CHAPTER I

GENERAL INTRODUCTION
Iron is one of the essential elements in human, animal and plant nutrition. Iron was regarded as source of strength and vigour for those suffering from many types of illnesses, long before its biological function was recognised. The use of iron by Assyrians and Egyptians was recorded in 4000 B.C. In 17th century the possibility of a connection between iron and human physiology was recognised. Later the iron content of animals was extensively analyzed by a French chemist, Bousinquant. After recognising iron as an essential nutrient, he continued to study the iron content of foods, wines and beverages. In those days physicians started using iron in the treatment of chlorosis, a type of anaemia found in women.

Iron metabolism and its relation to human disease has continued to interest many investigators. Investigations made during the past twenty years have provided data about the movements of iron into, within and out of the body (Moore, 1961). The rapid advances in different aspects of iron metabolism achieved during last three decades, and more so, after the availability and introduction of radioactive isotope of iron have given us a better understanding of the dynamics of iron in the body. Iron nutrition is still an important problem in human nutrition because of the important role of iron in biological processes and the unique way it is metabolised.
Biological role of iron

The central role of iron in the body is oxygen transport and in oxidative processes. Total amount of iron in the body varies with body weight, hemoglobin concentration, sex and size of the storage compartment. Two functional compartments of body are recognised.

1. As an essential component of hemoglobin, myoglobin, heme-enzymes and the iron transport protein transferrin and,

2. Non-essential storage iron found predominantly in liver, spleen and bone marrow as ferritin and hemosiderin.

It is found on analysis that 65% of total body iron is in hemoglobin, 3.5% in myoglobin, 30% in ferritin and hemosiderin and less than 1% in the form of enzymes (Moore, 1975).

Heme compounds and heme enzymes.

Hemoglobin. Hemoglobin accounts for the largest proportion of body iron. The molecular weight of hemo-
globin is approximately 67,000 and its iron content is 0.34 percent. Hemoglobin molecule consists of four heme prosthetic groups attached to one molecule of protein, the globin. The globin is comprised of four peptide chains, two of which are designated as alpha chains and two as beta chains. The synthesis of heme and its attachment to globin takes place in the terminal stages of erythropoiesis
in the bone marrow. Iron in heme is in the ferrous state and it is conjugated to imidazole nitrogen contained in two histidine residues within the globin. When hemoglobin combines with oxygen, the iron is displaced from one imidazole group and one molecule of oxygen is bound by one atom of iron forming oxyhemoglobin. This combination is reversed by exposing the oxyhemoglobin to a low oxygen tension.

Hemoglobin combines with carbon dioxide in the tissues, transports via blood, and releases it in the lungs. Hemoglobin is also an excellent biological buffer system. Hemoglobin content of blood is known to vary with age, sex and physiological state.

**Myoglobins.** These are respiratory pigments which occur in muscle cells of vertebrates and invertebrates. The molecular weight of myoglobin is 17000. It is composed of one heme prosthetic group, one protein molecule. It’s function is to store oxygen for utilization during muscle contraction. The amount of myoglobin iron in the adult body is about 20% of that of hemoglobin. When myoglobin is released into the bloodstream it is rapidly excreted through to the kidney, unlike hemoglobin, which when released from red cells is normally bound to plasma haptoglobin.

**Cytochromes.** Cytochromes, the electron transport enzymes, are located in the mitochondria as well as the
other cellular membranes. In the cytochromes iron atom oscillates between Fe** and Fe** during oxidation and reduction processes. Cytochromes accept initially the electrons from flavoproteins and transfer it to the final acceptor oxygen.

**Catalase.** It is iron porphyrin peroxidase enzyme containing four heme groups. Its function is assumed to be destruction of hydrogen peroxide formed by the action of aerobic dehydrogenase. It is particularly abundant in the red cell and in liver. Iron is present in catalase in the ferric state.

**Peroxidase.** Peroxidases occur chiefly in plant tissues. The protoheme enzymes also found in milk and leucocytes, reduce hydrogen peroxide at the expense of several acceptors such as ascorbate, quinone and cytochrome C. The peroxidase present in leucocytes is important for its anti-microbial action. The myeloperoxidase of the granulocyte is also known to contain iron.

**Non-heme iron containing enzymes.**

These are xanthine oxidase, succinic dehydrogenase and NADH-dehydrogenase. In the liver, xanthine oxidase plays an important role in the conversion of purine base to uric acid. Succinic dehydrogenase is concerned with the electron transport in or to the respiratory chain.

