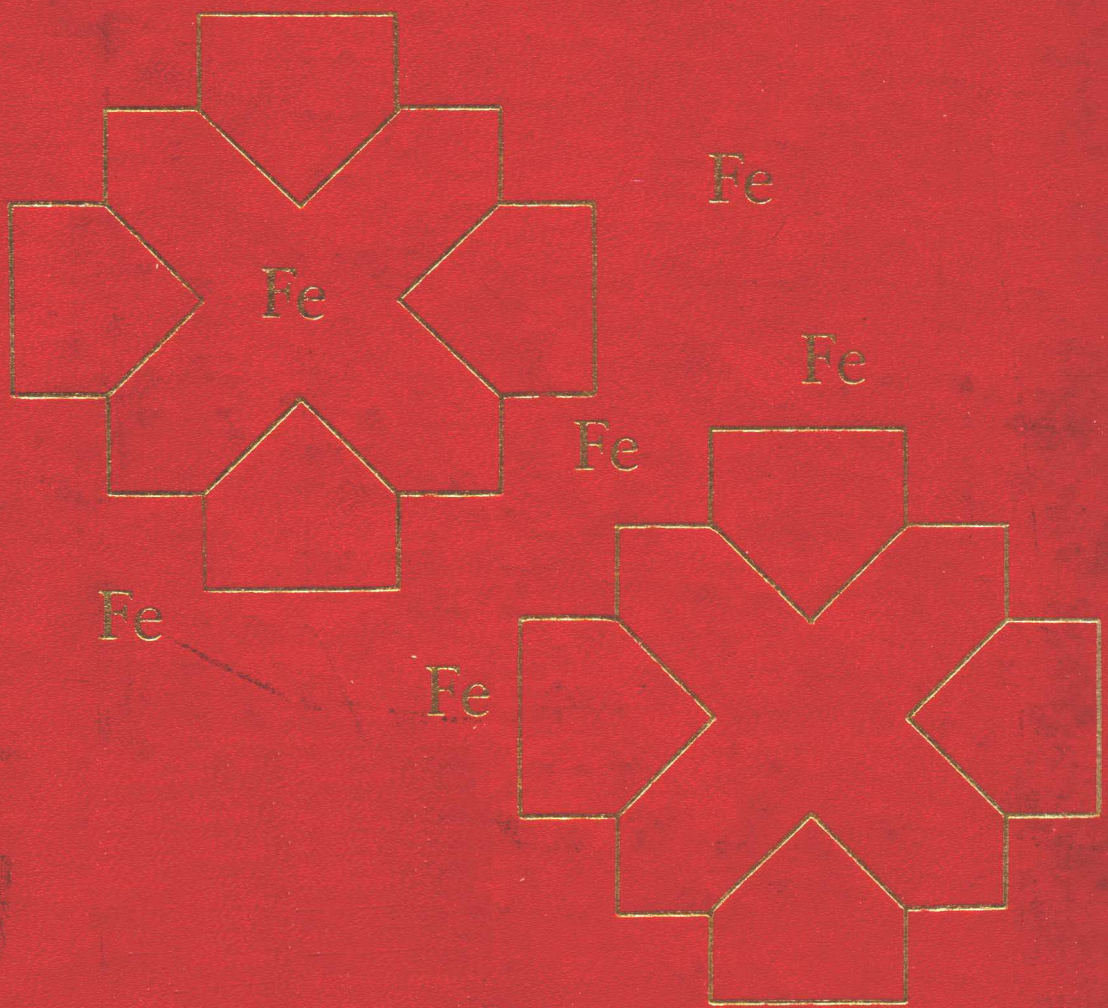
IRON EXCESS

ABERRATIONS OF IRON AND PORPHYRIN METABOLISM



Ursula Muller-Eberhard / Peter A. Miescher / Ernst R. Jaffé
Editors

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ABERRATIONS OF IRON AND PORPHYRIN METABOLISM

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Introduction

SEVERAL RECENT CONFERENCES AND REVIEWS have dealt with clinical aspects and biochemical advances in the areas of iron[2,3,7,8] and porphyrin[1,4–6,9] metabolism. States of iron deficiency are very common and states of iron overload are being recognized with increasing frequency. Still, many questions regarding these states are unanswered. While hematologists and nutritionists continue to confer about the advisability of fortifying food with iron, pediatric hematologists are convinced of its unquestionable value for infants because iron overload so rarely occurs in this age group. Why certain individuals acquire excess body iron after the third decade of life is largely unknown. The genetic and environmental factors offsetting balanced iron absorption, storage, and distribution are not yet delineated. The cause of iron accumulation associated with the disturbance of porphyrin metabolism in porphyria cutanea tarda is unknown. Likewise, we do not understand the pathognomy of sideroblastic anemia and erythropoietic protoporphyria, both of which exhibit aberrations of porphyrin metabolism.

In these two issues of *Seminars in Hematology,* an attempt is made to intertwine some of the current knowledge of iron and porphyrin metabolism. The first part reviews the distribution of iron in the organism, the structure and function of the specific iron-binding proteins of serum and tissues, the states of iron overload found in the adult and pediatric populations, and attempts at their therapeutic alleviation. The second part is devoted to basic considerations of heme biosynthesis, experimental porphyrias, recent developments in the methodology for porphyrin analysis, and protein binding of porphyrins. Not all aberrations of porphyrin metabolism are discussed. Rather, we will concentrate on a few the hematologist is likely to encounter: sideroblastic anemia, erythropoietic protoporphyria, and the dermatologist's puzzle, porphyria cutanea tarda. Amazingly, the remedy found effective by our Aesculapian forefathers for a variety of ailments, phlebotomy, provides a most effective therapy for iron excess in hemochromatosis and porphyria cutanea tarda.

