IRON METABOLISM AND ANEMIA

PAN AMERICAN HEALTH ORGANIZATION
Pan American Sanitary Bureau, Regional Office of the
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1969

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Symposium on

IRON METABOLISM AND ANEMIA

Moderator: Dr. Marcel Roche

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OPENING STATEMENT

Marcel Roche, Moderator

It would be safe to say, without any statistics at hand, that 1,000 million people in the world are iron deficient, and many of them actually have anemia. The problem is most acute in tropical areas, and anemias, particularly iron-deficiency anemias, are rampant in all the tropical and subtropical zones of the Americas.

The attempt in this symposium is to present, in a short time, a general view of iron metabolism, extending from the basic concepts of chemistry and biochemistry to the epidemiological and therapeutic aspects of the problem.

Specifically, we shall start by taking a look at basic aspects of hemoglobin transferrin-ferritin metabolism on the whole. Next, there will be four papers dealing with the question of absorption of iron—possibly the single most important influence on the production of iron-deficiency anemia. The subject will be approached from the point of view of mechanisms at the intestinal level; the effect of protein depletion—obviously very important to us, since many of the populations we are interested in are protein-depleted; intersubject and intrasubject variations; and findings to date on iron absorption from specific staples. After this, the old question of iron requirements will be dealt with.

Turning to epidemiology, the particular aspects of iron deficiency and anemia that bear on the Western Hemisphere will be considered. Also, these matters will be looked at as they affect pregnancy and infancy. The relationship between anemia and hookworm disease will then be touched on briefly. The concluding presentation will round out the matter by dealing with the treatment and prophylaxis of iron deficiency.

THE BIOCHEMISTRY OF IRON

Pauline M. Harrison

Structure and function in biological iron compounds

The biochemistry of iron may be divided broadly into three interrelated aspects: the structure and function of biological iron compounds, their synthesis and turnover, and the metabolic and functional relationships among the various iron compounds. The present symposium is largely devoted to human iron metabolism: the body's requirements for iron, its absorption from food, the turnover of iron compounds and the maintenance of an iron balance, and the effects of iron deficiency. This paper will concentrate on the structural and functional aspects of iron compounds, principally hemoglobin and myoglobin, ferritin and hemosiderin, and transferrin. Their syntheses and metabolic interrelationships will be touched on briefly.

Free ionic iron does not occur to any significant extent in living organisms because of its tendency to form complexes with many organic compounds. Since quite low concentrations of ionic iron are toxic, this has the biological advantage of enabling iron to be stored, transported, or utilized in nontoxic forms. The biochemistry of iron is therefore the biochemistry of the complexes of which it forms a part. The presence of iron atoms may confer on these complexes certain properties, such as the capacity to combine reversibly with oxygen or the ability to accept and donate electrons. These properties acquired through the presence of iron atoms may in turn be profoundly modified by the environment provided for the iron by the complex—reversible combination with oxygen being a case in point. The effect of the complex may be to allow the iron to function in a specific and controlled manner.

A neutral iron atom contains 26 electrons, 18 of which occur in the closed shells of the argon core. When ionized, two (ferrous) or three (ferric) of the outer electrons are removed, leaving the inner electrons unchanged. The spins of the outer electrons may be aligned in a variety of different ways, giving rise to differences in magnetic properties. Thus ferric iron, Fe^{3+}, has five outer electrons. In high-spin compounds, these have all their spins parallel to one another, giving a large magnetic moment. In low-spin compounds, four of the electrons are aligned in two antiparallel pairs, leaving a single unpaired electron and hence a small magnetic moment. Ferric iron with three unpaired electrons is less common. Ferrous iron normally has either four or no electrons unpaired.

The biological properties of iron complexes may depend not only on the presence of a localized center of positive charge but also on the tendency of iron to form directional covalent bonds with organic ligands. These ligands are often, but not always, arranged in the form of an octahedron around the iron atom. The nature and arrangement of the ligands affect the distribution of the outer electrons in the iron atom and, in particular, the way their spins are paired, enabling the iron to play a variety of roles. The correlation between electronic configuration and function has been discussed by Eichhorn (*43*). The atoms that are commonly linked to iron are

N (e.g. histidine or pyrrole), O (e.g. O_2 or tyrosine) and S (e.g. thiol). The nature of the chemical groups not in the immediate coordination sphere of the iron atom, but surrounding it, may also be of functional importance. This is shown dramatically by the difference in behavior of the heme iron in hemoglobin as compared with that in free heme. The protein in myoglobin and hemoglobin provides the heme group with a nonpolar environment, which allows the ferrous iron at its center to combine with molecular oxygen without itself becoming oxidized, a property not shared by free heme groups (*87, 128*) or by free Fe^{2+}.

The iron atoms may also affect the properties of the compounds to which they are attached. Thus iron-transferrin (*47*) and ferritin (*104*) are more stable to heat and other denaturing agents and more resistant to attack by proteolytic enzymes than are their apoproteins. This may help to prevent a build-up in the cell of the protein moiety in amounts greatly in excess of the available iron. The increased stability conferred by iron-binding may result from conformational changes in the protein. This is probably true for transferrin (*47, 94*) and for the bacterial nonheme iron protein, rubredoxin, in which an iron atom, coordinated to four thiol groups, forms a bridge between remote parts of the primary structure in a manner analagous to that of a disulphide bond (*11*). The addition of heme to globin alters the conformation of the latter, increasing its helix content by 10 to 20 per cent (*26, 76*). The binding of a gaseous ligand to hemoglobin iron causes a physiologically significant change in quaternary structure (*116*), as discussed below. Conformational changes in the protein of transferrin induced by iron-binding and by binding of the protein to cell membranes may play a role in the control of iron metabolism (*53*). On the other hand, the addition of 4,000 or more iron atoms to the molecule has little effect on the structure of apoferritin (*51, 68*).

Biological iron compounds form a very diverse group, both structurally and functionally. It is convenient to classify them into those that contain heme (*27, 30, 31, 45, 90, 101, 119*) and those that do not (*29, 47, 89, 100, 118, 147*). Free heme (Figure 1) does not occur in quantity in animal tissues; it becomes incorporated into proteins or is broken down.

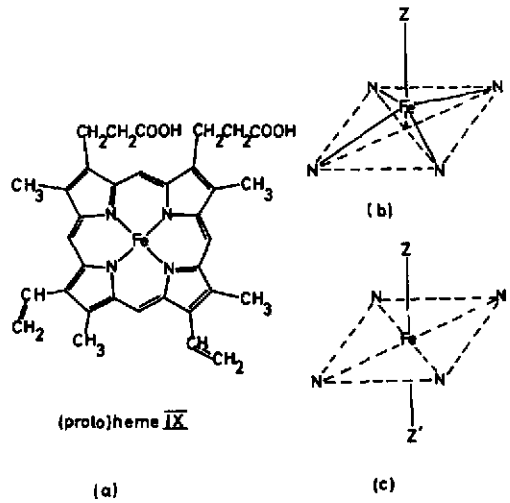


FIG. 1. (a) The structure of the heme group (ferroprotoporphyrin IX) as found in hemoglobin, myoglobin, catalase, and peroxidase. In cytochrome *c* a similar group is attached to the protein by addition of two cysteine thiol groups to the two vinyl groups of the protoporphyrin. The four protoporphyrin nitrogen atoms form a square planar arrangement to which the iron is coordinated. The iron atom may also be attached to other groups on either side of this plane. In deoxymyoglobin and deoxyhemoglobin the ferrous iron atom is coordinated only to five ligands in a square pyramidal arrangement, as shown in (b), with histidine nitrogen occupying position Z. The iron atom is displaced at least 0.3Å from the protoporphyrin plane in the direction of the histidine nitrogen. In oxymyoglobin and oxyhemoglobin the oxygen molecule occupies a position on the side of the plane opposite to Z (like Z' in (c)). In cytochrome *c* the iron atom is attached to six ligands arranged at the corners of an octahedron and is probably centered in the plane of the nitrogens as shown in (c) (*31*).

Heme proteins include the very widely distributed proteins of the cytochrome system (*90, 101*), which occur in virtually all organisms except anaerobic bacteria, and function as an electron transport chain associated with oxidative phosphorylation in mitochondria. Of the cytochromes, *c*, a protein of molecular weight 12,400 containing a single heme, is the best characterized. Unlike that of hemoglobin, its proto-

porphyrin ring system is covalently linked to the protein (by thioether links with two cysteine residues). The fifth and sixth sites of the octahedrally coordinated heme iron are occupied by histidine, and probably methionine, and the heme group is situated in a deep crevice normal to the surface of the molecule (*38*). Cytochrome *c* accepts electrons from cytochrome *b* and transfers them to cytochrome oxidase, with which it forms an active complex, the iron atoms being alternately oxidized and reduced. Other heme proteins include myoglobin and hemoglobin, discussed below, and the catalases and peroxidases that catalyze the breakdown of peroxides in the presence of a reducing agent (*85, 89, 119, 125*). It has been suggested that an intermediate in the peroxidase reaction may be one in which the fifth and sixth iron coordination sites are empty (*127*).

Nonheme iron compounds vary in size from the small ferric hydroxamic acid chelates found in aerobic bacterial cells, which appear to be involved in heme synthesis (*130*) and also possibly as iron carriers (*118*), to the large iron-storage complexes ferritin and hemosiderin. And they vary in function from electron transfer in the ferredoxins (*29*) (found in plants and microorganisms but not in animals) and in a number of iron-flavoproteins (*89, 147*) to oxygen carriers, as in the hemerythrins of sipunculids, and iron carriers, as in transferrins. The nature of the iron ligands is often difficult to determine in the absence of an easily recognizable group such as heme, and they may be sulphur (in ferredoxin and rubredoxin), oxygen (in ferrioxamine), or nitrogen (both nitrogen and oxygen in transferrin).

The present symposium is largely concerned with the metabolism of iron compounds in humans. Quantitatively, the most important iron compound in man is hemoglobin (*111*), which accounts for some 70 per cent of the total iron (about 4 g in a man weighing 70 kg), as compared to the related muscle protein, myoglobin, which constitutes about 3 per cent. Next to hemoglobin comes storage iron, located in ferritin and hemosiderin and accounting for about 26 per cent (*111*). Transferrin, at about 0.1 per cent, is quantitatively less important, but it plays a vital role in iron transport between sites of iron absorption and storage and hemoglobin synthesis, and probably in the control of iron absorption. The remainder of this paper will be confined to a discussion of these compounds. For other iron compounds the reader is referred to recent reviews (*27, 29, 30, 31, 45, 47, 89, 90, 100, 101, 111, 118, 119, 147*).

Oxygen carriers: Hemoglobin and myoglobin

Hemoglobin occurs in the red cells of all vertebrates. In higher vertebrates it is typically a tetramer of four polypeptide chains and four heme groups. The molecule is a compact ellipsoid measuring 64 x 55 x 50 Å (*129*) and having a molecular weight of about 65,000. Hemoglobin also occurs in many invertebrates, although its polymeric form may vary, e.g. a monomer with a single heme is found in the marine annelid worm *Glycera dibranchiata* (*122*) and in larvae of the insect *Chironomus thumni* (*78*), while polymeric forms with molecular weights of 3,000,000—the erythrocruorins—occur in the worms *Lumbricus terrestis* and *Arenicola marina* (*97*). A leghemoglobin has been reported in the root nodules of leguminous plants (*138*).

The importance of a supply of oxygen for the survival of man and other animals need hardly be emphasized. Since the body has a limited capacity for storing oxygen, a steady supply and an efficient means of circulating it to the tissues are vital. Hemoglobin fulfills the latter role efficiently both by increasing the capacity of the blood for oxygen by a factor of some seventyfold, and by binding oxygen strongly at the partial pressure of alveolar oxygen and unloading it readily at the reduced pO_2 of the tissues (*144*).

The relative ease with which tetrameric hemoglobin yields its oxygen at lower oxygen tensions as compared with monomeric myoglobin and monomeric forms of hemoglobin is illustrated by the oxygen dissociation curves in Figure 2. These curves suggest that the combination of oxygen

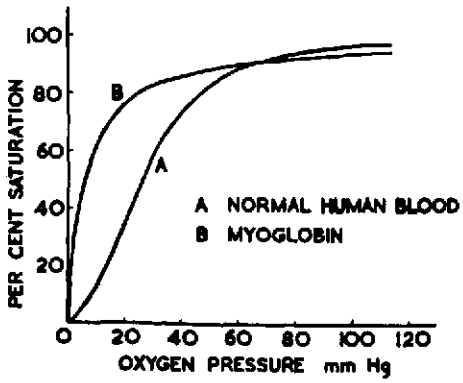


FIG. 2. A: Oxyhemoglobin dissociation curve of human blood at 38°C, pH 7.40. B: Oxygen dissociation curve of myoglobin under similar conditions. Reproduced by permission of F. J. W. Roughton (*144*, p. 775).

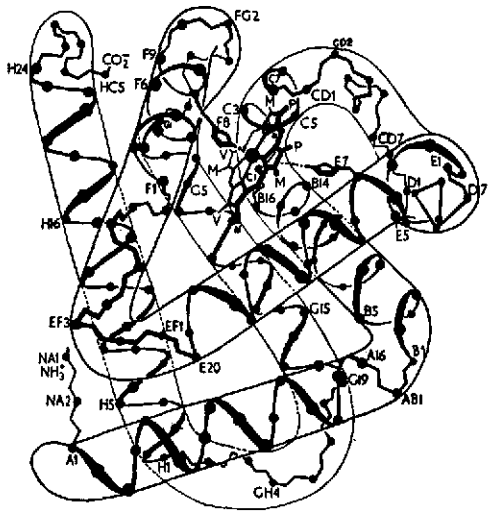


FIG. 3. α-carbon skeleton diagram of myoglobin molecule showing helical and nonhelical regions and the location of the heme group. Reproduced by permission of R. E. Dickerson, from H. Neurath (ed.), *The Proteins*, 2d ed., vol. 2, New York, Academic Press, 1964, p. 634.

with the iron atoms in the two proteins is not simply a function of the presence of iron in a particular local environment. The iron atoms in the two proteins are combined in the same protoporphyrin ring system, and the hemes have very similar surroundings (*87, 128*). The sigmoid dissociation curve for hemoglobin as against the hyperbolic curve for myoglobin is attributed to the presence of a "heme-heme interaction" in tetrameric hemoglobin and the lack of it in monomeric myoglobin. That is to say, in hemoglobin, combination with the four oxygen molecules does not occur independently. Another physiologically important property of hemoglobin not shared by myoglobin is that an increased partial pressure of carbon dioxide facilitates the unloading of its oxygen—the oxygen dissociation curve is shifted to the right (Bohr effect).

The configuration of deoxymyoglobin based on X-ray diffraction studies of Kendrew and his colleagues (*88, 120*) is shown in Figure 3. The coordination of the iron atom is approximately octahedral, but the sixth coordination site is unoccupied by any ligand. The ferrous iron atom is situated about 0.4 Å from the center of the protoporphyrin ring, which supplies four pyrrole nitrogen ligands. A fifth nitrogen ligand is supplied by a histidine side chain (F8) of the protein, while another histidine (E7) is situated close to the empty sixth coordination site. In oxymyoglobin, an oxygen molecule occupies this sixth site without altering the valence of the iron atom, although it changes from high-spin to zero-spin state. *In vitro*, the iron atom is readily oxidized, giving ferrimyoglobin, in which the sixth site is occupied by a water molecule or other ligands, while the position of the iron atom remains as in ferromyoglobin. The arrangement of ligands around the heme iron atom in deoxymyoglobin is identical with that in α-chlorohemin (*92*). Figure 3 shows the heme group in a crevice in the protein. An analysis of the protein side chains in proximity to the heme in myoglobin and hemoglobin shows that the protein provides it with a nonpolar surrounding (*87, 128*).

Hemoglobin consists of two pairs of chains with different primary structures (*25*) and can therefore be designated as a tetramer $\alpha_2\beta_2$. Each chain, with its attached heme, has a tertiary structure very similar to that of myoglobin. The chains are situated roughly at the corners of a tetrahedron. Models of oxyhemoglobin and deoxyhemoglobin molecules are shown in Figure 4. These structures show three important features: the chain conformations are unaffected by com-

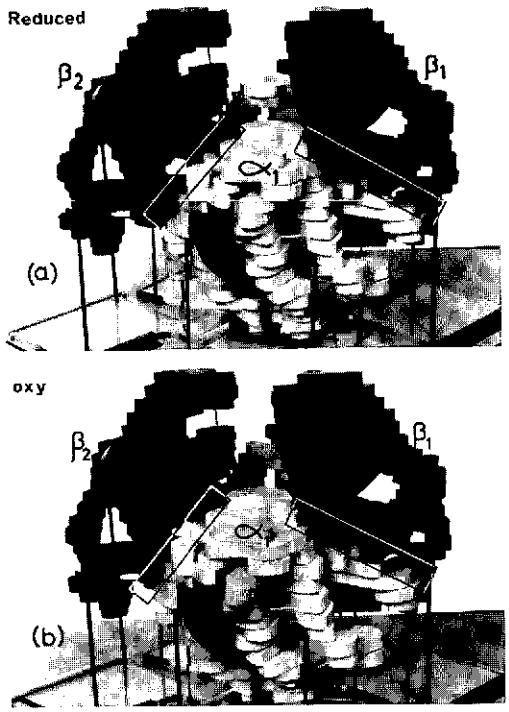


FIG. 4. Models of (a) deoxy and (b) oxyhemoglobin at 5.5Å resolution. The heme groups are represented by grey discs. The α chains are white and the β chains black. The α_2 chain lies at the back of the molecule behind the α_1 chain. The contact areas between chains are marked as boxes. On combination with oxygen the chains shift relatively by a few Å units along the $\alpha_1\beta_2$ and $\alpha_2\beta_1$ contacts, while contacts $\alpha_1\beta_1$ and $\alpha_2\beta_2$ alter very little. The β chains move closer together on oxygenation. Photographs kindly supplied by M. F. Perutz.

bination with oxygen (at least at the resolution, 5.5Å, of the models); the relative orientations of the four chains change on combination with oxygen; and the heme groups are not close enough for direct interaction, their iron atoms being 25Å or more apart (*21, 115, 116*).

Much experimental and theoretical work has been carried out in attempts to explain the sigmoid shape of the hemoglobin oxygen dissociation curve (*7, 8, 142, 171*). Kinetic measurements have shown that, while the rates of combination of hemoglobin with the first three oxygens are approximately the same, combination with the fourth oxygen is much more rapid (*145*). This suggests that the structural change associated with the deoxy-oxy transformation may have occurred before the fourth ligand combines. Most of this change occurs along the contact between the α_1 and β_2 chains shown in Figure 4, some of the atoms at the contact being displaced relatively by as much as 6Å, although distances between the heme iron atoms in these chains are very little affected. The iron atoms in the two β-chains, however, move about 6Å closer together on oxygenation (*115*). How the structural change is triggered off and relayed across the protein is not yet apparent, although this may become clearer when the conformations of both deoxy and oxyhemoglobin have been determined at atomic resolution. The transformation in quaternary structure seems to be a consequence of the change from 5- to 6-coordination of the iron atom rather than of the change in its spin state.

Structure-function relationships in hemoglobin are complicated by the fact that in solution a dynamic tetramer-dimer-monomer equilibrium exists (*8, 9, 142*). Symmetrical splitting into $\alpha\beta$ dimers probably occurs along the $\alpha_1\beta_2$ contact and also along the $\alpha_2\beta_1$ (*141*), while the $\alpha_1\beta_1$ and $\alpha_2\beta_2$ contacts remain unchanged (Figure 4). It has been suggested that conformational changes in the $\alpha\beta$ dimer are of prime importance in hemoglobin oxygenation and that the oxy-dimers induce a transformation to a more reactive conformation in any deoxy-dimers with which they combine (*10, 13, 64, 117*). Recent kinetic evidence, however, suggests that the tetramer and not the dimer is the prime unit of function, since combination with at least three ligands is necessary to produce a transformation to the rapidly reacting form (*56*). The X-ray crystallographic studies also support the tetrameric molecule as the functional unit in cooperation binding effects (*128*). In any event, the functional importance of the protein as well as the iron is evident. This is also shown by the fact that modifications in the protein may reduce or destroy both heme-heme interaction and the Bohr effect. Interestingly, these effects are absent on the reaction of ferrihemoglobin with ligands (*8*), and ferrihemoglobin has a conformation similar to that of oxyhemoglobin.

The effect of iron salts as stimulators of both heme and globin synthesis *de novo* from amino acids by reticulocytes *in vitro* is well known (*95*), while the rate of globin synthesis is decreased in the absence of heme or heme precursors such as iron (*131*). The observation that heme and globin are synthesized at approximately the same rates argues for a mechanism coordinating their syntheses (*95, 131*). This may be achieved by an inhibitory effect of hemin on its own synthesis coupled with a stimulatory effect of heme on the formation of globin (*28*). Oxygen concentration also plays a regulatory role in hemoglobin synthesis. Globin synthesis was found to be stimulated by low oxygen tensions and inhibited at the higher levels (*67*). Inhibition is relieved by the addition of heme, and the effect is believed to occur at the level of heme synthesis.

The regulatory effect of heme (and of iron as a heme precursor) on hemoglobin synthesis appears to be twofold. It is found to stabilize reticulocyte polyribosomes (*131, 162, 163*), and it also appears to promote the assembly of newly synthesized α and β globin chains (*156*).

Iron stores:
Ferritin and hemosiderin

Storage iron, amounting to about 700 to 1,000 mg in a normal man (*111*), represents a mobile reserve that can be drawn upon in iron deficiency or after hemorrhage, thus allowing supplies of "functional" iron compounds, such as hemoglobin, to be maintained or rapidly replaced (*22*). Iron deficiency in a clinical sense occurs only when iron stores are depleted. In iron overload, storage iron is increased, while hemoglobin iron usually remains normal. Iron released from hemoglobin in the normal breakdown of red cells is also stored temporarily in reticuloendothelial cells from which it can subsequently be released and reutilized for hemoglobin synthesis (*121*). Iron is stored in two forms, ferritin and hemosiderin, and can be mobilized from both (*22, 150*). The formation of ferritin, which occurs in response to the presence of iron (*48, 58*), may play a part in the mechanism regulating iron absorption in mucosal cells (*33, 37*).

In ferritin, the iron is associated with a well-defined protein moiety, apoferritin, forming a soluble red-brown complex (*57, 61*). The term hemosiderin was first applied to microscopically visible, Prussian-blue-staining, insoluble granules isolated from the liver and the spleen (*35, 59, 106*). It has also been used to denote massive iron-rich deposits seen in the electron microscope (*16, 132*). The need for a soluble and also nontoxic form of iron store seems to be generally widespread among living organisms. Ferritin has been found not only in many vertebrates (*109*) and invertebrates (*140, 160*) but also in the plant kingdom (*79, 139, 148*), including fungi (*126*). It would therefore seem to be of ancient evolutionary origin. Hemosiderin or hemosiderin-like deposits have been found in both vertebrates and invertebrates (*35, 159*). Under normal conditions in man and in experimental animals, most storage iron is in the form of ferritin, but in iron overload the amount of hemosiderin may greatly outstrip that of ferritin (*108, 149, 154*).