**Non-heme iron compounds.**

**Transferrin.** It can be regarded as a transport form
of iron present in plasma. It is a glycoprotein with an approximate molecular weight of 63,000. Each molecule of transferrin can bind two atoms of iron by ionic bonds (Beveridge et al., 1965). It has been suggested that tyrosine and histidine are the sites on the transferrin molecule to which the ferric iron ions are attached. The major function of transferrin is to act as the carrier of iron in the body. Under normal conditions all the plasma iron is coupled to transferrin. Since even very small quantity of free iron in the plasma is toxic, the prevention of circulation of free iron in plasma can also be regarded as one of the functions of transferrin.

The transferrin content of normal adult serum varies from 1.8 - 2.6 mg/ml with a mean of approximately 2.3 mg/ml (Taylor and Gatenby, 1966; Lane, 1966). This corresponds to total iron binding capacity (TIBC) of 250-400 μg/100 ml, with a mean of approximately 330 μg/100 ml (Laurell, 1947; Ramsay, 1958). The half-life of iron binding protein has been reported to be 8 to 10.4 days (Katz, 1961; Arai and Brown, 1961).

The degree of saturation of transferrin with iron does not appear to affect appreciably the rate of movement of iron from mucosal cell into the blood stream. Each transferrin molecule is capable of binding two atoms of ferric iron tightly until they are released at a receptor site on the surface of erythrocyte precursor (1.25 mg of ferric iron/gm of transferrin). The total amount of
transferrin present in plasma is small and forms only 3% of total plasma protein. Mitchell et al. (1960) suggested that there are two types of transferrin and these are designated as circulating transferrin, in the plasma and sequestered transferrin at the cellular site (Allen and Jandal, 1960; Badenoch and Callender, 1954).

Measurement of plasma transferrin is ordinarily indirect and involves the determination of latent iron binding capacity of plasma. Percent saturation is defined as the serum iron derived by the total iron binding capacity (TIBC). Normally, about one third of the total iron binding capacity is saturated with iron, while two thirds of the transferrin binding sites are available for additional binding and transport (Wallerstein and Mettler, 1956).

The major factors affecting the transferrin synthesis are: the level of iron stores, nutritional status and hepatic function (Bothwell and Finch, 1962).

Since iron in the plasma is bound to protein, it is normally not filtered by the glomerulus. When circulating transferrin is nearly saturated the excess iron is deposited in the liver.

Ferritin and hemosiderin. These two non-heme compounds are storage forms of iron in the body. Approximately 25 percent of iron in the body is in a storage form. The highest concentration of these compounds occurs in liver, spleen and bone marrow. Ferritin is an iron protein
complex made up of a homogenous protein portion apo-ferritin with a molecular weight of about 4,60,000. Iron in ferritin constituting about 20% is essentially protein free aggregate of ferric hydroxide.

Hemosiderin is the other storage iron complex. It contains aggregated ferritin molecules together with other structural elements.

According to Morgan and Walters (1963), when total storage of iron in liver and spleen was below 500 \( \mu g/g \) of tissue, more iron was stored as ferritin than as hemosiderin. When the level was above 1000 \( \mu g/g \), more iron was stored as hemosiderin.

Ferritin and hemosiderin have two metabolic functions. (1) To remove and store iron not needed by the body and (2) To provide a source of iron which can be drawn upon, when need arises.

Strugaeon and Shoden (1964) and Wapnick et al. (1970) have reported that iron can be exchanged between ferritin and hemosiderin in both directions. Shoden et al. (1953) have suggested that ferritin and hemosiderin are functionally related, as it was shown that iron can be mobilized from both fractions when needed.

**Total body iron content.**

Normal child (at birth) acquires 250-300 mg of iron. The total amount of iron in the body of the fetus is directly related to it's weight. Therefore, the iron content
at birth is determined by the birth weight. The body iron concentration at birth is higher than at any other time in life, but the low intake of iron by the infant during the first few months of life results in rapid decline in the concentration of the level found in adults. After the age of 2 years the growth rate slows down to about 2.5 kg/year. The iron requirement during this period is therefore only about 0.3 mg/day. Between the ages of 12 and 18 years there may be a weight gain of about 30 kg in boys and a little less in girls requiring an average daily iron absorption of 0.8 mg. An adult body contains about 4 gms of iron which varies with weight, hemoglobin concentration, sex and the amount of storage iron. Moore (1975) reported that under normal conditions body content of iron is about 50 mg/kg in adult men and 30 mg/kg in adult women (Finch et al., 1968).

