been on iron supplements. Iron supplementation was discontinued at least 3 weeks prior to the study. All but 3 patients had received

blood in the past but none was given for at least 3 weeks before and 2 weeks after the study.

The absorption of iron from a 3 mg dose of ferrous salt was measured in all subjects and a plasma iron turnover study was performed on the same day as the oral iron was administered. Seven subjects later had a repeat absorption test done in which the dose of iron was increased to 50 mg. Whole body retention of radioiron was again used to measure iron absorption (Chapter 3). On the same day as the absorption study was carried out, 2,5 μCi ^{55}Fe in 0,005 N HCl was bound to 0,05 ml 4% sodium citrate in 5 ml N saline and was then administered intravenously. Blood samples were taken regularly over the next several hours and the radioactive counts in each sample were determined in duplicate 1-ml plasma samples which were digested for counting of ^{55}Fe activity using the method of Katz and co-workers (1964) and counted in a Packard Tri-Carb AAA spectrometer (Model 3375). The plasma iron turnover was calculated from the plasma iron concentration and the half time (T 1/2) clearance of radioiron from the plasma, using standard formulae, and was expressed as mg iron/dl blood/day (Bothwell et al 1979). Suitable corrections were then applied in order to obtain the standard erythron iron turnover, which is an estimation of the proportion of the plasma iron turnover being utilised for erythropoiesis (Bothwell et al 1979). The absorption of iron from a 50-mg dose of ferrous sulphate was carried out in identical

fashion to that for the 3-mg dose, except for the fact that no added ascorbic acid was used in this part of the study.

5.2 Results

Haemoglobin concentrations of the patients varied between 5,8 and 14,9 g/dl (mean 8,8 g/dl) (Table 3). The plasma iron concentration varied between 64 and 172 /ug/dl (mean 89 /ug/dl), the transferrin saturation between 21 and 68% (mean 35%), the plasma ferritin concentration between 12 and 690 /ug/dl (geometric mean 173 /ug/dl), and iron absorption (3 mg dose) between 7,2 and 72% (geometric mean 20,5%). The T 1/2 of injected radioiron varied between 58 and 354 min (mean 111 min). The plasma iron turnover varied from 0,40 to 0,89 mg/dl blood/day, while the standard erythron iron turnover varied from 0,06 to 0,73 mg/dl blood/day.

The relationship between the haematological indices, the indices of iron status and the absorption of radioiron were calculated (Table 4). A significant negative correlation between the log of the plasma ferritin concentration and the absorption of the iron salt was noted at both dosage levels. None of the other indices of iron status correlated significantly with iron absorption.

In another comparison, the results for log iron absorption obtained with the 3-mg dose were plotted against log plasma ferritin concentrations (Fig. 4). In addition, similar results obtained in 50 subjects with normal renal function (described in Chapter 4) were also plotted on the

Table 3 Haematological, ferrokinetic and iron absorption data on the 15 patients studied

Patient No.	Haemoglobin (g/dl)	Plasma iron (µg/dl)	Saturation (%)	Plasma ferritin (µg/l)	Plasma radioiron half-time (min)	Plasma iron turnover (mg/dl blood/day)	Standard erythron iron turnover (mg/dl blood/day)	Iron absorption (mg)	
								3-mg dose	50-mg dose
1	8,0	64	24	210	65	0,77	0,62	0,67	3,45
2	9,9	67	28	500	64	0,77	0,63	0,22	0,55
3	9,1	70	30	690	58	0,89	0,73	0,35	1,95
4	7,9	79	35	270	89	0,69	0,50	0,44	-
5	7,6	68	25	53	115	0,47	0,31	2,06	-
6	5,8	72	31	105	150	0,40	0,21	0,93	7,35
7	14,9	72	32	12	52	0,78	0,66	2,17	10,85
8	11,1	172	69	500	354	0,40	0,06	0,22	0,45
9	11,1	135	56	90	100	0,94	0,66	1,93	-
10	5,8	76	26	290	82	0,77	0,58	0,35	-
11	6,7	68	21	94	79	0,70	0,53	0,98	-
12	12,5	68	32	230	95	0,46	0,33	0,62	3,15
13	5,8	94	45	555	103	0,76	0,52	0,31	1,1
14	6,8	135	47	680	144	0,76	0,43	0,32	0,55
15	8,9	89	23	27	122	0,55	0,35	0,83	-
Normal range	13,0-16,0	50-150	25-40	20-200	60-120	0,6-0,8	0,4-0,6	-	-

Table 4 Correlation between iron absorption and indices of iron status

	Absorption 50 mg iron (mg)	Haemoglobin concentration (g/dl)	Plasma iron concentration (µg/dl)	Transferrin saturation (%)	Log plasma ferritin concentration (µg/dl)
Absorption 3 mg iron (mg)	r + 0,95 P < 0,001	r + 0,39 P > 0,05	r - 0,12 P > 0,1	r - 0,11 P > 0,1	r - 0,83 P < 0,001
Absorption 50 mg iron (mg)		r + 0,40 P > 0,1	r - 0,44 P > 0,1	r - 0,41 P > 0,1	r - 0,96 P < 0,001
Haemoglobin concentration (g/dl)			r + 0,14 P > 0,1	r + 0,24 P > 0,1	r - 0,37 P > 0,05
Plasma iron concentration (µg/dl)				r + 0,92 P < 0,001	r + 0,26 P > 0,1
Transferrin saturation (%)					r + 0,35 P > 0,1

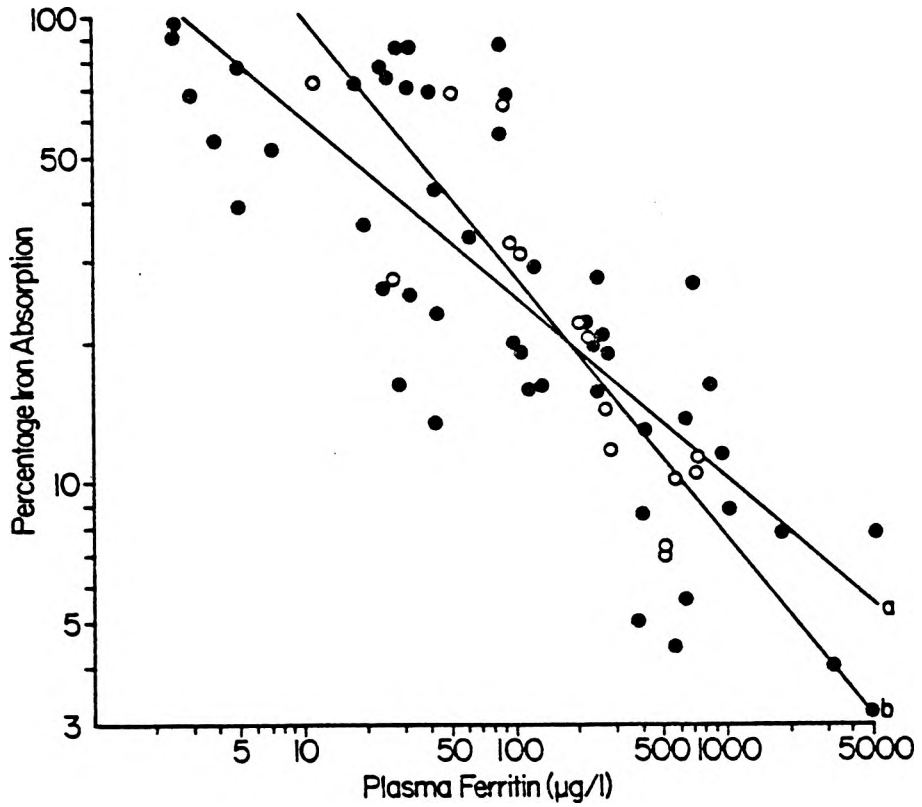

Figure-4. The relationship between the percentage absorption of a 3-mg dose of ferrous iron and the plasma ferritin concentration in a group of 50 subjects with normal renal function (closed circles) and in 15 patients on regular dialysis therapy (open circles). The regression line (a) for the subjects with normal renal function was: log ferritin = 0,14 log % iron absorption +0,40, while that for the patients on regular dialysis therapy (b) was: log ferritin = -0,17 log % iron absorption +0,44. Using analysis of variance, there was no significant difference between these two lines.

same graph. It was apparent that the relationship between iron absorption and the ferritin concentrations was the same in the two studies, thus indicating a normal absorptive mechanism in the patients with chronic renal failure. In a third analysis, a comparison was made between the amounts of iron absorbed from the 3-mg dose of iron and the 50-mg dose of iron. In order to find out whether this relationship was a normal one, it was compared with figures previously published in the literature, which had been obtained in normal subjects using differing doses of iron and which were collected together in graphical form (Bothwell et al 1979). Using covariance analysis, the relationship between the amount of iron administered and the amount absorbed in patients on regular dialysis therapy (log iron absorption = 0,48 log dose iron + 0,55) did not differ significantly from that in normal subjects (log iron absorption = 0,68 log dose iron + 0,54).

In a final analysis (Table 5), iron absorption at the 3-mg dose, was compared with various indices of erythroid activity. As might be expected, several of these indices correlated in various ways with each other but none of them, including the standard erythron iron turnover which is a measure of total erythroid activity, showed a significant correlation with iron absorption.

5.3 Discussion

The positive correlation between plasma ferritin and marrow iron concentrations and the negative correlation

Table 5 Correlation of iron absorption with indices of marrow erythroid activity

	Plasma iron concentration (µg/dl)	Plasma radioiron half-time (min)	Plasma iron turnover (mg/day/dl whole blood)	Standard erythron iron turnover (mg/day/dl whole blood)
Absorption 3 mg iron (mg)	r - 0,12 P > 0,1	r - 0,23 P > 0,1	r - 0,22 P > 0,1	r + 0,11 P > 0,1
Plasma iron concentration (µg/dl)		r + 0,80 P < 0,001	r + 0,79 P < 0,001	r - 0,40 P > 0,05
Plasma radioiron half-time (min)			r - 0,60 P < 0,05	r - 0,82 P < 0,01
Plasma iron turnover (mg/day/dl whole blood)				r - 0,82 P < 0,00

between each of these indices of storage iron and the rate of iron absorption has been discussed in Chapter 1, and again in Chapter 4. In the present investigation the plasma ferritin concentration, which was used as an index of the size of the stores, was compared with the rate of iron absorption in subjects with chronic renal failure. The inverse relationship which was found was virtually identical with the one previously noted in subjects with normal renal function. These findings, therefore, confirm recent reports from other workers (Eschbach et al 1970; Eschbach et al 1977) and underline the fact that the amounts of iron absorbed by subjects with chronic renal failure are appropriate to their storage iron status. In the present study a further point was established. It was possible to show that the normal inverse relationship between iron stores and the rate of iron absorption did not only occur with physiological doses of iron but also did so when a therapeutic dose of 50 mg was fed. This finding has some practical relevance, since it implies that iron-deficient subjects with chronic renal failure can be treated effectively with oral iron. While there have been some previous clinical studies in which improvement of haemoglobin levels has been noted on oral therapy (Wallace 1971; Strickland et al 1974; Baker et al 1975; Winney et al 1977), parenteral iron is still widely used by renal units throughout the world. The present results strongly suggest that equally satisfactory results can be obtained with oral

therapy and while it has been argued that patient compliance can be adversely affected by side effects, such side effects are dose related and can usually be eliminated by reducing the dose (Bothwell et al 1979). Oral treatment has the added advantage that it does not lead to iron overload, since the rate of iron absorption is regulated to match body needs. In contrast, iron overload has been shown to be a real hazard when parenteral iron is administered repeatedly to patients with chronic renal failure (Gokal et al 1979).

The ferrokinetic measurements which were carried out on all the subjects showed a uniform pattern, with an inadequate erythropoietic response to the stimulus of anaemia. Within the group, however, there were some variations in the degree of erythropoietic depression. No correlation was noted between total erythropoietic activity, as measured by the standard erythron iron turnover, and the rate of iron absorption. The fact that absorption was appropriate to the size of the body iron stores even in those subjects with virtually absent erythropoietic activity can be taken as strong evidence that the rate of iron absorption is not normally influenced by the rate of erythropoiesis to any significant degree.

5.4 Summary

Various haematological, ferrokinetic and iron absorption measurements were carried out on 15 patients with chronic renal failure who were undergoing regular haemodialysis. There was no evidence that the rate of iron

absorption was impaired significantly by the depressed erythropoietic activity present in these patients. In contrast, there was a significant inverse correlation between the rate of iron absorption and the plasma ferritin concentration, which has been shown to be an index of the size of the iron stores. This relationship, which was shown with both small (3 mg) and large (50 mg) doses of ferrous iron, was no different from that previously noted in subjects with normal renal function. These results suggest that iron deficiency in patients with chronic renal failure undergoing regular haemodialysis can be adequately prevented or treated with oral iron therapy, since the absorptive mechanism for iron appears to be normal.

Chapter 6

Patterns of Food Iron Absorption in Iron Deficient White and Indian Subjects and in Venesected Haemochromatotic Patients

The source of the body iron is the diet. Dietary constituents thus play an important role in iron economy and during the last few years considerable progress has been made in understanding the factors which influence the absorption of iron from the diet. The concept of dietary iron pools and the measurement of absorption from both the pools has been dealt with in Chapter 1.