Ferritin isolated from tissues (principally liver, spleen, and bone marrow) may contain a variable amount of iron, although this is usually around 20 per cent of its dry weight (*57*), or about 3,000 iron atoms per molecule. Each preparation contains a spectrum of molecules of different iron content (*143*), ranging from iron-free molecules to those containing up to 4,000 or 5,000 iron atoms (*50, 70*). The distribution of iron content among the molecules varies from one individual to another and depends on the state of iron-loading. In anemic animals, however, iron-free apoferritin is not present in quantity (*59, 60*). It is apparently degraded when not required to store iron, whereas iron stimulates its synthesis. Ferritin preparations consist mainly of monomeric molecules, but some polymers are also present—about 10 to 15 per cent by weight of the preparation (*39, 155, 166*). On ion-exchange chromatography (*155, 157*), heterogeneity has been observed in ferritin, but not in apoferritin (*155*). No differences could be

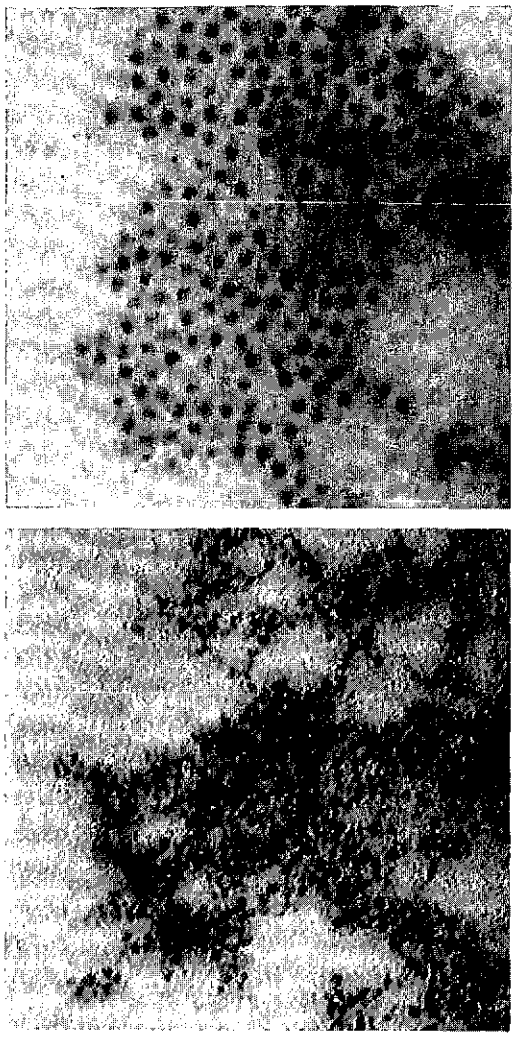


FIG. 5. Electron micrographs of ferritin (a) and hemosiderin (b) prepared from the same horse spleen by the methods of Granick (*57*) and McKay and Fineberg (*106*), respectively. The samples were unstained and unshadowed. The iron-containing micelles can be seen as electron-dense (grey) areas. Magnification × 250,000 (F. A. Fischbach, D. W. Gregory, P. M. Harrison and T. G. Hoy).

detected in the amino acid compositions of the chromatographic fractions (*155*). The heterogeneity may therefore be due to differences in surface conformation, or in bound ferric or other ions. Variations in the electrophoretic mobilities of ferritins isolated from different tissues within the same animal have also been found (*5, 54, 55*).

The iron of ferritin occurs in a "micelle" with a maximal diameter of about 70Å (*46, 50, 69, 91*), or less in molecules of low iron content (*50, 66*). Its composition corresponds roughly to the formula $(FeOOH)_8$ $(FeO:OPO_3H_2)$ (*70, 110*), but the phosphate present does not seem to be an integral part of the micelle and may be largely confined to its surface (*51, 72*). Ferritin and its protein-free micelles give X-ray (*51, 72, 158*) and electron diffraction patterns (*65*) typical of crystalline material of small particle size. The micelles, which can be seen without staining in the electron microscope, sometimes have the appearance of being subdivided into a few smaller crystallites (*46*), although this could possibly be an artifact. The appearance of four crystallites or "tetrads" in a micelle of average over-all diameter of about 60Å is taken by many electron microscopists to be diagnostic of ferritin. Ferritin can also be recognized—and distinguished from hemosiderin—by its tendency to form close-packed monolayers on electron microscope grids and by the fact that its micelles are surrounded by protein shells, which are not electron dense, and which prevent the micelles from coming into contact (Figures 5 and 6).

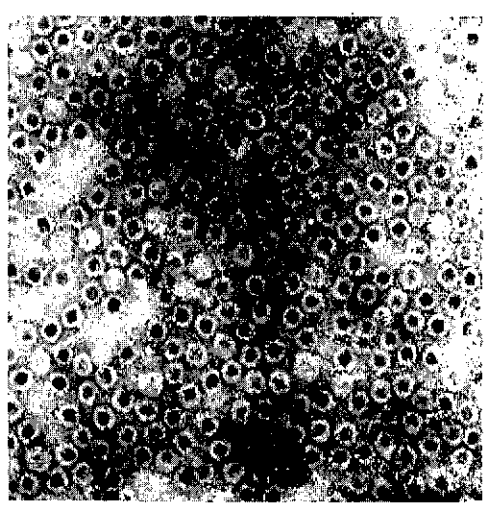


FIG. 6. Electron micrograph of ferritin negatively stained with sodium phosphotungstate kindly supplied by G. H. Haggis (unpublished). The ferritin iron-cores are surrounded by protein shells, which appear light against a background of negative stain. Magnification × 250,000.

Several different structures have been proposed to fit the "tetrad" appearance (*46, 114*). However, these fit only a small proportion of the views seen in the electron microscope, while in electronmicrographs taken close to true focus many of the micelles look rather uniform in appearance (*65*). In ferritin solutions (*50*) and in wet crystals (*69*), the micelles closely approximate spheres or polyhedra and are of uniform density.

The ferritin micelle diffraction patterns differ from those of the well-known ferric oxide or oxyhydroxide minerals (*65, 72*). Three alternative atomic structures have been proposed for the ferritin iron cores (*72*) or for closely related synthetic "hydrous ferric oxides," which give similar diffraction patterns (*24, 158*). These vary in the coordination of the oxygen atoms ($O^{=}$, OH^{-} or H_2O) around the ferric iron—they may be all octahedral (*158*), all tetrahedral (*24*), or mixed octahedral and tetrahedral (*72*). Owing to the poor quality of the diffraction patterns, it is not easy to decide between these alternatives. From measurements of the magnetic susceptibility of ferritin iron, values of about 3.8 Bohr magnetons were obtained for the magnetic moment. This value is close to that expected for ferric iron with three unpaired electrons (instead of the more usual five or one unpaired electron) and a square planar arrangement for the iron oxygen ligands (*59, 110*). Recently, however, a value of 5.08 μ_B has been reported (*20*), with evidence for antiferromagnetic ordering in the crystallites at low temperatures. This value is closer to that normally observed for high-spin ferric compounds with five unpaired electrons (5.9 μ_B).

The protein shell that surrounds the iron-containing core of ferritin is roughly spherical, with an average outer diameter of about 122Å (maximum 130Å) and an inner diameter of about 73Å under conditions in which the protein is hydrated (*19, 50, 69*). In the electron microscope these dimensions may be reduced by about 15 per cent (*166*) (Figure 6). Sedimentation studies and X-ray measurements of the molecular weight of apoferritin give values in the range 460,000 to 480,000 (*69, 143*), using the measured partial specific volume, 0.747 (*143*), or 440,000 to 460,000, using $\bar{V}$ calculated from the amino acid composition (*166*). The molecular weight based on the tryptophan content (*75, 153*) is 420,000 to 460,000, and on light scattering 430,000 (*137*). Ferritin containing its full complement of iron has a molecular weight of about 900,000 (*50, 70*). The protein shell is divided into about twenty subunits (*74, 77*), probably arranged at the apexes of a pentagonal dodecahedron (*69*) (Figure 7). This arrangement allows for the presence of channels connecting the inside and outside of the molecule. These channels are about 10Å to 15Å wide (*134*, and T. G. Hoy and P. M. Harrison, unpublished observations), thus allowing for the passage of hydrated iron atoms in and out of the molecule. Properties that depend on the size and/or external surface of the molecule—viscosity (*104*), electrophoretic mobility (*105*), crystal packing (*68*), gel filtration (*6*), antigenicity (*105*)—show that the protein is essentially unchanged on binding iron in

FIG. 7. Model of an apoferritin molecule, reproduced by permission of G. H. Haggis (*66*). It shows the arrangement of twenty protein subunits at the apexes of a pentagonal dodecahedron (*69*) with a pentagon face at the front of the model. At the center of each face there is a channel that connects the outside of the molecule with the central cavity in the protein and allows the passage of iron in and out of the molecule. The central cavity, which occupies about 22% of the total volume, is not visible in the model, but it can be seen in Fig. 6.

its interior, and this is borne out by a comparison of X-ray diffraction patterns of ferritin and apoferritin (*51, 68, 71*). Nevertheless, the presence of the iron-containing core renders the protein less susceptible to denaturation (*104*) and to attack by proteolytic enzymes (*104*). The atomic structure of the iron core is not specifically orientated with respect to the protein (*51*). It appears that the cores can grow in different directions inside the protein shell, their external shape complementing that of the protein when the latter is essentially full (*51, 65*).

The biosynthesis of ferritin has received considerable attention since it was discovered that *de novo* synthesis of ferritin protein from amino acids was induced by administration of iron salts (*48, 49*). Several workers have confirmed this finding in whole animals (*41, 99, 103*), in tissue slices (*146, 172, 173*), and in tissue cultures (*136*). Tracer experiments with ^{14}C-labeled amino acids show that apoferritin, or possibly a ferritin of very low iron content, is formed first, and that with time the radioactivity passes to iron-rich species, suggesting that the empty shells are gradually being filled (*41*). Reconstituted "ferritin" can also be produced *in vitro* from intact apoferritin molecules, ferrous salts, and oxygen or other oxidizing agents (*17, 72, 98*). The product resembles ferritin in electron microscopic appearance and in its diffraction pattern, despite the absence of phosphate (*72*). This observation, together with the results of the tracer experiments, suggests that ferritin may be formed from apoferritin by a similar mechanism *in vivo* (*40*), rather than by aggregation of apoferritin subunits around a preformed iron-core template (*123*). The protein shell may itself assist in the removal of electrons from the Fe^{2+} ions entering the molecule. Some "isoferritins" may be more active in incorporating iron than others *in vivo* (*54*).

The mechanism of induction of ferritin by iron is not yet known, although it seems fairly certain that it does not occur through control of transcription, but rather at some subsequent stage (*41*). Drysdale and Munro (*39, 41, 42*) conclude that messenger RNA for ferritin is stable and that the iron either causes it to be used more efficiently or assists in the release of apoferritin subunits from the ribosomes or their aggregation to completed shells. Iron, however, is not essential for aggregation of apoferritin subunits *in vitro* (*39, 73*). Since both ferritin and hemoglobin are induced by iron and both are found in erythroid cells, it seems likely that their biosynthetic control mechanisms are interconnected in these cells (*44, 103, 161, 174*).

Little is known about the mechanism of release of iron from ferritin *in vivo*. It can be removed as Fe^{2+} from intact molecules by reducing agents *in vitro* (*18, 61*), or, slowly, as Fe^{3+} by iron chelating agents (*124, 169*). An *in vivo* release mechanism involving xanthine oxidase has been proposed (*62*). Iron that has been more recently deposited as ferritin following red-cell breakdown is more easily mobilized than iron from older ferritin deposits (*121*). Possibly this is because the more recently formed ferritin has a lower iron content and the iron can be more readily removed from only partially filled molecules, or it might be due to conformational differences in the protein. Iron can apparently be released both from intact molecules and from those in which the protein has been degraded (*154*).

Hemosiderin is both chemically and metabolically related to ferritin. This term has been used to describe both amorphous and crystalline intracellular deposits seen in the electron microscope (*16, 133*), but it seems certain that the latter are ferritin. It has been suggested that the term hemosiderin should be restricted to those granules that are water insoluble (*151*). While this is a useful means of distinguishing hemosiderin from ferritin at a preparative level, it obviously cannot apply to iron-rich deposits seen in electron micrographs of tissue sections. Here the term should perhaps be restricted to deposits that are amorphous and in which the iron micelles are not clearly surrounded by well-defined protein shells. Such deposits are, however, of variable appearance, sometimes, but not always, membrane-bound (*14, 15, 132*). On the basis of morphological appearance alone, it may not al-

ways be possible to decide whether they contain ferritin or not.

Isolated hemosiderin granules have variable composition. Their iron, phosphorus, and sulphur contents are higher than those found in ferritin (*35, 106, 107, 135, 150, 168*), and they contain a number of different organic constituents, including protein and small amounts of apoferritin (*107, 135*). The magnetic moment of the iron in hemosiderin is similar to that in ferritin, but with a greater range of values (3.5 to 4.7 μ_B (*150, 154*). X-ray diffraction patterns obtained from ferritin and hemosiderin extracted from the same normal horse spleen show that the atomic structures of the two mineral components are similar, although the average particle size observed in hemosiderin is smaller (F. A. Fischbach, D. W. Gregory, P. M. Harrison, and T. G. Hoy, unpublished observations).

The biological origin of hemosiderin is of some interest. There is evidence that in liver parenchymal cells it is a breakdown product of ferritin (*154*), presumably resulting from digestion of the protein with intracellular proteases. The X-ray diffraction results mentioned above would be consistent with this conclusion. Hemosiderin-like material can also be produced from ferritin by denaturation followed by trypsin digestion (*102*). Studies on human siderotic livers show that above a certain level of iron-loading the ratio of hemosiderin to ferritin is nearly constant, thus suggesting a dynamic equilibrium between the two storage forms (*108*). In rabbits, the ferritin level reached a maximum limiting value in response to massive doses of iron, whereas the hemosiderin content appeared to be able to rise continuously (*154*). The implication in this case would be that the rate of ferritin turnover was increased at high iron levels, or that some of the hemosiderin was not formed from ferritin and possibly differed from "normal" hemosiderin. Thus, there may be two mechanisms for "hemosiderin" formation, one operating when the iron-level exceeds the cell's ability for ferritin synthesis and turnover. Since ferritin-like colloidal "iron oxide hydrates" can be produced *in vitro* in the absence of ferritin protein (*24, 158*), two such mechanisms appear to be plausible.

Iron transport and delivery: Transferrin

A group of closely related iron-binding glycoproteins occurs in vertebrate blood serum (transferrin), in mammalian milk (lactotransferrin), and in avian egg white (ovotransferrin or conalbumin) (*47*). These proteins are similar in size and iron-binding properties, although they differ in amino acid composition and carbohydrate content (*47*). The discussion that follows will be principally concerned with serum transferrin.

Transferrin accounts for 2 to 3 per cent of the dry weight of vertebrate sera. This represents about 100 times the amount of free iron in serum, but only 0.1 per cent of the total body iron (3 to 4 mg transferrin-bound iron in humans). Human serum transferrin has a molecular weight of about 86,000 [86,000–93,000 based on physical measurements (*12, 47*), 86,000 from iron-binding (*82*)]. It contains several disulphide bridges but no free thiol groups (*47*). Its attached carbohydrate, the function of which is uncertain, consists of four moles of sialic acid, eight moles of N-acetylglucosamine, four moles of galactose, and eight moles of mannose for every 90,000 molecular weight, joined to the protein as two branched glycopeptide chains (*80*). This suggests the presence in the protein of two peptide chains. Only one N-terminal residue (valine) has been found until recently (*12*), but Jeppsson and Sjöquist have observed N-terminal serine in addition to valine (*84*), and they have also reported the splitting of transferrin into two subunits in 8M urea (*83*). Greene and Feeney were unable to obtain evidence for dissociation under similar conditions and think that probably all transferrins are monomers (*63*.)

Metal-free transferrin is colorless, but when ferric iron is bound in the presence of bicarbonate a salmon-pink complex is formed. It also complexes less firmly with other metals, such as copper. Two metal ions are bound per molecule of transferrin. At physiological pH, transferrin

has a very high affinity for iron, the equilibrium binding constants being of the order of 10^{30} for both iron atoms (*2*). Electron spin resonance studies have shown that the iron is bound as Fe^{3+}, and that the two binding sites are approximately equivalent and at least 9Å apart (*2, 4, 167*). Under physiological conditions, three protons are displaced and one mole of bicarbonate is bound per iron atom (*4*). The bicarbonate was previously thought to be coordinated to the iron atoms (*167*), but recent evidence suggests it is probably attached to groups on the protein (*3*) and not directly to the iron. The metal ligands are probably three tyrosine oxygens and two nitrogens from the imidazole or guanidyl groups of histidine or arginine, respectively (*2, 93, 167*). Transferrin will bind iron or copper slowly in the absence of bicarbonate. Electron spin resonance studies suggest that more protein ligands are available for binding (three or four N and three O ligands) when bicarbonate is absent (*1*). Evidence of heterogeneity in the two binding sites in the pH range of 4 to 6 has been obtained in the absence of bicarbonate (*1*), although at pH 7 to 11 the sites were indistinguishable. Kinetic evidence indicates that the binding of the two atoms is cooperative, chelation of the first ion facilitating that of the second, presumably as a result of a conformational change in the protein (*170*). The two binding sites also appear to be functionally different. Release of bound iron to immature red cells occurred more readily from one site than from the other (*52*). Exchange of iron atoms between the two sites occurs only very slowly or not at all (*4, 52, 113*). As already noted, iron-binding stabilizes the protein (*47*) without substantially altering its shape. However, in the course of binding, alterations occur in the antigenic structure (*94*), indicating that some conformational change has taken place. Possibly the metal stabilizes the protein by forming crosslinks between separated regions of the primary structure.

The physiological function of transferrin is to transport iron. Most of the body's iron is conserved and reused after the erythrocytes and other iron-containing cells have been destroyed. Little iron is excreted and only a small proportion of dietary iron is absorbed. Transferrin acquires iron, derived from hemoglobin breakdown or entering the body through the intestinal mucosa, and delivers it to the erythropoietic bone marrow for incorporation into new hemoglobin molecules, to other tissues requiring physiologically "active" iron, and to the storage depots. Transferrin also transfers iron from the placenta to the fetus. This protein has been regarded as having an essentially passive role, i.e. it simply provides a convenient means of carrying iron in a nontoxic form. Recent work, however, suggests that it may play an active part in the mechanisms regulating both absorption and delivery of iron (*53, 86*). These control mechanisms appear to depend on structural changes in the protein, which result both from its chelation with iron and from its attachment to specific receptor sites on cell surfaces.

Every day plasma transferrin delivers from 30 to 40 mg of iron to the erythropoietic bone marrow—the tissue with by far the highest iron requirement. The protein is not consumed when its iron is released. It becomes available for further iron transport and is able to deliver about six to ten times its weight of iron in a day. Unlike free iron, which is taken up indiscriminately, transferrin iron is passed selectively to immature red cells, which are still actively synthesizing hemoglobin, rather than to mature cells in which hemoglobin synthesis has ceased (*81, 82, 86*). This suggests there are receptor sites on the surfaces of immature cells that become lost as the cell ages. These sites must be specific for transferrin protein and not for its iron. The relative ease with which transferrin iron can be transferred to reticulocytes, as compared with its removal *in vitro*, suggests that a conformational change in the protein occurs on binding. The uptake of iron by reticulocytes seems to occur as a three-stage process (*82, 112, 113*): (1) physical absorption of transferrin to receptor sites; (2) the formation of a tighter union between the transferrin molecule and the receptor site, probably involving an alteration in

the tertiary structure of the protein; and (3) transfer of iron to the cell.

Stage 1 is reversible, the molecules being comparatively easily displaced competitively by other molecules. At stage 2, iron-bound molecules have a greater affinity for the receptors than does apotransferrin (*82*), suggesting that iron-binding facilitates the conformational change required for attachment to the receptor. Transferrin molecules containing two bound iron atoms release their iron more readily than do those with only one (*52*). Probably only one of these atoms is taken up at a time, iron from one site being preferred over that from the other (*52*). Stages 2 and 3 are dependent on the active metabolism of the cell (*81, 112*). The iron does not become free during transfer and cannot be eluted by apotransferrin or other chelators (*81*).

The conformational changes associated with the binding of iron to transferrin and its delivery to reticulocyte receptors is summarized diagrammatically in Figure 8. As already indicated, these changes, and the ways in which the transferrin-binding sites are occupied by iron, may be factors controlling the rate at which iron is released into the serum from intestinal mucosal cells, and hence iron absorption from the gut, as well as the rate of delivery (*53*). Under normal physiological conditions, transferrin is only about one third saturated with iron (*22*). At high levels of saturation (above 60 per cent), much of the iron is deposited in the liver. The saturation may be normally kept at 30 to 60 per cent, so that the marrow, which has a high affinity for iron, can obtain an adequate supply while other tissues are not overloaded (*22*). Neither the percentage saturation of transferrin nor the level of iron in the stores appear to have a controlling influence on iron absorption (*34, 164*).

Absorption of iron is related to the rate of erythropoiesis, even under conditions in which iron-loading is abnormally high (*23, 164*) or low (*164*), and to the rate of plasma iron turnover (*165*). Not all the iron in the mucosa finds its way into the serum. At high iron levels some of this iron is sequestered in the mucosa in the

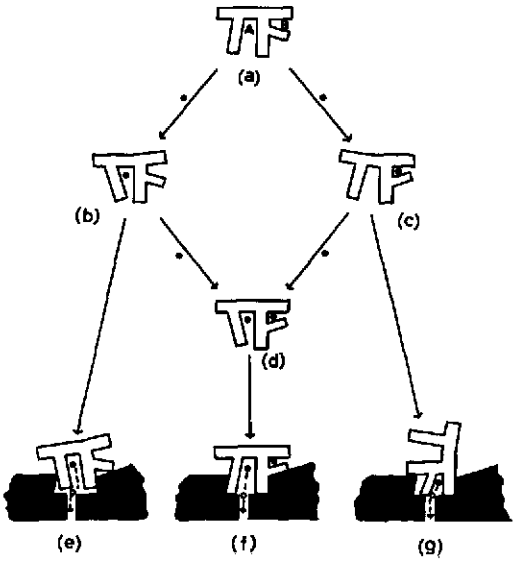
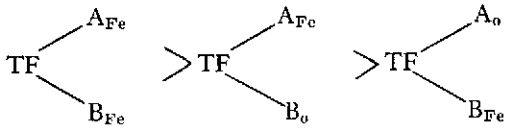


FIG. 8. The apotransferrin molecule TF, (a), has two iron-binding sites, A and B. On binding a single iron atom, •, at either site A or B, as in (b) and (c), the molecule undergoes conformational changes, which allow the first iron atom to be firmly bound and which facilitate the binding of a second atom (*170*). A molecule with both sites occupied is shown in (d). The shape of the molecule is not much affected by iron-binding (*47*). Iron, once bound, cannot easily be removed from transferrin molecules in solution, but the molecules can readily deliver their iron to receptor sites on red cell precursors, depicted in (e), (f), and (g), as a result of conformational changes on binding to the receptors (*81*). Molecules with two bound irons, (d), can transfer an iron atom (one at a time) more readily than those with a single iron atom (*52*), as can be seen by comparing (f) with (e) or (g). Iron atoms from site A can be rather more readily transferred, (e), than can those from site B, (g), (*53*).

form of ferritin (*32, 33, 37*), which is synthesized in response to the presence of iron (*152*). Much of this iron is lost to the body when the cells are exfoliated. Mucosal cells can both release iron to the serum and acquire iron from it, depending on the body's requirements. Conrad, Weintraub, and Crosby (*34*) proposed that absorption is controlled by the concentration of iron in the intestinal mucosa and that this in turn depends on the amount of "messenger" iron from the plasma entering these cells. This messenger iron would be free to enter the intestinal cells when not required elsewhere. When

erythropoiesis is stimulated, much of the mucosal iron would pass to the plasma and absorption would then be increased.

These ideas have been interpreted by Fletcher and Huehns (*53*) in terms of the distribution of iron on plasma transferrin. They point out that transferrin consists of four molecular species, two with a single iron atom at different sites (A and B), one with no iron atoms, and one with two iron atoms occupying both sites. The suggested order of ability to deliver iron to the erythroblasts (*52, 53*) would be as follows:



They propose that "messenger" iron is represented by those molecules that have two bound iron atoms. The amount of this species present would tend to be decreased during high marrow activity and increased when the body's iron stores are large. The quantity of iron entering the mucosa from the plasma would be determined by the number of transferrin molecules carrying two iron atoms, of which that at site B might be the more easily incorporated. Iron released from the mucosal cells to the plasma would, however, have a preference for site A on the transferrin molecule—that is, the same site from which iron is preferentially delivered to the red cell precursors.