**Iron absorption.**

Factors involved in iron absorption can be broadly classified as: (1) **Exogenous** and (2) **Endogenous**.

**Exogenous** or dietary factors will be discussed under dietary iron absorption.

**Endogenous** or physiological factors which regulate iron absorption will be briefly summarised here.

There is continuous change in concepts of iron absorption as the methods of investigations are improved. Regulation of intestinal absorption is more important for
Iron than other minerals, because of its limited capacity to excrete iron from the body. There is no single and satisfactory hypothesis today that can adequately explain the role of factors that are believed in regulating iron absorption.

The physiology of iron absorption in man and experimental animals has been a subject of intensive study for more than half a century. Hahn et al. (1943) reported that the gastro-intestinal mucosa is the chief regulator of iron absorption. Later Granick (1949) postulated a theory that iron in the gastro-intestinal tract combines with a protein apoferritin in the mucosa to form ferritin. The presence of ferritin in the mucosal cell was postulated to block further absorption of iron, until ferritin can give up its iron to transferrin in blood. This regulatory mechanism is known as "mucosal block theory". Granick (1951) further showed that very little ferritin and no apoferritin was normally present in the gastric and intestinal mucosa.

However, Witty and Crosby (1963) first postulated that serosal site of the cell was the major site of control of iron entry into the body. Recently, Linder et al. (1975) confirmed the importance of serosal transfer as a site of iron regulation. Based on these findings, Linder and Nkomo (1977) have summarized the present concept of mechanism of iron absorption as follows.

 Ionic iron in the lumen of the small intestine is
absorbed on to the receptors in the brush border of the mucosal cell, from where it enters the cell. Within the cell, the iron is transported to the absoral surface as a complex with a small molecular weight compound. Part of this iron is transferred across the absoral cell membrane and the remaining iron is returned to the gut. The iron status of the individual, his erythropoietic rate and other factors within the body regulate the process of iron absorption, especially at the level of transfer across the absoral membrane.

**Site of absorption.**

The duodenum and jejunum have the highest absorptive activity for iron (Wheby, 1966). The schematic outline of iron metabolism in the adult is given in figure-1. Though it is clear that maximum absorption takes place in the duodenum, it is difficult to determine the percentage of total amount of absorbed iron by different portions of the gastro-intestinal tract (Brown 1965; Brown, 1966). But recently, Ansari et al. (1977) injected labelled iron (Fe$^{59}$) to rats and determined the site of absorption. They reported that the duodenum is the primary site of iron absorption in rats; similar results have been obtained with mixed food source (Hussain et al., 1965).

**Iron excretion.**

A basic concept of iron metabolism is the body's limited ability to excrete iron, once iron is absorbed into the body. Iron has been classified among the poorly
Fig. 1. Schematic outline of iron metabolism in the adult.
absorbed and poorly excreted elements in human nutrition. Most of the iron is intracellular and the small fraction which transits through plasma is tightly bound to protein. Thus, normal kidneys appear to be incapable of excretion of iron from the body, less than 10% of the daily iron loss through the kidneys, the remaining quantity being lost through exfoliated cells and the secretions. Other routes of iron excretion includes desquamation of iron containing cells from the gastro-intestinal tract and the skin and loss in various secretions such as bile and sweat (Comar and Bronner, 1962).

The data about the daily non-heme iron loss are incomplete and are difficult to interpret. It is difficult to determine the amount of the metal excreted because extreme care is required to avoid contamination. No good method exists to differentiate between the unabsorbed and excreted iron in the faeces using the chemical method, and with radio iron, distribution of isotopes may not be uniform in the various body pools.

The total iron present in the faeces of a normal adult depends upon the amount ingested, since very little of the food iron is absorbed. The excretory iron estimated by chemical studies according to Ingalls and Johnston (1954) is about 0.2 mg per day. By radio iron technique, it was found to be 0.3 to 0.5 mg/day (Dubach, 1955; Dubach et al., 1946). This iron is derived from desquamated cells and from the bile. Most of the iron present in bile is derived from
the breakdown of hemoglobin and is thought that a large proportion of it is reabsorbed. In man the combined excretion through all routes is reported to be about 0.5 - 2.00 mg/day (Cubler, 1956; Moore and Dubach, 1956).

The mean urinary excretion of iron by normal adult men and women is given as 0.2 to 0.3 mg/day (Hawkins, 1964).