Bringing together two adjacent areas, such as iron and porphyrin metabolism, is done in the hope of improving communication and stimulating research in fields of mutual interest. Regrettably, not all investigators actively working in these areas have been able to participate in this endeavor, but I am grateful to those who kindly made their time available to summarize for us their findings and knowledge. I would also like to thank the editors, who with their constructive, generous, and appreciative attitudes made this task a memorable experience.

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The Clinical Biochemistry of Iron

By M. Worwood

IRON IS FOUND IN THE DIET in trace amounts; nevertheless, it is essential for growth. In the body it is present in larger amounts than the other trace metals, since it is a constituent of the hemoglobin molecule, the oxygen carrying pigment of blood. The widespread occurrence of iron deficiency anemia has stimulated the study of iron balance. This investigative work has shown that loss of iron from the body is normally extremely small and that the iron content of the body is maintained by changes in iron absorption. Iron overload is much less common, but patients with the rare hereditary disease hemochromatosis, or patients with refractory anemia who receive regular blood transfusions, demonstrate the toxic effect of iron. Research has been initiated into the body's ability to store excess iron, into the ways in which iron causes damage to tissues, and into ways of removing excess iron from the body. This chapter summarizes the biochemistry of iron, which is of clinical importance, and deals in particular with ways of assessing the amount of storage iron in the body. Further reviews of many aspects of the structure and function of iron containing proteins and of iron metabolism in medicine can be found in the book edited by Jacobs and Worwood.[75]

INORGANIC BIOCHEMISTRY

The transition metals, including iron, share two properties of particular importance in biology—the ability to exist in more than one relatively stable oxidation state and the ability to form many complexes. Iron is commonly found in two oxidation states, Fe^{2+} or Fe^{3+}. In acid solution these can exist in the hydrated form, as the free ion surrounded by six molecules of water, but neutralization of such solutions results in progressive hydrolysis which ends with precipitation of ferric hydroxide. Although Fe^{2+} is much more soluble than Fe^{3+}, oxidation of Fe^{2+} to Fe^{3+} takes place readily in air. If the water molecules are replaced by suitable "ligands," complexes can be formed which are both soluble and stable at pH 7. Some examples of biological iron complexes are shown in Fig. 1. Many iron complexes contain unpaired electrons; this is a property of iron which has been of great use in determining the structure of biological complexes. The use of electron paramagnetic resonance spectroscopy in studying membrane-bound hemoproteins and iron-sulfur proteins is a good example.