These ideas offer a simple explanation of the link between absorption and the body's need for iron. The degree to which transferrin molecules are saturated with iron also depends on the rate at which the protein is synthesized and catabolized, both of which have been found to be related to the erythropoietic rate (*36, 96*). Suppression of transferrin synthesis seems to be

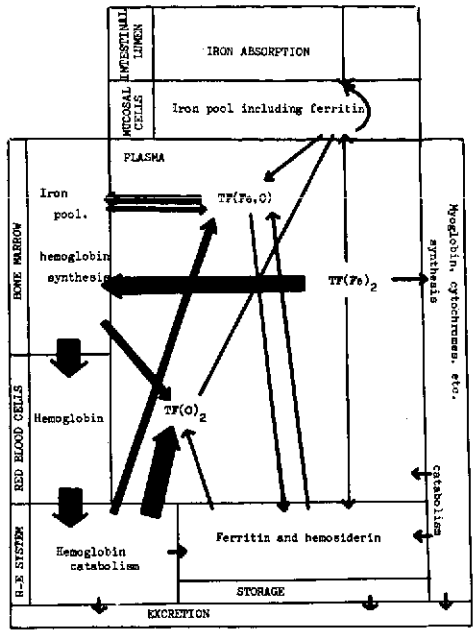


FIG. 9. Diagram of the central role of transferrin in iron metabolism. Plasma transferrin is represented by three species, $TF(o)_2$, $TF(Fe,o)$, and $TF(Fe)_2$, which have their two binding sites occupied by no, one, or two iron atoms, respectively. Differences between the nature of the binding sites (see Fig. 8) have been ignored. The arrows represent the directions of flow of iron atoms in and out of the various compartments. The sizes of the compartments are in no way related to the amounts of iron they contain.

associated with iron-loading, high hepatic ferritin formation, and low erythropoiesis. In iron deficiency, the concentration of transferrin in the plasma is abnormally high (*22, 96*). Thus, iron influences the synthesis of transferrin in a way that is different from its effect on ferritin formation and in a way that ensures that the body's main requirement for iron will be met.

The central role of transferrin in the metabolism of iron is illustrated schematically in Figure 9.

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THE CONTROL OF IRON BALANCE BY THE INTESTINAL MUCOSA[1]

William H. Crosby

Iron metabolism is under the control of a complicated system for keeping unneeded iron out of the body. It is unlike the metabolism of other nutrient metals, such as potassium, calcium, or copper, which depend upon excretory functions to remove any excess. The excretory capability for getting rid of iron is meager, so the balance is maintained primarily by adjusting the absorption of available iron (*17*). The small intestine of a normal man absorbs about 1 mg of iron a day (*18*), thereby offsetting similar losses (*14*), which are comprised of the traces of iron in all of the epithelial cells shed from the surfaces of the body. This 1 mg of iron is absorbed from the 15 to 20 mg in the daily diet. More than 50 per cent of dietary iron is available for absorption (*13*), but the normal intestine declines to accept unneeded iron. When the requirement is increased during growth or pregnancy or after blood loss, the intestine's mucosal block is relaxed to permit more of the available iron to enter (*9*). A normal man with a moderate-to-severe iron deficiency can absorb 6 mg of his dietary iron in a day—1 mg lost in desquamating epithelium and 5 mg incorporated into the expanding mass of circulating hemoglobin as the anemia is repaired (*6*).

The differences of behavior of the intestine in these two states, repletion and depletion of iron, provide a useful model for comparative studies and for speculation concerning the control of iron metabolism. They also provide some insight into the complexity of the system that exerts the control.

Iron deficiency is induced experimentally by removing blood from normal volunteers. One liter of blood with 150 g of hemoglobin contains 500 mg of iron, which is 10 to 15 per cent of the body's total. Following such a loss, the anemia is corrected by a temporary increase of erythropoiesis. The iron required for this spurt of hemoglobin synthesis comes from two sources —the physiologically uncommitted stores of iron in the liver, spleen, and bone marrow, and from the diet. Following this loss of blood and iron, the small intestine absorbs more than the usual amount of iron. It changes its behavior in an appropriate reaction to a changed requirement for iron. In other words, the intestine is acting upon information it receives concerning the requirement.

The iron absorption control system comprises a series of recognizable steps: (1) information concerning the requirement for iron must originate somewhere in the system; (2) this information must somehow be transmitted to the small intestine; (3) the intestine must somehow receive the information; and (4) the information must be translated into a pattern of iron absorption—an avidity that is appropriate to the requirement for iron. Though the nature of this system is known, information is still lacking on precisely how the several steps are accomplished.

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Sufficient clinical and investigational data are available, however, to provide the scaffolding for a working hypothesis.

Origin of information concerning the requirement for iron

The rat, after its iron stores have been overloaded by injections of iron, absorbs less of a test dose of radioiron than normal animals do. After a moderate bleeding—not nearly enough to deplete the excess stores—the animal behaves as though he were iron deficient, absorbing an increased amount of the test dose (*8*). This would indicate that the information concerning requirement for iron does not originate primarily within the iron stores. When there is iron-deficiency anemia and the amount of iron returning from the normal destruction of red cells is insufficient, the principle requirement for iron exists at the point of erythropoiesis. However, when the stores are depleted without anemia and without reduction in hemoglobin iron, the intestine permits iron to accumulate until the stores are replete. The signals generated by the loss of storage iron—following a hepatectomy for example—are not equal in intensity to those generated by the loss of a similar quantity of hemoglobin iron (*21*).

Nature and transmission of information concerning the requirement for iron

It is not known for certain what stimuli cause the intestine to increase or decrease its absorption of iron in accordance with the body's need. Anemia, hypoxia, erythropoietin, hypoferremia, and the rate of iron turnover in the plasma have been suggested as possibilities. These can all be ruled out, however, by observing the behavior of the intestine in a chronic situation and noting the increases in iron absorption and accumulation of unneeded iron (*12*). Care must be taken to eliminate diseases with an associated iron metabolism disorder that in and of itself permits the accumulation of iron—for example, Cooley's anemia and the acquired sideroblastic anemias, in which a syndrome resembling hemochromatosis develops even without transfusion. Also to be eliminated are acute situations in which the size of the red cell mass or the erythropoietic marrow changes, thus causing alterations in the availability and demand for iron—as in the onset of polycythemia, which is accompanied by a relative iron deficiency, and the onset of folate deficiency, where the dwindling red cell mass provides iron to be placed in storage.

Anemia, hypoxia, and excess erythropoietin are all present in the hypoplastic anemias that are not sideroblastic. Patients with these conditions do not absorb iron in amounts above the normal range, and, if they are not transfused, they do not accumulate iron. Permanent dwellers at high altitude live with a permanent hypoxia (although plethoric) and with increased erythropoietin, yet they do not accumulate unnecessary iron.

Plasma iron itself does not seem to be the channel of information to the gut. Hypoferremia is associated with increased iron absorption in iron deficiency, but not when the hypoferremia is due to inflammatory disease. In uncomplicated hemolytic disease (for example, hereditary spherocytosis), the turnover of plasma iron may remain perpetually at 5 to 10 times the normal rate, yet unneeded iron does not accumulate. Iron needed for accelerated erythropoiesis is easily available from equivalent hemolysis, and the patient with such a chronic disorder remains in a state of dynamic equilibrium similar, except for volume, to that of a normal person.

When iron becomes relatively unavailable, iron absorption increases. Erythroblasts remove the iron from plasma transferrin, and the transferrin must then recoup from other sources. When the requirement exceeds the amount made easily available by hemolysis, the stores of iron are tapped and additional iron is brought in from the intestine. In this situation, the concentration of serum iron is often, but not necessarily, low. When the iron stores are not exhausted, the marrow may repair a diminished red cell mass while the plasma iron concentration remains normal (*20*).

Iron removed from transferrin at an increased rate is replaced at an increased rate, yet the

concentration of plasma iron and the saturation of plasma transferrin tend to remain constant. This suggests that the transferrin's avidity for iron involves a homeostatic mechanism that maintains the iron-binding protein at about one third of saturation (*11*). When saturation falls below that level, the avidity of the protein seems to increase so that iron is drawn from the storage depots. When a normal man is injected parenterally with a modest amount of iron in excess of requirement—let us say 1 or 2 grams—within a few weeks that iron has been distributed to the storage depots, and the ratio of plasma iron to transferrin re-establishes itself at the evidently critical level of about one third saturation. The avidity of the transferrin is low enough to prevent the excessive storage iron from bulging into the plasma space.

The homeostatic behavior of transferrin, which shows avidity increased during iron deficiency and decreased during surfeit, suggests that this is the mechanism whereby information concerning iron requirement is transmitted from the place where iron is needed to the place where it is available.

The intestine's reception of information concerning the requirement for iron

The plasma transferrin behaves in response to the requirement for iron, bringing iron to the stores when there is an excess and cleaning out the stores when there is a deficit. It is evident that the several pools of uncommitted iron vary in their availability. For example, the iron derived from normal hemolysis seems to be most readily available, while iron stored in the liver is less so. As a consequence, the daily cycle of erythrocyte iron involves little of the liver's storage iron: about 90 per cent of the iron in new red cells comes from old red cells. When the requirement increases and the transferrin must recover the needed iron from available sources, the more labile, easily available iron pools are the first to go. Notable among these is the nonheme iron in the intestinal mucosa. We have found that the concentration of iron in the duodenal mucosa is low in the case of iron deficiency and high in experimental iron overload (*8*). For example, in rats, within a week after hemorrhage, the concentration of iron in the duodenum falls from about 15 to 8 μg per g of tissue. When 25 μg of iron is injected intravenously, the concentration of duodenal iron abruptly increases, then gradually subsides to normal within four days. There is evidence that the differences of intestinal iron concentration between normal, iron-deficient, and iron-loaded animals may not be so great in the epithelial cells as it is in the entire mucosa (*4*), and the differences may not be evident in man (*1*). The problem needs further investigation, but it is worth considering that in iron deficiency all tissues become iron deficient, and in the state of iron overload resulting from injections of iron or transfusions it is possible to see excess iron in the intestinal lamina propria (*2*).

The working hypothesis here is that the small intestine receives information concerning the body's requirement for iron through the medium of its nonheme iron pool. When iron is needed the pool is depleted; when iron is in excess the pool becomes overloaded.

Translating iron requirement information into an appropriate pattern of iron absorption

In both man and rats (*7, 10, 16*), radioautographs of the duodenum after ingestion of radioiron have demonstrated that the epithelial cells of the villi react differently in different states of iron repletion. In the iron-deficient state, the cells *in vivo* take up a great amount of the radioiron and pass all of it into the body, so that in the radioautographs the villi are free of activity. *In vitro,* the epithelial cells accept the iron freely but are unable to pass it on, so they are highly radioactive. Normal epithelium *in vitro* accepts less iron than that of the iron-deficient patient, while the epithelium of the iron-loaded subject accepts little or no iron at all. The *in vivo* experiment in iron-loaded animals does not reveal any radioactivity in the villous epithelium either. One may conclude that these cells have been conditioned to refuse available iron. In this situation, the mucosal block to absorption appears to

reside at the surface of the cell. On the other hand, in normal animals the radioautograph demonstrates that the epithelial cells sequester a portion of the absorbed iron, and that iron is lost into the lumen when the cells are shed at the end of their life cycle. Another portion of the absorbed iron, of course, is passed along to the body. Thus, in addition to the mechanism at the surface of the cell, there appears to be a second mechanism within the cell to prevent unneeded iron from entering the body. The epithelium has the ability to synthesize ferritin, and this ability is sharply diminished in iron deficiency (*5*). For this reason, it is believed possible that the accepted iron which becomes sequestered in the epithelium has been bound into ferritin. In this conception, ferritin is not a stage of iron absorption but rather a metabolic cul-de-sac to prevent iron absorption. For the sake of completeness, it should be noted that there is yet another block to the absorption of iron. Following a large dose of iron by mouth, it is possible within two or three hours to demonstrate the presence of iron-loaded macrophages in the duodenal villous tips. This occurs even in iron deficiency (*19*), thus indicating that the iron is not of internal origin. These iron-laden cells move across the epithelial cortex and return the iron to the intestinal lumen (*2*).

Conditioning of the epithelial cells results in the absorption of iron in consonance with the body's requirement. It is evident that the "front door" of the epithelial cells can be closed against dietary iron. There is also evidence to indicate that the transfer of iron from cells to the plasma is rate-limiting (*22*). It is reasonable to postulate that external information concerning requirement determines the attitude of the epithelial cells. According to the hypothesis, this attitude is determined by the concentration of nonheme iron in the lamina propria of the duodenal mucosa. As the cells are formed, they somehow receive a signal concerning the requirement for iron, and this impression dominates the ability to accept or reject available iron throughout the cells' life span. A cell informed of a surfeit cannot accept iron; a cell informed of a deficiency cannot reject it. According to this concept, the duodenum changes its attitude toward available iron by changing its epithelial covering.

The means whereby the cells are imprinted with instructions for absorbing iron may be related to a small amount of iron that is incorporated into the cell as it is formed. When a trace of highly radioactive iron is injected intravenously in normal and iron-loaded rats, the villous epithelial cells formed thereafter are radioactive; none of the radioactivity goes into cells already formed (*8*). In iron-deficient animals, none of the cells become radioactive. The iron involved represents a tiny investment. If it is this mite that controls iron absorption by the cell, it must be strategically positioned (*15*).

While the ability to excrete iron is limited, it is not entirely lacking. The macrophages of the intestinal lamina propria are loaded with iron in patients with transfusion siderosis, and these cells have been shown to intercept absorbed unneeded iron (*19*). It is not known if they play any part in normal iron metabolism.

Epithelial cells of the mucus-secreting glands are also capable of accepting more iron than they require and storing it as ferritin-hemosiderin (*3, 10*). These cells are located at the surfaces of the body, and when they are shed the iron within is lost. In the gut, the mucous glands of the stomach and Brunner's glands of the duodenum provide yet another means by which a small amount of excess iron can leak out.

The total excess iron that an iron-loaded man can lose may not exceed 4 or 5 mg a day (*13*). This seems small when the overload is measured in grams, as in a patient with transfusion siderosis, but it is adequate to help maintain iron balance under normal conditions. It is possible that the intestine may, from time to time, absorb some small quantity of iron in excess of the requirement. These excretory channels would be adequate to remove the excess. Thus, the macrophages and specialized epithelial cells may serve as a supplementary device in support of

the intestine's ability to refrain from absorbing available unneeded iron.

Summary

The small intestine controls the level of iron in the body, responding to the body's requirement and refusing to absorb unneeded available dietary iron. The intestine receives information concerning the requirement and acts upon this information, absorbing more or less dietary iron as indicated.

It is suspected that a chain of phenomena controls these responses in approximately the following manner: (1) bleeding stimulates erythropoiesis, which increases the marrow's requirement for iron; (2) this accelerates the turnover of plasma iron, thus hastening the removal of iron from the intestinal mucosa and causing the iron concentration in the mucosa to fall; and (3) the intestinal epithelial cells formed in this iron-poor environment do not have the ability to refuse available dietary iron. When the intestine becomes covered with such cells, iron absorption is increased. After the body's accumulation of iron is restored to normal, all these changes subside and the intestine once again can refuse to absorb available iron.

The injection of excessive iron has an opposite effect. The absorption of iron becomes less than normal. Some of the excess may be lost with the shedding of iron-laden phagocytes and epithelial cells.

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THE ROLE OF PROTEIN IN IRON ABSORPTION

Marcel E. Conrad

Iron deficiency is widespread among populations that consume a protein-deficient diet. Its etiology has been related to a variety of different factors in combination, some of them directly attributable to protein-depleted diet and others apparently unrelated to food but occurring with high frequency among people of limited means (*3, 4, 42*). In some geographic areas protein consumption is diminished by religious beliefs and customs that forbid the use of meat as food. However, in most parts of the world, a protein-depleted diet is not eaten by choice, it being a reflection of poverty rather than dietary idiosyncrasy.

Nutritional studies suggest that the iron content of the diet is the most important factor affecting iron repletion. A low incidence of iron deficiency is found in the United States, where the adult diet contains 10 to 20 mg of iron, whereas it is commonplace in areas of the world where the diet contains less iron (*42*). The problem is further complicated by marked differences in foods and the chemical form of dietary iron in various parts of the world. Significant differences in the quantity of iron absorbed from various radiolabeled foods attests to the importance of this factor (*24, 26, 27, 29*). These differences exist because foods contain various forms of iron and ingredients that serve to increase and/or decrease iron absorption. Ferrous iron is absorbed in greater quantities than ferric iron, and dietary ingredients such as certain sugars and amino acids solubilize iron to enhance iron absorption, whereas other constituents of the diet such as carbonates, oxalates, phytates, and phosphates cause precipitation or molecular aggregation of iron and hence decrease absorption (*2, 5, 8, 14, 21, 23, 28, 30, 32*).

Iron deficiency is not prevalent among people who consume meat as a major constituent of their diet. This can be attributed to both the high iron content of meat diets and the more efficient absorption of hemoglobin iron than dietary iron. Heme is absorbed from the intestinal lumen as an intact metalloporphyrin. It does not compete with intraluminal iron for absorptive sites in the mucosal cell, and in the presence of globin-degradation products it is less susceptible than inorganic iron to precipitating and aggregating chelators (Figure 1 and *1, 10, 35, 38, 41*).

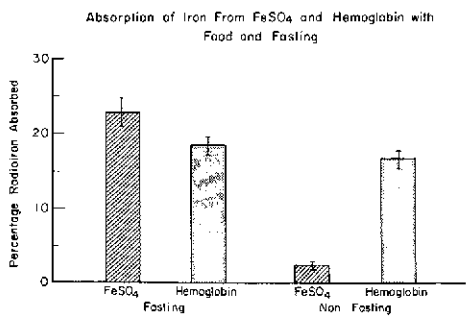

FIG. 1. In measuring the absorption of Fe^{59} from a test dose of ferrous sulfate or labeled hemoglobin containing one milligram of iron, each test dose was administered to human volunteers after a 16-hour fast or with food (*36*). More iron was absorbed by fasting subjects from ferrous sulfate than from hemoglobin. Addition of the test doses to a meal markedly diminished the absorption of iron from ferrous sulfate but had little effect on the absorption of hemoglobin iron. Bars represent the mean of eight absorption studies and brackets are the standard error of the mean.

Many other factors that are generally found in populations of limited means can contribute significantly to iron deficiency. For example, the histologic appearance of the small intestine in normal subjects from Southeast Asia contrasts sharply with biopsy specimens from Europeans and North Americans. There appears to be a marked diminution in the absorptive surface area of the gut, with alterations that resemble sprue (*9, 33*). Many other factors contribute to iron deficiency as well (Table 1). These include hookworm infestation, chronic infection, chronic diarrhea, and frequent pregnancy (*1, 13, 19, 20, 25, 31*). Diets often contain marginal amounts of other nutrients, and iron deficiency may be complicated by the coexistence of deficiencies in dietary needs such as folic acid, vitamin B_{12}, thiamine, and riboflavin (*34, 37*).

It is difficult to study the effect of protein deprivation on iron absorption in malnourished people because of the multiplicity of factors involved. For this reason, experiments with rats have been used to study the effect of starvation and dietary depletion of protein on iron absorption (*11*).

Previously, acute starvation was used to assay erythropoietin (*22*). Rodents starved for five days have a marked depression in the incorporation of intravenous doses of radioiron into red blood cells. Similarly, there is a notable decrease in the absorption of iron from oral test doses of ferrous sulfate. The animals lose body weight; they develop a relative erythrocytosis; and they show an elevated serum iron concentration, a significant decrease in their total iron-binding capacity, and a prolonged plasma iron clearance with diminished plasma iron turnover (Table 2).

Similar changes were observed in ferrokinetic studies of rats made iron-deficient by phlebotomy of 4 ml of blood at four and ten days before starvation when compared with iron-deficient rats that were fed. The starved animals lose 25 per cent of their body weight after five days of fasting. The packed cellular volume increases, because starved rodents voluntarily abstain from drinking normal quantities of water. Starvation decreases iron absorption and the incorporation of radioiron into red blood cells. Serum iron concentration was shown to be increased, and the total iron-binding capacity decreased. Differences in the plasma iron clearance and calculated plasma iron turnover were not significant (Table 3).

Ferrokinetic data from starved iron-deficient rats were similar to observations in normal unstarved animals. A comparison of iron absorption studies in normal, iron-deficient, iron-loaded, and phenylhydrazine-treated rats is shown in Figure 2. In groups of unstarved animals, iron deficiency and phenylhydrazine-induced hemolysis caused an increase in iron absorption, whereas iron-loaded rats absorbed less iron than normal control animals. A similar

TABLE 1. Factors affecting iron repletion in protein-deprived populations

Absorption:

1. Iron-deficient diet
2. Chemical form of dietary iron
3. Histology of small intestine
4. Limited erythropoiesis
5. Limited growth rate
6. Chronic infection
7. Chronic diarrhea
8. Geophagia

Excretion:

1. Enhanced excretion
2. Intestinal parasites
3. Frequent pregnancy

TABLE 2. Effect of acute starvation on iron metabolism in normal rats

	FASTED OVERNIGHT	STARVED 5 DAYS
Iron absorption (%)	20	5
Weight loss (%)	6	23
Hematocrit (%)	48	55
Serum iron (μg/100 ml)	116	192
TIBC (μg/100 ml)	495	416
Plasma iron clearance (T ½ min)	70	169
Daily iron turnover (μg/d)	115	79
RBC iron incorporation (%)	36	7

TABLE 3. Effect of acute starvation on iron metabolism in iron-deficient rats

	FASTED OVERNIGHT	STARVED 5 DAYS
Iron absorption (%)	48	24
Weight loss (%)	5	25
Hematocrit (%)	39	45
Serum iron (μg/100 ml)	51	89
TIBC (μg/100 ml)	540	428
Plasma iron clearance (T ½ min)	40	50
Daily iron turnover (μg/d)	89	110
RBC iron incorporation (%)	80	54

relationship was observed among groups of starved animals in the various states of iron repletion and with hemolysis. However, the starved animals absorbed significantly less iron than unstarved rats in the same state of iron repletion. Thus, iron-deficient and phenylhydrazine-treated starved rats absorbed approximately the same amount of iron from oral test doses of ferrous sulfate as did unstarved normal control animals. This indicates that starvation produces relative changes in iron absorption and that any human or animal studies must include appropriate control groups, or else differences will remain undetected.

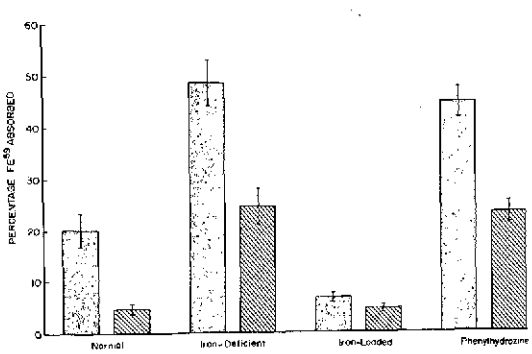

FIG. 2. In the various states of iron repletion and hemolysis, rats fed a normal diet (stippled bars) absorbed significantly more Fe^{59} from oral test doses of ferrous sulfate than comparable animals starved for five days (striped bars) (*16*). Fasted, iron-deficient rats absorbed as much iron as normal, unstarved animals but significantly less than the iron-deficient rats that received a normal diet. Similar changes were observed in acetyl-phenylhydrazine-treated animals. This suggested that there was a relative deficiency of substances required for hemoglobin synthesis but that the deficit was not absolute. Bars show mean levels and brackets indicate 1 SE for 10 rats.