Cutaneous loss of iron through sweat, hair and nail is also important especially in tropical countries. Quantitation of iron lost through skin has been a subject of controversy. Estimates of daily iron loss through the skin have ranged from 0.07 to 6.5 mg (Apte, 1963; Mitchell and Hamilton, 1945; Prasad et al, 1963; Dubach et al, 1955).

The total amount of iron lost daily in the sweat will depend upon the individual, the ambient temperature and the cell content. "Cell rich" sweat of healthy Indian adult male was reported to contain 1.15 to 1.61 mg of iron/ml compared with 0.34 and 0.44 mg of iron/ml for the cell free sweat of men and women respectively (Hussain and Patwardhan, 1959; Hussain et al, 1960). In the anemic Indian women, the average iron content of cell rich sweat was found to be only 0.44 mg/ml and no iron was detected in the cell free sweat. Some workers have reported that, loss of iron through sweat is 6.5 mg/day (Foy and Kandi, 1957). The average total loss of iron in Indians estimated as 1.7 mg/day (range 0.8 - 2.6 mg/day) (Apte and Venkateshalam, 1963). Several groups have found that anemic individuals
lose less iron through skin than did normal individuals under the same condition, (Cavill and Jacobs, 1971; Prasad et al., 1963). Wheeler et al. (1973) found no decrease in the average amount of iron lost in sweat (0.35 mg/day) when the iron intake was reduced from 36 mg to 17.5 mg/day.

The iron turnover or the rate of iron loss from the whole body is determined by estimating radioactivity in the whole body or in red blood cells over a prolonged period, after an oral or intravenous dose of labelled iron. The iron turnover rate is expressed as a percentage of the body iron pool lost each day. The actual daily iron loss is determined from the turnover rate and an estimate of the miscible iron pool. Bothwell and Finch (1968) recalculated the data of Green et al. (1968) and found that daily iron loss from studies in several geographical locations was 12 to 16 µg/kg/day.

Wheeler et al. (1973) determined 3-day balances with 6 healthy young men who were adapted to a hot climate and normally performed a moderate amount of activity. When their activity was increased by 2 hours of rhythmic stepping, the mean iron loss in sweat was unchanged (0.35 mg/day), inspite of an increase in loss of cutaneous plus respiratory water from 5.5 liters to 7.9 liters.

Additional routes of iron loss include hair, finger nails, saliva and bile. Estimates of iron content of hair range from 1 to 19 mg/100 gm (Johnston, 1958). Annual losses
of iron from hair were calculated as 1 mg/year by Johnston (1958). Jacobs and Jenkins (1960) studied the iron content of finger nails in 100 persons of 0 to 84 years. The iron content of finger nails was very high in infancy and dropped to adult levels (0-400 µg/g) by age 15. There was no relationship between the severity of the anemia and the iron content of finger nails.

Menstrual iron loss has been expressed as mg iron/menstrual period or as ml blood/period. Menstrual blood losses show large variation between women. According to a study by Hallberg and Nilsson (1964) on 12 young women over 12 months, the mean individual menstrual losses ranged from 4 to 26 mg iron per period. Apte and Venkatachalam (1963) found the menstrual loss in Indian women to range from 8 to 30 mg per period, thus, amounting to 0.2 to 1.0 mg of iron per day with an average of 0.5 mg per day.

An important factor in estimating menstrual loss is the variation in an individual from one period to the next. Widdowson and McCance (1942) measured menstrual loss in three women for 8 to 13 consecutive periods and concluded that individual’s loss was quite constant but that loss varied greatly among individuals. Kybo (1966) reported that in general, women with longer periods have a greater average loss than women with shorter periods. The frequency of the menstrual cycle is an important consideration in attempting to relate the relatively heavy iron losses,
incurred for a few days to an appropriate daily estimate for iron intake. Beaton et al (1970) suggested that with iron intakes below about 11 mg/day, menstrual loss may not be compensated by intake, and iron depletion results. Iron derived from every day diet should meet the normal requirements of the body.

The daily intake of iron must replenish the amount lost from the body, plus supply and additional amounts needs for growth and development. The daily requirements of iron as recommended by a WHO expert committee for individuals of different age and sex are as follows: (WHO Tech. Report Series 505, 1972; 452, 1970).

