PHYSIOLOGICAL CONSIDERATIONS
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In 1713 Lassar and Geoffroy first demonstrated the presence of iron in the tissues. In 1843 Liebig postulated that the red blood corpuscles contain an iron compound which helps in respiration. Hoppe-Seyler succeeded in crystallising haemoglobin in 1867. In 1925 plasma iron was demonstrated as different from haemoglobin iron by Bates and Thavelli. Two years later Barke (1927) observed that iron was bound to plasma proteins. Hilsberg and Laurit (1945) demonstrated the iron binding property of plasma. Surgenor, Kochling and Strong (1949) isolated siderophilin. Leuberger was able to isolate ferritin in 1937. Early in the twentieth century Warburg (1949) found out that molecular oxygen used by the cells for respiration of aerobic organisms did not react directly with the biological substrates, but only with the divalent iron combined in a complex. He postulated that iron of a higher valency is thereby formed, which is reduced back again to a divalent state by organic substances, the iron regaining its original state. A valency change was postulated to occur in a complex iron compound by which the
oxygen required for cellular respiration was transported. In other words, iron constitutes an oxygen transporting vehicle for respiratory enzymes.

In 1937, new light was shed on the physiology of iron by McCance, who observed that parental injection of iron was not followed by an increase in fecal excretion of the metal. This initiated the concept of a closed internal cycle of iron metabolism.

IRON METABOLISM

Although the amount of iron in the body is small (about 45 mg/kg, body weight), it is an essential constituent of haemoglobin, cytochrome, cytochrome oxidase, catalase and peroxidase enzyme systems. Its chief functions lie in the transport of oxygen to the tissues (haemoglobin) and in cellular oxidation mechanisms (cytochrome system).

A brief review of the various facets of iron metabolism are of some relevance and the succeeding paragraphs bring out the important aspects of work done on the subject.
DISTRIBUTION OF IRON IN THE BODY: The total amount of iron in a healthy adult male averages between 3.5 to 4.5 grammes. As would be expected of such a biologically active metal, virtually all of this is bound to protein, either as functional iron, such as haemoglobin and the iron containing enzymes or as storage iron or as iron in transport. The following table shows mostly iron is present in the form of haemoglobin, constituting 65 to 70% of the total (Diorgio, A.J., 1970).

Table I: Showing the distribution of body iron

<table>
<thead>
<tr>
<th>Compound</th>
<th>Iron in mg.</th>
<th>Total body iron</th>
<th>70 kg.</th>
<th>3.0 kg.</th>
<th>Adult male</th>
<th>New born</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>2,800</td>
<td>156</td>
<td>65-70</td>
<td>70-75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue iron, myoglobin and enzymes</td>
<td>150</td>
<td>21</td>
<td>4</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage</td>
<td>1,000</td>
<td>20-30</td>
<td>20-30</td>
<td>10-15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transport</td>
<td>3-4</td>
<td>1</td>
<td>Δ1</td>
<td>Δ1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total:</td>
<td>3,500-4500</td>
<td>200-225</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Reproduced from the Medical Clinics of North America Vol. 54 No.6, p. 1401 (1970).*
Haemoglobin contains only 0.34 percent iron (3.4 mg. iron per gramme). Thus at a haemoglobin concentration of 15 gm/100 ml, one millilitre of the whole blood would contain 0.5 mg. of iron. Most of the remainder (20-25%) of body iron is found in the ferric form in the reticuloendothelial system bound to storage proteins - as water soluble compound "Ferritin" and water insoluble molecules "Haemosiderin". Functional but non-haemoglobin iron including myoglobin and enzyme iron as well as the iron in transport bound to transferrin, together do not account for more than 5% of total iron content of the adult.

