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Absorption and Metabolism of Iron

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Iron was one of the first nonprecious metals to be known and used by man. Soluble iron salts were used medicinally in early times because it was thought that the iron would impart to the recipient the physical strength associated with the metal. Iron in various forms is still a very important medicinal, but its use is now based on a knowledge of its physiological functions and requirements. It has been common knowledge for many years that iron is an essential component of the hemin chromoproteins—hemoglobin, myoglobin, catalase, the cytochromes, and peroxidase—all of which play an essential role in the transport and utilization of oxygen for energy requirements.

In the years that have intervened since the discovery of the essential role of iron in these systems, a tremendous number of papers have appeared that deal with various aspects of this subject. Since it is not possible here (*1*) to discuss adequately this large volume of literature, the following remarks will of necessity be of a general nature; reference should be made to specific papers for more detail. References to these can be found in recent reviews of iron metabolism (*2–7*).

Dietary Sources of Iron

The total iron content of a foodstuff or diet can be readily determined, and such values for most common foods are found in numerous dietary tables (*8*). These figures, however, do not tell us what proportion of the total iron is potentially available for use by the body after ingestion of a particular food. The latter value is dependent on a number of factors that will be discussed here. Thus, a food high in iron may not necessarily be a good source of biologically available iron.

Dietary iron may be divided roughly into two fractions: (i) that which can be readily converted into ionized form by the action of dilute acids and (ii) that portion which resists ionization under these conditions. The latter fraction is composed chiefly of iron porphyrins and other complexes in which iron is firmly bound with organic molecules. These must be partially or completely broken down before the iron becomes ionizable. In the older terminology, the term *available iron* referred to that iron which was ionizable by treatment with dilute acid and, hence, would react with α,α′-dipyridyl on reduction (*9*). However, the "availability" of iron in a food, as determined by the *in vitro* α,α′-dipyridyl test, is of limited physiological significance. The iron in an inorganic salt, which would be rated as 100 percent available by this test, may be "physiologically available" to the extent of only 5 to 40 percent, depending on a variety of other conditions. It is also known that variable amounts of the so-called "nonavailable" iron may be absorbed and utilized (*10*).

Absorption of Iron

Factors influencing iron absorption. The chemical nature of the iron occurring in foods is probably of less importance in determining its physiological availability than a variety of other factors that exert a favorable or unfavorable influence on its absorption from the gastrointestinal tract. Some of the most important of these follow.

1) Divalent iron (ferrous) is generally absorbed to a greater extent than trivalent (ferric) (*11*). This is undoubtedly partially owing to the greater solubility of ferrous complexes with a variety of inorganic and organic anions and of ferrous hydroxide as compared with the corresponding ferric compounds. Thus, the availability of reducing mechanisms in the environment will determine the proportion of ferrous iron and, hence, the degree of absorption of the iron ingested. These reducing mechanisms may be supplied in the various digestive juices or they may come from the diet in the form of ascorbic acid, sulfhydryl compounds, and so forth (*12*). The sulfhydryl groups liberated by protein hydrolysis during digestion may also contribute substantially to this reducing medium.

2) There is an inverse correlation, other things being equal, between the size of dose and the percentage of the dose that is absorbed. On the other hand, the total amount of iron absorbed depends on the dose. Thus, if a 100-milligram dose of iron is fed, only 10 percent may be absorbed, but this would represent 10 milligrams of iron; whereas after the feeding of 10 milligrams of iron, 20 to 30 percent (or only 2 to 3 milligrams) may be absorbed. With small doses, then, the efficiency of absorption may be greater, but the total amount absorbed may be entirely inadequate to meet the requirements. Therefore, in clinical therapy with iron, the practice is to give as large a dose as can be tolerated. A common mistake in the treatment of iron deficiency states is the discontinuance of therapy with iron as soon as the hemoglobin attains normal levels but before the iron deficit in the tissues has been replaced.

3) In an acid medium (*p*H below 5), the iron in foods and in ferric hydroxide is converted to the soluble ionic form, and the formation of insoluble and undissociated complexes is inhibited. The reduction of ferric ions to the ferrous form by ascorbic acid, sulfhydryl groups, and so forth, also takes place more readily at acid *p*H. For these reasons, the acidity of the gastric juice may exert a favorable influence on iron absorption (*13*). However, if it exerts a significant influence, it must be looked upon as only one of several contributory factors since

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not all patients with achlorhydria become iron deficient, and not all cases of iron deficiency have achlorhydria. Thus, achlorhydria alone will rarely lead to iron deficiency without the intervention of other factors that either increase the requirements or decrease the amount of iron available (*7, 14*). Iron absorption takes place largely in the stomach and duodenum (*15*).