The elevated hematocrit observed in all groups of starved animals was caused by dehydration and a diminished plasma volume. Starved rats ingest about one half the volume of water consumed by fed animals, and Cr^{51} red blood cell volume studies showed the same red blood cell mass in normal animals and in rats starved for five days. To determine whether relative erythremia or dehydration affected iron absorption, water was removed from animal cages and rats were hydrated by hypodermoclysis of 10 ml 5% dextrose at six-hour intervals for five days. Half the animals were starved and the remainder were fed dried food. The hematocrit remained normal in the parenterally hydrated starved animals, but iron absorption and the red blood cell incorporation of Fe^{59} was notably depressed (Table 4).

Periodic studies following recovery from a five-day period of starvation showed that iron absorption became normal after six days of feeding. This coincided with the interval required

TABLE 4. Hydration and iron absorption

	NORMAL		STARVED 5 DAYS	
	WATER AD LIB.	HYPODERMOCLYSIS	WATER AD LIB.	HYPODERMOCLYSIS
Water intake (ml/day)	35	40	21	40
Food (g/day)	17	12	0	0
Weight (g)	+12	−4	−57	−44
Hematocrit (%)	48	47	56	48
Cr^{51} RBC mass (ml)	5.1	5.0	4.9	5.0
Fe absorption (%)	18	15	5	6
RBC Fe^{59} incorp. (%)	39	34	7	7

TABLE 5. Recovery from a five-day starvation

GROUP *	WEIGHT CHANGE FROM BEFORE STARVATION (GRAMS)	IRON ABSORPTION (%)	RBC INCORP. Fe^{59} (%)
Control	0	23	33
0	—62	6	4
1	—51	12	5
2	—39	14	8
3	—25	13	14
4	— 8	16	19
6	+ 2	26	33
9	+20	27	34

* Number of days fed following starvation period

for each group of rats to regain their prestarvation body weight and normal erythropoiesis (Table 5).

The effect of starvation on ferrokinetics in the duodenum was studied. The nonheme iron concentration of duodenal segments from starved animals was similar to chemical measurements

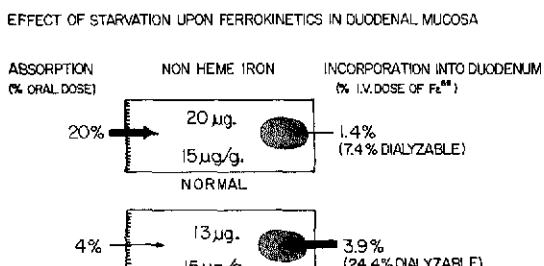

FIG. 3. This schematic diagram of the small intestinal mucosal cell in normal and starved animals shows a similar nonheme iron concentration for both groups (*17*). Starved animals sequester more iron from body stores in their duodenal mucosa than do normal animals, and this iron is more readily dialyzed from duodenal homogenates. This suggests that the mucosal iron of starved animals is either unbound or loosely bound to protein and may be more readily available to diminish the uptake of dietary iron from the intestinal lumen. Homogenates were dialyzed in 0.5 M Tris (pH 9) containing Tiron (1 g/liter) in 250 ml plastic bottles rotated at 4° C for 48 hours. Fe^{59} passing through the dialyzer tubing was bound to Tiron and collected in anion exchange resin (1 AG-1 × 8) (*11*).

in gut specimens from normal rats (Figure 3). These data seemed to conflict with the hypothesis that the iron content of intestinal cells regulates the quantity of iron absorbed from the intestinal lumen. It seems unlikely that generalized malabsorption caused the marked decrease in iron absorption that was observed in starved rats, because these animals absorbed normal quantities of glucose and had an intestinal mucosa of histologically normal appearance. However, there was increased incorporation of body iron into the intestinal cells of starved animals, and much of this iron was readily dialyzed from duodenal homogenates. This suggested that a larger amount of the intestinal iron in starved animals was either unbound or loosely bound to protein and might be available to regulate the uptake of iron from the intestinal lumen. Previous experiments have already suggested that body iron sequestered by intestinal mucosal cells is a more potent regulator of absorption than dietary iron. An intravenous dose of iron markedly decreases absorption, whereas the ingestion of iron only slightly reduces the quantity of iron absorbed from oral test doses of radioiron administered several hours later (*6, 12*).

The effect of dietary composition on ferrokinetics and iron absorption was studied by feeding food containing various amounts of protein and carbohydrate to rats (Figure 4). Each diet contained 10 per cent fat and 0.15 mg/g of inorganic iron. Rats weighing 125 g were selected and fed these diets for three weeks. Weight gain was proportionate to the protein content of the diet. Iron absorption and kinetic studies in rats receiving a protein-depleted diet were similar to those observed in starved animals. Iron absorption, the incorporation of Fe^{59} into red blood cells, and the concentration of iron-binding protein in plasma were decreased. The serum iron concentration was elevated, the plasma Fe^{59} clearance became prolonged, and the calculated plasma iron turnover was approximately half the level observed in rats that were fed a normal diet. Unlike starved animals, rats on a protein-depleted diet consumed relatively

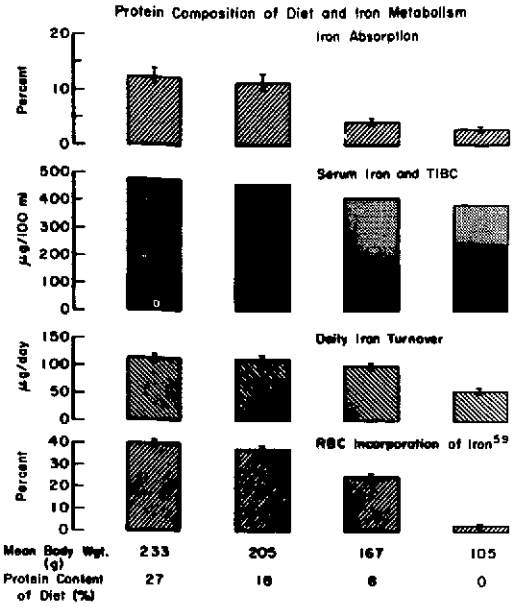

FIG. 4. Diets containing various quantities of protein were fed to 125-gram rats for three weeks. WRCF rats were used because they are unable to absorb heme iron (*40*). Each diet contained 0.15 mg/g of nonheme iron. Weight gain was proportionate to the protein content of the diet. Animals maintained on protein-depleted diets had a higher serum iron concentration but absorbed less iron from oral test doses of ferrous sulfate and had diminished incorporation of radioiron into red blood cells and a decreased plasma iron turnover. Testing was performed on groups of 10 rats fasted for 16 hours. Brackets represent the standard error of the mean.

normal amounts of water and did not become dehydrated. Rats maintained on an 8 per cent protein diet had ferrokinetic values intermediate between those of starved rats and animals receiving a normal protein-replete diet.

To determine the effect of various types of carbohydrate on iron absorption, cooked white rice and diets containing either sucrose or cornstarch were prepared and fed to rats. Vitamin-free casein was added to different diets in various quantities. Corn oil, vitamins, and iron were added to each diet in equal amounts. The rats weighed 125 g at the beginning of the study and were kept on each diet for three weeks. Testing was performed after an overnight fast. Rats on protein-deficient diets gained less weight, absorbed less iron from oral doses of ferrous sulfate, and incorporated less of an intravenous dose of radioiron into the red blood cells. Ferrokinetic data and iron absorption studies were similar in the rats that received the starch and sucrose diets (Figure 5).

The habitual ingestion of large amounts of laundry starch is a common practice among Negro females in certain geographic areas of the United States (*15, 18, 39*). Occasionally the amount consumed exceeds two pounds a day and causes severe iron deficiency. The severity of "starch eaters'" anemia seemed excessive for an iron-deficient diet alone and raised the question of whether the starch chelated iron within the intestinal lumen to make it unavailable for absorption. To test this hypothesis, rats were given 5 ml oral test doses of ferrous^{59} sulfate containing either 1 g of starch, 1 g of sucrose, or no additive. The absorption of similar amounts

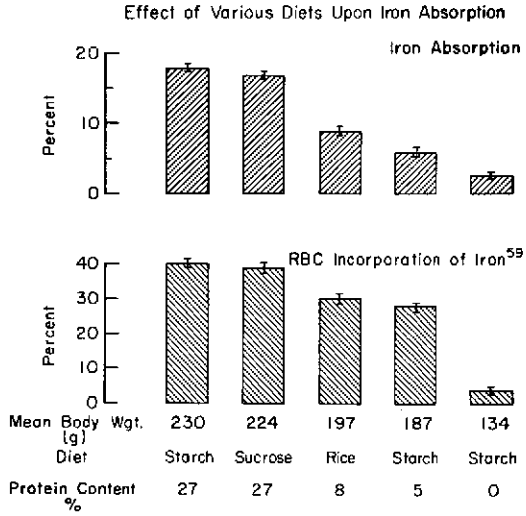

FIG. 5. Cooked white rice and diets containing either sucrose or cornstarch were prepared for rats. Vitamin-free casein was added to certain diets and equivalent amounts of corn oil and vitamins were added to each diet. Diets contained 0.15 mg of iron per gram of dry weight. The rats weighed 125 g at the beginning of the study and were kept on each diet for 3 weeks. Rats on protein-deficient diets gained less weight, absorbed less Fe^{59} from oral test doses of ferrous sulfate, and incorporated less of an intravenous dose of Fe^{59} into red blood cells. There was no significant difference between groups of rats fed starch or sugar. Bars represent the mean for 10 animals±1 SE.

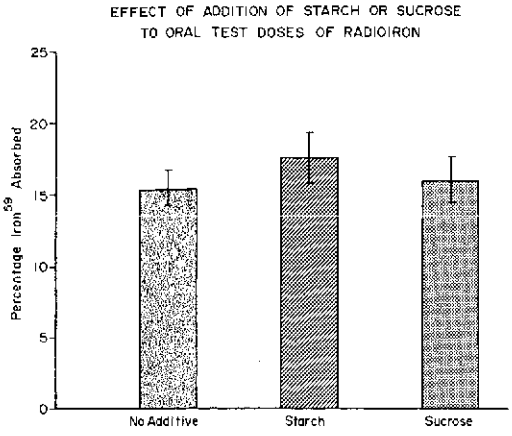

FIG. 6. The addition of 1 gram of either starch or sucrose to oral test doses of ferrous[59] sulfate had no significant effect on iron absorption (*18*).

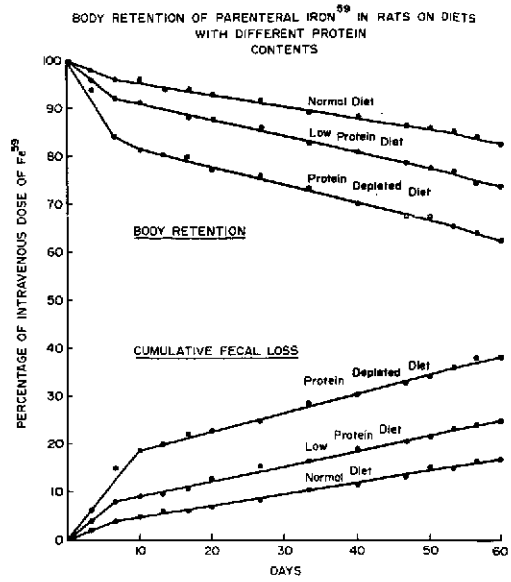

FIG. 7. Body retention of a parenteral dose of Fe^{59} was measured twice weekly in rats given diets containing either 27 per cent, 8 per cent, or no protein. The animals were fed the special diets for two weeks before they were injected with an intravenous dose of Fe^{59}-labeled plasma. Whole body radioactivity was measured in a small animal liquid scintillation detector (ARAMAC) and by assay of the radioiron in stool collections (*16*). Protein depletion caused increased loss of parenteral iron from animals. Each point represents the mean for a group of 10 rats.

of iron from each preparation indicated that starch had no direct intraluminal effect on iron absorption (Figure 6).

The effect on iron excretion of diets containing various amounts of protein was studied by measuring the whole body retention of a parenteral dose of radioiron in rats with diets containing either 27 per cent, 8 per cent, or no protein at all. After two weeks on the special diets, the animals received an intravenous dose of Fe^{59}-labeled plasma, and they continued to receive the same diets for another two months after injection. The rats that were fed the protein-depleted diets lost significantly more radioiron than the animals that received a normal diet (Figure 7).

The effect of prolonged protein depletion on anemia was studied in rats with either iron-replete or iron-depleted diets containing various amounts of protein. Six weeks of protein deprivation caused a moderate anemia in the rats receiving an iron-replete diet. However, the red blood cells remained normochromic and normocytic. All iron-depleted diets produced a microcytic hypochromic anemia, and the anemia was most severe in the animals that were fed a protein-depleted diet (Table 6).

TABLE 6. Hematologic data on rats fed various amounts of protein for six weeks

	RBC (10^6 CELLS/CMM)	HB (G/100 ML)	HCT (%)	MCV (μ^3)	MCH ($\mu\mu$G)	MCHC (%)
28% Protein with iron	8.6	14.5	44	50	17	33
Iron-depleted	8.1	10.5	32	40	13	30
8% Protein with iron	7.2	11.6	36	49	16	34
Iron-depleted	6.9	9.7	28	41	14	29
0% Protein with iron	5.1	8.2	24	49	16	34
Iron-depleted	4.9	6.7	21	43	14	31

Summary

Iron deficiency is widespread among populations consuming a protein-deficient diet, and it is found with greatest frequency in women during the childbearing years and in children during periods of maximal growth. These people frequently have other nutritional problems such as multiple deficiencies of the B vitamins. Among protein-starved humans the most important nutritional factors causing iron deficiencies are the low iron content of most protein-deficient diets and a lack of readily absorbed heme iron in the diet. Other factors that diminish iron absorption in protein-deprived populations are (1) histologic alterations of the small intestinal mucosa, (2) a diminished corporeal stimulus to absorb iron, (3) a decreased concentration of the amino acids in the diet that facilitate iron absorption from the intestinal lumen, (4) chronic diarrhea, and (5) chronic infection. Iron deficiency is worsened in many of these people because of a high incidence of hookworm infection and frequent pregnancies.

Studies of starvation and protein depletion in experimental animals showed ferrokinetic abnormalities and diminished iron absorption. This was not caused by a direct intraluminal effect of starch or sucrose diets on iron absorption. The abnormality was attributed to retarded growth rate and diminished hemoglobin synthesis in these animals. Protein-deprived animals seemed to attempt to re-establish and maintain a normal body iron concentration by decreased absorption and increased excretion of iron. Rats receiving an iron-replete, protein-deficient diet for prolonged periods developed a normocytic normochromic anemia. A microcytic hypochromic anemia occurred only when iron was not added to the protein-deficient food. The decreased absorption of iron resulting from protein depletion was not accompanied by increased iron content or concentration in the duodenal mucosa. However, in starved rats increased amounts of dialyzable iron were shown to be incorporated into the duodenum from body stores and may act to diminish the uptake of iron from the lumen of the gut into mucosal cells.

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INTERSUBJECT AND INTRASUBJECT VARIATION OF IRON ABSORPTION

James D. Cook

Measurements of iron absorption from the gastrointestinal tract have been a highly useful tool for studying the disorders of iron balance. The effectiveness of this tool, however, has been limited by the marked variations in absorption that have characterized measurements with present isotopic methods. In earlier reports, this variability was usually attributed to methodologic errors in determining the activity retained in the body. Tests were subsequently performed using the double isotope technique by comparing absorption of Fe^{55} and Fe^{59} administered as a single dose. The differences noted by dual isotope counting of whole blood taken 14 days after administration were within a range of plus or minus 2 per cent, whereas absorption measurements among normal subjects typically range from 1 to 50 per cent (*5*). Thus, it is apparent that the explanation of variability in iron absorption lies with the individual subject rather than with the method of assay.

It is important to examine variations in iron absorption and the factors that give rise to them in order to find ways of reducing or eliminating their effect and thereby improve the precision of the tests now being used. Before considering specific factors, brief mention should be made of the general nature of iron absorption data. The frequency distribution of large series of measurements obtained under comparable conditions have shown a highly skewed distribution that bears little resemblance to normality. By applying statistical methods based on assumptions of normal distribution, useful information may have been lost in the reports published to date. This loss of sensitivity in the analysis of absorption data can be avoided by transforming the data to a scale that normalizes the frequency distribution before applying classical methods of statistical analysis. It has been found that logarithmic transformation gives a distribution that is remarkably close to normal and permits more sensitive evaluations to be made (*5*).

In turning now to specific causes of iron absorption variation, it is useful to consider two major categories: *inter*subject variations, or differences among individuals, and *intra*subject variations, or differences in the same subject from day to day. Subject-to-subject variations can be determined by subtracting the differences in absorption of Fe^{55} and Fe^{59} administered to the same subject on two successive days from the over-all variation in absorption among the test group as a whole. Studies of this kind have shown intersubject and intrasubject variability to be roughly equal in magnitude among normal subjects.[1]

*Intra*subject differences represent an artifact of current isotopic methods that presumably arises from differences in the gastrointestinal motility or secretion at the time of dose administration. A useful approach to minimizing the effect of such variations is to administer the radioiron as a series of doses over a period of

[1] J. D. Cook, M. Layrisse, and C. A. Finch, unpublished observations.

several days (2). This method is useful for studies of inorganic iron, where the test dose can be easily prepared and taken by the subject; it offers little advantage, however, when more detailed preparation of the test dose is required, as in studies of dietary iron absorption. In the latter case, better information can be obtained by giving single doses to a larger number of subjects.

The importance of *inter*subject differences depends on the purpose for which the absorption studies are being performed. In certain cases, it is precisely these variations that are of interest, since iron absorption measurements provide a sensitive index of an individual's iron requirements. Women, for example, absorb more than twice as much as men (6), reflecting differences in iron requirements due to menstrual losses and the demands of pregnancy and lactation. The iron balance of an entire population may vary from one geographic region to another, presumably reflecting the type and level of dietary iron supply. Thus, in studies from England, absorption of a 5 mg dose of ferrous sulphate in normal subjects averages from 20 to 30 per cent (*1, 4, 8*), whereas comparable data for subjects living in the United States is of the order of 10 per cent (*3, 9*). A valuable feature of iron absorption measurements, therefore, is their ability to characterize the iron balance of an individual.

Another important kind of iron absorption study involves evaluating the availability of different forms of administered iron. Here, subject-to-subject differences are an unwanted variable that should be minimized in order to obtain optimal sensitivity. When only two iron compounds are being assessed, a useful approach is to compare them both in the same subject by means of a double tracer technique. When more than two compounds are being evaluated, however, it is important to eliminate possible differences in the over-all setting of the mucosa among different groups of subjects. The most suitable approach here is to relate the absorption of the test compound in each subject against a standard reference dose of inorganic iron (7).

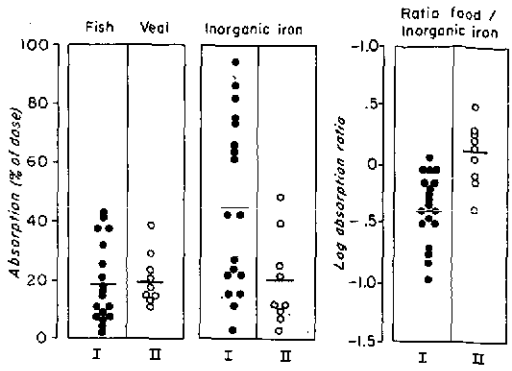

FIG. 1. Comparison of absorption from two types of biosynthetically tagged dietary iron

An example of this model is shown in Figure 1. Mean absorption from two forms of biosynthetically labeled dietary iron were roughly equal, whereas inorganic iron absorption revealed appreciable difference in the over-all level of iron absorption in the two groups of test subjects. When these were unmasked by relating the absorption of the food iron to the reference dose, highly significant differences in the availability of iron became apparent. Optimal sensitivity of the statistical comparison requires that the ratios be tested on the logarithmic scale.

In summary, the limitations in the precision of present iron absorption techniques can be explained in terms of day-to-day variations in the same subject and individual variations among different subjects. In all types of studies, precision can be improved by employing more appropriate methods of statistical analysis for the skewed distribution of iron absorption data, and also by using sufficient numbers of subjects to give statistical validity to the results. Studies with inorganic iron can be made more precise by administering multiple test doses to reduce differences between days. In studies of dietary iron assimilation, it is important to relate measurements to a reference dose of inorganic iron so as to eliminate any variations in the mucosal setting for iron that may exist among the different groups of test subjects.

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IRON ABSORPTION FROM FOOD[1]

Miguel Layrisse

Most of the knowledge on iron absorption is based on studies performed with radioiron in the form of iron salts. Investigations of iron absorption from food in which radioiron is biologically incorporated were first started in 1951 with the pioneer work of Moore and Dubach (*19*). However, despite the importance of this subject for nutrition, it has not been actively pursued since then. The large amount of radioiron necessary for biological tagging and the tediousness of cultivating the necessary plants have made progress very difficult. Only the absorption of hemoglobin iron has been studied in detail (*2, 3, 6, 7, 9, 23*).

Early contributions (*3, 5, 17, 18, 19*) have shown in general that food iron of animal origin is better absorbed than vegetable food iron, and that absorption rates vary widely according to the type of food and individual tested. In normal subjects, the mean iron absorption from animal food ranges from 2 to 6 per cent in eggs to 11 per cent in chicken muscle and 9 to 13 per cent in hemoglobin. Iron absorption from vegetable food (spinach, mustard greens, swiss chard, and beet greens) is shown to be generally lower than with animal food, but only a few samples of each food have been tested.

In 1964 the Department of Medicine and Botany of the University of Washington and the Department of Physiopathology at IVIC in Caracas started a program to study iron absorption with the staple foods consumed in tropical and temperate zones. A summary of the results of the last five years' work, which involved more than 500 subjects, will be presented here. Some of these findings have been the subject of recent publications (*8, 10, 12, 14, 15*), and the others, still in the process of analysis, are presented here as preliminary information.

Any discussion of iron absorption from food should take into account that, besides the variation among different individuals owing to the degree of iron storage, there is an intrasubject day-to-day variation that may be as high as 100 per cent at different times. Some authors have found a reduction in intrasubject variation with the administration of multiple doses over a period of 10 days (*1*), whereas others have found a similar variation regardless of whether dose administration was single or multiple (*13*). Methodological errors play a minor part in variation; they are estimated to be of the order of plus or minus 2 per cent.[2] This has been demonstrated by determining iron absorption in subjects fed with either inorganic iron tagged with Fe^{55} and Fe^{59}, or corn from cultures in which Fe^{55} was added to the nutrient and Fe^{59} injected into the stem. It is also important to mention that the skew distribution observed in the absorption of an iron salt (*8*), which is changed into a normal distribution by using the logarithm of the percentage of absorption, is also observed in the absorption of food iron.

[1] The studies performed in Venezuela were supported in part by U.S. Public Health Service Grant No. RO1-HE-06507.

[2] J. D. Cook, M. Layrisse, and C. A. Finch, unpublished observation.

This has been clearly shown with foods such as black beans and veal tested in a large number of subjects. Accordingly, the mean absorption and standard deviation of absorption from each food presented here is calculated from the logarithms of the percentage of absorption.

The results of 448 tests of six foods of vegetable origin and four foods of animal origin are shown in Figure 1. This material includes absorption tests already published (*10, 14, 15*) and new information collected in the last two years. For the most part, iron from vegetable foods is poorly absorbed (soybean was the only exception). The mean absorption, including normal and iron-deficient subjects, ranges from about 1 per cent in spinach to 5 per cent in wheat, with intermediate values of 3 per cent in corn and 4 per cent in black beans and lettuce. Iron absorption from animal food ranged from 7 per cent in ferritin to 22 per cent in veal, with intermediate values of 11 per cent in fish and 12 per cent in hemoglobin. The mean iron absorption from soybean was 11 per cent, which is close to the intermediate values observed in animal food. The mean absorption of each food, with the exception of veal, was lower than that reported in recent publications (*14*). These lower figures may be accounted for in part by the mathematical treatment of the data.