At birth, a somewhat greater proportion of iron is in the form of red cell haemoglobin (70-75%) while the tissue stores contain about 25% of the total iron in the body (Giorgio A.J., 1970). It is therefore not difficult to understand why infants who triple their birth weight during their first year of life (and appreciably enlarge their haemoglobin mass) are prone to develop iron deficiency, especially if nutrients other than milk - a notoriously poor source of iron - are not taken in sufficient amounts during this period of development.
IRON ABSORPTION: The quantity of iron in the body is controlled primarily by iron absorption from gastrointestinal tract. The average adult diet provides about 12-15 mg. of iron per day, but only about 5 - 10% of this amount (0.6 - 0.12 mg.) is absorbed.

Although it is well established that iron deficiency acts as a potent stimulus to the absorption of inorganic iron and food iron, recent studies of iron deficient children and dogs indicate that iron lack at tissue level may even impair iron absorption as a result of biochemical derangements of the absorbing epithelium. This may be somewhat akin to the occurrence of malabsorption to folate and B₁₂ demonstrable in the presence of a deficiency of these vitamins (Giorgio, A.J., 1970).

There are several methods which can be used for measuring the absorption of iron i.e. the chemical balance studies and radioactive labelled iron studies both have their advocates. A number of very elegant studies using radioactive inorganic iron added to food as well as naturally labelled food iron have served to elucidate some of the factors that influence iron absorption. As is the case with many complex physiologic processes, new knowledge
has brought into focus additional unknowns, and the relative importance of many of the findings to the overall process of intestinal iron absorption is far from settled.

**Dietary Requirements of Iron**—Daily requirements of iron in men vary greatly depending on the age as well as the sex. With respect to the total body iron, the daily requirements are proportionately the greatest during the first two years of life. During this period the growing child needs more than double his iron endowment at birth. The next period of stress on iron balance occurs during the pubertal growth phase, the requirements reaching a peak at the age of 14 to 15 years.

Since menarche imposes the extra burden of blood loss on the female at this time, one would expect that requirements in females during puberty would exceed those of the males, but as pointed out by Hawkins (1964), boys during this period also have an equally great if not greater iron requirement because their pubertal incremental needs to watch for body growth exceed those of the female. This is because boys not only undergo greater growth during puberty but also acquire a relatively greater haemoglobin mass at this time (Giorgio, A.J., 1970).
the end of pubertal growth period, women have a higher daily
iron requirement than men, and it remains so throughout the
child bearing period. After the age of 60, the iron require-
ment for both sexes becomes identical.

IRON TRANSPORT— Iron is transported in plasma as Fe+++ ions firmly bound to a beta sub 1 globulin, called transferrin
(or siderophilin), which is present in a concentration of
about 0.25 gm/100 ml. of plasma. Each molecule can combine
with two atoms of iron, which is very firmly bound to
transferrin and is resistant to cleavage even by the chelating
agents. But, in the bone marrow the reticuloendothelial cells
which line the sinusoids and the erythroid tissue which
surrounds these sinusoids are able to remove iron from the
iron-transferrin complex with remarkable ease. Transferrin
is therefore, considered to be transported from the functional
confines of the "vascular space" to the reticuloendothelial
cells and immature-erythroid precursors and, after giving
up its iron to return directly to the plasma pool. Such a
pool would account for the discrepant disappearance of Fe59
and the labelled protein from the plasma. It is also consis-
tent with the fact that, in the final analysis, the rate of
removal of iron from the plasma by the marrow is related to
the number of active red cell precursors present. The data of Pollycove and Mortium (1961) indicates that plasma perfusion of the marrow is sufficiently great to account for even the most rapid iron clearance, were it accomplished solely by the adsorption of transferrin to these cells.

There is experimental evidence to suggest that iron is delivered to the developing red cells by means of the specific adherence of the iron-transferrin complex to receptor sites at the surface of these cells. Their attachment permits the active removal of iron from the transferrin and its incorporation into the cells. The transferrin, freed of its iron, is displaced by other iron-bearing molecules which are also taken up directly from the plasma. The displaced transferrin reenters the plasma, where in the course of its circulation, it acquires more iron from storage sites, enabling it to repeat the cycle once more.