4) Since ferric iron readily forms insoluble and undissociable complexes with phosphate ions, the presence of much phosphate in the diet can materially reduce the absorption of iron. Conversely, diets lacking or very low in phosphates can lead to excess iron accumulation in the body (hemosiderosis) (*16*). Calcium in moderate amounts can have a favorable effect on iron absorption by combining with the phosphate ions. On the other hand, if excessive amounts of calcium are present in the diet, the absorption of iron is inhibited, which gives rise to an iron-deficiency anemia.

5) Phytic acid, by virtue of its ability to form insoluble iron complexes, also inhibits iron absorption. This may be reversed by calcium, which ties up the phytic acid. Many other organic acids occurring in foods or their digestion products form insoluble or undissociated iron complexes, chiefly with ferric ions, and thus exert an inhibiting effect on iron absorption (*17*). This is probably the reason that lower absorption of iron occurs when it is taken along with food than occurs when it is taken without food. It has been shown that pyridoxine influences iron absorption. In pyridoxine deficiency, the absorption of iron is increased (*18*). Copper is another dietary essential that influences iron absorption. A deficiency of copper lowers iron absorption in animals (*19*).

Regulation of iron absorption. The concept of the regulation of iron absorption is comparatively recent. Even as late as 1937, it was generally thought that iron, in common with most other heavy metals, was absorbed more or less indiscriminately from the gastrointestinal tract and that excretion through the colon maintained iron balance and prevented overloading of the body with iron.

McCance and Widdowson (*20*) first introduced the concept that the intestine in some manner controls the amount of iron absorbed. With the advent of more refined methods based on the use of radioactive isotopes of iron, it has been shown that the excretion of iron is negligible (*21*) and hence that there must of necessity be some mechanism for regulating iron absorption to prevent toxic accumulation of the element (*2–7*). In man and animals that have been made anemic by bleeding, the amount of iron absorbed from a given dose increases 5 to 15 times over the amount absorbed before bleeding. However, the degree of iron absorption is not increased until 6 to 7 days after an acute massive hemorrhage (*15*). This suggests that the intestinal mucosa does not respond immediately to a reduced hemoglobin level with an increase in absorption but must wait until the body stores have been depleted by accelerated hemopoiesis or until some other factors are activated. There is evidence that chronic low levels of hemoglobin do lead to increased iron absorption even in the face of adequate or excessive storage iron (*22, 23*).

Prolonged anoxia and the level of the plasma iron and iron-binding B_1-globulin have not been shown to play a significant role in the regulation of the absorption of iron. The regulatory mechanism normally seems to be most closely correlated with the body stores of and requirements for iron. This has given rise to the so-called selective or "intestinal intelligence" theory of the regulation of iron absorption, wherein the ability to select or reject iron according to the body needs is ascribed to the intestinal mucosa (*15*).

Since a single large dose of oral iron will inhibit or block iron absorption for a period of several days, the idea of a "mucosal block" was advanced to describe this phenomenon. According to this theory, an acceptor is present in the intestinal mucosa that is capable of combining with iron that comes into the mucosal cells. When the acceptor is saturated with iron, no more iron can pass through the cells until some of the acceptor is made available again by removal of the iron. It has been claimed that the protein, apoferritin, is such an acceptor (*24, 25*). This protein is able to accept iron in the ferric form to become ferritin. Normally, there is no demonstrable apoferritin and there are only small amounts of ferritin present in the mucosa. After iron-feeding, the amount of ferritin in the upper gastrointestinal mucosa, and particularly in the duodenal mucosa, is increased considerably (*25*). This disappears gradually over a period of several days.