<table>
<thead>
<tr>
<th>Group</th>
<th>Requirement (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants 5 to 12 months</td>
<td>0.7</td>
</tr>
<tr>
<td>Children 1-12 years</td>
<td>1.0</td>
</tr>
<tr>
<td>Boys 13-16 years</td>
<td>1.8</td>
</tr>
<tr>
<td>Girls 13-16 years</td>
<td>2.4</td>
</tr>
<tr>
<td>Men</td>
<td>0.9</td>
</tr>
<tr>
<td>Menstruating women</td>
<td>2.8</td>
</tr>
<tr>
<td>Pregnancy first half</td>
<td>0.8</td>
</tr>
<tr>
<td>-de- second half</td>
<td>3.0</td>
</tr>
<tr>
<td>Lactation</td>
<td>2.4</td>
</tr>
</tbody>
</table>

The above required amounts must be absorbed to maintain homeostasis. Disturbances in iron metabolism can lead to three kinds of disorders: iron deficiency, iron overload and impaired utilization. The first two are disorders of iron balance. Iron overload occurs in
conditions such as hemochromatosis due to hereditary defect, transfusion, hemosiderosis due to prolonged massive iron therapy in the form of repeated transfusion, hemosiderosis and cytosiderosis as a nutritional anomaly. Disordered utilization involves the inability of the body to allocate iron properly so that iron which is present in the body cannot be made adequately available for physiological requirement (Beutler et al, 1963). These disorders are extremely rare in India. The disorders of iron metabolism leading to iron deficiency would be discussed in the following paragraphs.

Iron deficiency.

Iron deficiency anemia is one of the most common nutritional deficiencies encountered in many parts of the world. A recent estimate indicates that 10-30% of world's population suffer from iron deficiency. The incidence of nutritional anemia in tropics is greater than anywhere else (Dasgupta, 1954; Woodruff, 1951, Venkatachalam, 1968; Mclean Baird, 1959; Soed, 1966; Cook, et al, 1971; Aungthan Bantu et al, 1972; Chandra, 1976). The results of numerous nutrition and hematological surveys carried out during the last three decades in India confirm the observation in other parts of the world, that nutritional anemia of various types is one of the most important nutritional diseases. These surveys further indicate that type of anemia prevalent in India is the microcytic hypochromic variety responding to treatment with iron. Despite of several publications on anemia in India, the actual incidence of hypochromic
anemia in the country is rather difficult to assess.

The peak incidence of iron deficiency is found in the physiologically vulnerable sections of population namely the women of reproductive age, infants and preschool children, especially those from the lower socio-economic strata. Iron deficiency anemia contributes directly to a high maternal mortality rate, high prematurity rate, still births and neonatal deaths and is therefore an important public health problem (WHO Report, 1975; Dasgupta, 1954; Smithburn et al, 1931). Hynes (1949) found iron deficiency anemia in India among soldiers from the north-west despite daily dietary iron intake of 60 mg. Ramalingaswamy and Patwardhan (1949) reported a high incidence of anemia among the South Indian plantation labour. Recent surveys of the entire population of village has indicated that incidence of anemia among rural population is higher than what was reported earlier (Annual Report, 1978) and that incidence is high even among men.

Normal individuals assimilate only enough iron to replace losses and this delicate balance is largely maintained by variations in the rate of intestinal absorption of dietary iron. The relationship between dietary iron absorption and iron deficiency is well known. One of the important causes of iron deficiency are inadequate intake of dietary iron and its absorption. The results of various diet surveys in India seem to indicate an adequate iron supply (Gopalan et al, 1971). However, availability of
iron for absorption appears to be poor (Hussain et al., 1965; Layrisse et al., 1969). Lack of available iron is therefore an important factor in pathogenesis of iron deficiency in population subsisting on diets in which the staple food stuffs are cereals and vegetables. Therefore, dietary iron content and its availability are the two important factors determining the iron nutritional status of an individual.

Iron absorption studies are useful as an indicator of iron balance in individuals. The iron absorption from the whole meal which contain both inhibitors and promoters is more important than iron absorption from individual foods.

**Dietary iron intake and iron balance.**

Results of diet survey in India indicate that the mean intake of dietary iron is in fact not low. The intakes however vary over a wide range (NMNB diet survey, 1975; Mitra, 1955). The average daily intake of iron in the low socio-economic group of urban population in India is about 20-30 mg per consumption unit (Prabhavathi, 1976).

Mean intakes of iron do not reveal the correct picture with regard to the adequacy of intake by all the segments of population because of wide individual variation in intakes.