Iron-containing Proteins of the Body

Almost all the iron in the body is bound to protein; most of the known iron-containing proteins are listed in Table 1.

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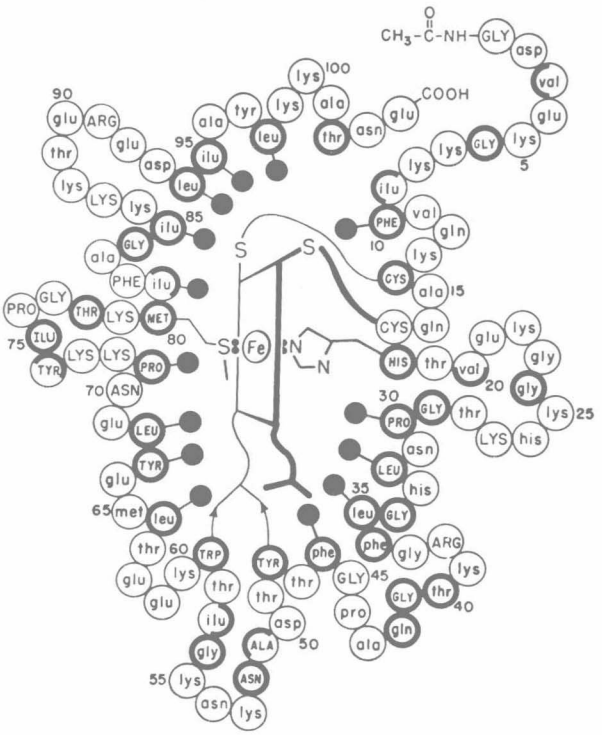

Fig. 1A. Diagram of the cytochrome c molecule. Heavy circles indicate side chains that are buried on the interior of the molecule, and attached black dots mark residues whose side chains pack against the heme. Light circles indicate side chains on the outside of the molecule, and dark half-circles show groups that are half buried at the surface. Arrows from residues 48 and 59 represent hydrogen bonds. Residues designated by capital letters are invariant among the proteins of 29 species. From Dickerson et al.,[39] with permission.

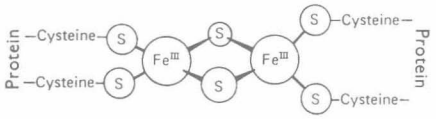

Fig. 1B. Model of the iron-sulfur group in plant ferredoxins in its oxidized form. From Rao et al.,[130] with permission.

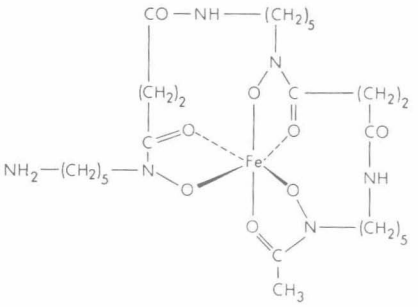

Fig. 1C. Atomic structure of ferrioxamine B. Desferrioxamine B is an iron chelator from *Streptomyces pilosus*, available commercially as the methane sulfonate. From Bock and Lang,[19] with permission.

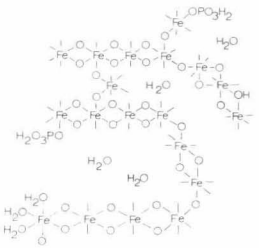

Fig. 1D. Model illustrating some features of the polynuclear iron complex of ferritin. From Gray,[48] with permission.