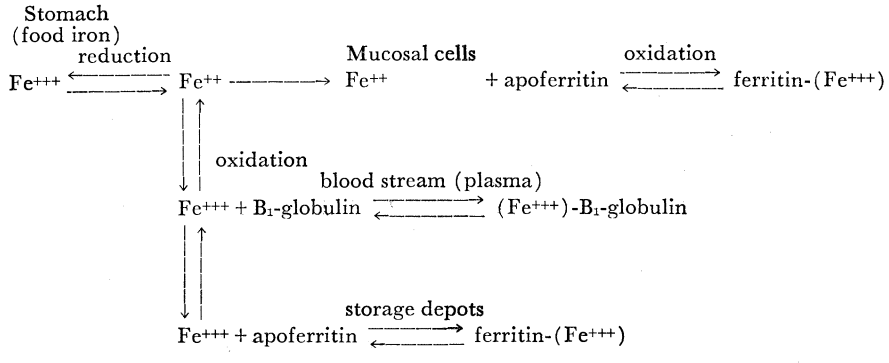
This sequence of events closely parallels the development of the "mucosal block" after an oral dose of iron. The acceptor, apoferritin, does not accumulate in anticipation of iron availability but is synthesized as the need arises. Whether the iron itself is the agent that initiates this synthesis or whether it is some other substance has not been clarified. Granick (*3*) explains this theory diagramatically as shown at the bottom of the page.

Iron passes into the mucosal cell in the form of ferrous ion. Within the cell the ferrous ion (Fe^{++}) is in equilibrium with the ferric iron stored as ferritin. When more iron is required in the body as a result of depletion of the iron stores, ferrous iron is drawn from the mucosal cell. Only when the mucosal cell ferritin-Fe^{+++} has decreased to a point where the cell is no longer "physiologically saturated" with Fe^{++} does absorption commence. This suggested mechanism provides the most satisfactory explanation available at present for the regulation of iron absorption. However, it must be regarded in relative rather than absolute terms because persons with adequate and even excess iron stores may absorb appreciable amounts of iron.

Pathology of iron absorption. The afore-mentioned mechanism of selective iron absorption breaks down under a variety of pathological conditions. Thus, in hemolytic and pernicious anemia, pyridoxine deficiency, and in some other conditions where the storage iron is known to be adequate or even excessive, iron absorption continues at an increased rate (*18, 22*). The condition known as hemochromatosis is probably the result of inborn error of iron metabolism that allows iron to be absorbed in excessive amounts despite a plethora of iron in the tissues. This results in the accumulation of massive quantities of iron over a period of years. The exact nature of the defect in the regulatory mechanism in this condition is not known (*26, 27*).

Excretion of Iron

Iron is normally excreted in very small quantities. In man, the combined excre-



tion by all routes (urine, bile, sweat, intestines, loss of hair, desquamation of body cells, and so forth) amounts to about 0.5 to 1.5 milligrams per day (*14*). In nephrosis, the excretion of iron in urine may be as high as 1.5 milligrams per day, depending on the quantity of protein excreted. This iron is excreted chiefly as an iron-B_1-globulin complex (*28*).

Iron Transport

Absorbed iron passes through the mucosal cells directly into the blood stream. Very little is absorbed by way of the lymphatic system (*29*). The plasma is the chief medium for iron transport. Ferrous iron entering the plasma is quickly oxidized to the ferric form by the dissolved oxygen (*30*). This ferric iron then forms a complex with a specific B_1-globulin (siderophillin, transferrin, metal-binding globulin) in which form it is transported to various parts of the body as required. This combination can take place either *in vivo* or *in vitro* after addition of iron to plasma (*26, 31, 32*).

The Fe^{+++}-B_1-globulin complex has a characteristic salmon-pink color with a maximum light absorption at 520 millimicrons, and this property has made possible the development of simple methods for the measurement of the available iron-binding capacity of the plasma (or serum). Iron that is added to the plasma in excess of this physiological iron-binding capacity is bound more loosely than the B_1-globulin-iron and in a nonspecific manner with other plasma proteins, and the complexes formed do not absorb light at 520 millimicrons. This loosely-bound iron is very rapidly removed from the plasma *in vivo* and it is this fraction that causes toxic reactions in the organism after the injection of larger doses of ionizable iron compounds.

The mean of the plasma (or serum) iron values taken from 13 different groups of normal human subjects is 129 micrograms per 100 milliliters for men and 110 micrograms per 100 milliliters for women (*33*). The specific iron-binding B_1-globulin makes up about 3 percent of the total serum proteins, or 0.26 gram per 100 milliliters of serum. Since two molecules of iron combine with a single molecule of protein (molecular weight, 90,000), this makes a total iron-binding capacity of 300 to 350 micrograms of iron per 100 milliliters of serum. Thus, the B_1-globulin is normally about 33 percent saturated with iron. In cases of iron need (iron-deficiency, chronic hemorrhage, and so forth), the total capacity to bind iron increases and the degree of saturation decreases markedly. On the other hand, a plethora of iron in the storage depots is associated with a decrease in the total iron-binding capacity and a marked increase (up to 100 percent) in the degree of saturation. In conditions such as infection and malignancy, the total iron-binding capacity is decreased and the serum iron is also decreased. This results in a degree of saturation only slightly below normal.