A comparison of iron absorption from each food, based on the three parameters used to estimate body iron stores in studies of this kind,

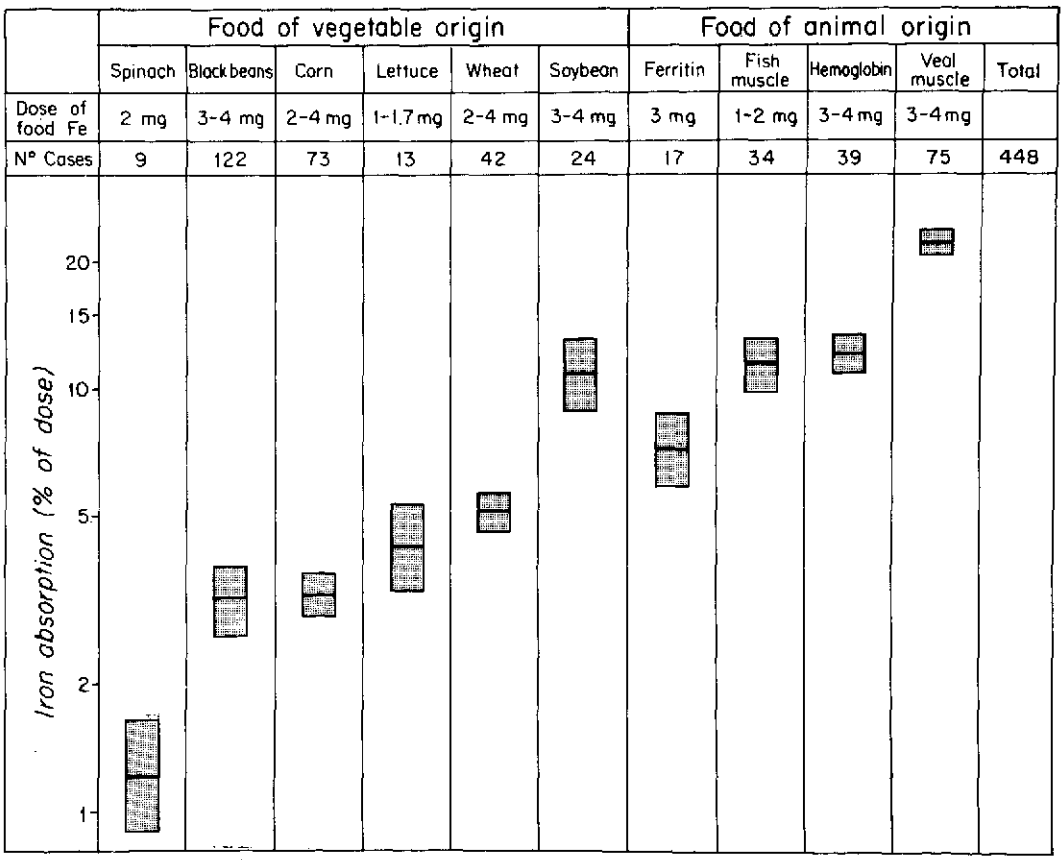

FIG 1. Findings on iron absorption from foods; the result of a collaborative study conducted by the Department of Botany and Medicine at the University of Washington, Seattle, and the Department of Physiopathology, IVIC, Caracas, Venezuela. The mean absorption and standard error is calculated from the logarithms of the percentage of absorption.

showed that there is a poor correlation between the absorption of food iron and either serum iron concentration or percentage of transferrin saturation. However, a significant correlation was observed between absorption of food iron and inorganic iron. Accordingly, a ratio of food iron and inorganic iron absorption could be used, instead of food iron absorption alone. This made it possible to compare differences in absorption in the various foods independently of variations in individual iron stores. The mean ratio was below 0.20 in black beans, spinach, and corn; from 0.20 to 0.29 in wheat and lettuce; from 0.30 to 0.39 in soybean and hemoglobin; 0.52 in fish; and 0.97 in veal. On the basis of these results, the iron absorption from these foods may be predicted in a subject whose inorganic iron absorption is known. For example, a normal male with an absorption of 7 per cent will absorb less than 1 per cent from corn, spinach, or black beans; between 1 and 2 per cent from lettuce or wheat; between 2 and 5 per cent from fish, soybean, or hemoglobin, and about 8 per cent from veal muscle. In subjects with iron-deficiency anemia and diminished iron stores, inorganic iron absorption will be 50 per cent and above in most cases, and, proportionally, the level of food iron absorption will be at least two or three times as great.

Previous studies (*10, 12*) have shown that absorption of food iron (wheat, corn, and ferritin) is enhanced by ascorbic acid and markedly depressed by desferrioxamine. The only two foods studied so far that do not follow this pattern are hemoglobin (*23*), in which absorption is not affected by reducing or chelating agents, and soybean, whose absorption is not increased with ascorbic acid (*12*). In view of the wide range of iron absorption levels among different subjects, which is more pronounced than the daily intrasubject variation, the iron absorption from a single food given alone and combined consecutively with ascorbic acid and desferrioxamine was determined in each individual. The results showed that iron absorption from veal muscle is not affected by these agents, whereas iron absorption from fish muscle is markedly reduced by desferrioxamine and increased by ascorbic acid. These preliminary results may suggest that the myoglobin iron is still attached to the porphyrin ring during the absorption, as was already demonstrated with hemoglobin iron, and that fish iron goes by a different route.

The possible interacting effect of several foods administered in the same meal, as in the usual diet, has also been studied. Experiments with animal and vegetable foods mixed in a single meal (*15*) have shown that iron absorption from veal is significantly lower when veal is combined with either black beans or corn than when it is given alone. Contrariwise, corn and black beans give higher absorption levels when mixed with veal or fish than when administered alone.

Further studies have been conducted to find out whether the amino acids present in fish muscle are responsible for the enhancement of vegetable iron absorption in the same way that some amino acids affect the absorption of inorganic iron (*11*). In the first experiment, black beans were mixed with amino acids in the same number and proportion as those present in 100 g of fish. The resulting iron absorption level was more than double the figure obtained with black beans alone. Further experiments showed that the sulfur-containing amino acids—cysteine plus methionine, or cysteine alone—enhance vegetable iron absorption. However, there was no significant increase in absorption with the other amino acids grouped according to their chemical properties (*16*).

Although there is no further information in the literature concerning the interacting effect on iron absorption of foods administered in the same meal, studies have been performed to determine the effect of a single food or standard meals on the absorption of tagged inorganic iron. The goal in most of these studies has been to investigate the possible benefit of feeding a population with staple foods enriched with inorganic iron. They have shown that either a single food, such as wheat (*4, 22*), or a standard meal (*20, 21*) result in a much lower inorganic iron absorption level than that of iron given

alone. Absorption is improved when ascorbic acid is administered with the meal (*4, 20*). In connection with this point, the two groups of the University of Washington and IVIC have found that inorganic iron absorption is markedly reduced when the iron is combined with either soybean or corn, but it is not affected when administered with veal muscle.

The information presented in this paper centers around two aspects of iron metabolism that are of great importance in public health: namely, absorbability of iron from diet, and absorbability of iron supplementing the diet. The studies of iron absorption from food have shown how poor some of the national staples really are. This is the case with corn, for example, which is consumed as a large part of the diet throughout Central America. The findings also indicate that food of animal origin is important not only for its high rate of iron absorbability but also for its effect on vegetable iron absorption. The great difference between vegetable and animal food may explain why the prevalence of iron-deficiency anemia (in the absence of blood loss) is less frequent in temperate zones than in tropical areas; even though iron intake in the latter is higher, most of it comes from vegetable sources.

Studies on the absorption of inorganic iron administered with a single food or with a diet show variations according to the accompanying food. Thus it may be concluded that food iron fortification programs should first be evaluated by isotopic methods in a pilot study using a sample of the population in question and exposing them to the same environmental conditions.

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IRON LOSSES

Clement A. Finch

Iron exchange between man and his environment is limited. It is important, however, to define these losses in as quantitative a fashion as possible, since this is the best way to establish absorptive needs for iron. Figure 1 shows the various routes by which iron may leave the body. Types of iron loss include surface desquamation, secretion, and loss of internal tissue. Surface loss includes exfoliation of both the skin and the intestinal mucosa.

Losses from the skin have been the subject of some controversy because of the technical difficulties in their measurement. Chemical analysis of the iron content of exfoliated skin is suspect because of surface contamination. As might be expected, such analyses have given larger estimates of loss than seem consistent with other information concerning body iron turnover. Methods of testing have included isotopic measurement of a collection of sweat and desquamated skin after the intravenous injection of radioiron, measurement of isotope localization in the skin with assumptions as to the rate of desquamated turnover, and injection of radioiron directly into the skin with determinations of the rate of disappearance of the isotope by *in vivo* counting. It appears that the pathway of excretion is directly from transferrin iron to the epithelium, with subsequent exfoliation, and that the level of plasma iron influences skin loss. Average dermal loss is about 0.2 mg a day (*6*).

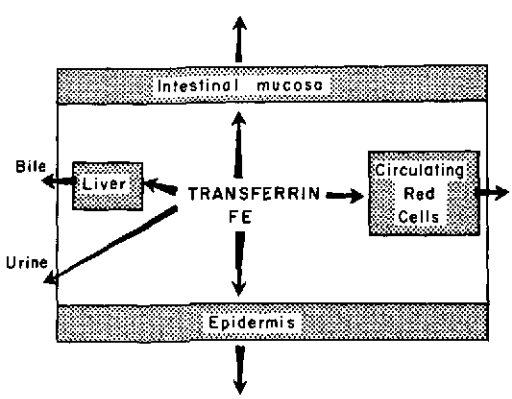

FIG. 1. The various routes by which iron can leave the body

The gastrointestinal mucosa has been of considerable interest in animal studies because approximately 10 per cent of plasma iron has been demonstrated to localize there and to be subsequently lost through exfoliation (*3*). However, isotope studies of mucosal iron turnover in man suggest that this amount is less than 0.2 mg a day (*6*).

Only limited amounts of iron are lost in the urine and bile. Urinary losses have been extensively studied and there is general agreement that they constitute less than 0.1 mg a day in a normal person (*4*). Biliary losses are much more difficult to estimate. It would seem they are derived from an hepatic iron pool that is only slowly miscible with radioiron. Chemical studies carried out in people with biliary fistulae suggest that the mean daily iron content of bile is about 0.2 to 0.3 mg (*6*).

The only internal body tissue of significance with respect to iron losses is the red cell mass. Even in normal individuals there are appreciable losses of red cells in the gastrointestinal tract,

and this "physiologic" bleeding from the gut has been estimated at 0.35 mg a day (*6*). To this might be added the day-to-day losses of blood that may occur from trauma to the body exterior. All these individually estimated iron losses in the adult male are summarized in Table 1.

There are obvious difficulties in estimating iron losses in such a piecemeal manner. It would seem more accurate to measure the turnover of body iron in man by isotopic methods. There are two general approaches to this; one involves total body counting and the other involves the measurement of the specific activity of the circulating red cells.

In the first technique, Fe^{59} must be used to permit external counting. This method has obvious drawbacks, since the isotope's relatively short half-life of 45 days limits body loss measurement to about a year. Moreover, assumptions have to be made concerning the degree of mixing of body iron with the isotope injected.

The second method involves the intravenous injection of Fe^{55}, an isotope with a half-life of three years (*5*). The specific activity of the red cells is then measured over a period of several years. Approximately 90 per cent of the injected iron is found in the circulating red cell mass at 90 days; thereafter, the level of circulating activity declines. This occurs more rapidly during the first year, presumably because of some dilution in body stores. In subsequent years the level of circulating activity falls at an exponential rate. In the interpretation of this late curve, certain assumptions must be made. Total body iron must be constant over the period of study, and the total miscible pool must be estimated.

TABLE 1. Estimated iron losses in the adult 70 kg male (mg/day)

Total	0.93
Gastrointestinal	
Blood	0.35
Mucosal	0.10
Biliary	0.20
Urinary	0.08
Skin	0.20

With these assumptions in mind, total daily body iron turnover has been calculated in the adult male to be 0.95 mg, or 13 μg per kilogram of body weight, in subjects studied in Seattle, Caracas, and Durban (*6*). These figures are in good agreement with the tabulation of the individual losses previously discussed.

The information concerning iron losses in the normal adult male may be considered highly precise. Iron losses in the adult female are less consistent owing to the variability of menstrual blood loss. Figure 2 shows the results of measurements by Hallberg *et al.* (*7*) of losses incurred by menstruating and pregnant females. The mean daily menstrual loss is 0.5 mg, but 5 per cent of the women go up to more than 1.4 mg. Pregnancy losses are also highly significant, with a mean daily loss of 2.5 mg, and an even greater requirement during the last months (*1*).

Virtually nothing is known about iron losses in infancy. It is apparent, however, that appreciable requirements result from rapid growth of the child and expansion of his body tissues. For every kilogram of lean body weight produced there is an iron requirement of approximately 35 mg. Isotope labeling of the fetus *in utero* permits measurement after birth of the dilution of the body iron pool by absorbed iron (*10*).

In addition to physiologic losses of iron, the numerous mechanisms by which pathologic iron loss occurs must be taken into account as well. These relate to the red cell mass and usually to bleeding. In most instances, bleeding occurs from the gastrointestinal tract, and in terms of its global significance hookworm infestation represents the leading cause. The outstanding studies of Roche and Layrisse (*8*) have identified many aspects of hookworm blood loss. They have demonstrated a daily iron loss per worm of 0.03 mg for *Necator americanus*, 0.05 mg for *Ancylostoma caninum*, and 0.15 mg for *Ancylostoma duodenale*. Iron may also be lost by infestation of other intestinal or blood-sucking insects, by pathology of the gastrointestinal tract, by uterine bleeding in the female—to mention a few of the more common ways. A single blood

TABLE 2. Iron balance data on three population groups

	LOSSES (MG/D)	STORES (MG)	INCIDENCE OF IRON-DEFICIENCY ANEMIA (%)
Males	0.9	1,000	< 5
Menstruating females	1.5	400	5–10
Pregnant females	2.5	200	20–30

donation requires an increased daily iron absorption of 0.6 mg for one year to replace the amount removed. Unusual causes of iron deficiency include hemosiderinuria (*9*) and intrapulmonary bleeding (*2*).

The significance of physiologic iron losses is only apparent when matched against iron available in the diet. Since iron absorption and dietary iron are discussed elsewhere, this direct comparison will not be made. Table 2 points to the significance of physiologic iron losses in another way—by relating them to iron stores and to the incidence of iron-deficiency anemia. Iron stores portray the result of balance between absorption and loss. The incidence of iron-deficiency anemia indicates the state of iron balance in the population at large. In the man, iron balance appears satisfactory in that iron stores are ample for any foreseeable needs, including blood donation, and iron deficiency is limited to pathologic bleeding and rare instances of malabsorption. The requirement of the menstruating female compromises her iron stores and is associated with an incidence of 5 to 10 per cent of iron-deficiency anemia. In pregnancy, a negative iron balance is inevitable. These data indicate that the physiologic losses of about 1 mg in the male are of no consequence but that losses of 2 or more mg a day in the menstruating or pregnant female are almost certain to create a negative iron balance.

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GENERAL DISCUSSION

Moderator: We have heard about the basic aspects of iron deficiency, and we will now take a few minutes for a discussion of the session so far. A question has been raised about the role of erythropoietin in iron absorption, which Dr. Crosby will answer.

Dr. Crosby: Erythropoietin does play a role in iron absorption, but probably it is entirely indirect. By stimulating the requirement for hemoglobin iron, it establishes a state of iron deficiency, and this then is reflected in altered iron absorption by the intestine. Erythropoietin itself probably does not cause any change in the behavior of the intestine.

Dr. Cohen: There have been reports in the literature on the relative turnover rates of the various iron compounds; viz., the cytochromes, hemoglobin, myoglobin, and the peroxidases. From the standpoint of iron deficiency, it is crucial to know where the rate-limiting concentration will have its greatest impact. If cytochromes have high turnover rates and there is only a limited amount of iron available, a very serious problem could exist, obviously, for cell function. What is the current state of knowledge on the relative rates at which the different iron pools turn over? Does cytochrome turn over more rapidly than hemoglobin, myoglobin, or the other iron heme compounds?

Dr. Finch: Since the hemoglobin cycle is so overwhelming from a quantitative standpoint, it is very difficult in the kinetic studies to see the changes in the rates of these other pools. There is some evidence clinically that the erythroid marrow is the most avid tissue in taking up iron, and even at extremely low plasma levels the erythron does better than other tissues. In states of very low plasma iron, other tissues may well suffer. There have been findings such as spoon nails and certain red cell and tissue enzyme measurements showing decreases. The functional significance of such changes is far from clear.

Dr. Crosby: I suspect there is one organ whose individuality exceeds even that of the erythroblast—the placenta. A child can be born with a normal complement of iron from a woman who is iron deficient.

Dr. Waterlow: I would like to ask a question and to show a couple of slides. As several speakers have said and will say, infantile anemia is a tremendously important problem throughout the area. Failure to absorb iron may be one of the causal factors. Therefore in Jamaica, in collaboration with Dr. Paul Milner and Dr. Ann Ashworth, we have made measurements in infants of the absorption of iron from labeled foods supplied by Dr. Finch.

Figure 1 shows the absorption of corn iron compared with that of ferrous ascorbate. The corn iron was very poorly absorbed—on the average only 2.5 per cent, compared with 28 per cent from ascorbate. Three measurements were made on each patient, either two with corn and one with ascorbate, or vice versa. The point I want to emphasize is the variability of repeat measurements—a point on which many authors working with iron absorption have commented.

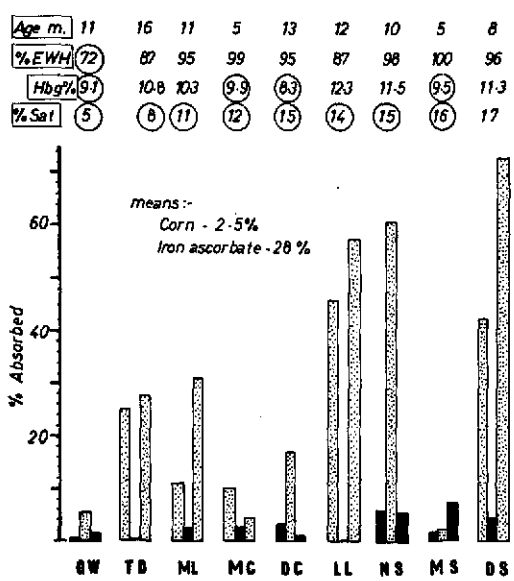

FIG. 1. Absorption of corn iron compared with that of ferrous ascorbate

We observed that children who happen to have a fever at or near the time of the test absorb very poorly. Figure 2 shows some measurements of iron absorption in relation to body temperature. Fever seems to depress the absorption almost to nothing. I want to stress that a child doesn't have to be clinically ill to show this effect. In one case we picked up an undetected subclinical infection because the child failed to absorb iron. This effect of even a mild infection may be one of the causes of the variability mentioned above, and it may in practice be an important cause of poor iron absorption by people in tropical countries.

The question I want to pose is, what is the mechanism of this effect?

Moderator: Do you say it is the fever that causes poor absorption, or just the infection?

Dr. Waterlow: All we know is that there is a rise of temperature. Usually we can find no cause for the infection. Sometimes these effects are produced simply by routine immunization.

Colonel Conrad: If you inject endotoxin into an animal, it will immediately have a marked diminution in its capability to absorb iron. Unlike most known factors, which take several days to affect iron absorption, endotoxin produces a significant effect within one hour after injection. The animal does not die, but it appears slightly lethargic. This is similar to the results reported with injections of turpentine. I believe experiments with endotoxin are more definitive. If you studied your febrile children you would find their serum iron concentration was quite low in comparison to measurements at the time they were absorbing iron in normal quantities.

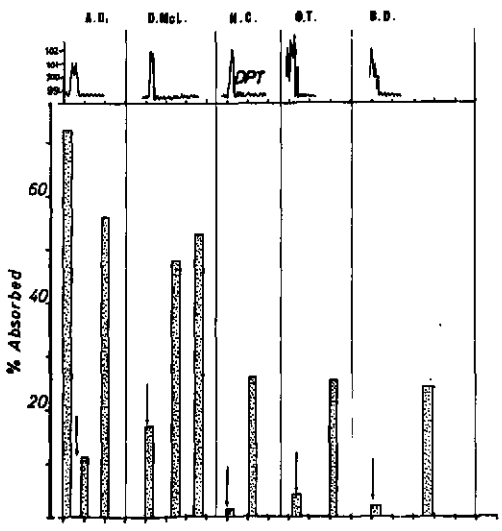

FIG. 2. Effect of pyrexia on iron absorption

Dr. Moore: Dr. Crosby, what do you think happens to the mechanism controlling intestinal absorption when it seems to break down? For instance, in pyridoxine-deficient animals, Dr. Wintrobe and his associates have demonstrated increased absorption, even though these animals have some degree of iron overload. In individuals with ineffective erythropoiesis, it has been demonstrated that iron overload develops in part because increased amounts of iron are absorbed. The same thing must happen with hemochromatosis. Persons who develop iron overload as a result of drinking wine, or as a result of either pancreatic or hepatic disease, also seem to absorb increased amounts of iron even in the presence of the overload.

Dr. Crosby: The very nature of your question helps me to answer it. In posing a question with so many facets, you indicate that the problem is a complex one. In my presentation I stated that the control of iron balance appears to reside in a complicated system for keeping iron out of the body, and I simplified it into four steps, but there may actually be more. In a complicated system for doing anything, disruption can occur in many places. For this reason, in the present instance, I suspect that the disorders that permit unneeded iron to come into the body are numerous and varied. There is some support for this in the rather large number of hereditary disorders that are clinically identifiable as different; in all of these, the syndrome which we might call hemochromatosis does occur.

Dr. Caldeyro-Barcia: I would like to know whether the increased iron needs in pregnant women are entirely due to the iron that is being transferred to the fetus, or if the maternal

needs for her own metabolism are increased. Also, I would like to know whether the placental transfer from mother to fetus is accomplished by simple diffusion or facilitated diffusion, or whether it is an active transfer.

Dr. Moore: There is probably an increased requirement during pregnancy, caused by the demands of the fetus and by the sizable increase in circulating red cell mass that occurs during the second half of this period. As I will point out shortly in my paper, however, the iron that goes into the increased circulating red cell mass is largely conserved by the mother and returned to her body economy when the pregnancy is terminated. I cannot answer the question about the mechanism of iron transfer across the placenta. Dr. Elmer Brown in our department tried very hard to find evidence for an active transfer system, but he was not able to do so. Dr. Finch has also worked on this problem. Perhaps he can supply additional information.

Dr. Finch: I can comment that there are certainly large amounts of iron going across the placenta, in spite of marked deficiency on the part of the mother. Fetal plasma does not go back across the placenta. It is a one-way street, and an extremely effective one, able to compete successfully with all maternal needs for iron.

Moderator: I have a small comment to make in regard to Dr. Conrad's statement that the iron content of the diet is usually low when the protein content is low. The fact is that in our hemisphere this is not necessarily true. In most of the Latin American countries, the iron content of diet is quite high. In Venezuela, for example, it is of the order of 25 to 50 mg per day. In spite of the high intake in our countries, iron-deficiency anemia is widespread, so there must be difficulty with absorption, even more so than in Asia.

Dr. Cohen: I would like to go back to the question of the dynamics of the system. Obviously, one tends to focus on hemoglobin, because it represents the largest mass of iron, and also because it is relatively easy to get at. However, in consideration of the dynamic steady state, we recognize that the distribution of iron to the different pools is going to be, within certain limits, a function of the concentration of transferrin iron, or whatever pool is rate-limiting. The avidity factor that has been alluded to in the erythropoietic system is very illuminating. We know that there is considerable reserve capacity of the circulating hemoglobin concentration from the standpoint of oxygen transport and buffering. Thus, there should be some recognition of the fact that in this steady state process there are iron compounds of far more critical significance to cell function than the mere transport of oxygen or the buffering of the blood.