Table 1. Some Mammalian Iron-containing Proteins

Protein	Mol. wt.	No. of Fe Atoms Per Molecule	Distribution	Function	Ref.
Heme containing					
Hemoglobin	65,000	4 protoheme	Red blood cells	O_2 carrier	Text
Myoglobin	17,000	1 protoheme	Muscle	O_2 carrier	Text
Cytochrome aa_3	180,000*	2 heme a	Mitochondria	Terminal oxidase	89
b	18,000–30,000*	1 Protoheme	Mitochondria	Electron transport	89
c_1	37,000*	1 heme c	Mitochondria	Electron transport	89
c	12,000	1 heme c	Mitochondria	Electron transport	89
b_5	12,000	1 protoheme	Endoplasmic reticulum	Electron transport	89
P-450	—	protoheme	Endoplasmic reticulum	Steroid, drug, hydroxylation	89
Catalase	240,000	4 protoheme	Red blood cells, peroxisomes	Peroxide breakdown	21
Lactoperoxidase	93,000	1 protoheme	Milk	Peroxide breakdown	21
Tryptophan pyrrolase		heme dependent	Liver cytosol	L-tryptophan → formylkynurenine	3
Nonheme					
Aconitase	66,000	2 Fe 3 S	Pig Heart†	Citric acid cycle	51
(Phenylalanine hydroxylase)	100,000	2 Fe	Rat liver†	Phenylalanine → tyrosine	51
Adrenodoxin	12,500	2 Fe 2 S	Adrenal mitochondria	Steroid hydroxylation	51
(Complex III Fe-S protein)	30,000	2 Fe 2 S	Mitochondria	Electron transport	51
(Succinate dehydrogenase Fe-S protein)	27,000	2 Fe 2 S	Mitochondria	Electron transport	51
(Succinate dehydrogenase flavoprotein)	70,000	4 Fe 4 S 1 FAD	Mitochondria	Electron transport	51
NADH dehydrogenase		23–28 Fe + S FMN	Mitochondria	Electron transport	51
Xanthine oxidase	275,000	8 Fe 8 S 2 FAD 2 Mo	Milk,† tissue	Hypoxanthine → uric acid	51
Transferrin	77,000	2	Plasma	Iron transport	Text
Lactoferrin	77,000	2	Milk, secretions	Iron transport	Text
Ferritin	450,000–900,000	0–4000	All tissues	Iron storage	Text
Hemosiderin		Up to 37% Fe (dry wt.)	Liver, spleen, bone marrow	Iron storage	Text

A number of iron-dependent enzymes or processes have not been included.

*Soluble preparations of membrane-bound cytochromes.

†Enzyme isolated from this source. Found in other tissues.

Hemoproteins. These consist of an iron-porphyrin complex (Fig. 1) and a protein. Hemoglobin has a molecular weight of 64,500 and consists of four heme groups linked to four polypeptide chains. Each heme can bind one molecule of oxygen. In deoxyhemoglobin four of the ligands in the iron complex are from the porphyrin ring and there is a fifth ligand involving a histidine residue from the polypeptide chain, which completes a pyrimidal complex of Fe^{2+}. In oxyhemoglobin oxygen provides the sixth ligand and the complex is roughly octahedral. A description of the function of iron in hemoglobin is given by Perutz.[114,115] Myoglobin has a molecular weight of 17,000 and consists of a single polypeptide chain with one heme group. It has a higher affinity for oxygen than hemoglobin and is found in muscle cells, where it functions as an oxygen store, releasing oxygen to cytochrome oxidase when the supply of oxygen is insufficient for the needs of the tissues.