In addition to the intraluminal factors which affect the absorption of food iron, the behaviour of the intestinal mucosa itself has also to be considered. Normal individuals assimilate only enough iron to replace losses, and this delicate balance is largely maintained by variations in the rate of absorption. If the body is short of iron the absorption rate is increased, while the opposite occurs when body stores are larger than normal. In certain individuals, however, the controlling mechanisms fail and a state of positive balance persists until the tissues are greatly overloaded with iron. The prime example of this is the genetic disease idiopathic haemochromatosis, and in spite of investigation by a number of workers, the reason for the inappropriate absorption of iron has not been established. The biochemical lesion could theoretically operate at either

the luminal or the mucosal level. While there is some information concerning the absorption of non-haem food iron in this disease (Chodos et al 1957; Pirzio-Biroli et al 1958; Smith et al 1969; Powel et al 1970; Walters et al 1975), there is no reported study in which the total absorption of iron from a mixed diet containing both haem and non-haem iron has been categorized. Since it is now possible to do this by labelling differentially with ^{55}Fe and ^{59}Fe the haem and non-haem iron pools, a study was undertaken in a group of patients suffering from idiopathic haemochromatosis. The majority were investigated at a time when body iron stores had been greatly depleted by repeated venesections, as it was felt that more valid information about the underlying disorder of iron balance could be obtained if the absorptive pattern was not being modified by the presence of massive iron stores. Two control groups were studied at the same time; the one was made up of iron depleted or iron deficient but otherwise healthy Indian housewives and the other of a miscellaneous group of hospital patients whose iron stores had been depleted by therapeutic venesections or pathological blood loss.

6.1 Subjects and Methods

There were 12 patients with idiopathic haemochromatosis, the diagnosis being based on the finding of the clinical, biochemical and histological features which are generally recognised to be typical of the disease (Bothwell and Finch 1962). None of them had ever received

iron therapy or blood transfusion and there was no evidence of excessive alcohol intake. At the time of diagnosis, pigmentation and a firm hepatomegaly had been present in all subjects, the plasma transferrin saturations had been greater than 85% and the desferrioxamine-induced urinary iron excretions in 6 h more than 7,Ø mg. Liver biopsies had revealed the presence of cirrhosis and grossly excessive haemosiderin deposits located predominantly in hepatocytes. Diabetes mellitus had been present in five of the 12, and three had been in cardiac failure. Venesection therapy had been completed in eight of the patients, and was in progress in three of the remaining four, but no venesections were performed from 6 weeks before the start of the absorption studies until their conclusion. In the eight fully treated subjects the plasma ferritin concentrations at the time of the study were below 25 /ug/l (Table 6); they were therefore actually iron depleted, although none of them was anaemic. In the remaining four the plasma ferritin concentrations were greater than 1ØØØ /ug/l.

The second group was made up of 12 multiparous housewives of Indian descent living in poor socio-economic circumstances in Chatsworth, Durban. The diet of these people consists very largely of vegetable foodstuffs and iron deficiency has previously been shown to occur commonly amongst them (Mayet et al 1972). The subjects taking part in the present study were selected from 22 randomly chosen volunteers on the basis of plasma ferritin concentrations

that were lower than 25 /ug/l. In 10 of the 12 the transferrin saturation was 20% or less (Table 5).

Finally, 18 white hospital patients (five males and 13 females) aged between 17 years and 84 years were studied. The subjects selected for study all had plasma transferrin saturations lower than 20% and plasma ferritin concentrations lower than 25 /ug/l, but not all were anaemic. Seven had been subjected to venesection therapy for polycythemia vera but in no case had venesections been performed more recently than 6 weeks prior to the absorption study. The remainder suffered from menorrhagia or chronic gastrointestinal bleeding; none had undergone abdominal surgery and there was no clinical or biochemical evidence of steatorrhoea. Treatment with iron was delayed until the absorption studies had been completed.

6.1.1 Standard Meal

The meal consisted of a thick slice of bread, a hamburger with gravy, a piece of fresh lettuce and a glass of tomato juice. The bread was baked from a 1:3 mixture of 100% extraction flour and 70% extraction flour. The 100% extraction flour had been made from wheat intrinsically labelled with ^{55}Fe by hydroponic culture (Hussain et al 1965) and the 80 g bread consumed by each subject contained 2,5 /uCi ^{55}Fe and 4,2 mg iron. This figure was more than double that which would be anticipated from food tables (Documenta Geigy Scientific Tables, 1970); the difference presumably represented extraneous contamination with iron.

The hamburgers were made from minced lamb seasoned with salt and pepper and fried in vegetable oil. Each weighed 80 g and contained 2,4 mg iron. The gravy consisted of a mixture of a cooked haemolysate of ^{59}Fe-labelled rabbit erythrocytes and gravy prepared from a commercial gravy concentrate ('Brono', Hind Brothers, Durban, South Africa). The haemolysate was prepared by alternately freezing and thawing washed erythrocytes from a male New Zealand white rabbit which had been given an intravenous injection of $^{59}FeCl_3$ 2 weeks previously. Each person received 5 ml haemolysate containing 2,5 μCi ^{59}Fe and 0,6 mg iron mixed with 20 ml gravy containing 0,3 mg iron. The 12 g lettuce eaten by each subject contained 0,2 mg iron and 0,8 mg ascorbic acid and the 175 ml bottled tomato juice (Schweppes Minerals Ltd., Johannesburg, South Africa), 0,3 mg iron and 8,8 mg ascorbic acid.

The meal was eaten at 11.00 hours following an overnight fast. Two weeks later the subjects reassembled again after an overnight fast, and blood samples were taken for determination of ^{59}Fe and ^{55}Fe, haemoglobin, plasma ferritin, serum iron concentration and total iron-binding capacity. They then drank 50 ml of a freshly prepared solution containing 30 mg ascorbic acid and 3 mg iron as $FeSO_4.7H_20$ labelled with 2,5 μCi $^{59}Fe\ Cl_3$. After a further 14 days a second blood sample was collected and its ^{59}Fe activity measured. The absorption of the iron salt was determined by difference.

The isotopic chemical and immunological methods used to obtain the results in this investigation are outlined in Chapter 3.

6.2 Results

The ferritin and iron absorption data was log transformed in order to normalise the distributions. Geometric means and SD ranges are given for these parameters. Arithmetic means were used for the haematologic and plasma iron data. Iron absorption by the four haemochromatotic subjects whose plasma ferritin and plasma iron concentrations were still markedly above normal was found to be a function of the number of venesections which had been performed, whether the iron was administered as food iron (haem and non-haem) or as a solution of ferrous ascorbate (Table 6). It is possible that the absorptions were actually higher than the calculated figures, since the calculation was based on the assumption that all the absorbed iron was present in circulating haemoglobin and this has been shown to be invalid when the plasma iron concentration is high (Bothwell and Finch 1962). Results from these patients were consequently not used for calculations of the means. This problem did not arise in the remaining eight haemochtomatotic subjects, since they had been fully treated by repeated venesection and the percentage absorptions of each form of iron were high. The geometric means and standard deviation ranges were 36,8% (32,4-41,9%) for the haem iron and 33,3% (21,Ø-52,7%) for

Table 6 Absorption of haem and non-haem food iron from a standard meal and of ferrous ascorbate by patients with idiopathic haemochromatosis, iron deficient Indian housewives and iron deficient white hospital patients

	Age (yr)	Sex	Venesections (ℓ) or diagnosis	Hb (g/dl)	Plasma iron (μmol/l)	Saturation %	Plasma* ferritin (μg/l)	% Iron absorption* Haem food iron	Non-haem food iron	Ferrous ascorbate	Absorption ratios* Non-haem: haem	Non-haem: ferrous ascorbate
Idiopathic haemo-chromatosis	59*	M	0,0*	15,6*	43,6*	91*	4778*	3,7*	0,3*	9,6*	0,08*	0,03*
	55*	M	4,0*	15,9*	48,8*	92*	4603*	9,6*	0,1*	8,6*	0,01*	0,01*
	68*	F	10,0*	15,6*	48,8*	90*	2513*	19,4*	5,6*	13,0*	0,29*	0,43*
	54*	F	31,5*	16,3*	61,1*	94*	1015*	30,6*	20,3*	11,7*	0,66*	1,73*
	38	M	43,0	14,1	22,7	31	14	37,7	16,8	95,4	0,45	0,18
	38	M	50,0	15,2	7,0	13	13	41,5	25,9	75,3	0,62	0,34
	56	M	55,0	14,1	16,6	26	11	33,5	20,6	65,6	0,61	0,31
	58	M	59,5	14,1	13,0	18	4	46,0	62,4	88,4	1,36	0,71
	57	M	61,5	18,1	19,1	34	20	30,7	34,9	75,3	1,14	0,46
	58	M	75,0	16,3	13,9	21	12	35,6	32,4	58,4	0,91	0,55
	54	M	98,0	15,1	7,0	8	19	33,9	53,7	76,8	1,58	0,70
	48	M	140,0	15,9	7,0	14	22	38,0	44,6	58,4	1,17	0,76
Mean	50,9	—	72,8	15,4	13,3	20,6	13,9	36,8	33,3	73,1	0,90	0,45
Iron deficient Indian housewives	49	F	—	11,8	12,1	15	3	35,7	0,1	31,5	0,003	0,003
	48	F	—	12,7	9,5	13	1	38,4	22,3	56,2	0,58	0,40
	35	F	—	13,3	13,0	12	3	34,7	2,0	58,0	0,06	0,03
	55	F	—	12,9	13,0	19	14	20,9	2,2	45,0	0,11	0,05
	43	F	—	13,1	12,7	20	7	43,3	3,6	12,9	0,08	0,28
	38	F	—	11,0	7,9	8	1	31,6	2,4	80,5	0,08	0,03
	27	F	—	13,0	5,5	8	3	24,5	0,8	32,3	0,03	0,02
	52	F	—	13,7	21,1	32	23	22,2	3,0	52,5	0,14	0,06
	49	F	—	8,4	19,1	25	2	20,4	4,0	16,0	0,20	0,25
	30	F	—	13,3	15,7	16	10	42,5	6,6	75,9	0,16	0,09
	43	F	—	11,4	8,8	10	3	16,8	6,8	90,7	0,40	0,07
	48	F	—	12,9	10,5	11	9	47,6	15,5	93,1	0,33	0,17
Mean	43,1	—	—	12,3	12,4	15,8	4,2	29,9	3,0	37,7	0,10	0,06
Iron deficient white patients	62	F	P. vera-11,0	17,5	3,6	9	19	26,2	31,5	57,4	1,20	0,55
	55	M	P. vera-16,5	16,3	8,9	16	14	12,8	22,4	24,8	1,75	0,90
	58	F	P. vera-24,0	16,1	8,4	12	22	38,4	19,3	34,9	0,50	0,55
	71	F	P. vera- 4,5	12,2	3,6	5	17	20,7	4,3	46,9	0,21	0,09
	84	F	P. vera- 5,0	11,8	5,6	7	9	24,3	7,4	17,9	0,30	0,41
	46	M	P. vera-18,0	15,4	10,4	17	24	24,2	29,3	64,8	1,21	0,45
	58	F	P. vera- 3,5	11,4	4,5	6	17	21,6	22,4	70,4	1,04	0,32
	73	F	Gastro-intestinal haemorrhage; ?site	16,3	5,6	8	8	21,7	6,7	39,8	0,31	0,17
	69	F	Chronic gastritis	10,2	4,3	6	17	45,9	3,9	29,3	0,08	0,13
	64	F	Menorrhagia to age 59	8,4	3,6	5	10	19,1	29,9	18,7	1,57	1,60
	67	F	Gastric ulcer	11,7	7,7	10	10	35,5	6,1	19,4	0,17	0,31
	59	M	Duodenal ulcer	8,4	4,5	6	15	20,4	27,1	79,0	1,33	0,34
	20	F	Menorrhagia	9,5	3,6	4	22	24,8	5,4	63,6	0,22	0,08
	17	F	Undetermined	11,2	4,6	6	3	34,5	12,3	67,3	0,36	0,18
	63	M	Diverticulosis	16,0	8,0	11	7	14,1	15,7	16,8	1,11	0,93
	73	F	Hiatus hernia	10,9	6,1	12	22	35,9	11,0	44,1	0,31	0,25
	56	M	Doudenal ulcer	11,9	7,3	8	24	54,9	56,4	66,8	1,03	0,84
	53	F	Menorrhagia	10,4	7,9	11	9	60,9	29,2	80,3	0,48	0,36
Mean	58	—	—	12,5	6,0	8,8	13,3	27,0	14,4	41,0	0,53	0,35

* Geometric means * Not included in means

the non-haem iron in the meal, and 73,1% (61,2-87,5%) for the ferrous ascorbate. The group of Indian housewives absorbed 29,9% (27,1-33,1%) the haem food iron, but only 3,Ø% (Ø,74-12,3%) of the non haem iron in the meal and 37,7% (17,1-83,3%) of the ferrous ascorbate. In the group of white hospital patients these figures were respectively 27% (17,7-41,9%), 14,4% (6,5-32,Ø%) and 41,Ø% (23,5-71,5%). By analysis of variance there were no significant differences between the haem iron absorptions by the three groups but the absorption of non-haem food iron by the Indian women was significantly less than that of both the haemochromatotic subjects and the white hospital patients, with a p value < Ø,ØØ5 in each instance (haemochromatosis: Indian females, F = 28,4 Indian females: white hospital patients, F = 18). Although the mean ferrous ascorbate absorptions by the white hospital patients were less than those of the haemochromatotic subjects (F = 6,4, p < Ø,Ø2), the Indian females did not differ from either the haemochromatotic subjects or the white patients in this regard.

Both the non-haem:haem food iron absorption ratios and the non-haem food iron:ferrous ascorbate absorption ratios (Table 6) were significantly lower (by analysis of variance) in the group of Indian women than in the treated haemochromatotic subjects or in the hospital patients (in the case of non-haem:haem ratio, F = 21,8, p < Ø,ØØØ2 and F = 18,5, p < Ø,ØØØ2; for the non-haem: ferrous ascorbate ratios, F = 2Ø,1, p < Ø,ØØØ3 and F = 21,3, p < Ø,ØØØ1).