The pool size of hemoglobin is at least two orders of magnitude greater than perhaps that of the cytochromes or other systems which may be rate-limiting. I am suggesting, therefore, that within a fairly small range of hemoglobin change there might be much larger changes in specific iron compounds that are crucial, particularly for the fetus during pregnancy, or in certain pathologic states. I feel that these important areas, though much more difficult to get at, warrant thorough study.

Dr. Gandra: I should like to hear from Dr. Layrisse something about the common causes for impairment of iron absorption that might be present in black beans and spinach, such as phytates and oxalates.

Dr. Layrisse: Black beans and corn probably contain a large amount of phosphate and phytate, which could partially block the iron present in these seeds. However, we do not know yet if this is the case, or whether there are other chelating agents in these foods.

Moderator: Do we have any idea of the chemical state of the iron in these foods?

Dr. Layrisse: No.

Dr. Waterlow: Perhaps we should call on the physical chemists. Obviously, standard chemical analysis is not going to tell us much about the state of iron in vegetables. Would Dr. Harrison have anything to add?

Dr. Harrison: Unfortunately, for X-ray analysis, you have to crystallize your material first.

Dr. Cohen: I would like to ask Dr. Harrison whether the ligands or chelates that are formed between ascorbate and iron can be formed with both the divalent and trivalent forms of iron.

Dr. Harrison: I don t know specifically in relation to ascorbate.

Colonel Conrad: The compound formed with ferric iron and ascorbate is totally different from that formed with ferrous iron and ascorbate. It has different solubility characteristics, different characteristics by infrared spectroscopy, and a different chemical composition. On theoretical grounds, the complex formed by ferric iron and ascorbate is probably more available for absorption than the ferrous ascorbate complex. The ferric iron probably becomes ferrous iron when it combines with the ascorbate. However, it forms a tighter complex, which remains soluble over a much wider range of pH.

Dr. Caldeyro-Barcia: It has recently been reported that when the interval between two successive pregnancies is very short (less than 12 months between births) the second newborn has a lower birth weight and poorer motor and psychological performances later in life than babies from comparable groups in which the interval between pregnancies has been longer. I wonder if the question of iron deficiency may be related to this problem.

Moderator: I don't know whether anybody can answer that.

It appears not.

If there are no further comments, I think we will go on with the program and ask Dr. Moore to speak to us on human iron requirements.

HUMAN IRON REQUIREMENTS

Carl V. Moore

Nutritional requirements for iron are determined by the amount lost from the body, the amount needed to support growth and pregnancy, and the efficiency with which food iron is absorbed from the intestinal tract. Efforts to define requirements have brought out the following facts: (1) information about iron losses and iron absorption from whole diets is incomplete; (2) variations among people, determined by factors such as body size and differences in menstrual loss, cover a relatively broad range; and (3) needs during infancy and pregnancy have probably been underestimated (*5, 21, 23*). Recent studies of the kind already summarized by previous speakers at the present symposium have focused on these problems and have added sufficient data so that intelligent estimates of iron requirements can now be made.

Requirements can be stated in one of two ways. The first takes into account differences among people and states the estimates as a range. The second approach, often preferred by public health workers, states requirements in terms that are broad enough to meet the needs of a large segment—often 90 or 95 per cent—of any given population.

Iron needed for excretion, pregnancy, and growth

The best and most recent measurements of obligatory loss of body iron by adult male subjects (Table 1) indicate a daily excretion rate of 0.5 to 1.63 mg; mean values for three groups of subjects varied from 0.95 to 1.02 mg (*8*). A total of 41 subjects, from Seattle, Venezuela, and South Africa (Indian men), was included in the group. A breakdown of the data indicated that about 0.1 mg of the gastrointestinal loss occurred through desquamated mucosal cells and about 0.4 mg through blood; bile delivered to the small intestine each day contained about 0.26 mg, but there was no way to determine how much of this amount was reabsorbed. The daily iron content of urine was only about 0.1 mg. The estimated daily iron loss from skin at normal transferrin saturations was between 0.2 and 0.3 mg, but this figure could rise to as much as 0.6 or 0.7 mg when transferrin saturation was high. It was postulated that the values for iron-deficient subjects would be about half these figures. In two groups of Bantu subjects with high iron stores, the total body iron loss was as high as 4.4 mg a day, with mean values of 2 and 2.4 mg. No

TABLE 1. Iron required to replace physiological losses and to support growth and pregnancy (mg/day)

	AVERAGE	RANGE
Loss in feces, urine, and sweat	0.9–1.0	0.5 –1.6
Menstrual loss	0.5	0.1 –1.4
Pregnancy	2.4	1.68–3.75
Growth (average)		
Males	0.5	0.3 –0.7
Females	0.37	0.3 –0.45

[1] The investigations by the author and his associates described in this presentation were supported in part by U.S. Public Health Service Research Grant No. H–22 from the National Heart Institute.

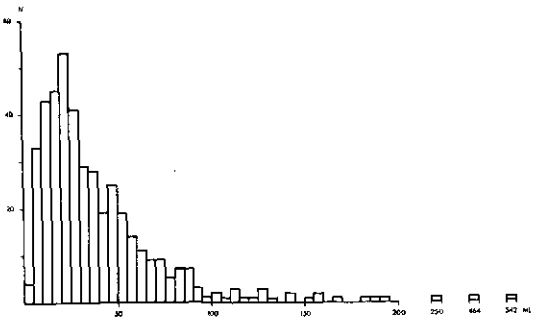

FIG. 1. Distribution of menstrual blood loss in a population sample of women living in Göteborg, Sweden. Loss in ml of blood is indicated along the horizontal scale; number of subjects along the vertical scale. Reproduced from Hallberg *et al.* (*9*).

similar data are available for children. In nonmenstruating women, the figures were 0.64 ± 0.05 mg (*4*).

The most extensive studies of menstrual blood flow indicate that the loss is fairly constant from month to month in the individual woman but varies widely from one subject to the next (*9*). It may be as small as 10 ml a month, but may be greater than 60 ml a month in 15 to 25 per cent of a presumed normal population. The mean loss in a group of Swedish women was found to be 43.4 ± 2.3 ml a month, or about 0.5 mg a day (Figure 1). In 95 per cent of the women studied, menstrual loss was found to be less than 1.4 mg a day. Consequently, the range of iron needed to compensate for menstrual blood loss in healthy women extends from as little as 0.1 to as much as 1.4 mg a day.

The amount of iron necessary to compensate for losses sustained through pregnancy is relatively large and varies widely (Table 2 and *5, 21, 23*). The average obligatory excretion of body iron has conservatively been estimated at 170 mg, with a range of 150 to 200 mg. The iron contained in the fetus varies from about 200 to 370 mg, with an average of about 270 mg, while that contained in the placenta and cord varies from about 30 to 170 mg, with an average of roughly 90 mg. Blood lost at delivery has been estimated to contain 90 to 310 mg, or an average of 150 mg. These are quantities actually lost from the body; they vary from 470 to 1050 mg, with an average of 680 mg. Stated in terms of daily losses, they range from 1.68 to 3.75 mg, with an average of 2.4 mg. This figure does not include the 200 to 600 mg required for the expanded red blood cell mass which develops late in pregnancy, because that iron is largely conserved when the erythrocyte mass returns to normal after delivery. Lactation after delivery produces additional iron losses of approximately 0.5 to 1 mg a day, but that amount is usually balanced by the absence of menstruation during the period in question.

TABLE 2. Iron required for pregnancy (mg)

	AVERAGE	RANGE
External iron loss	170	150–200
Fetal iron	270	200–370
Iron in placenta and cord	90	30–170
Blood loss at delivery	150	90–310
(Expansion of red blood cell mass	450	200–600)*
Total requirement *	680	470–1050
Av. requirement per day *	2.4	1.68–3.75

* Expansion of red cell mass not included.

The iron required for growth varies with the rate of growth and the body weight to be eventually attained at adult stature. The fully grown man has approximately 50 mg of iron for each kilogram of body weight; the adult woman, about 35 mg (*5*). Thus, a man who stops growing when he reaches an adult weight of 50 kg above his birth weight will normally have assimilated 2500 mg. Spread over the 20 years that males take to reach adult stature, this figure amounts to 125 mg a year, or about 0.35 mg a day (Table 1). A large man who ends up weighing 100 kg above his birth weight would require twice as much iron—250 mg a year or 0.7 mg a day. Similar figures for human females, based on the assumptions that growth is completed by the age of 15 years and that normal adult weight varies from 45 to 70 kg above birth weight, would be 100 to 160 mg a year, or 0.3 to 0.45 mg a day. These average values would vary from year to year depending on growth spurts. During the first year of life, for instance,

TABLE 3. Estimated iron needs and dietary requirements

	IRON NEEDED (MG/DAY)					
	FECES, URINE, DERMAL	MENSES	PREGNANCY	GROWTH	TOTAL	DIETARY IRON REQUIREMENTS (MG/DAY)*
Men, nonmenstruating women	0.5–1.6				0.5 –1.6	5–16
Menstruating women	0.5–1.6	0.1–1.4			0.6 –3.0	6–30
Pregnancy			1.7–3.75		1.7 –3.75	17–37.5
Adolescent boys	0.5–1.6			0.35–0.7	0.85–2.3	8.5–23
girls	0.5–1.6	0.1–1.4		0.3 –0.45	0.9 –3.45	9–34.5
Children (average)	?0.1–0.8			0.3 –0.7	0.4 –1.5	4–15
Infants (3–12 months)	?			0.8 –1.5	1.0 –1.5	10–15

* Assuming 10% absorption.

it is estimated that the need is approximately 0.8 to 1.5 mg a day for normal term infants (*19, 26, 30, 31*).

The data summarized above have been used to construct the range of values presented in Table 3—namely, the estimated average amounts of iron that must be accumulated in a day to meet the needs of various segments of the population. They are of the same magnitude as those previously published by the Committee on Iron Deficiency of the American Medical Association's Council on Foods and Nutrition (*5*) but differ slightly to reflect the new data on obligatory body iron loss (*8*) and a different method for calculating the needs associated with pregnancy (inclusion of blood loss at delivery plus omission of the iron required for expansion of red blood cell mass).

Absorption of food iron

The task of determining how much food iron must be consumed in order to provide for these needs is particularly difficult (*21, 22*). In recent years, most studies on food iron absorption have used foods grown or prepared so as to contain radioiron. The foods are then cooked and fed as they would be under normal consumption except that they are given alone.

Figure 2 summarizes most of the published data on the absorption of radioisotope-tagged foods (*1, 3, 7, 12, 13, 20, 27, 29, 33*). The

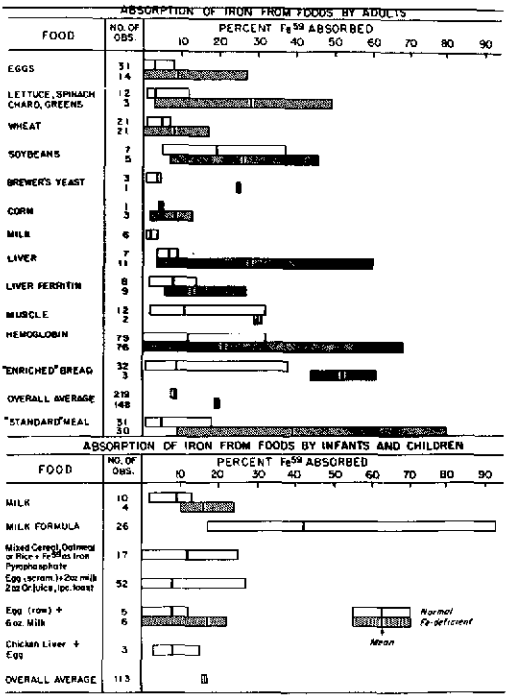

FIG. 2. Radioiron measurement of the absorption of iron from foods by adult subjects and children. The length of the bars indicates the degree of variation in results obtained from each food; the heavy vertical line across each bar indicates the average value. Cross-hatched bars = iron-deficient; clear bars = normal. The amount of iron in each feeding varied from 1 to 17 mg. Data compiled from the following references: *1, 3, 7, 12, 13, 20, 24, 27, 29, 33.*

length of the bars indicates the variation among different subjects for each food; the heavy

vertical line across each bar indicates the average value; the cross-hatched bars represent the results with iron-deficient subjects. The amounts of iron in each vary from 1 to 17 mg. Several facts are evident: variation among subjects is large; iron-deficient patients tend to absorb food iron better than normal subjects do; and assimilation from wheat and corn is less efficient than from most other foods, particularly those of animal origin. In all the studies taken together, the over-all average is 8.5 per cent for normal subjects and 18.9 per cent for iron-deficient persons.

Several deficiencies in these data should be pointed out. The test dose was administered on a one-shot basis and does not, therefore, illustrate the day-to-day variation that may occur in the same individual. Furthermore, the food was fed by itself, so that the effect of other foods administered in combination was not evaluated. Layrisse and his associates (*16*) have elegantly demonstrated that absorption from corn and black beans is increased by feeding animal food simultaneously; that absorption from veal (but not from fish) is reduced when fed together with corn or black beans; and that certain amino acids, especially the sulfur-containing group of amino acids, enhance the absorption of iron from vegetable sources.

Several groups of workers have tried to circumvent some of these problems by feeding a tracer dose of radioiron along with a standard meal. The results of one such study from Dr. Finch's laboratory are also included in Figure 2 (*24*). This method saves the trouble of labeling the foods themselves, avoids the artificiality of measuring absorption from only one food at a time, and permits evaluation of a number of intra- and extraluminal factors that affect iron absorption. It assumes, however, that the tracer dose of ferric chloride will admix or interchange sufficiently with the iron in food to provide an index of iron absorption, whereas there is evidence to suggest that, in the case of hemoglobin at least, such interchange does not occur (*10*). Results with the four different standard meals agree reasonably well with each other (Table 4 and *6, 24, 28, 32*). The average

TABLE 4. Absorption of radioiron from standard meals

	Fe	NORMAL			Fe DEFICIENT		
	mg	NO.	Range %	Av.%	NO.	Range %	Av.%
Sharpe et al (adolescent boys)	7.5 - 6	10	?	5.76			
	8	10	?	8.3			
Turnbull	8	7	1-10	3.4	2*	17 - 33	25
	8				22	14 - 80	46.2
Pirzio-Biroli et al	4.6	8	1.7 - 16.6	6.35	8	9.9 - 38.7	21.8
	4.6	12**	0.5 - 10.5	4.6			
	4.6	4***	0.6 - 8.8	5.3			
Goldberg et al	12.5				8	17 - 88.5	57.5
					7†	4.1 - 29.8	18.5

* Fe deficiency without anemia
** Non-hematologic patients
*** Pernicious anemia in remission
†Patients with Achlorhydria

Composition of the standard meals as follows:

[1] Sharpe, Peacock, Cooke, and Harris (1950): Milk, 200 g; cooked rolled oats, 285 g; white bread, 34 or 56 g; tomtao juice, 150 g.

[2] Turnbull (1965): Canned stewing steak, 50 g; potato powder, 15 g; frozen peas, 50 g; butter, 10 g; orange, 100 g; cream, 20 g.

[3] Pirzio-Biroli, Bothwell, and Finch (1958): Canned corned beef hash, 200 g; apple sauce, 200 g; tomato juice, 200 g; soda crackers, 13 g; cocoa, 3 g.

[4] Goldberg, Lochhead, and Dagg (1963): Corned beef, 100 g; apple sauce, 100 g; tomato juice, 200 g; cream crackers, 13 g; cocoa, 3 g.

values for healthy or normal subjects range from 3.4 to 8.3 per cent, and for iron-deficient patients from 18.5 to 57.5 per cent. The strong possibility exists, however, that these data represent the absorption of inorganic iron and are not an accurate measure of the assimilation of iron from food.

There is a tendency to ignore the results of early balance studies because technical errors were significant, because the difference between oral intake and fecal loss was so small that precise measurement was difficult, and because differentiation between excreted and unabsorbed fecal iron was not possible (*22*). Five of these studies, however, were done with such great care that they merit attention (Figure 3 and *11, 14, 17, 25, 34*). They have the advantage of having been performed, with one exception, over a period of at least 28 days, so that daily variation was minimized. In addition, a varied diet was used. In these healthy subjects, the absorption range, calculated on the basis of the positive balance, was from 7.3 to

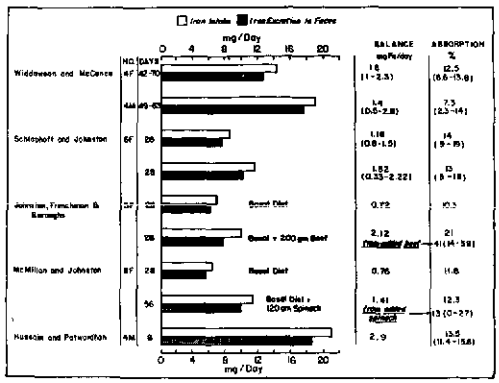

FIG. 3. Iron balance studies of iron absorption

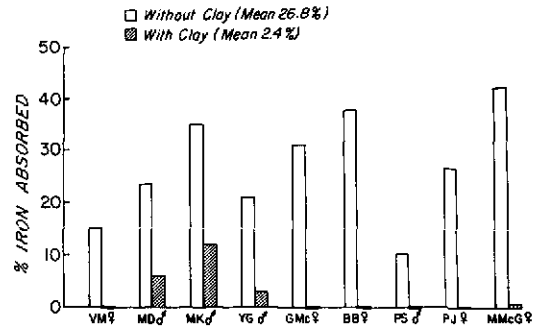

FIG. 4. Effect of 5 grams of Turkish clay (pH 10.3) on $Fe^{59}SO_4$ absorption in nine normal subjects. Reproduced from Minnich *et al.* (*18*).

21 per cent. If one assumes that 0.5 mg of fecal iron was excreted iron, then the proportion of food iron absorbed from the basal diets would be increased to from 11.3 to 27.5 per cent.

There are several other dietary factors that must be considered in attempting to arrive at an average figure for food iron absorption:

1. Achlorhydria may impair the absorption of food iron (*6, 22*).

2. In countries where wine is taken with meals, the wine serves as a source of iron and its alcohol content may enhance absorption as well (*2*).

3. Ascorbic acid will increase absorption from certain foods (*15, 22*).

4. The use of iron cooking utensils may add considerable amounts of iron to foods (*21*).

5. Geophagia, or clay eating, is more frequent, particularly among economically deprived groups, than is commonly recognized. Alkaline clays with a high cation exchange capacity are particularly instrumental in depressing iron assimilation (Figure 4 and *18*).

For all these reasons, it is impossible at the present time to draw any firm conclusions about the composite percentage of iron that will be absorbed from a given diet. Furthermore, it is difficult to judge exactly how iron absorption will increase or decrease on a sliding scale as iron deficiency develops or is corrected. One can do no better than to guess that a figure of 10 per cent might represent a fair approximation for diets that contain a generous amount of animal protein; the estimate of 10 per cent is probably too high when food is derived largely from vegetable sources, particularly cereals.

Dietary iron requirements

Application of the 10 per cent absorption figure to the estimates of iron needs gives the dietary estimates indicated in the right-hand column of Table 3.

Since in most diets there is only about 6 mg of iron per thousand calories, it is evident that the higher values in the various ranges would be difficult to achieve, particularly in infants, adolescent girls, menstruating women, and pregnant women. It is not surprising, therefore, that these are the groups in which iron deficiency is most common. The estimates are higher than formerly believed and cause consternation among nutritionists. The greatest chance for error would come from underestimating the efficiency of absorption, but at this time the recommendations seem reasonable. It is doubtful that they are off by a factor of more than plus or minus 25 per cent.

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IRON-DEFICIENCY ANEMIA IN LATIN AMERICAN AND CARIBBEAN POPULATIONS

Yaro R. Gandra

Iron-deficiency anemias have long been identified as a major public health problem in different areas of Latin America and the Caribbean. The frequency of anemia in these populations and its negative effect on their progress and development are recognized. The infections and reduced work capacity associated with anemia result in educational and economic losses that eventually affect the social behavior of the hemisphere's population. The problem is most acute among vulnerable groups, whose physiological iron requirements are particularly high.

Although many reports on iron-deficiency anemia in different areas of the hemisphere have been published, the real extent of this disorder is still unknown. In recent years a coordinated effort to explore the problem fully has been carried out under the auspices of WHO, PAHO, and FAO (*20, 21, 22, 23*). In various meetings studies have been recommended to clarify the role of dietary deficiency and parasitism in the pathogenesis of nutritional anemias and also to learn more about anemias associated with pregnancy.

In 1963 a regional center was established under the auspices of PAHO/WHO at the IVIC, in Caracas, to train investigators and technicians for work in this field throughout Latin America and the Caribbean area.

In August 1968 a meeting of the PAHO Scientific Group on Research in Nutritional Anemias was held at Caracas. Its main purpose was to study and discuss a WHO nutritional anemias program in Latin America and the Caribbean; it also examined parameters for the reproducibility of data from different cooperating laboratories and made appropriate recommendations.

Iron-deficiency anemia

Iron-deficiency anemia is the most common kind of anemia in the Americas, and, as malnutrition, it is second only to protein-calorie malnutrition. Since dietary iron intakes in this area of the world are not significantly lower than they are elsewhere, where iron-deficiency anemia is not a major problem, it is probable that other factors enter into play—the amount of iron available for absorption, the interference of nutrients in the common diet, abnormalities of small bowel architecture, or abnormal blood loss. Other possible causes of iron-deficiency anemia, such as low dietary calcium, high phosphorus in the diet, or iron loss from the skin through excessive perspiration or due to increased intestinal desquamation have not been investigated in detail in this area.

The literature on this subject is extensive, but the population samples analyzed, the methods used, and the criteria of normality have not been homogeneous and do not allow for an adequate analysis of the results. Thus, emphasis has been focused on the need for data that are comparable rather than for information from field studies in general.

The reports from different surveys carried out in Latin America and the Caribbean area show

hemoglobin averages varying from 11.8 g to 16.0 g/100 ml of blood, the level for males being 1 or 1.5 g higher (Table 1). Some areas, like the West Indies, showed low over-all averages—12.5 for males and 11.8 for females (*19*), whereas other places, such as Uruguay (*17*), had general averages that were somewhat higher —14.7 for men and 13.5 for women. A better analysis of this situation, however, is obtained when one considers the percentage of individuals in the population classified as "low" or "deficient." In Venezuela, for instance (*18*), although the male population had an average of 13.7 g/100 ml, 41.7 per cent had hemoglobin rates considered "low," and 14.2 per cent were under 12 g/100 ml; and in the same country, 18.9 per cent of the women were below this last-mentioned level. The percentage of total population having less than 12 g of hemoglobin varies in the different countries from 1.7 to 38.6 for males and from 3.6 to 49.1 for females (*12, 17, 20*).

Quite often generally elevated hemoglobin averages have been found among populations living at high altitudes. It is evident that a more meaningful interpretation of the hematological data is obtained when the figures are adjusted taking altitude into consideration. In Bolivia, for instance (*12*), a general average of 16.4 was obtained for persons over 15 years of age and 14.9 for those 15 and under. Seven study areas located at altitudes ranging from 6,700 to 13,467 feet presented generally high hemoglobin averages, but when these data were corrected for altitude, most of the subjects fell into the "acceptable" or "low" category. The same thing happened with the studies in Colombia (*14*), Ecuador (*15*), and Mexico (*2*).

The mean corpuscular hemoglobin concentration (MCHC) was around 32 per cent in all the countries but Venezuela, where it was about 36 per cent. The proportion of the general population having an MCHC of less than 30 per cent varied from 1.5 per cent for Venezuela (*18*) to 18.0 per cent for the West Indies (*19*). This is a partial suggestion of the presence of iron-deficiency hypochromic macrocytic anemia.