Mitochondria contain an electron transport system which transfers electrons from substrates to molecular oxygen with the simultaneous generation of adenosine triphosphate (ATP). The cytochromes are components of this pathway, and in the process of electron transfer their iron atoms are alternately oxidized and reduced. The cytochromes are mostly membrane bound and are low spin complexes of iron with additional ligands above and below the heme plane involving imidazole nitrogen or sulfur in cysteine or methionine (Fig. 1). Cytochrome P-450 and cytochrome oxidase (cytochrome aa_3) react directly with oxygen. In the absence of oxygen they are probably high spin complexes, but they are nevertheless called cytochromes. Recent reviews of the structure, function, interrelationships, synthesis, and degradation of the cytochromes are given by Lemberg and Barrett[89] and Nicholls and Elliott.[112]

Iron-sulfur proteins. Cytochromes are not the only iron-containing proteins of importance in oxidative phosphorylation. A number of "iron-sulfur" proteins also take part. These are proteins with "non-haem iron in the active centre which is covalently associated with either acid-labile sulphide or cysteinyl sulphur."[51] Iron-sulfur proteins were first recognized by a characteristic electron paramagnetic resonance signal ($g = 1.94$); the most widely studied proteins are the ferredoxins, which function in bacterial electron transfer reactions and in photosynthesis in plants. Some mammalian iron-sulfur proteins of similar structure are listed in Table 1 along with the iron-sulfur flavoproteins. The structure of the iron-sulfur center in a two-iron ferredoxin is illustrated in Fig. 1. The structure and function of the nonheme iron proteins has been reviewed recently.[51]

Transferrin and lactoferrin. These are members of a group of iron-binding proteins which also includes conalbumin or ovotransferrin found in egg white. Since the properties of transferrin have been reviewed recently[109] and are dealt with later in this issue, only a brief summary will be given here. Plasma transferrin is a β globulin of molecular weight approximately 80,000 and pI 5.8. It consists of a single polypeptide chain and is a glycoprotein containing about 6% carbohydrate in the form of two identical branched side chains, each terminating with a sialic acid residue. Each molecule can bind two atoms of iron at specific sites on the protein. Transferrin will bind many metals, but iron will displace other metals from the complex, so that, except for iron, the physiological significance of the metal binding is uncertain.[165]

There are at least 21 variants of transferrin in man which have been separated by electrophoresis in starch or polyacrylamide gels.[127] Turnbull and Giblett[154] found no evidence for differences in the plasma clearance or utilization of radioiron bound to four different transferrins or in the iron binding in vitro. Transferrin synthesis occurs mainly in the liver in adult animals, although synthesis has been demonstrated in a wide variety of tissues.[109] The protein is synthesized on ribosomes of the rough endoplasmic reticulum with attachment of carbohydrate during passage through the smooth endoplasmic reticulum and golgi vesicles before eventual secretion into the plasma. No single tissue appears to predominate in transferrin breakdown. Distribution and turnover studies on transferrin have been reviewed by Morgan.[109] The transport function of transferrin is dealt with here and elsewhere in this issue, and its bacteriocidal activity has been covered in a recent review.[127]

Lactoferrin is a transferrinlike protein found in milk and other secretions[103] and in neutrophils.[104] Both lactoferrin and transferrin have the same molecular weight and iron-binding properties, although the binding constant for one iron per molecule is greater for lactoferrin than transferrin.[2] There are differences in amino acid sequences[107] and there is little or no immunological cross reaction between the two proteins.[38] The function of lactoferrin is still uncertain, although it may have a bacteriostatic activity related to its very powerful iron-binding activity.[103] It has been suggested that lactoferrin is involved in the reduction of the plasma iron concentration found in acute inflammation.[143]