6.3 Discussion

The underlying metabolic abnormality in idiopathic haemochromatosis has so far eluded definition. Although it is the excessive absorption of dietary iron which is responsible for the development of iron overload in this disease, absorption is probably not increased to any marked degree, if at all, in untreated subjects (Chodos et al 1957; Pirzio-Biroli et al 1958; Smith et al 1969; Powell et al 1970). Presumably absorption is depressed in such individuals by the massively increased stores, since it has been shown that iron absorption is inversely proportional to the quantity of storage iron in idiopathic haemochromatosis (Walter et al 1975) as it is in normal subjects (see also Chapter 4). The opportunity to study iron absorption at the beginning of the disease has rarely, if ever, presented itself. It is not unreasonable to suppose, however, that after treatment by means of venesection therapy absorption returns to levels comparable to those which were present at the onset of the disease. Walters and co-workers (1975) noted that patients with idiopathic haemochromatosis absorbed more non-haem food iron (chicken soup labelled extrinsically with ^{59}Fe ferric citrate) than did normal subjects with similar plasma ferritin levels. If confirmed this finding might have yielded a valuable clue to the nature of the metabolic error responsible for the disordered iron balance. Since this study did not, however, include an assessment of haem-iron absorption, it was felt that further

information might be gained by measuring iron absorption from both dietary iron sources in these subjects. In particular, since haem-iron and non-haem iron are absorbed through different mechanisms it was felt that the investigation might help to differentiate between a mucosal or luminal abnormality, if such an abnormality exists in haemochromatosis. Since the factors which influence food iron absorption operate at either the mucosal or at the luminal level the differential absorption of haem iron and of non-haem iron can be expected to provide information about each of these phases respectively.

In order to provide a suitable yardstick for assessing iron absorption in venesected subjects with idiopathic haemochromatosis, two other study groups with depleted body iron stores were chosen as controls. The iron status of the study populations was established by determining the plasma ferritin concentrations, which have been shown to reflect the quantity of storage iron in the body in idiopathic haemochromatosis (Beamish et al 1974) as well as in normal subjects and in other varieties of disordered iron balance. The plasma ferritin values of the haemochromatotic subjects (geometric mean 13, SD range 8-22) were similar to those of the white hospital patients (geometric mean 14; SD range 8-23), although the Indian females had levels which were significantly lower (geometric mean 4; SD range 2-12) than those of either of the other two study groups ($F = 20.4$, $p < 0,005$). However, all three study groups were felt to be iron

depleted and the demand for iron should reflect these low ferritin values. Indeed, although the mean ferrous ascorbate absorption of the white iron deficient patients was somewhat less than that of the other two groups, the absorption of this form of iron was good in all three groups of subjects and the difference was small. The Indian females and the haemochromatotic subjects absorbed similar quantities of ferrous ascorbate. The absorption of haem iron did not differ significantly between any of the study groups. This was not, however, the case when non-haem iron absorption was considered. Although the haemochromatotic subjects absorbed significantly more non-haem iron than the Indian females, they did not differ in this regard from the iron deficient white subjects. This makes it unlikely that the haemochromatotic individuals were abnormally efficient absorbers of iron. Powell and co-workers (1970) found no difference in either mucosal uptake or eventual absorption of non-haem food iron (ferric citrate added to a meal) between eight fully treated haemochromatotic subjects and eight patients with iron deficiency anaemia. It should be noted, however, that although the statistical analysis of the non-haem iron absorptions failed to show a difference between the haemochromatotic subjects and the white patients, there were a number of individuals in the latter group who absorbed this form of iron as poorly as did the Indian females. The anomaly in the present results is therefore not the high absorption of non-haem food iron by

the haemochromatotic individuals but the poor absorption of the major dietary iron fraction by so many iron-depleted individuals, especially Indian women, but also some of the white patients.

If the present findings do not provide support for the possibility that some intraluminal abnormality is responsible for the excessive absorption of iron in idiopathic haemochromatosis, do they offer any other clue to the nature of the biochemical defect? They certainly underline the efficiency with which such subjects do assimilate iron once their accumulated stores have been depleted, at a time when the haemoglobin concentration is normal and the plasma iron concentration normal or raised. Iron absorption still appears to be subject to the same influences that act in normal individuals, but presumably the 'homeostat' is set at a much higher level than normal. Although various explanations have been offered, the reason for this remains obscure. Indeed the mechanism by which the body stores regulate absorption in normal subjects has not yet been established.

The most striking observation in the present study is the poor absorption of the non-haem food iron in the standard meal by the majority of the Indian housewives and some of the white hospital patients, although their assimilation of the haem iron was efficient (Fig. 5). Variable absorption of food iron by iron deficient subjects is familiar enough. For example, Hussain and co-workers

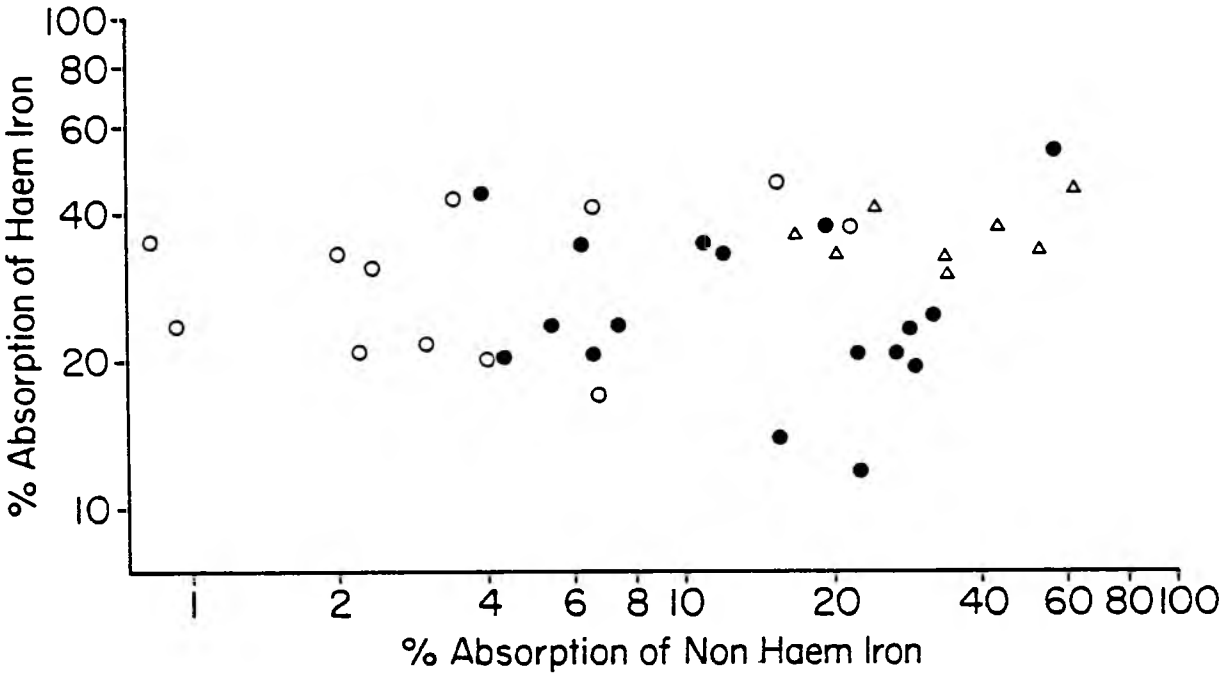

Figure 5. A comparison of the absorptions of the haem iron and the non-haem iron in a standard meal by venesected patients with idiopathic haemochromatosis (Δ) and by Indian (o) and white (●) iron deficient subjects.

(1965) found that six of a group of 21 patients with iron deficiency anaemia absorbed between 11,3% and 16,3% of the wheat iron in bread, while five of them absorbed only between Ø,4% and 4,Ø%. It is the marked discrepancy between the ability of some individuals to absorb the non-haem iron and the haem iron in a meal, to which attention does not appear to have been drawn previously. The discrepancy is highlighted by calculating the non-haem:haem food iron absorption ratios (Table 6). In the treated haemochromatotic subjects the mean ratio was Ø,9Ø, whereas in the group of Indian women the highest figure was Ø,58 and in nine of the 12 it was less than Ø,2Ø (Fig. 6). The white hospital patients were not significantly different to the haemochromatotic subjects. This malabsorption of non-haem food iron must contribute to the pathogenesis and persistence of iron deficiency in such subjects, even if abnormal blood loss also plays a part.

The reason for the malabsorption will be dealt with in more detail in the following chapter. However, the good absorption of haem iron suggests that intestinal mucosal function was adequate, and that the fault was therefore intraluminal. This view is strengthened by the fact that high percentages of ferrous ascorbate were also absorbed by these subjects, since a deficiency at the luminal level would be expected to have little effect on the absorption of iron in this form. One obvious possibility was gastric hyposecretion with hypochlorhydria or achlorhydria. In

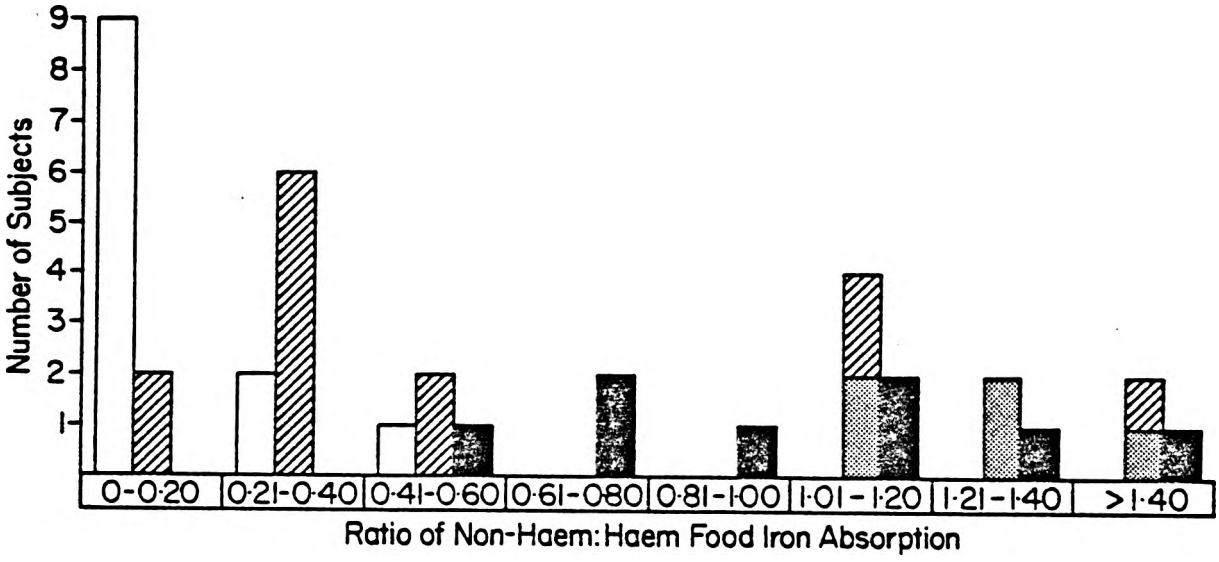

Figure 6. Ratios of non-haem:haem food iron absorption in patients with idiopathic haemochromatosis (solid bars), iron deficient Indian housewives (open bars), male iron deficient white patients (obliquely cross-hatched bars) and female iron deficient white patients (horizontally cross-hatched bars).

several studies the absorption of a radio-iron tracer added to a meal has been shown to be significantly lower in achlorhydric individuals than in normal subjects (Pirzio-Biroli et al 1958; Goldberg et al 1963; Cook et al 1964), while Jacobs and co-workers (1966) noted that absorption by a group of patients with iron deficiency anaemia was a function of their gastric acid secretion in response to histamine stimulation. Although only an extrinsic label was employed in these investigations, there is now good evidence that absorption from the non-haem food iron pool can be validly assessed in this way (Cook et al 1972). It has also been shown that the absorption of haemoglobin iron is not defective in achlorhydric subjects (Callender et al 1957; Jacobs et al 1964), and furthermore that their absorption of ferrous ascorbate is good, although possibly improved by hydrochloric acid (Jacobs et al 1964). A recent study of unselected Indian patients admitted to hospital in Durban revealed that 20% had hypochlorhydria or achlorhydria on pentagastrin stimulation (Mayet and Paruk, 1972). The present results suggest that the prevalence among iron deficient Indian housewives from the Chatsworth community in Durban may be even higher.

One final point merits comment. Layrisse and co-workers (1969) reported that the correlation between the absorptions of ferrous ascorbate and various forms of iron was close - sufficiently so to permit the latter to be predictable from the former. For example, those subjects

with iron deficiency anaemia who absorbed 60% of the ferrous ascorbate would be expected to absorb 18,6% of the iron in wheat pancakes, 8% of that in corn pancakes, 12,8% from lettuce and 27,8% from soybean cakes. Scrutiny of their results, however, reveals the presence of a few individuals whose food iron absorptions fell far short of the predicted values. Widely divergent absorptions of vegetable iron and ferrous ascorbate can also be noted in a small proportion of the iron deficient individuals in other reports (Hussain et al 1965; Mameesh et al 1970; Cook et al 1972). The striking feature of the present investigation is the high prevalence of such subjects, particularly among the Indian women. Indeed, the ratios of non-haem food iron absorption to ferrous ascorbate absorptions in this group were significantly lower than in either of the other groups (Table 5). Many other Indian housewives from this community have taken part in previous studies from this laboratory, and on reviewing the data, several of them in virtually every group exhibited poor non-haem iron absorption in relation to their ability to absorb ferrous ascorbate (Sayers et al 1973; 1974a,b; Disler et al 1975). The greater prevalence in these subjects, compared with those studied by Layrisse and co-workers (1969), may well explain the absence in all but one of our studies of the significant correlation they found between the percentage absorptions of ferrous ascorbate and non-haem food iron.

6.4 Summary

The absorption of radioactive iron from a solution of ferrous ascorbate, and from a standard meal containing intrinsically labelled haemoglobin and wheat, was measured in 12 Indian housewives, 18 white hospital patients and 12 subjects with idiopathic haemochromatosis. Eight of the latter had been fully treated by multiple venesections, so that their plasma ferritin concentrations were below 25 /ug/l. Since the plasma ferritin concentrations of the treated haemochromatotic subjects and the hospital patients were comparable, their body iron stores were considered to be depleted to a similar degree while the Indian women were somewhat more iron depleted. The absorptions of ferrous ascorbate and of the haem iron in the standard meal were comparable but the housewives in spite of having lower plasma ferritin concentrations absorbed significantly less of the non-haem food iron than the other two groups. The mean non-haem food iron absorptions were 33,3%, 3,Ø% and 14,4% for the treated haemochromatotic subjects, the Indian housewives and the white hospital patients respectively. The discrepancies between the absorptions of the different forms of food iron were highlighted by calculating the ratios between them. The geometric mean non-haem:haem food iron absorption ratio for the group of treated haemochromatotic subjects was Ø,9Ø, and for the Indian housewives only Ø,1Ø. The white hospital patients did not differ from the haemochromatotic subjects with a geometric mean non-haem:haem iron absorption ratio of Ø,53. The

results of this study suggest that malabsorption of non-haem iron from a meal containing bread, presumably due to a defect at the luminal level, may be an important factor in the pathogenesis of iron deficiency in some subjects. The abnormality appears to be particularly prevalent among Indian women living in Durban.