Serum iron was not determined in most of the studies. In Trinidad (*5*), the serum iron values ranged between 22.92 to 274 μg/100 ml, with a mean value of 87.2 μg/100 ml; 43 per cent of the subjects studied had values under 80 μg, and 15 per cent were below 50 μg/100 ml.

All the data that have been cited refer to the general population, and therefore they do not always give a clear picture of the vulnerable groups, where the problem of anemia is of greatest magnitude.

In Venezuela (*18*), for example (Table 2), children under one year of age have a mean average hemoglobin of 10.27 g/100 ml, and one third of them have hemoglobin concentrations of less than 10 g/100 ml. In Brazil (*16*), the children in this same age group have an average hemoglobin of 12.1 g/100 ml, and half of them have levels below 12 g/100 ml. In the West Indies (*19*), almost 90 per cent of children under two years of age have hemoglobin levels below 12 g/100 ml.

Preschool children in all these countries except Uruguay are deficient. In this age group, the average hemoglobin levels in the different Latin American countries has varied, with general averages of from 10.8 g/100 ml (and 77.4 per cent with less than 12 g) in the West Indies (*19*) to 13.2 g/100 ml in Bolivia (*12*).

Many authors found "foci" of anemia in schoolchildren in certain tropical areas. In Iquitos, Peru (*6*), for example, the schoolchildren had an average hemoglobin of 10.6 g/100 ml, with 56 per cent having less than 10 g/100 ml. A recent examination of schoolchildren from villages in the coastal region of the State of São Paulo (Brazil), showed general hemoglobin averages in many villages of around 9.5 g/100 ml, with more than half the children having less than 10 g/100 ml. Throughout almost all of Latin America and the Caribbean area a large percentage of the children have inadequate hemoglobin concentration levels.

Pregnant and lactating women are another group in which anemia is an important public health problem (Table 3). Some of the surveys by the U.S. Interdepartmental Committee on

TABLE 1. Hemoglobin levels and average daily iron intake per person in Latin America and the Caribbean area

HEMOGLOBIN (G/100 ML)	BOLIVIA (12)*		BRAZILIAN NORTHEAST (16)		CHILE (13)		COLOMBIA (14)*	ECUADOR (15)*	MEXICAN COAST (2)		TRINIDAD (5)		TRINIDAD AND TOBAGO (19)		URUGUAY (17)		VENEZUELA (18)	
	♂	♀	♂†	♀†	♂	♀	♂+♀	♂+♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Mean	16.0	15.2	14.3	12.8	13.9	13.0	14.1	13.7	16.4	12.4	15.0	13.2	12.4	11.8	14.7	13.5	13.7	12.9
% <12.0	3.2	3.6	9.5	nc	7.5	15.3	6.9	13.3	nc	33.0	6.4	24.2	38.6	49.1	1.7	6.1	14.2	18.9
% 12.0–13.9	9.9	16.2	23.8	66.9‡	43.8	64.9	32.4	28.1	nc	nc	nc	nc	40.1	47.8	33.9	60.2	41.7	66.5
Daily iron intake (mg)																		
Questionnaire method	19.8		17.4		18		7.4	19.3	17.0		—		8.3		17§	16 #	16	
Calculated intakes; "recipe method"	26.7		nc		17.6		12	15.5	—		—		6.6		17	19	18	
Food analyses	58.6		nc		30		17	27.2	—		—		11.0		23	22	32.3	

* Data not corrected for altitude
† > 17 years
‡ < 14.0 g/100 ml
§ For Montevideo
# Interior of Uruguay
NC Not calculated

TABLE 2. Hemoglobin levels for infants and children in Latin America and the Caribbean area

HEMOGLOBIN (G/100 ML)	BOLIVIA (12)		BRAZILIAN NORTHEAST (16)		CHILE (13)		ECUADOR (15)		MEXICAN COAST (2)	TRINIDAD AND TOBAGO (19)		URUGUAY (17)		VENEZUELA (18)	
Infants	(under 1 year)		(under 1 year)							(under 2 years)		(under 1 year)		(under 1 year)	
	♂	♀	♂+♀							♂	♀	♂+♀		♂+♀	
Mean	14.0	12.8	12.1		—		—		—	9.6	9.6	11.2		10.27	
% <10.0														33.3	
% <12.0	nc	nc	50.0		—		—		—	88.9	91.7	nc		50.0	
% 12.0–13.9	nc	nc	33.3		—		—		—	11.1	8.3	nc		nc	
Preschool children	(1–4 years)		(1–4 years)						("Preschool children")	(2–4 years)		(1–4 years)			
	♂	♀	♂+♀						♂+♀	♂	♀	♂+♀			
Mean	13.2	12.9	12.2		—		—		11.1	10.8	11.1	12.2		—	
% <10.5									27.4						
% <12.0	nc	nc	nc		—		—		nc	77.4	78.9	nc		—	
% 12.0–13.9	nc	nc	nc		—		—		nc	22.6	21.0	nc		—	
Schoolchildren	(3–9 years)		(6–16 years)		(4–9 years)		(<15 years)		("School children")	(5–14 years)		(5–14 years)		(5–9 years)	
	♂	♀	♂	♀	♂	♀	♂	♀	♂+♀	♂	♀	♂	♀	♂	♀
Mean	14.7	14.6	12.4	12.6	12.6	12.9	11.7	12.6	12.4	11.9	12.0	13.3	13.4	12.3	12.5
% <10.0			8.3	5.7					3.8						
% <12.0	5.2	2.1	28.4	27.2	19.2	12.0	50.0	33.3	16.9	nc	nc	4.5	2.0	40.7	28.1
% 12.0–13.9	20.7	29.8	nc	nc	73.1	72.0	27.8	44.4	—	nc	nc	75.0	73.5	55.6	68.8
	(10–14 years)				(10–14 years)									(10–14 years)	
Mean	15.2	15.2	—	—	13.0	13.3	—	—	—	—	—	—	—	13.2	13.4
% <12.0	3.2	3.8	—	—	9.3	—	—	—	—	—	—	—	—	10.0	3.8
% 12.0–13.9	11.1	7.7	—	—	67.4	80.6	—	—	—	—	—	—	—	65.0	69.2

NC = Not calculated

TABLE 3. Hemoglobin levels for pregnant and nonpregnant women in Latin America and the Caribbean area

HEMOGLOBIN (G/100 ML)	ARGENTINA (10)	BOLIVIA (12)	BRAZILIAN NORTHEAST, SÃO PAULO (10, 16)		COLOMBIA (10)	MEXICO (11)	PERU (10)	TRINIDAD (5)	TRINIDAD AND TOBAGO (19)	URUGUAY (17)	VENEZUELA (10, 18)	
Nonpregnant women												
Mean	12.9	15.2	12.8	13.5	14.1	14.3	13.1	13.2	11.8	13.5	12.9	12.8
% <10.0	4	nc	nc	nc	nc	(<11.0) 5.4	4	3.5		nc		4
% <12.0	13	3.6	nc	nc	nc	8.3	12	20.7	49.1	6.1	18.9	12
% 12.0–13.9	nc	16.2	66.9	nc	nc	nc	nc	nc	47.8	60.2	66.5	nc
Pregnant women												
Mean	10.4	14.6	—	12.1	12.6	12.5 *	11.4	10.4	—	12.2 *	12.1	11.1
% <10.0	26	nc	—	nc	nc	(<11.0) 12.6	11	31.1				12
% <12.0	42	nc	—	nc	nc	27.9	48	83.9	—	60.0	57.9	50
% 12.0–13.9	—	nc	—	nc	nc	nc	nc	nc	—	30.0	36.9	nc

* Third trimester only

NC = Not calculated

Nutrition for National Defense (ICNND) include these groups in their studies. In Venezuela, for instance, where the hemoglobin average of pregnant and lactating women is practically the same as that of nonpregnant, nonlactating females, the proportion of cases with less than 12 g in pregnant women is 57.9 per cent, whereas in the total nonpregnant female group it is 18.9 per cent. The same situation was found in other areas. Even in Uruguay (*17*), where anemias are not a very serious health problem, 60 per cent of the third-trimester pregnant women were found to have less than 12 g/100 ml, as against 6.1 per cent in the nonpregnant group. In Trinidad (*4*), an examination of over 500 pregnant women selected at random showed that 34 per cent had hemoglobin concentrations of less than 10 g/100 ml. In this study it was shown that the rate of hemoglobin values decreases as the pregnancy progresses. Women in the first five months of pregnancy have an average of 11 g/100 ml, and those in the sixth month and onward have 10.6 g/100 ml. In Saltillo (*1*), a city in the north of Mexico with an altitude of over 5,200 feet, 50 per cent of the pregnant women under medical supervision had hemoglobin levels of less than 10.5 g/100 ml, and 15.2 per cent had less than 8.5 g/100 ml; 68 per cent of all the pregnant women studied had less than 50μg/100 ml of serum iron.

The 1968 PAHO Scientific Group on Research in Nutritional Anemias, meeting at Caracas, reviewed the pilot studies on the prevalence of nutritional anemia in pregnancy that had been recommended in 1963, and the results of 900 protocols from cooperating laboratories in Mexico City, Caracas, Medellín, São Paulo, Lima, Corrientes (Argentina), and Port of Spain led to the conclusion that 21 per cent of the women in the last trimester of pregnancy are definitely anemic and have hemoglobin levels below the lower limit of normality (10 g/100 ml at sea level, or corrected for altitude).

The foregoing data demonstrate the seriousness of anemia among vulnerable groups of infants, preschool children, schoolchildren, and pregnant and lactating women. If analyzed samples could be extended to all the population, one would realize that millions of people in Latin America and the Caribbean area have clear-cut anemia.

The most frequent types of anemia in Latin America and the Caribbean

As far as the type of anemia is concerned, many of the studies mentioned (*1, 9, 11, 16, 19*) seem to indicate that the most frequent anemia in Latin America and the Caribbean area is produced by the deficiency of available iron for the body. The values of MCHC in these areas are generally "acceptable," but in several countries the proportion of the population with average levels of less than 30 per cent was high—Ecuador (*15*), 42.7 per cent; Chile (*13*), 26.7 per cent for men and 42.8 per cent for women; Brazil (*16*), 23.5 per cent for men and 17.5 per cent for women—to cite a few. In Uruguay (*17*), where iron-deficiency anemia is not a public health problem and the general average of MCHC is similar to the level mentioned above, there were no cases below 30 per cent.

Serum iron has been shown to be generally low. In Trinidad (*5*), for instance, 43 per cent of the population has less than 80 μg/100 ml, and in 15 per cent of the population the level is less than 50μg/100 ml. In Mexico, too (*9*), the serum iron levels were generally low; in Huamantla (*11*) and in Saltillo (*1*), for example, a large proportion of the pregnant women had low serum iron and high MCHC, suggesting that at least 60 per cent of the cases of anemia in pregnant women were caused by iron deficiency.

It was concluded at the Caracas meeting in 1968 (*10*), that iron deficiency accounts for approximately 75 per cent of the cases of anemia in the third trimester of pregnancy, and in approximately one third of these cases an additional nutritional deficiency can be observed. In female control subjects, iron deficiency accounts for approximately half of the anemia found. Studies of low-income pregnant women

in the last trimester of pregnancy, nonpregnant women, and men conducted by IVIC in Caracas showed hemoglobin levels of less than 11 g/100 ml for 37 per cent of the pregnant women; low serum iron and decreased transferrin saturation indicated iron deficiency in nearly 60 per cent of the pregnant women and 19 per cent of the nonpregnant women (*23*). Finally, projects that included the administration of iron to the anemic population of these areas (*3, 6*) confirmed that iron deficiency is the most general and important cause of anemia in Latin America and the Caribbean area.

Etiology of iron-deficiency anemia in Latin America and the Caribbean

It is recognized that iron-deficiency anemia can be caused by the following factors: (1) inadequate ingestion; (2) poor absorption and utilization; (3) increased losses or requirements, as in growth, lactation, and pregnancy. All these factors are present in Latin America and the Caribbean area.

Inadequate ingestion

The idea that iron-deficiency anemias occur chiefly because of insufficient dietary iron intake has not necessarily been confirmed by surveys carried out in Latin America and the Caribbean area. The different reports on anemia and dietary iron intake from dietary surveys or food analysis show that in most cases there is no correlation between the amount of dietary iron and the degree of anemia (Table 1). In Bolivia (*12*), Brazil (*16*), Chile (*13*), Mexico (*2*), and Venezuela (*18*), the amounts of dietary iron were substantially above the NRC allowances and ICNND reference guides, and still iron-deficiency anemia is a problem in these areas. The biological availability of iron from dietary sources has not always been taken into consideration. In the surveys of Chile and Uruguay (*13, 17*), the average daily dietary iron intake was shown to be nearly 17 mg per capita in both cases—and yet anemia is a problem in Chile but not in Uruguay. These examples suggest that most anemia in these areas is not necessarily a consequence of dietary iron deficiency, but rather that other factors are interfering.

Poor absorption and utilization

Iron-deficiency anemia may result from poor absorption and utilization. Some reports have shown that dietary iron derived from animal sources is better absorbed than that from vegetable sources, even in an iron-deficient patient. In these subjects, the absorption of iron derived from animal products such as meat and fish is about 20 per cent (*8*), whereas absorption from staple foods such as wheat and corn is not as high as 10 per cent. However, diets containing large amounts of animal protein may be more efficient than vegetable diets as far as iron absorption is concerned. Layrisse *et al.* (*7*), working with food of animal and vegetable origin, found that the interaction of both may change the rate of iron absorption and that the mixture of a vegetable food such as corn or black beans with food of animal origin enhances the absorption of iron by threefold.

It should be kept in mind that for the great majority of people in Latin America and the Caribbean area the proportion of animal foods in the diet is not adequate. In Brazil (*16*), for instance, a dietary survey showed that iron intake is high chiefly because of the high consumption of legumes. Animal food, such as meat and eggs, is not commonly eaten by the population.

Malnutrition may well be an important cause of anemia in Latin America and the Caribbean area. Also, deficiencies of proteins, folate, vitamin B_{12} and other nutrients can result in anemia.

Increased losses

Epidemiological studies on losses of iron from the body through sweat, skin exfoliation, urine, and gastrointestinal processes are not available for Latin America and the Caribbean, and consequently it is not possible to give consistent figures for these areas. It is generally accepted

that one of the most important causes of body iron loss in tropical zones is hookworm infection. *Ancylostoma duodenale, Necator americanus, Trichuris trichiura, Schistosoma hematobium,* and *Schistosoma mansoni* infections can produce chronic blood loss resulting in iron-deficiency anemia.

Nutritional surveys carried out in Latin America and the Caribbean have shown that intestinal parasitic infections and anemias frequently constitute a serious public health problem (*4, 5, 14*). In the survey of Northeast Brazil (*16*), for example, 99.4 per cent of the results of fecal examination for helminths was positive. A comparison of hemoglobin levels in subjects with and without hookworm infection showed that those with hookworm had a mean hemoglobin consistently lower than those without.

In the study of an Amazon basin community (*6*), it was reported that 56 per cent of the schoolchildren had hemoglobin levels below 10 g/100 ml, and 96 per cent harbored more than one kind of intestinal parasite. Hookworms were found in 93 per cent of the schoolchildren. In one subgroup that received iron but not antihelminthic treatment, the mean hemoglobin concentration increased from 8.3 ± 2.1 to 10.8 ± 1.1 g/100 ml six weeks after treatment had been initiated. In another subgroup that received both iron and antihelminthics, the mean hemoglobin concentration increased from 8.2 ± 2.2 to 11.5 ± 1.0 g/100 ml during the same period. The response of the group that had previously been given antihelminthics was significantly greater ($p > 0.05$) than that of the group that did not receive the treatment.

A Mexico study (*2*), in which the frequency of anemia was analyzed both in coastal communities and in the highlands, revealed an average hemoglobin of 11.1 g/100 ml on the coast and 12.1 in the highlands. Hookworm infection was very common in the former area but not in the latter.

Studies of infected and noninfected rural communities in Venezuela have demonstrated that 30 per cent of the anemic conditions could be traced directly to hookworm infection. Parasitic blood losses had to reach a certain level of intensity, however, before they significantly influenced hemoglobin levels in the blood.

Since parasitic infection is a very widespread public health problem in Latin America and the Caribbean area, it can easily be surmised that it has an important role in enhancing the prevalence of iron deficiency in these areas. Some exception to this, of course, can be found in the literature. In Saltillo, for instance (*1*), where hookworm is not prevalent, 50.9 per cent of the pregnant women had hemoglobin levels below 10.5 g/100 ml, and 68.0 per cent of the same group had less than 50 μg of serum iron. Thus, although anemia is a serious problem in this area, the authors conclude that hookworm infection has little to do with the situation.

Final considerations

In all the available reports, a high percentage of the general population and of specific groups have blood parameters that are considered "deficient" or "low." If the groups that have been analyzed in these different surveys are really representative of the general population of the area, this means that millions of people in Latin America and the Caribbean have unsatisfactory hemoglobin levels.

For the most part, dietary iron deficiency does not stand out as a factor among these populations, though poor, unbalanced diets could have an important role in the absorption of iron.

The increase of intestinal hemoglobin iron loss as a consequence of the prevalence of parasitic infection might also be another important factor in the frequency of iron-deficiency anemia, chiefly in the tropical areas of Latin America and the Caribbean.

The reports that have been made to date on the subject of anemias are not sufficient enough to give a clear idea about their frequency, severity, or etiology, and more studies in different areas should be stimulated. Moreover, the lack of standardized techniques in these surveys, and the variation in criteria used for random sampling, make comparisons difficult; greater encouragement should be given to the PAHO/

WHO efforts to standardize methods and techniques for obtaining accurate data on the prevalence and etiology of anemias.

It is important also to consider that, despite the many surveys that have been made on iron-deficiency anemia, very few programs for the distribution of iron to the general population or to specific vulnerable groups have been carried out to date. It would be useful to stimulate more preventive programs in this area.

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IRON DEFICIENCY IN PREGNANCY AND INFANCY

Luis Sánchez-Medal

From the standpoint of iron nutrition, pregnancy is the most critical period in a woman's life. In a normal pregnancy, the total iron requirement is estimated to be from 580 to 1340 mg, the average being about 980 mg (*52*). This amount is the sum of the ordinary external losses, the requirements for red cell mass enlargement, and the supply to the fetus and the placenta.

The needs are unevenly distributed during the period of pregnancy, rising exponentially with the passing months. Thus, whereas in the first trimester they amount to about 0.6 mg a day, which is less than those of the nonpregnant woman, they go up to about 8 mg a day during the third trimester (Table 1). These estimates are based on the following data: (1) the iron content of the fetus, which is 5 mg at four months, 65 mg at seven months, and 265 mg at ten months (*37*); (2) increases observed in the mother's red cell volume during pregnancy, which range from 315 to 485 ml, with an average of about 425 ml, equivalent to 500 mg of iron (Table 2 and *4, 8, 10, 29, 40*); and (3) the apparent uneven distribution of this rise throughout pregnancy. Though Caton and associates (*8*) reported similar increases in the three trimesters, the data of De Leeuw *et al.* (*10*) and Berlin *et al.* (*14*) indicate that the increase starts after the sixth and seventh month, respectively. It seems likely that most of the red cell volume increase takes place during the last trimester.

Diet does not provide absorbable iron in sufficient amounts to meet the third trimester requirements. Common diets in Western societies provide less than 2 mg of absorbable iron per day to the iron-deficient subject (*10, 17, 25, 42*).

Iron stores are usually insufficient to cover the difference between what is required and what is absorbed. Estimates made using different methods have shown that all over the world the average woman has limited iron stores—

TABLE 1. Distribution of iron requirements (mg/day) during pregnancy

	FIRST *	SECOND	THIRD
External losses	0.5	0.5	0.5
The fetus	0.04	0.7	2.4
Mother's RCV expansion	0	1.4	4.2
Placenta	0.04	0.3	0.5
Totals	0.58	2.9	7.6

* Trimester

TABLE 2. Mean increase in red cell volume during pregnancy

Berlin *et al.* (*4*)	315 ml
De Leeuw *et al.* (*10*)	405 ml
Lund and Donovan (*29*)	439 ml
Pritchard (*39*)	485 ml
Caton *et al.* (*8*)	495 ml

Mean of all series: 425 ml = 500 mg of iron, distributed as follows (mg):

	FIRST *	SECOND	THIRD
Total	0	125	375
Per day	0	1.4	4.2

* Trimester

TABLE 3. Prevalence of anemia and iron deficiency in pregnancy

AUTHORS	PLACE	SUBJECTS	% FREQUENCY OF		
			ANEMIA	IRON DEFICIENCY IN THE ANEMICS	BOTH IN ALL CASES
Evers (*11*)	Holland	1000	—	—	>90
Benjamin *et al.* (*3*)	New York, U.S.A.	1052	49 * †	80	—
Hunter (*20*)	Indianapolis, U.S.A.	4744	20 †	98	—
Lund (*28*)	New Orleans, U.S.A.	4015	50 †	>99	—
Giles and Burton (*13*)	England	983	55 ‡	78	—

* Hb below 12 g; in the others Hb below 11 g
† First prenatal consultation
‡ At term

in most cases less than 400 mg (*6, 10, 39, 45, 54*).

In the light of these facts, it is not surprising that iron deficiency in pregnant women is a standing problem all over the world, even in the most industrialized countries (Tables 3 and 4 and *3, 11, 13, 20, 27, 28, 43*). The Bantus seem to be the only exception, which may be explained by the large iron content of their usual diet (*12*). Thus, the World Health Organization has made the general recommendation that "a dose of ferrous salt containing at least 60 mg of elemental iron [be] given once daily during the second and third trimesters of pregnancy and the first six months of lactation" (*56*).

TABLE 4. Prevalence of anemia and iron deficiency in pregnant women in WHO collaborative studies (*38, 56*)

	ANEMIA * (%)	IRON DEFICIENCY * (%)
Ramalingaswami: New Delhi (rural)	80 †	51.7
Baker: Vellore	56	99.0
Rachemilewitz: Upper Galilee (rural)	47 †	46.3
Layrisse: Caracas	37	59.7
Sánchez-Medal: Tlaxcala (rural)	28	64.5
Lawkowicz: Warsaw	21.8	40.0
Gutnisky: Corrientes	63.0 ‡	—
Layrisse: Caracas	52.0	57.0
Viteri: Guatemala	38.0	73.0
Sánchez-Medal: Mexico City	36.0	33.1
Sánchez-Medal: Mexico City	19.0	23.1 §
Reynafarje: Lima	35.0	—
Vélez: Medellín	26.0	11.0 #
Jamra: São Paulo	13.0	17.0

* Criteria: Hb below 11.0 g at sea level; saturation index below 15%
† Second and third trimesters
‡ Uncinariasis
§ Sampled during labor
# 43% of cases with SFe below 50 μg

The prophylactic administration of iron during pregnancy is useful even in populations without widespread iron deficiency (*35*). This was demonstrated in a study of women injected intravenously with 1200 mg of iron dextran* during the first trimester of pregnancy (Table 5). In the third trimester all the subjects had serum iron levels of over 60 μg/100 ml, transferrin saturation over 15%, and a higher hemoglobin (0.4 g/100 ml) than they had had during the first three months.

Iron nutrition is also seriously compromised during infancy. During the first two to three months of life, iron requirements of the normal infant are practically zero; the total hemoglobin level remains lower than that present in the newborn. From then on, however, total hemoglobin increases rapidly as the infant gains weight and his concomitant blood volume in-

* Iron dextran (Imferon) was kindly supplied by Farmacéuticos Lakeside, S.A.