Ferritin. Ferritin is the soluble iron storage protein found in all cells of the body as well as in plants and fungi. The most exhaustive structural investigations have been carried out on the protein from horse spleen (from which ferritin was first isolated by Laufberger in 1937). The structure and function of ferritin has been reviewed recently by Harrison et al.[56] and is discussed in other sections of this issue. Horse spleen ferritin consists of an apoprotein shell of molecular weight 460,000 surrounding a core of iron present as ferric hydroxyphosphate (Fig. 1). The core may contain over 4000 atoms of iron, but the average iron content of horse spleen ferritin is about 2500 atoms per molecule. Both X-ray crystallography and analysis of the subunits obtained after dissociation of apoferritin in sodium dodecyl sulphate indicate that horse spleen ferritin consists of 24 identical subunits. Different tissues contain different ferritin molecules, which can be distinguished by polyacrylamide gel electrophoresis, isoelectric focusing,[123] their amino acid compositions, and by peptide mapping.[35] A more controversial proposition[174] is that ferritin from any mammalian tissue consists of a mixture of different molecules called "isoferritins," which are found in differing proportions in each tissue.

Ferritin is a ferroxidase, catalyzing the oxidation of Fe^{2+} to Fe^{3+} during its incorporation into the iron core. The uptake and release of ferritin iron is discussed by Harrison.[175]

Administration of iron to the whole animal, to cells, or to cell-free extracts stimulates the synthesis of apoferritin. The increase in ferritin protein concentration is not dependent on the synthesis of new mRNA (synthesis is not inhibited by the administration of actinomycin D), but the mechanism of stimulation of apoferritin synthesis is still uncertain and may involve both an increase

in the amount (or availability) of apoferritin mRNA on polysomes[173] and a posttranslational action.[40] Although ferritin is generally considered to be a protein synthesized on free polysomes for the cells' internal use, synthesis on ribosomes bound to endoplasmic reticulum also takes place,[125] and this may be one of the ways in which ferritin enters the plasma.[77]

Hemosiderin. This term is usually applied to iron which, after staining with potassium ferrocyanide, can be seen as blue granules in sections of liver or bone marrow. Under the electron microscope it can be seen that such granules may contain anything from closely packed and well-ordered ferritin molecules to amorphous deposits of iron.[176] Attempts have been made to isolate such insoluble iron free of ferritin. The preparations contain more iron than ferritin (about 30% dry weight), with less nitrogen and a higher proportion of phosphorus.[56] The origin of hemosiderin, its function as an iron store, and its relationship with ferritin are not clearly understood.[176]

Iron Content of the Body

The estimation of the total amount of iron in the body, or of the concentration of iron in a particular tissue, is difficult and has been attempted in a number of ways. Few direct measurements of the total body iron have been reported,[108] and measurements of tissue iron concentrations tend to include iron from the variable amount of blood trapped. Table 2 is taken from the data of Tipton and Cook[151] and gives concentrations determined by emission spectroscopy in tissues from a large number of American men and women who died as a result of sudden accidents. The high concentrations of iron in lung and spleen are due to trapped blood. In order to estimate the amount of tissue iron (i.e., tissue free from hemoglobin) it is necessary to perfuse the tissue thoroughly. Where this is not possible measurement of the nonheme iron concentration has traditionally been carried out and a wide variety of extraction meth-

Table 2. Concentration of Iron in Tissues. From Tipton and Cook[151]

Tissue	No. of Samples	μg/g Wet Tissue (mean ± SD)
Adrenal	10	38 ± 20
Aorta	93	56 ± 36
Brain	108	58 ± 17
Diaphragm	91	47 ± 23
Heart	123	55 ± 18
Intestine, duodenum	60	41 ± 18
jejunum	84	38 ± 28
ileum	78	27 ± 13
Kidney	123	76 ± 31
Liver	127	195 ± 113
Lung	120	319 ± 176
Muscle	120	42 ± 14
Ovary	16	59 ± 50
Pancreas	119	51 ± 42
Spleen	124	336 ± 210
Skin	19	15 ± 9
Testis	68	29 ± 14
Thyroid	14	62 ± 29

Table 3. Tissue Storage Iron in Singapore[28]