The iron content of the plasma is 60 - 100 \( \mu \text{gms/100 ml} \), averaging 45 \( \mu \text{gms/100 ml} \) in women and 52 \( \mu \text{gms/100 ml} \) in men. Practically all of this is in organic form, as hemoglobin which contains about 0.338\% iron (Fe++) and all of which is in the red blood cells.
IRON BALANCE - ADULT MEN— The intracellular iron containing enzymes and myoglobin are stable substances, the iron of which cannot be called upon for other purposes. Blood haemoglobin on the other hand is continuously undergoing destruction. The iron content of haemoglobin being 0.33%/100 ml. of blood containing 15 gm. of haemoglobin would contain 50 mgm. of iron. With the normal red cell life span of about 100 days; 1% of the total blood is destroyed daily releasing about 25 mg. of iron. The entire amount of this endogenously released iron is eventually reutilised for fresh haemoglobin synthesis, because hardly any iron is normally excreted from the body. An adult male on an adequate iron intake excretes only about 0.4 mg. in the urine and about 0.8 mg. in the bile, while practically none is lost through the mucosa of the alimentary canal. It follows therefore, that the iron which is released from the destruction of senescent red cells after being temporarily stored in the reticulo-endothelial system, is used again for fresh haemoglobin synthesis. Only a fraction of the food iron is actually absorbed from the intestines which may be required in excess of the endogenously available iron. Thus, to complete the normal balance, the normal requirement
of an adult male amounts only to 1 mg/day which is easily available from a diet providing 10 - 15 mg of iron (as only 10 - 15% of the total available food iron is normally absorbed).

**ADULT WOMEN** - The iron loss (and therefore the food iron needs) of women is greater because of two reasons (1) the blood loss during the monthly menstrual period (2) The iron drain on the mother during pregnancy and labour.

**Menstruation** - The monthly blood loss in women averages 50 ml. (Grenick, 1954) corresponding to 25 mg of haemoglobin iron. It therefore necessitates an additional requirement of 1 mg of iron/day during the reproductive period of a woman's life to compensate, for the loss of blood in menstruation. When monthly menstrual blood loss exceeds 140 ml (70 mg of iron per month or 2-3 mg of iron daily) anaemia develops, if the food iron content is not able to match the demand.

**Pregnancy and Lactation** - Although menstruation is in abeyance, during this period, considerable iron drain is taking place as shown by the following data:-

<table>
<thead>
<tr>
<th>Description</th>
<th>Iron Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron content of fetus at term</td>
<td>400 mgs.</td>
</tr>
<tr>
<td>&quot; placenta &amp; uterus</td>
<td>150 mgs.</td>
</tr>
</tbody>
</table>
Iron content of blood loss after delivery = 170 mg.
" milk during 6 months lactation
= 180 mg.
Total iron loss in 15 months i.e. 450 days = 900 mg.
Average daily drain = 2 mg.

If provision is made for the absence of iron loss (1 mg./day) due to lack of menstruation during this period, an average of extra 1 mg./day of iron requirements is readily understandable to match for the iron drain during pregnancy and lactation.

The main iron demand on the mother occurs in the last two months of pregnancy when fetus and placenta grow rapidly, this is followed by the blood loss during labour.

Growing Children—During the period of growth the blood must grow to keep pace with the rest of the body; positive iron balance must therefore be maintained to enable the necessary additional haemoglobin to be formed. At birth, if a baby weighs 3 kg (7 lb.) and it contains 300 ml. of blood; the total iron content of the body is 400 mg. At one year it weighs 10 kg. (22 lb.) and contain about 1000 ml. of blood. To produce the necessary increase in haemoglobin and body iron a positive iron balance of 500 mg. of iron is required.
which is equivalent to 1.3 mg./day, over and above the iron needed to compensate for normal wastage. Even when mother and infant are on the satisfactory diet the infant's haemoglobin level falls from a birth value exceeding 100% to 75% at 3 months, and 85% between six and twelve months. Haemoglobin formation cannot therefore, even under good circumstances keep pace with the increase in plasma volume, subsequently, as the rate of growth slows down, the blood picture improves steadily during childhood. A fresh strain develops in girls with the onset of puberty and menstruation (Gamick, 1954).