Although the plasma iron makes up only 0.1 percent of the total iron circulating in the blood, it is a very important fraction and serves as a sensitive index of the state of iron metabolism in the body as illustrated by Fig. 1.

As shown in Fig. 1, the plasma iron level at any moment is the resultant of a number of factors and will depend on which of these factors assumes the greatest relative importance at the time. For example, if absorption is decreased, the plasma iron level will decrease unless this change is compensated for by decreased utilization, increased destruction, or decreased storage. On the other hand, if blood destruction is increased, the plasma iron will increase unless this is counterbalanced by increased utilization and/or storage. Excretion plays an insignificant role under most circumstances.

Whole blood normally contains 40 to 50 milligrams of iron per 100 milliliters. Of this 99.9 percent is in the form of hemoglobin, an iron-porphyrin (heme)-protein complex with a molecular weight of 68,000 and iron content of 0.34 percent. Each molecule is composed of four units of 16,000 molecular weight each containing one heme and one molecule of globin. Hemoglobin has the unique property of combining reversibly with oxygen at the O_2-tension in the lungs and giving up 70 to 90 percent of this oxygen at the O_2-tension of the tissues. Thus, its chief function is the transport of oxygen to the sites where it is required. In the muscle, this oxygen is accepted by another heme protein, myoglobin, which has a molecular weight one-fourth that of hemoglobin. Myoglobin functions in oxygen transport and as an oxygen reservoir in the muscle and delivers its oxygen to the cytochrome system and other energy-producing systems of the cell as required. This storage function becomes particularly significant during intense muscular activity.

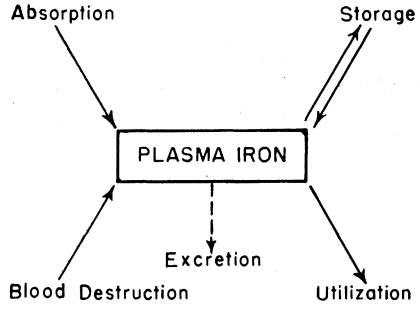


Fig. 1. The plasma iron level is the resultant of a number of factors.

The cytochrome system is composed of the four iron-porphyrin-protein complexes, cytochrome oxidase, and cytochromes *a, b,* and *c*. These serve in the stepwise oxidative transfer of electrons through the first steps of their passage from molecular oxygen to the substrates by way of intermediate energy-transfer systems of the cells. This makes possible the utilization of the energy in the foodstuff for the various physiological processes in the body. It is, therefore, evident that iron plays a key role in the most vital processes of the body. Catalase and peroxidase are two more iron-porphyrin-protein enzymes that are present in nearly all tissues. Their exact physiological functions are not clear. Presumably they serve to prevent toxic accumulation of peroxides within the cells.

Iron Storage

The average human body contains approximately 4.5 grams of iron. This can be divided roughly into four main fractions: The hemoglobin, which is found almost entirely in the blood, comprises 72.9 percent of the total. Myoglobin, which is found in muscle tissue, comprises 3.3 percent. Parenchymal iron (including the iron of the cytochromes, catalase, and peroxidase), which is found in all tissues, comprises 0.2 percent. The storage iron (ferritin, hemosiderin, and iron unaccounted for) comprises 23.5 percent, most of which is found in the liver, bone marrow, and spleen (*6*). In the dog, Hahn and coworkers (*4*) found a somewhat different distribution—hemoglobin, 57 percent; myoglobin, 7 percent, parenchymal iron, 16 percent, and storage iron, 20 percent. The myoglobin and parenchymal fractions of iron are less labile than the hemoglobin and storage fractions (*34*).

In the normal adult human being, about 27 to 28 milligrams of iron are released per day by the breakdown of hemoglobin (*6*). In contrast to the porphyrin moiety of hemoglobin, which is further degraded and excreted, very little of the iron is excreted. It is carefully husbanded and reutilized for new hemoglobin synthesis. Iron newly liberated by hemoglobin breakdown is used for resynthesis of hemoglobin in preference to that present in the stores or that which has been newly added by absorption (*35*). Similarly, newly absorbed iron is more readily utilized for hemoglobin synthesis than that already in the storage depots. The preferential utilization of these two fractions of the available iron may best be explained by the existence of a "labile

iron pool" that has been postulated (*7*); it represents a small fraction of the total available iron that is more readily mobilized and selectively utilized for current metabolic needs.