Chapter 7

The Importance of Gastric Hydrochloric Acid in the Absorption of Non Haem Food Iron

While body iron needs are the major factor which determine an increase or a decrease in the amount of iron absorbed, luminal factors play a major role making iron available for absorption. Consideration of these luminal factors involves the study of both the nature and amount of iron presented to the gastrointestinal tract as well as the effect thereon of intestinal secretions. Of particular interest in this regard is the effect of gastric secretion on the availability of food iron.

A number of studies have aimed at defining the role of the various constituents of the gastric juice in the absorption of iron. These studies have been reviewed in Chapter 1, but in view of the conflicting results of previous absorption studies there appeared to be room for further study. The problem was approached by comparing the absorption of the non-haem iron in bread with its solubilization on incubation in vitro with aspirated gastric juice or with HCl of similar molarity.

7.1 Subjects and Methods

A total of 48 subjects in four groups was studied. Nine white volunteers whose blood counts, plasma iron concentrations, and transferrin saturations were within the normal ranges formed the first group. The second consisted

of seven white patients with idiopathic haemochromatosis, diagnosed by the criteria set out previously. The excess iron in these haemochromatotic subjects had been removed by repeated venesections and their plasma ferritin concentrations at the time of the study ranged from 4 to 22 /ug/l. The third group comprised 14 white subjects with iron-deficiency anaemia, as evidenced by haemoglobin concentrations ranging froom 6,4 to 11,9 g/dl, the morphologic features of iron-deficient erythropoiesis, transferrin saturations below 15%, and the absence of storage iron on histological examination of bone marrow aspirates. In nine subjects the iron deficiency was due to chronic bleeding but in the remaining five the cause was not identified. The 18 patients of Indian origin in the fourth group were from the Chatsworth area near Durban and had been hospitalized for investigation of anaemia (haemoglobin concentrations 3,2 to 10,9 g/dl). Iron deficiency was established in each by a transferrin iron saturation below 15% and a plasma ferritin concentration below 10 /ug/l. Two of these patients were bleeding chronically from duodenal ulcers but the cause of the iron deficiency was not identified in the remaining patients.

Basal gastric juice, collected via a nasogastric tube, was discarded before stimulating gastric secretion by injection of 6 /ug of pentagastrin per kilogram of body weight (Peptavalon; ICI Pharmaceuticals, Millbank, London, England). Gastric juice was then collected until the volume

collected in a 15 min period was less than 50 ml. The pH was measured with a combination glass electrode (GK 2013 C; Radiometer, Copenhagen, Denmark), and the gastric juice was then stored at -20°C until required for study. Absorption studies in these patients were carried out within 2 weeks of collection of the gastric juice.

A 3:1 mixture of unlabelled 70% extraction flour and 100% extraction flour made from wheat intrinsically labelled with ^{55}Fe by hydroponic culture was used (Hussein et al, 1965) to bake bread. The bread contained 4,2 mg of iron and 2,5 μCi ^{55}Fe per 100 g. In one in vitro experiment a tracer amount of $^{59}FeCl_3$ in 0,05 N HCl was added prior to baking.

One hundred g of bread were homogenized with 400 ml of distilled water, and after preincubation of each to 37°C, aliquots were mixed with 2 volumes of either gastric juice or HCl (10^{-4}M to 10^{-1}M) and incubated at 37°C on a turntable (20 rpm). This gave a ratio of food to gastric juice of 1:15, which we calculated to be of the same order as would be found in the stomach following a light meal such as that used in the in vivo studies. Incubations were done in duplicate. The supernates were separated by centrifugation at 2700 x g for 30 min at 4°C.

Absorption of both haem and non-haem food iron was measured in 24 gastric juice donors, all of whom were iron deficient. After an overnight fast the subjects were given a standard meal consisting of 100 g of ^{55}Fe-labelled bread,

an 80 g hamburger steak with 25 ml of gravy, 12 g of fresh lettuce, and 175 ml of bottled tomato juice (Schweppes Minerals, Ltd., Johanneburg, South Africa). The hamburger steaks were made from minced lamb seasoned with salt and pepper and fried in vegetable oil. A cooked haemolysate of ^{59}Fe-labelled rabbit erythrocytes was mixed with gravy prepared from a commercial gravy concentrate ("Brono"; Hind Brothers, Durban, South Africa). The haemolysate was prepared by alternately freezing and thawing washed erythrocytes from a male New Zealand white rabbit which had been given an intravenous injection of $^{59}FeCl_3$ two weeks previously. Each person received 5 ml of hemolysate containing 2,5 μCi of ^{59}Fe and 0,6 mg of iron plus 20 ml of gravy containing 0,3 mg of iron. Each portion of lettuce contained 0,2 mg of iron and 0,8 mg of ascorbic acid, and the tomato juice, 0,3 mg of iron and 8,8 mg of ascorbic acid.

Iron absorption was measured by the appearance of radioactivity in the red cell mass 14 days after the ingestion of the meal as described in Chapter 3. In addition to studying the absorption of food iron a "reference" absorption of a readily available iron salt (3 mg ferrous sulphate + 30 mg ferrous ascorbate) was performed in each subject as previously outlined. The isotopic and chemical methods used as well as the radioimmunometric assay for ferritin are all described in Chapter 3.

7.2 Results

7.2.1 Solubilisation of bread iron on incubation in vitro Solubilisation of ^{55}Fe from intrinsically labelled bread on incubation with either gastric juice or Ø,1 M HCl solution proceeded rapidly and was almost complete after 5 min but for convenience a 3Ø min incubation time was adopted. Altering the temperature had little effect. When nine gastric juice samples were incubated with bread homogenate, the mean ratio of the percentages solubilised at 37°C and at 4°C was 1,12 (S.D. Ø,11). In 24 experiments the effect of varying the proportions of gastric juice and bread homogenate was examined. Increasing the gastric juice had no effect, the mean ratio of the percentages solubilised by 4:1 mixtures being 1,Ø5 (S.D. Ø,Ø9), but reducing the proportions to 1:1 diminished the solubilisation, the mean ratio being Ø,49 (S.D. Ø,Ø9).

When $^{59}FeCl_3$ was mixed with the dough prior to baking to serve as an extrinsic label for the bread iron and the bread was incubated with either gastric juice or HCl solution, there was a very close relationship between the solubilisation of the ^{59}Fe and the solubilisation of the intrinsic ^{55}Fe label, the latter always being smaller (^{55}Fe solubilised = (Ø,874 x ^{59}Fe solubilized) - 1,76Ø7; r = Ø,996, $p < Ø,ØØ1$).

7.2.2 Relationship of solubilisation to pH Gastric juice samples with pH values higher than 2,5 solubilised less than 2% of the radioiron in the bread (Fig. 7). Below pH 2,5 there was a linear relationship between pH and the

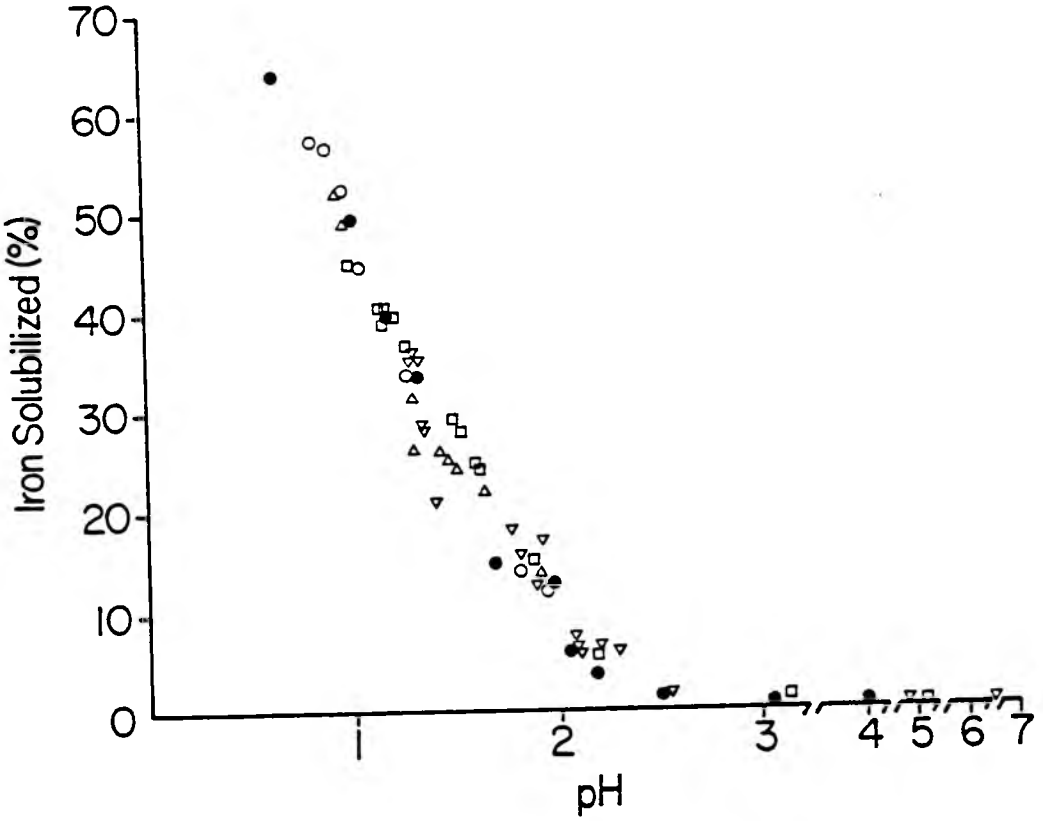

Figure 7. Effect of pH on the solubilization of the radioiron present in intrinsically labelled bread. Gastric juice was obtained from normal (Δ), haemochromatotic (o), and iron-deficient white (□) and Indian (▽) subjects HCI solutions (●) were also used.

percentage solubilised (percentage solubilised = 80,0 - 34,2 x pH; r = 0,96, p < 0,001). In this regard, covariance analysis revealed that there was no significant difference between the four groups (between slopes: F = 3,4; p < 0,05). However, all the normal subjects and those with idiopathic haemochromatosis had gastric juice pH values below 2, whereas the pH was greater than 2 in three of the iron-deficient whites and eight of the Indians (Fig. 7). The amount of radioiron solubilised by gastric juice after its pH had been altered by addition of $NaHCO_3$ or HCl could be predicted from the relationship between pH and solubilisation. This relationship was also valid for pure HCl solutions. The bread appeared to have little buffering effect, since the rise in supernatant pH after incubation could largely be accounted for by dilution. If the supernatant after incubation was removed and replaced with fresh gastic juice, a maximum of 80% of the radioiron could be solubilised. The amount of extra radioiron solubilised could be predicted from the relationship between iron solubilisation and pH.

7.2.3 Relationship of solubilisation to other ingredients The removal of molecules larger than 10000 daltons from the gastric juice samples by filtration through an Amicon PM 10 membrane (Amicon Corp., Lexington, Mass.) had no significant effect on the solubilisation of bread iron. Furthermore, the addition of 0,5% pepsin (B.D.H. Chemicals) to 0,1 M HCl solution had no effect. Adding 0,06

/uM ascorbic acid, Ø,Ø8 /uM fructose, or Ø,Ø4 /uM maltose was also without effect.

7.2.4 Nature of solubilised bread iron On dialysation of the supernates obtained after incubating the bread homogenate with gastric juice samples or HCl solution against Ø,1 M HCl (5 ml of supernate:15Ø ml of HCl), 95% passed through the membrane. This figure was reduced to a mean of 23,7% (S.D. 3,1) in 12 experiments in which the supernate after incubation was neutralized with $NaHCO_3$ before being dialysed against distilled water.

Gel-permeation chromatography through 4Ø by 1,5 cm columns of Sephadex G25 (Pharmacia Fine Chemicals, Uppsala, Sweden) was performed on the supernates after incubating the bread homogenate with each gastric juice sample. With samples of pH lower than 2,5, the mean recovery of the radioactivity was 85,1% (S.D. 7,1), but with higher pH values, the figure was only 45,9% (S.D. 12,6). In every case two fractions of radioactivity were found, one which eluted in the exclusion volume (fraction 1) and the other retarded by the gel (fraction 2). This pattern was obtained whether the eluant was Ø,Ø5 M HCl or distilled water, but when the supernate was neutralized with $NaHCO_3$ before chromatography, the second fraction disappeared and the recovery was reduced to 12% (S.D 4,3). When elution was attempted with Ø,Ø7 M phosphate buffer (pH 7,Ø), all the radioiron was retained in a brown band at the top of the column.

The distribution of radioiron between the two fractions varied according to the pH of the gastric juice sample. The percentage of the activity in the bread homogenate eluting in fraction 2 increased progressively from about 5% at pH 2,5 to 50% below pH 1 ($r = 0,955$, $p < 0,001$), whereas the percentage in fraction 1 was virtually unchanged at about 4% (Fig. 8).

Both fractions were also obtained on chromatographing gastric juice incubated with $^{59}FeCl_3$. However, $^{59}FeCl_3$ in 0,1 M HCl gave only a peak in the region of fraction 2. If the pH of the latter was raised above 3 with $NaHCO_3$, the iron could not be eluted from the columns with distilled water unless ascorbic acid (2 /umol/l /umol Fe) or fructose (3 /umol/l /umol Fe) was added to the solution prior to neutralization.