*EXCRETION OF IRON*—The efficiency of reutilisation of endogenously released iron is such that only small quantities of iron are excreted under normal conditions. The total iron excretion averages about 0.5 to 1.5 mg./day in men and approximately double this amount in women (due to menstrual loss). Small amounts are lost in the sweat (<0.5 or 1.0 mg.); minute quantities in the hair and the remainder largely in the faeces 0.3 to 0.75 mg.). No iron loss occurs in urine. The faecal iron loss is contributed to by (1) true excretion; (2) iron lost in the desquamated mucosal cells (3) incompletely reabsorbed biliary iron.
INGESTED IRON (10 - 20 mg/day)

(NON HEME FOOD IRON) ↓
GUT LUMEN Fe³⁺, Fe²⁺

DUODENAL MUCOSAL EPITHELIUM

DESCAMATION FERRITIN

EXCRETION 0.5 - 1.5 mg/day

ABSORPTION 0.5 - 2.5 mg/day

TRANSPORT 3 - 4 mg
TRANSFERRIN Fe³⁺

FUNCTIONAL NON-HEMOGLOBIN IRON
150 - 200 mg
HEMOGLOBIN
HEMEN ENZYMES
NON HEME IRON
DEPENDENT ENZYMES

BONE MARROW
HEMOGLOBIN BIOSYNTHESIS
25 - 40 mg Fe/day
Fe + PROTOPORPHIRIN → HEME

CIRCULATING ERYTHROCYTES 2800 mg

STORAGE HEMOSIDERIN
AND FERRITIN
1000 mg

RBC DESTRUCTION
♂
♀

5 mg/day 1.5 - 2.5 mg/day

PHYSIOLOGIC AND OCCULT BLOOD LOSS

IRON METABOLISM IN A 70 KG ADULT

Reproduced from Medical Clinics of North America Vol. 54 No. 6, p. 1441, 1970.
STORAGE IRON: In an adult male, storage iron constitutes about 1.0 to 1.5 gm. (20 - 25% of the total body iron). Obviously this is the amount of iron held as reserve in the body in excess of the immediate iron needs. Thus the exact amount contained in the stores at one time would be dependent on the requirements for functional iron at that particular period. It is believed that the iron absorbed from the gastrointestinal tract is stored chiefly in the liver, while that released by the breakdown of senescent red cells is deposited in the spleen, bone marrow and other reticuloendothelial tissues. Reserve iron is found mainly in the liver, spleen and bone marrow, although in smaller amounts, it is widely distributed throughout the body. Part of it occurs as an easily mobilisable (labile) pool (Wintrobe, 1967) but in the main, it is held in a somewhat less mobile form in the reticuloendothelial cells. Only small amounts are present in parenchymal cells of the liver. It is believed that the deposits in the reticuloendothelial cells are derived chiefly from the breakdown of senescent red cells and transferrin, while iron in the hepatic parenchymatous cells appears to be derived from that absorbed from the gastrointestinal tract.
Storage iron exists in two major chemical forms - Ferritin and Hemosiderin. Ferritin molecule is characterized by the presence of a core probably made up of six micelles of ferric hydroxide surrounded by a protein shell (Bothwell and Finch, 1962). Chemically ferritin is an iron protein complex made up of a homogenous protein portion with Apoferritin, and an iron containing portion with an approximate composition of ferric hydroxide. Because it is water soluble, it is not detectable histologically with iron stains.

Hemosiderin is the other iron containing storage complex. It differs from ferritin in that it is insoluble in water and can be visualized as golden yellow granules in smears and tissue sections in which it is easily demonstrable histochemically by Prussian blue reaction. Electronmicroscopy has shown this to consist of several complex molecules with the same characterization of the iron hydroxide micelles as are present in the ferritin molecule. A detailed description of storage iron is given in the "review of literature".