Iron is stored chiefly in the two forms, ferritin and hemosiderin (*24*). Ferritin is the more soluble of the two and can be readily extracted from tissues by water or dilute saline solutions. It consists of a protein moiety, apoferritin, of molecular weight 465,000, that may combine with up to 20 to 23 percent of its weight of iron. The iron in ferritin exists as micelles of ferric hydroxide of peculiar magnetic properties (3 unpaired electrons per iron atom) and configuration. It has the approximate composition $(FeOOH)_8$-$(FeOOPO_3H_2)$. Ferritin, as isolated, is not homogeneous with respect to iron content. The liver contains the highest concentration and the greater part of the total body ferritin; the spleen and bone marrow also contain considerable amounts. The postulated role of ferritin in the regulation of iron absorption has been discussed in a previous section. Ferritin-iron is readily available for synthesis of hemoglobin and other important iron-containing complexes of physiological importance. Ferritin has also been shown to act as an inhibitor of the vasoconstrictor action of epinephrine (*36*).

The other storage form of iron, hemosiderin, was the first to be recognized in tissues, but less is known about its composition and properties. It too contains the iron as ferric hydroxide stabilized by protein. Its iron content may vary from 9 to 55 percent. Hemosiderin remains insoluble under the conditions used for the extraction of ferritin (*37*). Extensive use has been made of this property as a means of separating these two forms of storage iron. The iron of hemosiderin is readily extracted from the insoluble fraction by the use of dilute solutions of strong acids. After the iron has been thus freed, it can be detected by the formation of prussian blue on the addition of potassium ferrocyanide. This is the basis of a commonly used method for the staining of hemosiderin in tissues. Ferritin also reacts in a similar manner, but it is not ordinarily visible microscopically because of its diffuse distribution. Hemosiderin occurs as discrete yellow-brown granules that can often be seen in unstained preparations.

The exact relationship between these two forms of storage iron is still not completely understood. Both forms are present normally, but unless the quantity of storage iron is normal or greater, the hemosiderin cannot be detected by staining methods. When small amounts of iron are presented to the storage tissues, ferritin is probably formed preferentially. When larger amounts of iron are introduced, the ability to synthesize apoferritin and to combine with iron to form ferritin is exceeded, and more iron is stored as hemosiderin. Some of this may later be converted to ferritin. After excessive hemoglobin breakdown and when large amounts of iron are injected parenterally, the storage of iron as hemosiderin is considerable. The amount of hemosiderin in bone marrow aspirates or liver biopsy samples has been used extensively as a rough index of the state of the iron stores in the body (*38*). Only when no hemosiderin can be detected can one expect a response to iron therapy.

Physiological Requirements for Iron

Since iron already present in the body is utilized over and over again, the amount of iron used daily for hemoglobin formation (26 to 27 milligrams) is far in excess of the actual daily requirement for iron in the diet (*6*). This requirement represents only that iron which is lost to the body through excretory channels. Naturally, any iron lost through acute or chronic bleeding will greatly increase the requirement. Barring blood loss, the normal human male excretes about 1.2 milligrams of iron per day. Normal women lose an average of 1 milligram per day in the menses and hence have nearly double the daily iron requirement of men. During pregnancy the loss through menstruation no longer exists, but an average of 2.7 milligrams of iron per day is supplied to the fetus, making a total daily loss to the mother of 3.8 milligrams per day.

Since infants, children, and adolescents are expanding their blood volume and tissue by growth, their iron requirements are much greater than those indicated by the small amounts lost by excretory routes. The increment required for growth in the first 20 years of life amounts to 0.3 to 0.6 milligram per day. As the result of a careful study of iron losses and requirements and multiplication by a factor of 10 to allow for the relatively low absorption of food iron, the following daily dietary allowances for iron have been recommended (*6, 39*): infants, 1 milligram per kilogram of body weight; children, 0.6 milligram per kilogram of body weight; normal adult men, 10 milligrams; normal adult women, 12 milligrams; pregnant women, 15 to 20 milligrams. These are conservative estimates and must be increased considerably if there is evidence of blood loss or of conditions that impair the absorption of ingested iron.

References and Notes

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