**Source of iron in the diet.**

The source of iron in the diet is an important
consideration in as much as it will determine the total intake and the extent of its absorption from the diet. The main source of iron in the diets commonly consumed in India and many other developing countries particularly among the low income groups is cereals. Nearly 72% of dietary iron (NNMB, 1975) is contributed by cereals. This is in contrast to developed countries where cereals contribute only 30% of dietary iron.

**Dietary iron absorption.**

The most important constraint on meeting iron requirement through dietary means is the limited absorption of dietary iron. Daily requirement of iron ranges from 0.5 mg for infant to 2.8 mg for women of reproductive age. Diet surveys done in India have shown that only 10% of the family is taking less than 20 mg of iron. Moore (1968) reported that iron is relatively poorly absorbed. Large proportion of the population in every part of the world is reported to have a low storage of iron in liver. This has lead to extensive studies in man on absorption of iron from different dietary sources. The physiological factors affecting iron absorption have been discussed earlier.

Increased iron absorption in those with poor iron status has been reported in many studies with patients with iron deficiency with or without anemia. These observations have been made in studies using test doses of iron fed alone (Brise and Hallberg, 1962; Conrad et al., 1967; Hard and Escalbergh, 1956; Heinrich et al., 1974;
Hoglund, 1971; Hoglund et al, 1970) or fed with test meals (Bothwell et al, 1958; Pirzio-Biroli et al, 1958; Pirzio-Biroli and Finch, 1960), and in studies with mixed food sources (Chodos et al, 1957). Greater proportion of the test iron was absorbed when it was fed to fasting subjects than when it was fed following a meal (Brisse, 1962; Hausmann et al, 1969).

It has been shown in some of these studies that women absorbed more iron from test doses than did men (Kuhn et al, 1968).

There is some evidence also that the amount of iron absorbed is related to age. In a group of children 7 to 10 years old, Derby et al (1947) observed a strong trend toward increased iron absorption as the age of the children increased. Schulz and Smith (1958) reported that the absorption of naturally occurring iron in milk was higher in children below the age of 5 years, than in children between the ages of 5-10 years old. Bonnet et al (1960) reported a slight decline in iron absorption in men and women between the ages of 20 and 34 years. Freiman et al (1963) however found no evidence for an impairment of iron absorption with age in a group of 69 to 87 year old men and women.

**Form of iron in the diet.**

The form of iron has a marked influence on availability for absorption. Heme iron is apparently absorbed by a different mechanism than that for inorganic iron.
In humans, iron administered as ferrous salts appears to be utilized more efficiently than that from ferric salts (Brise and Hallberg, 1962; Moore et al, 1944). Pritz and Pla (1972) tested a variety of iron compounds in animal by hemoglobin reflection test and found wide differences.

The absorption of iron from foods also varies widely. Moore (1968) summarized results of several reports with a tentative conclusion that assimilation tends to be better from muscle, liver and hemoglobin than from cereals, milk and eggs. Generally, animal sources of iron are better utilized than plant sources (Cook, 1970; Bowering et al, 1976). If animal and plant sources are combined and fed, one source tends to modify the availability of the other (Layrisse et al, 1968).

Factors affecting the absorption of iron.

There are two main factors causing variations in the percentage of dietary iron absorbed.

1. Physiological
2. Dietary

Some physiological factors like age, sex, have been discussed earlier.

Dietary factors.

The effects of several dietary factors on iron absorption are quite impressive. Variations can be caused by individual foods, specific nutrients or by the general
composition of the meal. Some of the dietary factors known to imbalance iron absorption are:

1. Oxalates, phosphates and phytates (Moore et al., 1943; McCance et al., 1943; Apte and Venkatachalam, 1962; Heinhold, 1975; Hussain and Patwardhan, 1959), tannins (Dialer et al., 1975) form insoluble complexes and reduce iron absorption.

2. Calcium which binds phosphates (Apte and Venkatachalam, 1964) increases iron absorption.

3. Metabolism products like lactic acid, pyruvic acid and malic acid can form iron complexes and facilitate transcellular passage (Pollack et al., 1964).


5. Heme iron is better absorbed than non-heme iron (Cook et al., 1969).

6. Ascorbic acid enhances the iron absorption (Pirzio Birolli et al., 1958; Wapnic 1970; Williams, 1959).

Since both foods and the nutrients, they appear to affect iron absorption it is not surprising that the composition of a meal profoundly influences the amount of dietary iron available for uptake by the mucosal cells.