7.2.5 Relationship between solubilisation of bread in vitro, gastric juice pH, and iron absorption The absorption of the ^{55}Fe in the bread was determined in 24 of the iron-deficient subjects who were given the test meal. A close correlation ($r = 0,9311$, $p < 0,001$) was found between the percentage absorbed and the percentage solubilised on incubation of the bread homogenate with the individual's gastric juice in vitro (Fig. 9). The correlation between the absorption of the bread iron and the pH of the gastric juice was also close ($r = 0,834$, $p < 0,001$), as was the correlation between the absorption of the bread iron and the percentage present in fraction 2 after gel-permeation

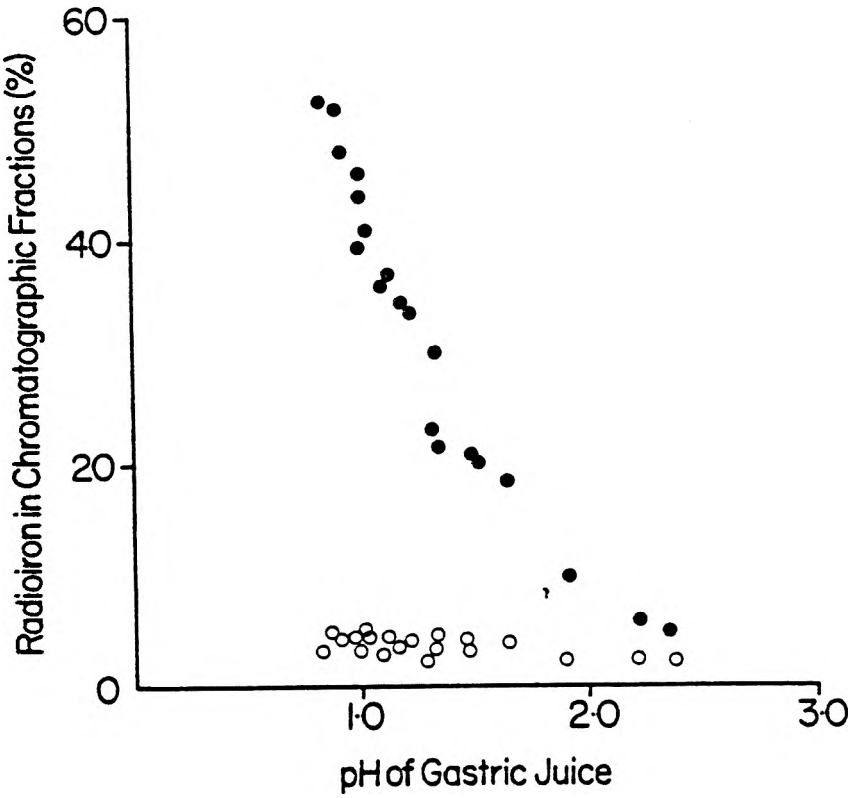

Figure 8. Effect of gastric juice pH on the elution pattern obtained when radioiron solubilized from intrinsically labelled bread was chromatographed on a 40 by 1,5 cm column of Sephadex G 25. Two peaks of radioactivity were obtained: fraction 1 (o) which eluted in the exclusion volume of the column and fraction 2 (•), a low-molecular-weight peak which was retarded in the gel.

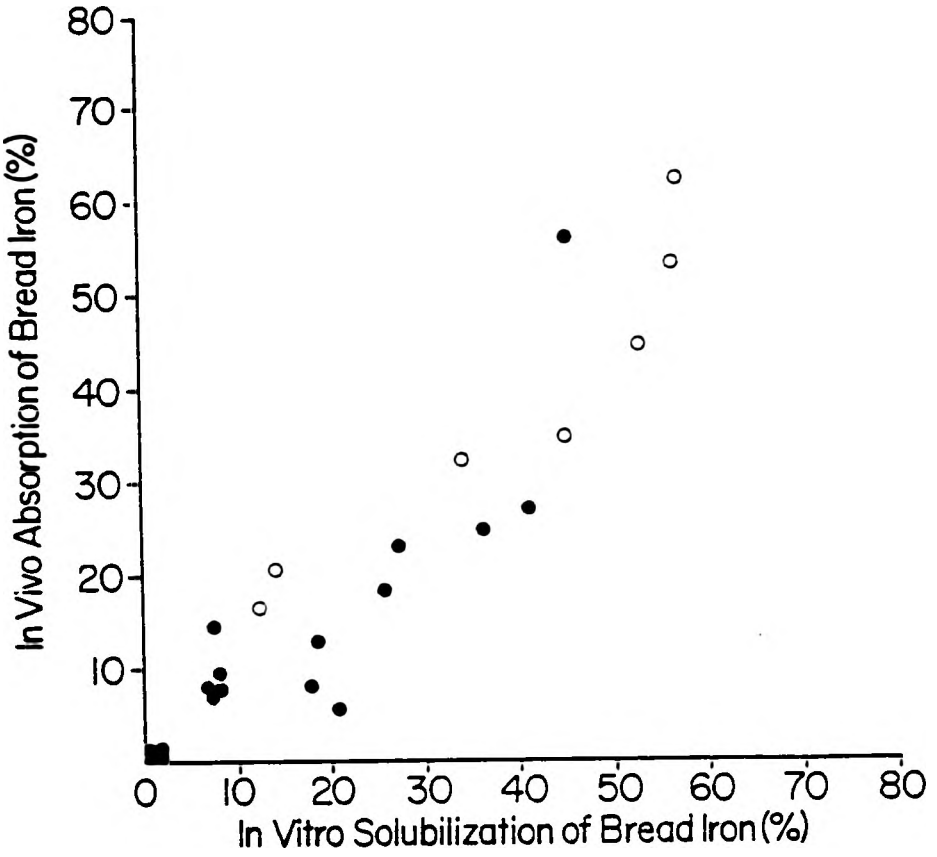

Figure 9. Relationship between the in vivo absorption of radioiron from intrinsically labelled bread and the ability of gastric juice from the same subject to solubilize the radioiron present in the bread in vitro. Iron-deficient white and Indian subjects (•) and phlebotomized idiopathic haemochromatotic patients (o) were studied.

chromatography ($r = 0{,}0845$, $p < 0{,}001$). There was no significant correlation with the percentage in fraction 1 ($r = 0{,}3332$, $p < 0{,}1$).

In contrast to the bread iron, there was no correlation between the percentage solubilised and the absorption of either the haem iron in the meal ($r = 0{,}16$, $p > 0{,}1$) or the $FeSO_4$ plus ascorbic acid administered on a subsequent occasion ($r = 0{,}38$, $p > 0{,}05$).

7.3 Discussion

The solubilisation of the iron in bread on incubation with gastric juice in vivo seemed to be due entirely to its HCl content. Not only was there a close relationship between the pH and the percentage solubilised, but the same relationship was also revealed when HCl solutions of differing strength were incubated with the bread (Fig. 7). Moreover, solubilisation by HCl solutions was not increased by adding pepsin, nor was solubilisation by the juice diminished by removing constituents larger than 10000 daltons. Even small-molecular-weight ligands such as ascorbic acid did not increase solubilisation over and above that achieved by 0,1 N HCl.

The close correlation between the solubilisation of the intrinsic ^{55}Fe label in the bread and that of ^{59}Fe mixed into the dough before baking was in keeping with the considerable body of evidence that an extrinsic label of this type provides a valid measure of the absorption of non-haem food iron. However, the fact that the mean ratio of

extrinsic to intrinsic iron in the supernate after incubation was Ø,82 and not 1,Ø indicates that the isotopes were not extracted to the same extent under the conditions of these experiments. Other recent evidence suggests that formation of a common pool of intrinsic and extrinsic non-haem iron is only completed in the duodenum (Thein-Thau et al, 1977).

Since the test meal in the present study was administered to subjects who were avid for iron as a result of iron deficiency, the major limiting factor for absorption was almost certainly the availability of the iron within the gastrointestinal lumen. In this regard, the paramount importance of the HCl in gastric juice as a determinant for the absorption of non-haem iron was established by the close correlation between the percent absorption and the in vitro solubilisation of bread iron by the relevant gastric juice. The inverse relationship between bread iron absorption and the gastric juice pH confirms such a conclusion. It is noteworthy that there was no such correlation between the pH and the absorption of the haem iron in the meal. Only one of the individuals with a gastric juice pH value greater than 2 absorbed less than 3Ø% of the haem iron, yet none of them absorbed more than 8% of the non-haem iron. These observations, which support the previous finding that haem iron is absorbed independently of intraluminal factors (Turnbull et al 1967) underline the significance of the haem iron content of the diet in iron nutrition. Since the

staple diet in many parts of the world contains little or no meat, iron deficiency in such areas is particularly likely to develop in those individuals with hypochlorhydria or achlorhydria. For example, there is very little meat in the diet of the people of Indian descent living in Chatsworth, (Mayet et al 1972) and the fact that as many as eight of the 18 iron-deficient individuals in the present study had gastric juice pH values higher than 2 probably explains why there is frequently malabsorption of non-haem food iron in such subjects (Bezwoda et al 1976).

Most of the iron solubilised from the bread by gastric juice samples appeared to be ionic in nature. After incubation of bread homogenate with gastric juice having an initial pH lower than 2,5 and subjection of the supernate to gel-permeation chromatography, more than 80% of the solubilised radioiron eluted in the same position as did $^{59}FeCl_3$ in HCl and in each case this peak was abolished by prior neutralization with $NaHCO_3$. In addition, the free passage of radioiron through a dialysis membrane was reduced to a mean of 23,7% at neutral pH. These observations suggest that the major fraction was present as Fe^{3+}. Virtually all the remaining iron eluted from the gel appeared to be in a form larger than 10000 daltons, since it appeared in the exclusion volume. A similar peak was found when chromatography was performed on $^{59}FeCl_3$ added to gastric juice, but not with $^{59}FeCl_3$ in HCl solution. This peak may consist either of larger polynuclear iron complexes

or, as has been previously suggested, of iron bound to large-molecular-weight components of gastric juice (Beutler et al 1963; Davis et al 1966; Jacobs and Miles 1969). That the iron is maintained in solution by such binding was also suggested by the observation that neutralization before chromatography did not affect this peak. The easy reversibility of the binding was demonstrated by the fact that after dialysis at low pH, the concentration of radioiron on either side of the membrane was approximately equal.

Although all these findings would be compatible with the hypothesis of Jacobs and Miles (1969), that large-molecular-weight components in gastric juice play a part in the process of iron absorption by holding iron in solution, other observations suggest that their role is not an essential one. Swan and Glass (1973), proposed that ligands in the ingested food might function in the same way and support for this view was provided by the finding of a similar large-molecular-weight peak on chromatography of the supernate obtained after incubating bread homogenate with an HCl solution. This peak was also not eliminated by raising the pH to neutral levels and dialysis at low pH established that the binding was once again easily reversible. If, therefore, such binding does preserve the availability for absorption of ingested ferric iron, extrinsic ligands may serve this purpose as well as those in the gastric juice. However, the absence of any relationship between the

phenomenon of iron binding to large molecules and the quantities absorbed raises doubts as to its significance. In contrast, the absorption of bread iron was closely correlated with the quantity of solubilised iron that was unbound. Since this was a function of the pH of the gastric juice, the results of the present study suggest that it is only the HCl in the gastric juice which plays any significant part in the absorption of bread iron from a meal of this type.

One final point merits comment. There have in the past been suggestions that the abnormally high absorption of iron in idiopathic haemochromatosis is due to the presence of a promoting factor in the gastric secretions. Although other evidence has discounted this hypothesis, the relevant observations in the present study should be pointed out. Like the other subjects, the absorption of the non-haem in the test meal by the individuals with idiopathic haemochromatosis was closely related to the pH of their gastric juice, and the nature of this relationship appeared to be identical to that found in the non-haemochromatotic group. No special gastric factor was therefore demonstrated in subjects with idiopathic haemochromatosis. However, as might be anticipated, all such individuals had gastric juices with a low pH (Fig. 8). If this had not been so, it would have been extremely unlikely that they would have accumulated sufficient iron from their diets to develop the clinical disease.

7.4 Summary

In an investigation of the gastric factors responsible for modifying the absorption of non-haem iron in food, gastric juices from normal subjects, iron-deficient white and Indian patients, and patients with idiopathic haemochromatosis were tested for their capacity to solubilise bread iron in vitro. In addition, in vivo absorption studies were performed to establish whether there was any correlation with the in vitro release of iron by gastric juice. Gastric juice with pH values above 2 had a very limited capacity to solubilise the iron in bread; below pH 2 solubilisation increased linearly with decreasing pH. The relationship between pH and solubilisation was independent of diagnosis and also held for HCl solutions. Gel chromatography of the gastric juice extract showed a small fraction of high-molecular-weight iron; the size of the fraction was independent of pH. In addition, there was a low-molecular-weight peak which increased markedly as the pH decreased below 2. The behaviour of this fraction on gel chromatography was similar to that of Fe^{+3}. The in vivo absorption of non-haem iron from bread correlated well with the ability of the patient's gastric juice to solubilise the iron in bread and more particularly with the amount of small-molecular-weight compound in the gastric juice extract. These results suggest that pH is the only factor in gastric juice that is of importance in modifying the absorption of non-haem iron.

Chapter 8

Absorption of Iron from a Complete Meal: Influence of Composition of Diet and Body Iron Store on Absorption

Previous investigations of dietary haem-iron absorption are outlined in Chapter 1. The studies described in both Chapters 6 and 7 indicated that haem-iron absorption was adequate even in subjects in whom significant malabsorption of non-haem food iron was present. In addition, in both investigations it appeared that the percentage absorption of haem-iron from the meal remained relatively constant compared with the wide variations in percentage absorption of both non-haem iron and of ferrous ascorbate. It was therefore thought to be of some interest to define more closely the contribution of haemoglobin to the overall absorption of iron from a meal of fixed iron content.

8.1 Patients and Methods

The four test meals, of which two were given to each of 2 groups of subjects, consisted of 120 g potato mash (non-haem iron source) plus gravy made with the addition of ^{55}Fe labelled haemoglobin (haem-iron source). Each meal was given after an overnight fast, 2,5 g of salt was allowed for seasoning and each subject was allowed 200 ml of water to drink. The potato mash was prepared by boiling the peeled potatoes and then mashing them after draining the water.