Group	Mean Concentration Nonheme Iron ± SD (μg/g Wet Liver)					
	No. Estimated	Liver	No. Estimated	Spleen	No. Estimated	Kidney
< 1 mo	4	343 ± 103	4	334 ± 146	2	43 ± 7
1 mo–2 yr	6	108 ± 91	5	61 ± 25	5	27 ± 8
3–14 yr	5	144 ± 115	5	99 ± 78	2	36 ± 7
Adult male	77	233 ± 123	53	243 ± 152	25	35 ± 9
Premenopausal female	27	130 ± 87	18	140 ± 83	8	33 ± 9
Postmenopausal female	6	303 ± 217	5	343 ± 253	2	28 ± 1

ods have been described with measurement of iron concentration either by colorimetry or atomic absorption spectroscopy. One example of the concentrations of nonheme iron found (mostly ferritin and hemosiderin) is given in Table 3. Some conclusions can be drawn from this and similar studies. Nonheme iron concentrations in liver and spleen are very high in the first few weeks after birth, then decline and remain low throughout childhood. Concentrations in men are higher than in premenopausal women. Charlton et al.[29] measured nonheme iron concentrations in 3983 specimens of liver from 18 countries, and again lower concentrations of "storage" iron were found in women under 40 years of age.

An estimate of the distribution of iron in the body is given in Table 4. It must be realized that several different methods of estimation have been used and that many people have less storage iron than indicated by these calculations.

IRON METABOLISM

Iron Absorption

In 1937 McCance and Widdowson[97] assembled the already extensive literature on iron balance and concluded that the iron content of the body was regulated by variation in the amount absorbed and not by variation in the amount

Table 4. Distribution of Iron in the Body of a 70 kg Man. From Jacobs and Worwood[76]

Protein	Tissue	Total Iron (g)	Per Cent of Total Body Iron
Hemoglobin	Red blood cells	2.60	57.6
Myoglobin	Muscle	0.40	8.9
Mitochondrial cytochromes		0.017	0.4
Catalase		0.005	0.1
Other cytochromes and heme-proteins		?	
Nonheme-iron (including ferritin and hemosiderin)	Liver	0.35	7.8
	Spleen	0.02	0.4
	Muscle	0.86	19.0
	Bone marrow	0.26	5.8
	Other tissues	?	
Transferrin	Plasma	0.004	0.1

excreted. Since that time the mechanism and regulation of iron absorption has been the subject of much experimental work and even more speculation. Recent reviews have given an account of this clinical and experimental work.[46,153]

Release of nonheme iron from food. In the stomach peptic digestion and the presence of hydrochloric acid releases nonheme iron from food. Gastric juice also contains mucoproteins of high molecular weight, which can bind the ionized iron in a form which remains soluble at neutral pH.[73] Most of the iron arriving in the jejunum after a meal is in such a high molecular weight form, but iron probably enters the small intestinal epithelial cells bound to low molecular weight chelators such as sugars and amino acids.[73]

Iron absorption from food. When iron absorption from biologically labeled foods is measured, there is a considerable variation between different foods. For example, in normal subjects absorption varies from 1% of the dietary iron from some vegetables to 10%–25% for meats.[88] Furthermore, interaction between the various components of a meal make it impossible to estimate the amount of iron absorbed by adding up the expected absorption from the constituents. A practical solution is the "extrinsic tag" method by which dietary iron absorption is considered as taking place from two independent pools—heme and nonheme iron—which can be separately labeled with different radioisotopes of iron. The "extrinsic tag" approach is a considerable help in assessing iron absorption from whole diets, but not all food iron exchanges completely.[164] Such practical methods for comparing food iron absorption in different parts of the world have been considered in a WHO Technical Report.[164]

Uptake of iron by epithelial cells. Iron is absorbed by the upper small intestine, and control of the amount absorbed appears to be exercised at this point. Once taken up by the mucosa some of the iron passes rapidly into the circulating plasma.