Methods used for studying iron absorption.

The methods most commonly used in the investigation of food iron absorption are:
1. Chemical balance method
2. Plasma iron tolerance test
3. Radio iron balance
4. Isotope technique.

**Chemical balance method.**

A large number of balance studies have been conducted for the purpose of determining iron retention from different diets. In the chemical balance studies, iron losses from faeces and urine are subtracted from dietary iron intake, without considering the losses from skin and sweat which are not normally measured. It is assumed that if intake and output are equal, the subject is in equilibrium. The chemical balance method was considered important to determine the amount of iron intake and to establish the limits of iron retention from diet. The intake and output run closely parallel (McCance and Widdowson, 1957). The merits of this method are; (i) exact replication of normal physiological conditions, (ii) the source of iron is administered as food iron, which is distributed over the daily meals, (iii) observations can be made over long enough period.

The disadvantages of the method are; (i) incomplete collection of faeces, (ii) analysis of bulky materials such as ingested food and faeces.

The chemical balance studies are laborious and time consuming and therefore expensive. They can be
carried out only in a limited number of subjects.

The general opinion about this method is that, it is especially suitable for measuring absorption of food iron as normally consumed. It is believed that (Josephs, 1958) the absorption values obtained by this method are higher when compared to those obtained with the isotopic method.

Radio iron balance.

This is a modification of chemical balance method. In this, radioiron is given by mouth as an inorganic salt or as labelled food stuff (Dubach et al., 1948; Moore 1955). Faeces samples are collected till less than 0.5 percent of the administered dose is present in a 24 hr fecal specimen. This normally occurs between 7 to 10 days after the administration of the test dose. The advantages of this method are: (i) it is more accurate than the chemical balance method, (ii) it is more specific and does not involve laborious chemical analysis. The disadvantages of this method lies in the prolonged faecal collection which requires co-operation from the subject. The normal iron absorption reported by using this method is about 6% and the error in measurement is estimated to be 5 to 10%.

Plasma iron tolerance test.

Since the absorbed iron is transported in plasma, an iron absorption test based on the measurement of plasma iron was designed in 1937 (Moore et al, 1937). In this
method, a fairly large amount of iron salt is administered orally to fasting subjects and the plasma samples are analysed for iron after 4 to 6 hours. Here the assumption is that a greater than normal rise denotes increased absorption, and subnormal rise denotes depressed absorption (Huff, 1950). A number of factors are involved in the rise of plasma iron after an oral ingestion of iron. Some of the factors are, the amount of iron absorbed, the rate of absorption, the amount of unsaturated transferrin binding capacity in plasma, and the rate of removal of iron from the plasma (Pinch and Hoss, 1952). The interpretation of the plasma iron curve should therefore take into account contribution of all these factors.

The plasma iron tolerance test can not be regarded as a physiological measurement, because large doses of iron must be employed. Josephs (1956) reported that around 50 mg of iron is necessary to rise the plasma iron level in normal man. The amount of iron that is usually taken in the normal diet does not affect the plasma iron level and hence this method is not suitable for measuring dietary iron absorption. Correlation between the results of plasma iron tolerance test (Jeffrey et al, 1955) and more quantitative estimates of iron absorption (Henley et al 1956) are not impressive.

Plasma iron tolerance test may be employed to measure altered absorption in a patient who has adequate
transferrin and not having conditions that can increase plasma clearance such as infection, iron deficiency and hemolytic anemia.

**Isotope technique.**

Iron has two commonly available isotopes: Fe$^{55}$, a low energy X-ray emitter with a half-life of 2.9 years and Fe$^{59}$, a β and γ-ray emitter with a half life of 45 days.

The liquid scintillation counter is required to measure both Fe$^{55}$ and Fe$^{59}$, while Fe$^{59}$ can be measured in a γ-counter. A whole body counter can be used for in vivo determination of Fe$^{59}$ retention. The ideal isotopic method for studying food iron absorption is the one in which biosynthetically labelled foodstuffs are used. Moare and Dubach (1951) were the first to study food iron absorption by employing biosynthetically labelled food. However, it is impossible to label all the components of a diet.