Iron in the form of ferrous sulphate as well as ^{59}Fe of high specific activity (3 /uCi per portion) was added at the time of mashing. The intrinsic iron content of the potato mash was 1,23 mg per 120 g portion. Ferrous sulphate was added in quantities sufficient to give a final non-haem iron content for each of the four meals of 5,72 mg, 4,48 mg, 3,76 mg and 1,52 mg per portion respectively. The gravy consisted of a cooked haemolysate of ^{55}Fe - labelled rabbit erythrocytes and gravy prepared from a commercial gravy concentrate ('Brono', Hind Brothers, Durban, South Africa). The haemolysate was prepared from ^{55}Fe labelled erythrocytes as described previously. Each person received a mixture of labelled and unlabelled haemolysate adjusted so as to contain 5 /uCi ^{55}Fe and a final haemoglobin iron concentration of 0,28, 1,12, 2,24 and 4,48 mg for each of the 4 meals respectively. The gravy was made up to a final volume of 10 ml with the gravy powder, which contributed 0,08 mg of iron per portion. The ascorbic acid content of each meal was 10,3 mg. The haem iron:non-haem iron ratios of the meals were 0,28:5,72, 1,12:4,48, 2,24:3,76 and 4,48:1,52 respectively. Each group of subjects ate 2 of the meals, with an interval of 14 days between meals. Prior to eating the second meal, blood was taken to determine iron absorption from the first meal and to provide suitable background values for the calculation of absorption from the second meal. A further 14 days later blood samples were again taken and after an overnight fast a 'reference' dose

of iron (3 mg $FeSO_4$ + 2,5 μCi $^{59}FeCl_3$) plus 30 mg of ascorbic acid was administered in 50 ml water. Two weeks after the last dose of radioactive iron, blood samples were taken for the last time for determination of ferrous ascorbate absorption and for estimation of haemoglobin content, plasma iron and transferrin values and plasma ferritin levels.

The subjects studied were multiparous females of Indian descent living in Chatsworth, Durban. The isotopic, chemical and radioimmunoassay methods used were those described in Chapter 3.

Geometric means and standard deviation ranges were used as the data was positively skewed. Statistical evaluations were carried out using Pearson correlation co-efficients, Student's t test for paired data, and analyses of variance and covariance and multiple regression analysis.

8.2 Results

The haematologic, biochemical and iron absorption data of the subjects studied are shown in Table 7. Of the 9 subjects in group 1, 4 showed significant evidence of iron deficiency, with plasma ferritin concentrations of less than 10 μg/l and transferrin saturation below 12 percent. The absorption of ferrous ascorbate ranged from 19,1 to 50,9 percent. Of the 8 subjects in the second group, 3 had plasma ferritin levels of less than 10 μg/l, together with transferrin saturation of less than 12 percent. The percentage absorption of ferrous ascorbate by this group of

Table 7 Absorption of haemoglobin and potato iron from meals of similar iron content but differing ratios of haem and non-haem iron

				Meal 1 - 0,28 mg Haem iron + 5,72 mg Non-haem iron				Meal 2 - 1,12 mg Haem iron + 4,88 mg Non-haem iron				Ferrous ascorbate
				Absorption				Absorption				Absorption
				Haem iron		Non-haem iron		Haem iron		Non-haem iron		
Hb (g/dl)	Plasma iron (µmol/ℓ)	Saturation (%)	Plasma ferritin (µg/ℓ)	%	Amount	%	Amount	%	Amount	%	Amount	%
GROUP 1												
12,4	13,9	18	23	20,9	0,06	6,8	0,39	15,6	0,71	8,3	0,41	30,7
11,8	12,8	19	12	30,4	0,09	14,5	0,83	29,5	0,33	10,1	0,49	34,2
12,2	9,1	11	7	22,9	0,06	6,2	0,35	23,9	0,27	8,2	0,40	42,1
13,0	13,5	17	33	22,4	0,06	4,8	0,27	27,3	0,31	10,1	0,49	19,2
11,7	9,7	12	14	14,6	0,04	3,2	0,18	16,8	0,19	5,1	0,25	20,1
10,9	8,8	8	9	12,9	0,04	2,7	0,15	19,3	0,22	6,5	0,31	50,9
12,3	15,1	25	28	29,2	0,08	4,3	0,25	17,8	0,20	9,7	0,47	27,3
11,0	9,2	9	7	20,7	0,06	12,8	0,74	13,7	0,15	10,3	0,53	39,8
10,8	8,7	8	3	17,9	0,05	12,2	0,69	13,4	0,16	16,1	0,79	40,9
		Geometric mean		20,6	0,06	6,3	0,36	19,0	0,21	49,0	0,44	32,4
		SD range		14,9-28,4	0,04-0,07	0,19-11,7	0,19-0,67	14,2-25,3	0,15-0,28	6,5-12,5	0,32-0,61	23,1-45,3
				Meal 3 - 2,24 mg Haem iron + 3,76 mg Non-haem iron				Meal 4 - 4,48 mg Haem iron + 1,52 mg Non-haem iron				
GROUP 2												
11,3	13,1	12	37	21,4	0,48	12,9	0,49	16,8	0,75	17,4	0,26	29,4
10,4	9,9	8	6	24,8	0,56	8,5	0,32	25,8	1,16	17,1	0,26	43,8
10,7	8,7	9	3	13,0	0,29	3,8	0,14	18,7	0,84	14,6	0,22	41,7
13,1	19,4	27	42	21,6	0,48	13,1	0,49	19,6	0,88	15,7	0,24	19,6
9,4	8,3	13	12	25,3	0,57	5,3	0,20	21,7	0,97	16,9	0,26	31,9
8,9	7,1	7	1	17,6	0,39	9,6	0,36	9,8	0,44	19,1	0,29	55,2
13,7	15,9	19	51	12,0	0,27	3,1	0,12	22,9	1,03	17,1	0,26	17,1
12,8	11,1	21	13	27,7	0,62	6,7	0,25	26,9	1,21	29,4	0,45	23,3
		Geometric mean		21,4	0,48	7,0	0,26	19,5	0,87	18,0	0,27	30,5
		SD range		14,3-32,1	0,32-0,72	4,1-12	0,15-0,45	14,2-26,8	0,63-1,2	14,6-22,3	0,22-0,34	20,2-40,6

subjects varied from 17,1 to 55,2 percent. The two study groups were not significantly different in terms of haematologic values or biochemical indices of iron nutrition nor did they differ significantly in respect of the 'reference' iron salt absorptions.

The percentage absorption of haemoglobin iron remained constant for all 4 meals despite the differing quantities of this form of iron: geometric mean and SD range, meal 1; 20,6% (14,9 - 28,4%) meal 2; 19,0% (14,2 - 25,3%) meal 3; 21,4% (14,3 - 32,1%) meal 4; 19,5% (14,2 - 26,8%). In contrast, the percentage absorption of non-haem iron was significantly higher from the meal containing the smallest amount of non-haem iron (1,52 mg) than from the other 3 meals: geometric mean & SD range, meal 1; 6,4% (3,4 - 11,8%) meal 2; 9,0% (6,5 - 12,5%) meal 3; 7,0% (4,9 - 11,9%) meal 4; 18,0 (14,5 - 22,3%), (by analysis of variance $F = 8,7$, $p < 0,0005$).

Since the study was performed with each of 2 groups of subjects eating 2 of the meals, Student's t tests for paired data was also used to determine intra group differences in the results of absorption studies of each dietary component. This analysis revealed that the log percentage absorption of non-haem iron differed significantly for the two meals fed to subjects in group 1 ($t = 2,43$, $p < 0,05$). Similarly, the subjects in group 2 also absorbed a greater percentage of the non-haem iron from the meal in which the quantity of this form of iron was smaller ($t = 4,77$, $p < 0,005$).

The quantities of haem iron and of non-haem iron absorbed from all 4 meals are shown graphically in Figure 10. A highly significant correlation was found between the amount of haemoglobin iron absorbed and dose ($r = 0,94$, $p < 0,001$). There was a weak correlation between the amount of non-haem iron absorbed and dose ($r = 0,31$) which failed to reach statistical significance.

Other statistical correlations are shown in Table 8, and include significant correlations between haemoglobin, plasma iron, percentage saturation and log plasma ferritin concentrations as well as a significant correlation between the log plasma ferritin concentration and the amount of ferrous ascorbate absorbed. The correlations between the log of the amount of haemoglobin iron and log of the total iron absorbed from the meal is not unexpected, since haemoglobin iron absorption is part of the equation for calculating total iron absorption. The most significant correlation, however was between the log of the amount of haemoglobin iron absorbed and the log of the amount of haemoglobin administered.

Multiple regression analysis was used to evaluate the contribution of haematologic indices (plasma iron, percentage saturation, haemoglobin, log amount reference salt absorbed and log plasma ferritin) to the relationship between the log of the amount of haemoglobin iron absorbed and the dose administered. Although there was a minor improvement of the statistical correlation (simple $r = 0,94$,

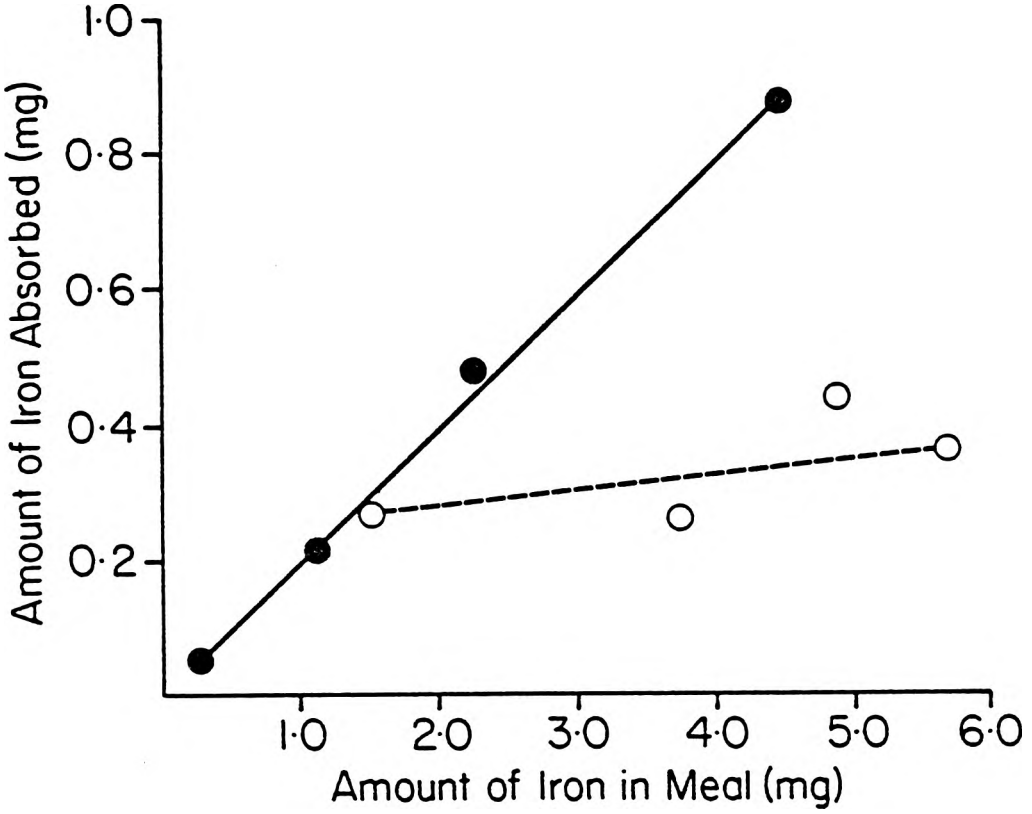

Figure 1Ø Absorption of haem (●) and non-haem (o) iron from a meal of gravy and potato in which the total iron content was kept constant but the relative amounts of haem and non-haem iron were varied.

Table 8 Correlation between iron absorption from gravy and potato meal and haematologic indices

	Plasma iron concentration (µg/dl)	Saturation %	Log plasma ferritin concentration	Log amount reference salt absorbed	Log amount of non-haem iron absorbed	Log amount of haemoglobin iron absorbed	Log total amount of iron absorbed from meal	Ratio of haemoglobin iron to non-haem iron in meal
Haemoglobin concentration (g/dl)	+0,78 P < 0,001	+0,74 P < 0,001	+0,77 P < 0,001	+0,77 P < 0,001	+0,01 P > 0,1	+0,07 P > 0,1	+0,07 P > 0,1	-0,18 P > 0,1
Plasma iron concentration (µg/dl)		+0,87 P < 0,001	+0,83 P < 0,001	-0,73 P < 0,001	+0,01 P > 0,1	+0,15 P > 0,1	+0,12 P > 0,1	+0,06 P > 0,1
Saturation %			+0,74 P < 0,001	-0,73 P < 0,001	+0,02 P > 0,1	+0,16 P > 0,1	+0,14 P > 0,1	+0,02 P > 0,1
Log plasma ferritin concentration				-0,82 P < 0,001	-0,12 P > 0,1	+0,07 P > 0,1	-0,02 P > 0,1	-0,03 P > 0,1
Log amount of reference salt absorbed					+0,18 P > 0,1	-0,15 P > 0,1	-0,03 P > 0,1	-0,07 P > 0,1
Log amount of haemoglobin iron absorbed							+0,78 P < 0,001	+0,94 P < 0,001
Log total amount of iron absorbed from meal								+0,68 P < 0,05

multiple r = Ø,96) when these other variables were included, it is clear that the major correlation is between the amount absorbed and dose. Eighty eight percent of the variation in haemoglobin iron absorption can be explained by variation in administered dose alone. With the addition of the haematologic variables, which correlated with the iron status of the individuals, 92% of the variation was explained.

A similar analysis of the non-haem iron absorption showed that only 22,3 % of the variation in the amount of iron absorbed from this source could be explained, even with the inclusion of the haematologic variables in a multivariate analysis.