The binding capacity for iron of the intestinal epithelial cell brush border varies with the iron status of the animal and with location of the cell, brush borders prepared from jejunal cells binding more iron than ilial cells.[50] Binding of metals such as cobalt, which compete with iron for uptake, appears to be less specific.[46] Little is known about the transfer of iron across the brush border membrane. With low concentrations of iron and in iron-deficient animals there is a "carrier" mechanism of limited capacity, and metals such as cobalt and manganese compete with iron.[150] At high concentrations transfer is a passive process of unlimited capacity.

Biochemical fractionation of the upper small intestine of rats shortly after giving ^{59}Fe has demonstrated concentration of radioactivity in a soluble form.[67,166] This radioactivity was almost all protein bound, mostly to ferritin in iron-replete rats, but to a protein with many of the properties of transferrin in iron-deficient rats.[54,68,119,140,167] This protein differs from plasma transferrin in isoelectric point and iron-binding ability[66] and in aminoacid composition,[118] but preparations may also include extracellular transferrin which has obtained radioiron from less powerful chelators during homogenization of the tissues. None of the other soluble iron-binding components described has been isolated or further identified. It has been proposed that the transferrin-type protein is an intracellular iron carrier on the absorption pathway.[68] Twelve to 18 hr after

giving ^{59}Fe, activity was concentrated in the particulate fractions, particularly the mitochondrial fraction.[166] This subcellular localization was similar to that found after giving radioiron intravenously,[168] and mitochondria appeared to be not only a major destination of iron entering the cells from the plasma but also the site of highest iron concentration.[132]

Electron microscopy autoradiography. Bédard et al.[15,16] examined iron absorption in mice. The dose of iron given was larger than that in the biochemical experiments described above, and only the proximal duodenum was studied. They found that much of the ^{55}Fe in the cells during the first 3 hr after feeding was present in areas rich in rough endoplasmic reticulum and free ribosomes. There was very little uptake by mitochondria, nuclei, and the Golgi apparatus or by morphologically identifiable ferritin, even up to 24 hr after feeding radioiron. Somewhat different results were obtained by Humphrys et al.,[69] who studied iron absorption in the rat intestinal epithelial cell by both electron microscopy autoradiography and differential centrifugation. They confirmed previous biochemical findings that the mitochondria played a quantitatively significant role in iron metabolism within the cell but not in the process of iron absorption.

Iron transport within the epithelial cell. The epithelial cells obtain iron for synthesis of cellular proteins both from the plasma[31,168] and directly from the gut lumen. Uptake from the lumen is demonstrated by the rapid decrease in iron concentration[177] and the increase in iron absorption[120] observed after placing rats on an iron-deficient diet, and by the dependence of intestinal cytochrome P-450 (a "microsomal" enzyme) on dietary iron.[65] Iron from the lumen destined to enter the plasma may be carried across the cell by an intracellular transferrin. Further evidence is needed to establish that such a protein is synthesized within the epithelial cell. It should be realized that membrane-bound iron may also take part in iron transfer and that the mechanism of iron exchange across the cell membrane remains obscure.

Absorption of heme iron. The ability to absorb heme iron varies widely in different species.[32] In man, heme is split from globin within the lumen of the intestine[30] and enters the intestinal epithelium, where iron is presumably released by heme oxygenase.[128] From this point the iron may follow the same metabolic pathway as iron which has entered the cell after administration as a salt.

Regulation of iron absorption. Iron absorption is influenced by many factors, some of which, such as the availability of dietary iron, have already been discussed. The amount absorbed increases with the amount presented to the intestinal mucosa, but the per cent of the dose absorbed decreases with increasing quantity of both nonheme and heme iron.[7,32] The amount absorbed is also controlled by the iron requirements of the body.

There have been three main theories describing the regulation of iron absorption,[26] relating this to the iron content of the intestinal epithelial cell, the different characteristics of the two iron binding sites on the transferrin molecule,[178] or to humoral factors. Recently Cavill et al.[26] suggested that the regulation of iron absorption is built into the way iron exchanges between plasma and the tissues. Circulating transferrin obtains iron from every tissue in the body, but the