Iron absorption studies have been done on food stuffs, measuring either the native food iron labelled intrinsically (Elwood et al., 1968; Elwood et al., 1970) or added radiociron (extrinsic tag) to the carrier food (Hallberg and Bjorn Rasmussen, 1972; Layrisse et al., 1972; Layrisse et al., 1974) or by measuring both extrinsic and intrinsic iron (Cook et al., 1972; Bjorn Rasmussen et al., 1972; Bjorn Rasmussen et al., 1973; Bjorn Rasmussen, 1973; Hallberg et al., 1974; Sayers et al., 1974; Narasinga Rao and Vijayasarathy, 1975). An alternate simplified isotopic
technique to the biosynthetic labelling is to spike the entire meal with extrinsic tracer dose of radioiron and the absorption measured. The principle of this procedure is that the extrinsic tracer, exchanges completely with the iron in the gastro-intestinal tract.

Iron from radioiron can be used to spike non-heme iron and labelled hemoglobin for heme iron in the diet. Iron isotopes absorbed would be in the same proportion as the iron in food. The ratio of food iron (intrinsic tag) to added iron (extrinsic tag) has been found to be close to unity in a wide spectrum of absorption percentages (Cook et al., 1972; Layrisse et al., 1973; Hallberg et al., 1974; Sayers et al., 1974; Cook 1977). The spike technique has shown to be valid for investigation of iron absorption from cereals, legumes and composite meals.

In the double isotope technique to measure iron absorption two isotopes are administered, one orally and the other intravenously and iron absorption is calculated from the ratio of radioactivity of the two isotopes in a blood sample after 12 days. The chief assumption here is that, the absorbed iron is transferred to tissues and the distribution of iron entering from the gut is similar to that of iron given intravenously. This technique has been used to study the absorption of different iron compounds (Briss and Hallberg, 1962; Saylor and Finch, 1953) and to measure plasma iron turnover (Hallberg and Solvell, 1960).
A number of methods for determining food iron absorption are discussed above. None of these considered as an ideal one. Each method has its own advantages and disadvantages.
SCOPE OF WORK
The results of several diet surveys in India indicate that the average Indian diet contains apparently adequate amounts of iron and yet iron deficiency anemia is widespread (Gopalan, 1967). The results of diet surveys carried out in different parts of India indicate that the mean intake of dietary iron is 30.3 mg iron/day/consumption unit (NNMD, 1975). The principle sources of iron in Indian dietaries are cereals, pulses and vegetables. Cereals with 2 to 8 mg percent iron and pulses with 2 to 10 mg percent together contribute a major proportion (80%) of daily iron intake.

A majority of anemia in India is iron deficiency type. The important fact is iron balance that cannot be maintained either due to inadequate amount of iron or enough iron is not absorbed, the latter appears to be more important.

It is apparent from the available data that poor availability of iron from diet is an important etiological factor in the development of iron deficiency anemia. The different methods used for studying food iron absorption have been discussed in the earlier sections of this chapter. Each method has been shown to have its own advantages and disadvantages. It has been realized in recent years that isotopic method is the most reliable method for measuring food iron absorption in humans (Hallberg, 1974).
Though isotope method gives reliable data, they are time consuming for screening large number of samples. Co-operation from the human subjects is another problem in conducting the experiments. In using isotopic method the question of radiation hazard to the subjects used for absorption study must be taken into consideration. To overcome some of these problems, an animal model for studying iron absorption from human diets has been proposed recently (Darasinga, 1977). Even this is not suitable and expensive for large scale routine screening of foods.

A method which is simple and rapid is therefore needed to estimate the available iron from foods and diets. Hence investigations were initiated in this direction. The results of these investigations are divided into five parts in this thesis.

The development of an in vitro method for the measurement of availability of iron is described in Chapter-III. The results obtained by in vitro method were compared with the in vivo method.

In Chapter-IV, comparison of results obtained by in vitro method and extrinsic tag and chemical balance methods after following the experimental design of classical chemical balance method were presented. The absorption of iron has been determined from the whole day's meal.
In Chapter-V, the application of the in vitro method for determining the effect of simple processing on the availability of iron was described. Foods are subjected to processing before they are consumed. Hence an attempt was made to study the effect of processing like germination, cooking, baking etc. on the available iron.

In Chapter-VI, studies on tannin as a factor in modifying iron absorption was described. Inhibiting substances like phytates, phosphates have been studied by many workers. Since not much is known about the role of tannin in dietary iron absorption, the effects of tannin on available iron were studied.

In the next (Chapter-VII) the availability of contaminant iron was investigated. Foods are contaminated during processing and preparation. The availability of that contaminant iron is not known. Hence, studies are carried out to know the extent of contamination and its availability.

In the final (Chapter-VIII) results of various investigations presented in this thesis were summarised.