8.3 Discussion

In the investigation discussed in Chapter 6 the absorption of non-haem dietary iron was found to be highly variable and dependant to a large extent on luminal factors. Of major importance in making non-haem food iron available for absorption is the role of gastric hydrochloric acid (Chapter 7) and achlorhydric individuals demonstrate significant malabsorption of wheat iron given in the form of bread. In contrast to these results the absorption of haem iron was found to be relatively constant at between 25-3Ø% in the two studies described in chapters 6 and 7, even in achlorhydric individuals who absorbed wheat iron poorly (Bezwoda et al 1976; Bezwoda et al 1978). The dose of iron administered in the form of haemoglobin in these studies was

2,4 mg and the good absorption of haem-iron held true for Indian females with overt or latent iron depletion as well as for venesected haemochromatotic subjects and for iron deficient white patients. Since the haemoglobin iron absorption appeared to be independent of the luminal factors which determine the availability of non-haem iron the relative proportions of haem and non-haem iron in the diet could have a major effect on the total amount of iron absorbed. The aim of the present study was to define more precisely the effect on iron nutrition of varying the amounts of the 2 different dietary sources of iron. Previous studies by other workers have shown that significant quantities of haemoglobin iron can be absorbed from the diet (Walsh et al 1955) and that the iron is taken up into the mucosal cell as an iron-porphyrin complex (Conrad et al 1966). In addition it has been established that luminal factors, such as the presence or absence of ascorbic acid (Callender et al 1957; Turnbull et al 1962; Hallberg & Solvell 1967) or of food or phytate (Turnbull et al 1962), have little effect on the absorption of haemoglobin iron. Valence of the iron within the porphyrin ring also does not appear to alter the availability of haemoglobin iron (Heinrich et al 1971). Although a number of studies have suggested that there is a relationship between the percentage absorption of haemoglobin iron and body iron status (Bannerman 1965; Conrad et al 1966; Hallberg & Solvell 1967; Martinez-Torres & Layrisse 1971),

for amounts in excess of 5 mg, there is relatively little information concerning the absorption of smaller amounts of haemoglobin iron such as might be present in the diets of peoples of lower socio-economic status.

Haemoglobin iron can contribute from 30 percent (Hallberg 1981) to as much as 60 percent of the amount of iron absorbed each day (Layrisse et al 1974), depending on the quantities of haemoglobin iron and non-haem iron present in the diet. However, the relative contribution of these two dietary components to the total amount of iron absorbed from a single mixed meal has not previously been studied systematically. In the present investigation the percentage absorption of haemoglobin iron did not vary significantly at the 4 dose levels studied i.e. 0,28 mg, 1,12 mg, 2,24 mg and 4,48 mg, when these amounts of haemoglobin iron were given as part of a mixed meal with a total iron content of 6 mg. In this respect, the data agree with the only other study which has investigated the absorption of haemoglobin iron over this range, namely, that of Hallberg and co-workers (1979), who found a relatively constant percentage absorption over the range, 0,2 to 5 mg haemoglobin iron. The results of this study do, however, differ in a number of respects from those of Hallberg and co-workers. In the present study the mean percentage absorption of haemoglobin iron was considerably higher (20,4%) than that found in the investigation by Hallberg, where the mean percentage absorption was below 10 %. A number of factors may go to

explain these differences. The iron status of the patients is the first factor to be considered. Although there is good evidence that body iron depletion does increase the percentage absorption of both non-haem iron and of ferrous ascorbate, and although a number of subjects in the present study were found to be iron depleted, covariate analysis did not reveal iron status to have a major influence on the haemoglobin iron absorption in the present investigation. Another factor to be considered is the influence of the other constituents of the meal. Both Hallberg and co-workers (1979) and Martinez-Torres & Layrisse (1971) have demonstrated a specific effect of meat, which causes an increase in the absorption of haemoglobin iron. It has been suggested that the effect of meat is due to the release of primary amines during the digestion of animal proteins which can maintain the haem in a monomeric form in which it is more available for absorption (Conrad et al 1966). Martinez-Torres and Layrisse (1971) found that veal approximately doubled the absorption of haemoglobin iron, while Hallberg and co-workers (1979) found that the absorption of haem iron from wheat rolls containing haemoglobin as an iron fortificant was increased to about 60% if a hamburger was eaten with the wheat roll. Martinez-Torres and Layrisse (1971) suggested that the additional protein degradation products from the meat were enhancing the action of the globin degradation products in preventing haem polymerisation. However, Hallberg and co-workers

pointed out that meat enhances the absorption of non-haem iron in a meal to approximately the same extent as it enhances the absorption of the haem iron and concluded that it was more likely that the effect was a non-specific one, namely a stimulation of the process of digestion. This would have to be independant of the effect on the secretion of gastric juice, since Bjorn-Rasmussen and Hallberg (1979) have shown that the absorption enhancing effect of meat is seen also in subjects with achylia gastrica. It seems that the nature of the mechanism has not been firmly established. Indeed there is some debate as to whether haem iron is absorbed as such when ingested as part of muscle. Some in vitro evidence suggests that under such circumstances well absorbed non-haem iron compounds of low molecular weight are the major degradation products that are released during digestion (Hazell et al 1978; Hazell et al 1981). It might therefore be necessary to distinguish between the absorption of haemoglobin iron when fed alone and when fed as part of meat. In the present study there was a small amount of animal protein present in the globin moiety of the haemolysate from which gravy in this meal was made. The source of the remaining iron in the meal was potato, together with ferrous sulphate. Could the non-haem in the meal have influenced haem iron absorption? Although the effect was not explained, the studies of both Callender and co-workers (1957) and of Turnbull and co-workers (1962) show that the absorption of iron in haem is enhanced when the

haem iron is given together with other dietary constituents. It is possible that other dietary constituents play a similar role to those derived from the digestion of meat. The results of this study are not incompatible with either hypothesis since the mean percentage absorption of the haemoglobin iron was higher (31,8%), when the meal contained hamburger (Chapter 6), as compared to the present investigation where haemoglobin was given together with potato (mean 20,4%). Dietary constituents other than meat may well cause a more modest enhancement of haemoglobin iron absorption. In addition, it should be pointed out that the studies of Heinrich and co-workers (1969, 1971) revealed haemoglobin iron absorptions which were of the same order as those in the present study. Furthermore, these investigators found that the absorption of a 5 mg dose of haemoglobin iron was considerably higher in subjects with gastric atrophy as compared with the absorption in normal volunteers and that this finding was independent of iron status. Although the reason for the higher haemoglobin iron absorption in patients with gastric atrophy has not been explained, it is of interest to note that in the population group investigated in our study, namely Indian women, hypochlorhydria was identified as a major factor in the poor absorption of non-haem iron.

The other difference between this study and that of Hallberg is that in the present study total iron absorption from the meal was measured, whereas Hallberg and coworkers

evaluated only haem iron absorption. The finding that the percentage absorption of non-haem iron falls with increasing dose is not unexpected. It was expected, however, that the lower percentage absorption would be compensated for by an increase in the amount of non-haem iron absorbed at the higher dose and, indeed, the paired comparison of the amounts of non-haem iron absorbed by the subjects in group 2 did show the expected rise. However, when all the results were considered together, no consistent increase in the quantity of non-haem iron absorbed could be demonstrated, at least not in the dose range studied.

In contrast, the amount of iron absorbed from the haem fraction increased progressively as the haemoglobin content of the meal rose (Figure 1Ø). The results of this study emphasise the importance of the haem iron content of the diet. The amounts of haemoglobin iron used in this study are within the range that might be expected to be present in Western type diets. Haem iron absorption accounted for the greater proportion of the iron absorbed, while non-haem iron absorption even after addition of $FeSO_4$ remained low. Dietary supplementation with haemoglobin, iron which is often at present discarded by abbatoirs, and which could be incorporated into a suitable vehicle, may be feasable in selected population groups, particularly for children of low socio-economic status whose home diet often consist, almost exclusively of vegetable staples. Relatively small amounts of haemoglobin iron (for example 5 mg) could supply a

considerable proportion of the daily iron requirement of these children.

Finally some consideration has to be given to the question of iron absorption and iron status. In this study the percentage absorption from the readily available iron salt correlated closely with the plasma ferritin concentration and this correlation again points to the influence of body iron content on iron absorption. In spite of this there was poor correlation between the absorption of either form of food iron and the indices of iron nutrition. Haem iron absorption from these quantities of haemoglobin is probably occurring at maximal rates and by a different mechanism from that responsible for non-haem iron absorption. Although the percentage absorption of non-haem iron from the potato was not particularly poor when compared to that from other vegetable staples, the iron requirements of these subjects were greater and it is probable that differences in the availability of non-haem iron, caused by individual variation in the luminal factors which play such an important role in making non-haem iron available for absorption, are responsible for the poor correlations obtained.

8.4 Summary

In an investigation of the influence of dietary composition (haem:non-haem iron) on iron accumulation, the percentage absorption of haem iron was found to be independant of dose in the range of approximately Ø,5 to 5

mg. The result was a progressive increase in the amount of iron absorbed from the haem fraction of the meal with increasing dose. The non-haem iron absorption on the other hand was not only considerably less in percentage terms than haem iron absorption but also failed to show any significant increase in the amount absorbed with increasing dose. The implications of these findings for the mechanisms of haem iron and non-haem iron absorption as well as for dietary fortification programmes are discussed.

Chapter 9

Effect of Diet on the Rate of Iron Accumulation in Idiopathic Haemochromatosis

The development of successful programmes of iron fortification of staple foodstuffs may in the future have far reaching consequences on the prevalence of both clinical and subclinical iron deficiency. However, it is not only populations that suffer from a high prevalence of iron deficiency that must be considered. The potential impact of fortification programs on iron balance in individuals with a genetic predisposition to develop iron overload is discussed in Chapter 1.

The purpose of this study was to obtain more information on the rate of iron accumulation in subjects with idiopathic haemochromatosis and so gain further insight into the control of iron absorption. The information obtained was also used to test various hypotheses of a mathematical model, developed to explain various aspects of iron balance in subjects with iron overload (Bothwell et al, 1978).

9.1 Subjects and Methods

Absorption of iron at 2 different dosage levels was studied in 7 patients suffering from idiopathic haemochromatosis. The diagnosis of idiopathic haemochromatosis was based on the usual laboratory and clinical findings (see Chapter 1). All patients had been

treated by repeated venesection therapy and at the time of the study more than 30 l blood had been removed from each of them. That their iron stores had been effectively depleted was confirmed by the fact that all plasma ferritin concentrations were less than 33 /ug/l (mean adult male value > 100 /ug/l). Venesection therapy was discontinued for at least 4 weeks prior to the study and was not resumed until it was finished.

Each subject ate 2 separate maize porridge meals on 2 successive mornings. The meals were fortified with 5 and 10 mg iron respectively. The maize porridge was prepared using 1 part of finely milled maize to 4 parts of water (w/v). The extra iron was added as ferric chloride in 0,01 N HCl just prior to cooking. In addition, the porridge was labelled on each morning with a different isotope of iron (2,5 /uCi ^{59}Fe or 5 /uCi ^{55}Fe in 0,01 N HCl) also added just prior to cooking. Care was taken to ensure even mixing of the radioisotopes with the unlabelled iron. The amount of cooked porridge given at each meal was weighed and was approximately 100 g. Each meal was given together with 1 dl pasteurised milk, 20 g sugar and a glass (2 dl) of fresh orange juice, after an overnight fast. The total iron contents of the meals (including the milk) were approximately 6,2 mg on day 1 and 11,3 mg on day 2 (Table 8) and the ascorbic acid content of the orange juice was 60 mg per 2 dl portion. Blood was drawn for radioactive counting 14 days after the second meal, and following this another

absorption study was carried out, in which a readily available iron salt was given. In this second part of the test, 3 mg iron as ferrous sulphate labelled with 2,5 /uCi ^{59}Fe was made up in 3 ml Ø,Ø1 N HCl, 3Ø mg freshly dissolved ascorbic acid was added and the total volume was made up to 5Ø ml.

The chemical, isotopic and immunoassay methods used were those described in Chapter 3.

9.2 Results

The haemoglobin concentrations varied from 12,9 to 15,3 g/dl (mean 13,9 g/dl) (Table 9). The plasma iron concentration ranged between 41 /ug/dl and 68 /ug/dl (mean 54 /ug/dl), the transferrin saturation between 12% and 25% (mean 17%) and the plasma ferritin concentration between 18 and 32 /ug/l (mean 25 /ug/l). The percentage absorption of iron from meal 1 varied between 17 and 34% (mean 25%) and from meal 2 between 15 and 23% (mean 2Ø%). Absorption of iron from the reference salt varied between 55% and 95% (mean 72%), thus indicating that all the subjects were iron depleted and therefore avid for iron at the time of the study.

In order to find out whether the pattern of iron absorption at the 2 dosage levels was the same in the subjects with idiopathic haemochromatosis as it was in normal individuals, the results were compared with those previously reported by Layrisse and co-workers (1973), in normal subjects (Fig. 11). These workers added varying

Table 9 Haematologic and iron absorption data in treated haemochromatotic subjects

Sex	Venesection (ℓ)	Haemoglobin (g/dl)	Plasma iron (µg/dl)	Saturation (%)	Plasma ferritin (µg/l)	Dosage of iron (mg)	Amount absorbed (mg)	Dosage of iron (mg)	Amount absorbed (mg)	Reference* absorption (%)
M	30	13,2	46	14	32	6,5	2,0	11,2	2,6	95
M	32	14,7	59	19	20	6,1	1,3	11,2	2,2	77
M	47	13,9	68	16	24	6,2	2,1	11,3	2,4	68
M	31	15,3	66	25	28	6,0	1,0	11,3	1,7	73
F	30	12,9	43	16	18	6,0	1,5	11,4	2,3	75
M	63	13,1	54	16	24	6,1	1,1	11,4	2,2	63
M	49	14,0	41	12	28	6,3	1,9	11,2	2,2	55

* 3 mg iron fed in the fasting state as ferrous ascorbate

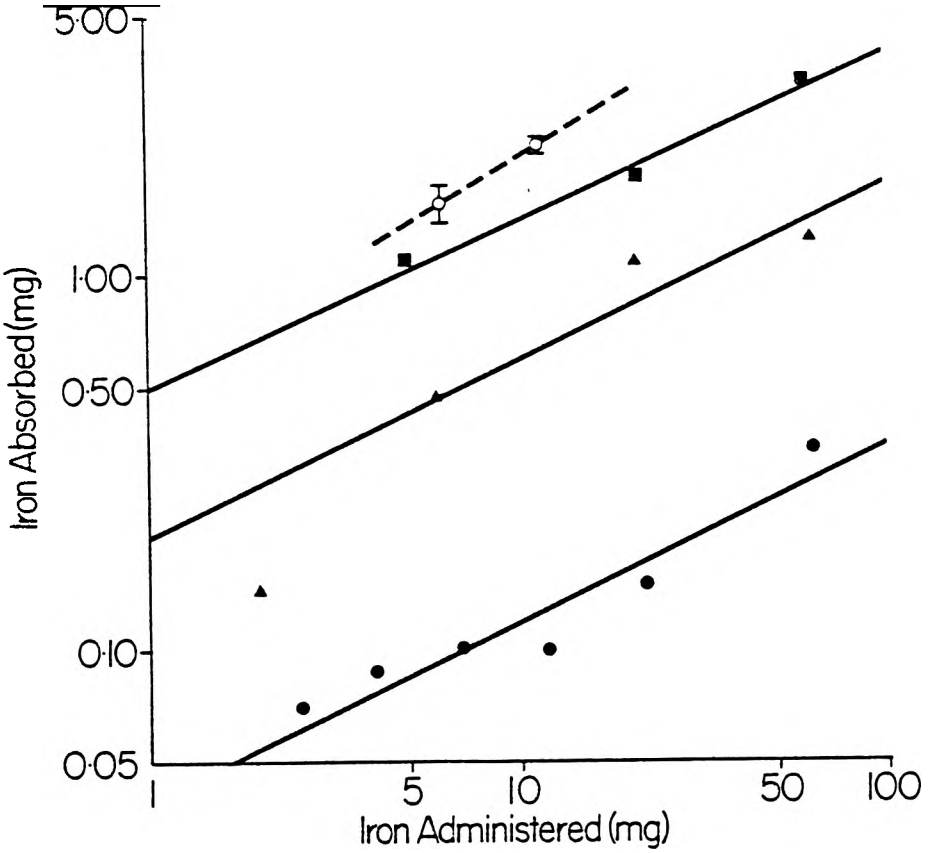

Figure 11. Absorption of non-haem iron from 3 different meals containing varying amounts of added iron ($FeCl_3$). The basic meals were as follows:- maize (closed circles); meat + maize (closed triangles); meat (closed squares). (After Layrisse and co-workers. Redrawn by permission of the Editor, Blood (41:333-352, 1973)). Included on the same graph are the results (mean ± SD) obtained in the present study in 7 venesected subjects with idiopathic haemochromatosis who on two occasions were fed a meal (maize porridge, milk and orange juice) to which different amounts of iron ($FeCl_3$) had been added (interrupted line).

quantities of iron as ferric chloride to 3 types of meal and measured the total quantities absorbed. In each instance the amount of iron absorbed increased progressively as the iron content of the meal rose. While the absolute amount of non-haem iron that was absorbed was dependent on the nature of the diet, being greatest with meat and least with maize, the rate at which iron absorption increased with increasing dose was independent of the nature of the meal. The relationship between absorption and dose could be expressed as follows:-

log iron absorption = Ø,47 x log dose of iron.

When the results obtained in the present study were plotted in the same way as the data of Layrisse and co-workers (1973) the relationship between the amounts fed and the quantities absorbed was as follows:-

log iron absorption = Ø,67 x log dose of iron.

This was not significantly different from that noted in normal subjects ($F = Ø,35$; $DF_1 = 1$; $DF_2 = 63$). While the actual quantities of iron absorbed by our subjects were higher than those in the subjects studied by Layrisse and co-workers, the patients in the present study were more iron-deficient and, in addition, the meal they ate was not the same, especially in terms of its ascorbic acid content.

9.3 Discussion

A model of iron balance in individuals with haemochromatosis is shown schematically in Figure 12. This model was worked out on the basis of data appearing in the

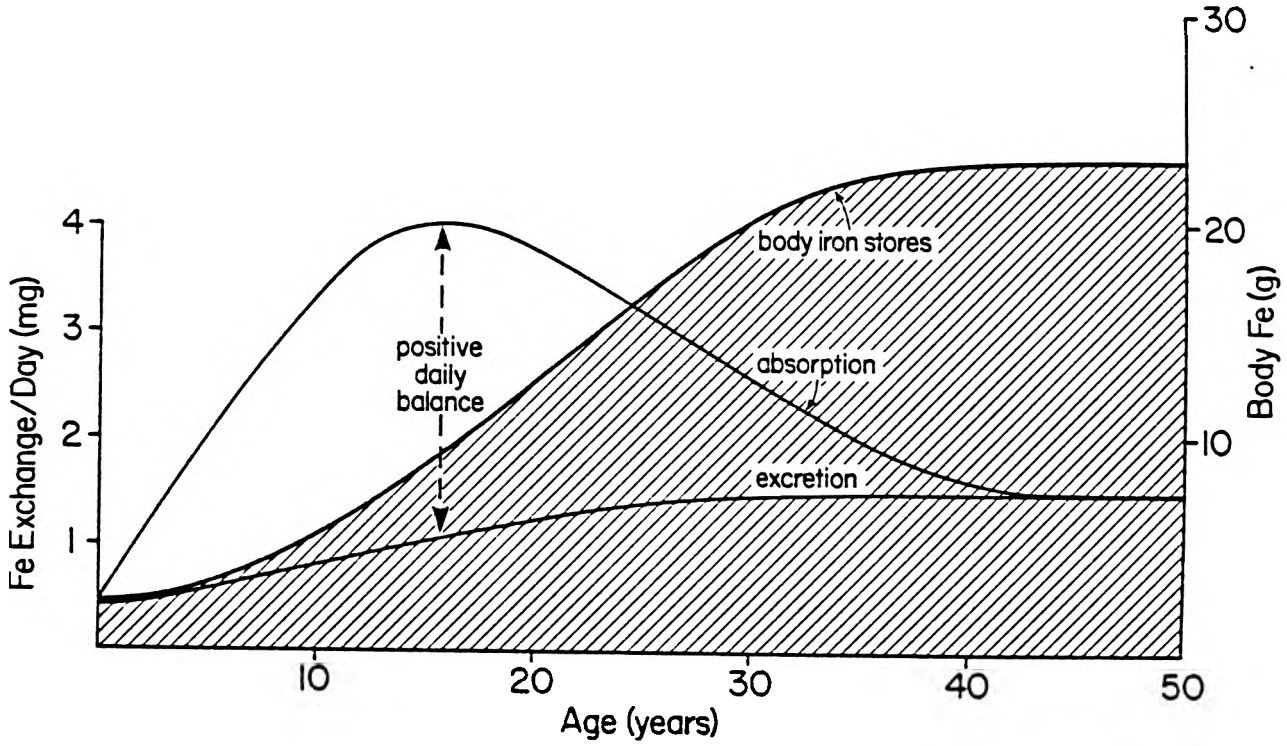

Figure 12. A schematic and idealised representation of how the body iron content increases over the years in idiopathic haemochromatosis. Note that the rate of iron absorption decreases as stores reach massive proportions, while the rate of iron excretion rises but to a lesser extent. Eventually equilibration is reached, but by this time the body iron content is very large. When the initial absorption rate is a good deal lower (e.g. in subjects heterozygote for the 'haemochromatotic' gene), equilibration would be expected to be reached before iron stores had become very large.

literature and which related the amount of iron absorbed from the diet to the quantity administered. The model is based on several premises, the most basic being that there is a significant negative correlation between the quantity of iron absorbed and the size of the body iron stores. Such a relationship has in fact, been demonstrated over a very wide range of iron stores (Cook et al 1974; Bezwoda et al 1979). It is possible that a similar relationship exists in idiopathic haemochromatosis but that the 'absorbostat' is set at a much higher level. At the time that patients with the disease present clinically, the iron absorption is usually normal, being about 1 mg daily (Bothwell et al 1979). However, after the excess iron has been removed from these patients by repeated phlebotomies, their iron absorption rises to high levels. For example, in the present study the average absorption from a daily diet containing 18 mg of iron would have been about 4,7 mg. It seems reasonable to suppose that this quantity is similar to that prevailing at the onset of the disease. This implies that there is a progressive fall in the amount of iron absorbed as the stores enlarge in idiopathic haemochromatosis.

In this model it is also assumed that iron excretion varies with the size of the body iron stores, being somewhat greater in those with increased stores, but that there is an upper limit to excretion. Such a relationship has in fact been demonstrated in black South Africans with iron overload

(Green et al 1968).

While there is reasonably firm experimental data to back up the presumptions made in working out this model the quantitative aspects remained to be investigated, particularly in regard to whether or not the data pertaining to normal subjects fed increasing amounts of iron could be applied to those in whom an inborn error of metabolism exists which is responsible for an excessive accumulation of iron. The results obtained in the present study indicate that the slope of increased iron absorption with rising iron intake in venesected haemochromatotic patients is very similar to that in normals. On this basis, it seems reasonable to believe that the rate of iron accumulation in subjects with the genetic predisposition to develop idiopathic haemochromatosis would be somewhat faster were national diets to be fortified with iron and that clinical manifestations would, as a result, occur at a younger age. However, the same relationship between iron absorption, iron excretion and the size of the iron stores would still exist, so that there is no reason to believe that the ultimate size of the body iron burden would necessarily be much greater than previously. The major change would be that the equilibration point where iron absorption and excretion equalled each other might be expected to occur sooner.

While idiopathic haemochromatosis is a relatively rare disease, there are a large number of people who are heterozygous for the mutant gene (Simon et al 1977;

Cartwright et al 1979). Such individuals appear normal or may show minor derangements of iron metabolism (Cartwright et al 1979). The plasma ferritin concentration, which reflects the size of the iron stores, is usually within the normal range (Simon et al 1977; Cartwright et al 1979) and iron stores rise during life from an average of Ø,2 g to 1,3 g (normal adult males less than 1 g) (Bothwell et al 1979). These findings imply that initial absorptive levels of iron are only slightly raised in heterozygotes and that the absorption and excretion rates usually equilibrate when stores are only modestly raised. Fortification of natural diets at the level proposed in the United States would therefore be expected to have only a limited impact. While the stores of heterozygotes would almost certainly increase, the increase would not be expected to be anywhere near the figures encountered in full-blown idiopathic haemochromatosis. If, in fact, the model previously suggested is a valid one, and the data presented in the present study are certainly compatible with it, then a heterozygote with an initial absorption of 2,Ø mg per day would start with an absorption of 2,5 mg per day were iron fortification as proposed in the U.S.A to be introduced. This might be expected to lead to a storage depot of at most 4,3 g instead of one of 1,6 g by the age of 5Ø years (Bothwell et al 1978).

While it is realised that a good deal of the previous discussion is conjectural, it does underline the sort of

information that is required if meaningful predictions on the effects of iron fortification are to be obtained. For example, it would be extremely important to know something concerning dietary iron absorption and stores in subjects with idiopathic haemochromatosis during a period of iron reaccumulation (i.e. after the completion of venesection therapy). Since there are a number of unanswered questions relating to the effects of food iron fortification on subjects with a tendency to absorb iron excessively, it is obvious that any projected fortification programmes must be carefully monitored. In this connection, the most important group to be followed would be heterozygotes carrying the mutant gene for idiopathic haemochromatosis. These subjects can, in fact, now be identified amongst the relatives of patients with the disease by HLA typing (Simon et al 1977; Cartwright et al 1979). Fortunately it is now possible to carry out monitoring on such individuals using a non-invasive technique, since repeated measurements of the plasma ferritin concentration should provide an accurate index of enlarging iron stores.

In South Africa, the situation is different from most other countries, since large numbers of the population, namely adult black males, have varying degrees of iron overload (Bothwell et al 1979). Fortification of the national diet with iron could therefore prove potentially deleterious for those many individuals who already have too much iron in their bodies. At the same time it must be

realised that there are other members of the population, notably Indian females (Mayet et al 1972) and underprivileged infants (Derman et al 1978), in whom iron deficiency occurs. Programmes to improve iron nutrition must therefore be specifically directed to the target groups. In infants this can be achieved in part at least by fortifying infant cereals with iron and ascorbic acid (Derman et al 1980), while in the Indian populations it may prove possible to fortify some key dietary constituent, which is regularly consumed in large quantities by this group, but not by others.

In addition this study demonstrates that although the level of absorption in venesected subjects with idiopathic haemochromatosis is generally higher for any given level of dietary iron content, the same factors which influence absorption in normal individuals appear to be present in these patients. The basic similarity in the process of food iron absorption in normal and in haemochromatotic, individuals was also dealt with in the investigation of patterns of food iron absorption in different groups of subjects (see Chapters 6 & 7).

9.4 Summary

There is still controversy concerning the effects on iron nutrition of increasing the dietary intake of iron. This debate has not only centred on the question of efficacy but also on safety. At particular, potential, risk are those individuals with disorders of iron metabolism such as

idiopathic haemochromatosis, who absorb iron excessively from the diet. Data obtained in the present study and in several other investigtions suggest that subjects homozygous for the mutant gene responsible for the disorder would develop clinical features of the disease at a younger age were the dietary iron intake to be increased. Iron stores in affected heterozygotes would be greater than they are at present but the size of these stores would be expected to equilibrate long before they had reached massive proportions. While these conclusions are based on data obtained in a number of studies, there are enough unanswered questions to make it mandatory for any future fortification programmes, whether they be directed at whole populations or only at certain segments of a population, to be carefully monitored. This can currently be achieved using serial plasma ferritin measurements, since the concentrations mirror the size of iron stores in the body.

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