TABLE 5. Effect of prophylactic administration of iron to pregnant women without evidence of iron deficiency in the first trimester *

	CONTROLS	IRON-TREATED
Data on first trimester		
Mean hemoglobin (g/100 ml)	—	13.0
Mean serum iron (μg/100 ml)	—	131.0
Cases with SFe below 60 μg (%)	—	0
Mean transferrin saturation (%)	—	34.8
Cases with saturation below 15 (%)	—	0
Data on third trimester		
Mean hemoglobin (g/100 ml)	12.4	13.4
Cases with hemoglobin below 12(%)	36	4.5
Mean serum iron (μg/100 ml)	97.3	121.0
Cases with SFe below 60 μg (%)	10.5	0
Mean transferrin saturation (%)	20.2	30.4
Cases with saturation below 15(%)	33	0

* The iron-treated group was randomly selected from the same population as the controls. In the latter, no laboratory determinations were performed in the first trimester, but it may be assumed that at that time they had values similar to those in subjects injected with 1200 mg of intravenous iron dextran.

creases. During the second three months of life, about 1.0 mg of iron a day is required to increase hemoglobin, myoglobin, and iron-containing enzymes, and to compensate for external losses estimated at 0.1 to 0.15 mg a day (*52*). The requirements decrease progressively from the third trimester on, so that in the second year of life they are probably of the order of 0.4 to 0.5 mg. The requirements of infants growing faster than average, and particularly those whose weight was low at birth, are even greater.

The infant's usual diet, with milk as the main component, plus meat, fruits, and cereals, cannot provide sufficient iron to meet these requirements. Recognition of this fact has led to the use of iron-enriched cereals, which at present are the main source of iron in the first-year diet of infants in upper-income families (*2*). However, many babies refuse to eat these processed foods (*2*), and low-income families cannot afford them. Moreover, the foods currently on the market do not seem to provide sufficient iron. It has been estimated that the usual diet with iron-enriched cereals contains from 4 to 6 mg of iron a day (*45, 50*). These considerations appear to be correct and significant. Guest and Brown (*15*) state that in 1957 iron deficiency in infancy in Cincinnati was as widespread as it had been 20 years before, and Moe (*33*) found a high deficiency rate in Norwegian infants. In addition, several studies have shown that in infants from upper-income families the blood hemoglobin and serum iron levels can be raised by supplemental iron (*33, 51*).

At birth the infant has two main sources of iron reserves: excess hemoglobin and tissue stores. The average newborn has about 20 g of excess hemoglobin, equivalent to 70 mg of iron. This excess, which accounts for the large blood volume and high concentration of hemoglobin in the newborn (*34*), is derived partially from placental transfusion. Early clamping of the cord decreases the amount of hemoglobin excess; the difference in total hemoglobin between early and late clamping has been estimated in 4.6 to 6.0 g per kilo (*36*). Thus, in the average newborn, early clamping will decrease the hemoglobin iron reserve to less than half the value mentioned above.

As to tissue stores, it may be assumed that the average newborn has a reserve of about 80 mg. This figure is calculated by subtracting 190 mg of hemoglobin and other functional iron from the 270 mg total iron content of the newborn (*52*).

Despite these reserves, it is a fact that iron deficiency is widespread not only among infants from families with limited economical means, in whom definite anemia is observed with frequencies of even greater than 70 per cent (*1, 9, 23, 44*), but also among those from upper-income families (Table 6 and *15, 50*). The prevalence of anemia in the latter is not as great, but in general these babies still exhibit low serum iron and MCHC, high TIBC and erythrocyte protoporphyrin (*49*), and increased absorption of iron (*47*). The deficiency becomes apparent in the second trimester of the infant's life (*21, 47*) and decreases after the age of 18 months, except in premature babies and those from very poor homes, in whom it appears earlier and may

TABLE 6. Iron-deficiency anemia in infants (Hb below 10 g)

AUTHOR AND PLACE	PREVALENCE (%)	SOCIOECONOMIC CONDITION
Currimbhoy:		
Bombay (*9*)	92.8	Poor
Andelman:		
Chicago (*1*)	76.0	Poor
Lanzkowsky:		
Cape Town (*23*)	65.4	Medium (colored)
Schulman:		
Chicago (*44*)	44.0	Poor
Lanzkowsky:		
Cape Town (*23*)	29.5	Poor (white)
Guest: Cincinnati (*15*)	20.0	Variable

continue without improvement until as late as three years of age (*9*).

Serum iron determinations in infants have shown that the level decreases starting at birth, and that throughout infancy the mean values are definitely lower than those seen in adults. In infants supplemented with iron, higher values have been obtained, but they are still below normal levels for adults (*1, 31, 33, 49-51*), which suggest that they are not yet iron sufficient (Figure 1). A hemoglobin level of 11 g per 100 ml is not always indicative of iron sufficiency. In Sturgeon's series of infants given 250 mg of parenteral iron (*51*), not one subject had a hemoglobin level lower than 11 g, and still the group as a whole was probably iron deficient, since the mean serum iron and MCHC were below the normal adult range and the TIBC was above.

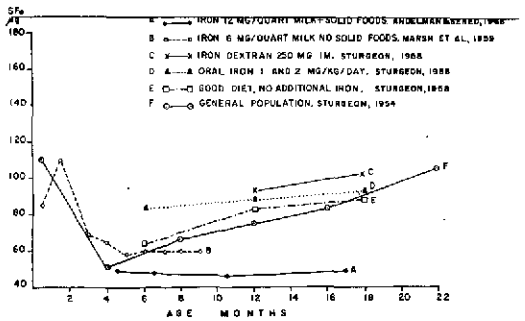

FIG. 1. Mean iron serum values found in infants with and without iron supplementation

Two complications seen in markedly iron-deficient infants add to the problem: the occurrence of microscopic but significant gastrointestinal bleeding (*18*), and the impairment of iron absorption (*22*).

The current impression is that the tissue stores of the infant do not play an important role in the development of iron deficiency in infancy and that the fetus gets all the iron it needs from the mother, regardless of her iron status (*5, 46*). It would appear that further studies on these aspects are needed, since it is reasonable to assume that the iron stores at birth can make up for the difference between iron requirements and iron absorption during infancy, and that such stores probably depend on the iron nutrition status of the mother. The amount of iron stored in the liver of stillborns has been shown to vary greatly from one case to another (Table 7 and *7, 14*). Similarly, variations in the total body iron content ranging from 200 to 370 mg for fetuses of similar weight have been observed (*55*). Consequently, infants with low iron stores have to depend solely on their diets to meet their iron requirements,

TABLE 7. Amount of iron found in liver of stillborns weighing over 3 kg

AUTHOR	SUBJECTS	MG/100G *	TOTAL MG
Gladstone (*14*)	1	72.0	140.0
Gladstone (*14*)	1	10.6	15.6
Widdowson and Spray (*55*)	1	10.4	—
Widdowson and Spray (*55*)	1	16.7	—
Widdowson and Spray (*55*)	1	21.1	—
Widdowson and Spray (*55*)	1	25.5	—
Widdowson and Spray (*55*)	1	37.0	—
Buchanan (*7*)	16	9.7–79	36±21
Wainwright (*53*)	4 (E)	18–110	
Wainwright (*53*)	17 (B)	81–666	

E = Europeans; B = Bantus

* Dry weight in Wainwright's cases and wet weight in the others

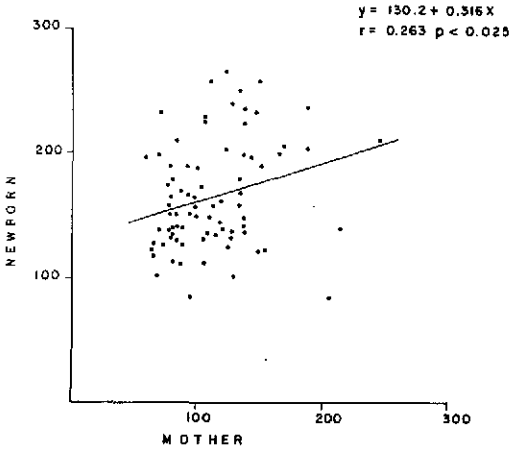

FIG. 2. Relationship between serum iron values in the mother and in the cord in 82 mother-infant pairs. A significant relationship was observed in the whole group, which became even more significant (p less than 0.001) when the pairs in which the mother had abnormally low folate values were excluded from the analysis.

whereas the infant born with large iron stores will be able to withstand the demands.

It is most likely that the magnitude of the infant's iron stores depends on the nutritional status of the mother. The present author's team has found (*26*) a direct relationship between serum iron values in the mother and in the cord (Figure 2). Earlier results reported by others (*19, 24*) are in agreement with this finding. More important is Wainwright's observation (*53*) that Bantu infants have five times more storage iron than European babies do, although most Bantu females of child-bearing age do not show histological evidence of siderosis. In fact, in over three fourths of the women under 40 years of age studied by the same author the liver iron was scored as zero. In 14 Bantu stillborns weighing more than 3 kg the mean iron content in the liver was 0.175 per cent of the dry weight, whereas in 4 European stillborns it was 0.035 per cent. Furthermore, though in general the mother's deficiencies do not influence the red cell values of the newborn, exceptions have been observed. In a study of 82 mother-infant pairs (*26*), a cord hemoglobin level below normal limits was found in three instances, and in all of them the infants' mothers showed megaloblastic changes in the bone marrow.

The practical implication of these findings is that among low-income families it will probably be easier to prevent iron deficiency in infants by prenatal measures than by medical supervision of the infant. These measures, moreover, will also benefit the mother.

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THE RELATIONSHIP BETWEEN HOOKWORM INFECTION AND ANEMIA

Marcel Roche

In the tropical and subtropical zones, where both hookworm infection and iron deficiency are widespread, cases both of hookworm without anemia and of anemia without hookworm can be readily found. That hookworm may be a major cause of iron-deficiency anemia can be stated on the basis of three lines of evidence: (1) the demonstrated blood and iron loss caused by hookworm; (2) the statistically significant correlation between circulating hemoglobin levels and hookworm load; and (3) the slow rise in circulating hemoglobin, without added iron, when hookworms are removed and the patient remains on his usual diet. The conclusions arrived at in the second and third lines of evidence are only known to apply in certain rural areas of Venezuela. In other countries, where a different nutritional status may prevail, the deductions are only tentative.

Blood and iron loss caused by hookworm

Attempts at estimating blood loss by isotopic and nonisotopic methods have been reviewed by Roche and Layrisse (*24*). Hahn and Offutt (*14*) were the first to use isotopic methods in animals. Two infected dogs received Fe^{59}-tagged erythrocytes from donor dogs, and the decline in circulating radioactivity during periods of constant hematocrit reading was subsequently determined. Gerritsen *et al.* (*12*) were the first to use an isotopic method in man (Fe^{59}). Since then, a number of authors have utilized either Fe^{59} or Cr^{51} (*26*). Daily blood loss by *Necator* is of the order of 0.03 ml per worm, while *Ancylostoma duodenale* gives losses of the order of 0.15 to 0.4 ml.

Since the review by Roche and Layrisse (*24*), several additional reports have appeared on blood loss by hookworm. Bloch and Ruiz (*2*) determined losses by means of Cr^{51} in ten patients (*N. americanus*) and Fe^{59} in eleven (*N. americanus* and *A. duodenale*). The average loss was 0.1 ml per parasite, with wide variations. Rep (*23*) studied blood loss due to *Ancylostoma ceylanicum* by means of Cr^{51} in two dogs and ascertained an average daily loss of 0.0137 ml per worm. Mahmood (*18*) found a daily blood loss of 0.032 ± 0.035 ml per worm in twenty patients infected with *N. americanus* and 0.152 ± 0.124 in ten patients infected with *A. duodenale*. Georgi (*10, 11*) utilized whole-body counting for the measurement of blood loss due to *Haemonchus contortus* and to *A. caninum* (*11*) and found considerable losses due to these parasites. Miller (*20*) reported a daily blood loss for *Ancylostoma caninum* of 0.08 to 0.20 ml per worm for light infections and 0.01 to 0.09 for heavy infections. Worms from irradiated larvae produced worms that caused little or no blood loss, and *Uncinaria stenophala* and *Ancylostoma braziliense* caused little or no blood loss. In two chimpanzees, *Ancylostoma duodenale* caused daily blood losses of 0.04 and 0.02 ml, respectively, per worm.

It is abundantly clear, therefore, that hookworm gives rise to blood loss, and therefore to

iron loss. By tagging circulating red blood cells simultaneously with Fe^{59} and Cr^{51}, Roche and Pérez-Giménez (*25*) showed that an average of 44.1 per cent of the iron lost into the intestine of infected subjects was probably reabsorbed and reutilized. Layrisse *et al.* (*16*) found a 36.3 per cent reabsorption. The average on all subjects reported in the aforementioned two reports, plus fifteen more subjects (*24*), was 39.2 per cent reabsorption of intestinal iron loss. In spite of this reabsorption, which tends to reduce the net loss, iron loss is still considerable, as may be seen in Table 1.

Of the two isotopes utilized, Cr^{51} and Fe^{59}, the former gives a more accurate and direct idea of actual losses, since iron is in part reabsorbed. The figures given for iron loss calculated from Cr^{51} measurements are probably too high, and should be reduced by approximately one third.

The loss of iron for a given amount of blood varies, of course, with the hemoglobin level. Eventually, as this level decreases, an equilibrium is established, at which point loss equals intake. To obtain an idea of the magnitude of such loss, it may be assumed that a subject infected with 500 hookworms and initially with a normal hemoglobin level will have a daily intestinal blood loss of the order of 16 ml. This means, in the presence of normal circulating hemoglobin, a daily iron loss of 7.9, and, if reabsorption is taken into account, a loss of approximately 5.0 mg.

There is a rough but significant correlation between total blood loss and number of eggs in the stools, which has been determined to be 2.74 ± 1.50 ml/1000 eggs per gram of stool by Roche *et al.* (*26*) in twenty-one cases; 2.59 by Bloch and Ruiz (*2*) in ten cases; and 2.14 ± 1.01 by Martínez-Torres *et al.* (*19*) in fifty cases.

Correlation between hookworm load and anemia

The association between hookworm and anemia was mentioned as early as 1880 (*22*), but the matter was not studied thoroughly until the twenties when Darling *et al.* (*6, 7*), by relating hookworm load to hemoglobin levels, showed that these were negatively correlated, in a general manner. The standardization of a method to count hookworm eggs in the stools (*27*) made the matter easier, and a number of studies have been carried out in that direction since then, several of them with statistical analysis (*24*). Of the 32 papers reviewed by Layrisse and Roche, 16 reported a definite correlation, and the rest showed uncertain correlation or none at all; 14 of the papers contained statistical analysis, and all of these but one (Chernin, *4*) reported significant correlation.

In making a statistical analysis of the correlation between hookworm load and anemia, a sufficient number of cases must be studied, since variations in individual subjects may be extreme. This figure will depend on the number of severe cases in the series and on the

TABLE 1. Iron loss in human hookworm infection studied by isotopic methods *

AUTHOR	YEAR	ISOTOPE USED	NO. OF SUBJECTS	IRON LOSSES (MG/DAY)
Gerritsen *et al.*	1954	Fe^{59}	3	3.4–6.2–8.3
Roche *et al.*	1957	Cr^{51}	21	1.2–29.1
Ventura *et al.*	1957	Fe^{59}	7	0.85–6.84
Roche *et al.*	1959	Fe^{59}	14	Average: 5.25 Range: 1.80–16.24
Nabekura	1959	Cr^{51}	47	0.8–7.5
Tasker	1961	Cr^{51}	20	1–40 (calculated on basis of "normal hemoglobin levels")
Layrisse *et al.*	1961	Fe^{59}	11	2.55–7.91
Aly *et al.*	1962	Cr^{51}	39	Average: 3.35
Farid *et al.*	1965	Cr^{51}	12	Average: 6.06 Range: 3.56–9.94

* From Roche and Layrisse (*24*)

background of iron balance in the community. In addition, the series studied should include a sufficient spectrum of both severe and light infections. Since light infection may not affect hemoglobin values, a relationship between load and anemia should not be expected when only small loads are present. Similarly, when only small samples of cases with large loads are analyzed (*13*), no relationship may be apparent because of the sample size and the many variables involved.

More recently, Topley (*30, 31*) found a statistically significant negative correlation between hemoglobin and hookworm load in Gambian men, but not in women—possibly because other sources of iron loss (pregnancy, lactation, menstruation) in women masked the milder effect of hookworm.

In most studies, small loads of worms have no apparent effect on hemoglobin. For example, Carr (*3*) and Stoll and Tseng (*28*) found that the drop in hemoglobin values began when the load was greater than 1000 eggs per gram of stool; Layrisse and Roche (17) found the "break" at 2000 eggs per gram in women and children, and 5000 eggs per gram in adult men. Figure 1 gives the relationship curve found by Layrisse and Roche in their rural communities in Venezuela.

Layrisse and Roche (*17*) attempted to make a general estimate of the contribution of hookworm as an anemia-producing factor in given rural communities. They studied two communities, geographically close to each other, one with hookworm and the other practically without. The results are shown in Table 2.

It is presumed that the difference in anemia

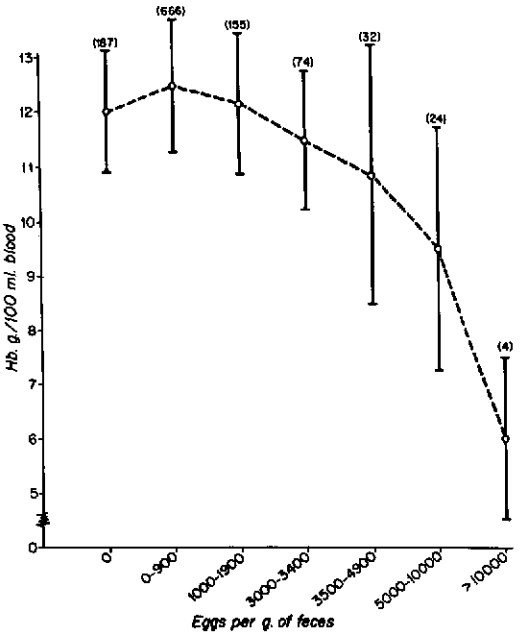

FIG. 1. Relationship between hemoglobin levels and severity of hookworm infection in various Venezuelan rural communities. The number of cases in each group is shown in parentheses. Vertical lines indicate one standard deviation. The differences between the negative group and both groups with less than 2000 eggs/g of feces are not statistically significant. In all groups with more than 2000 eggs/g of feces, the difference with the negative group is significant ($P<0.01$). From Layrisse and Roche (*17*).

prevalence among the *noninfected* groups between one area and the other is due to an alimentary factor, probably the quantity of available food iron. The difference between the prevalence of anemia among the infected and noninfected groups in the endemic zone presumably indicates the percentage of individuals

TABLE 2. Prevalence of anemia in two rural Venezuelan communities *

ZONE	CLIMATE	NUTRITIONAL STATE	NO. OF SUBJECTS	INFECTED	PREVALENCE ANEMIA	NONINFECTED
Endemic	Hot humid	Fair; little animal protein	1142	Abundant	46%	30%
Nonendemic	Hot humid	Fair; good animal protein (fish)	482	No		16%

* From Layrisse and Roche (*17*)

TABLE 3. Iron utilization from food measured in anemic subjects by the increase of total circulating hemoglobin and iron loss due to hookworm infection *

CASES	AGE IN YEARS	INITIAL HEMOGLOBIN (G/100)	INITIAL HEMOGLOBIN (TOTAL G)	FINAL HEMOGLOBIN (G/100)	FINAL HEMOGLOBIN (TOTAL G)	HEMOGLOBIN IRON INCREASE (MG/DAY) A	IRON LOSS DUE TO HOOKWORM INFECTION (MG/DAY) B	TOTAL IRON USED (MG/DAY)† A+B
			Children from 2 to 6 years					
1. E.A.	6	10.5	126	12.0	183	0.34	3.36	3.70
2. I.A.	6	8.6	89	14.4	152	0.37	1.34	1.71
			Children from 7 to 14 years					
1. L.M.	10	11.1	166	15.5	272	0.63	0.53	1.16
2. J.M.C.	9	10.5	164	12.6	246	0.49	0.20	0.69
3. J.R.V.	7	11.6	155	11.9	187	0.19	0.10	0.29
4. R.B.	9	8.5	113	10.6	179	0.39	1.11	1.50
5. E.G.	8	11.5	142	12.4	218	0.45	0.00	0.45
6. L.V.	12	11.7	205	13.3	311	0.63	2.54	3.17
7. A.M.	7	10.9	142	10.6	165	0.14	1.09	1.23
8. C.R.P.	7	8.0	130	11.4	237	0.54	1.14	1.68
9. C.R.M.	13	9.6	206	11.3	316	0.55	3.55	4.10
Average		10.4	158	12.2	237	0.45	1.03	1.25
			Men from 15 to 45 years					
1. P.A.	18	4.6	151	11.4	437	1.70	1.02	2.72
2. D.R.C.	38	12.5	422	15.2	494	0.42	0.18	0.60

* From Layrisse (*15*)

† Minimum estimate

with anemia due to hookworm as a paramount factor (roughly one third of the cases).

It has thus been abundantly shown, in a number of communities, that groups with varying degrees of hookworm infection have an average level of circulating hemoglobin significantly below that of uninfected groups living under similar conditions in the same community. Such groups could properly be said to exhibit *hookworm anemia.*

Rise in circulating hemoglobin after removal of the worms

Cruz (*5*) emphasized that hemoglobin responded rapidly to the administration of iron in iron-deficient hookworm-infected subjects, whereas patients in whom hookworms had been removed but to whom iron was not given did not respond in such a fashion. An examination of these data, however, shows clearly that such cases do respond, albeit very slowly. Increments in circulating hemoglobin were measured over a period of 365 days in thirteen endemic-zone anemic subjects, in whom repeated worming was performed, while they remained in their usual habitat and consumed their usual diet (*15*). The results are seen in Table 3. The increase in hemoglobin iron ranged from 0.14 to 1.70 mg a day but, in addition, there was a fecal iron loss, due to residual infections or reinfections, in spite of repeated worming. From egg counts, this fecal iron loss could be estimated to range from 0.10 to 3.55 mg a day, so that food iron utilization was probably at least of the order of 0.29 to 4.10 mg a day—values not unlike those that may be calculated from the data presented by Cruz (*5*) and those given by Finch *et al.* (*9*) in phlebotomized subjects with

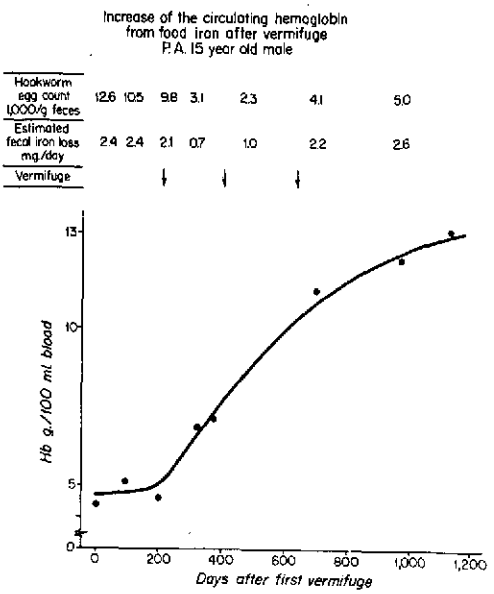

FIG. 2. Response of circulating hemoglobin to the administration of vermifuge. The values are near normal after 3 years even though there is continued fecal blood loss. Iron utilized from food for hemoglobin synthesis was calculated to be 2.7 mg/day. The patient, P.A., is a 15-year-old male from a Venezuelan rural area. He remained in the same locality throughout the study and consumed his usual diet. From Roche and Layrisse (*24*).

polycythemia vera. The fact that hemoglobin rises to, or at least towards, normal after worming, on local diet only and despite residual infections, suggests that in the absence of hookworm infection the food iron in the area studied (Pequín, Venezuela) should be adequate to sustain hemoglobin levels.

Figure 2 shows the hemoglobin curve in one patient in whom the study was carried out for a longer time. The curve may be seen to rise from 4.5 to 13.0 g/100 ml over the 1200 days during which the patient was studied.

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PREVENTION OF IRON-DEFICIENCY ANEMIA

Joginder Chopra

Introduction

Nutritional anemia has long been considered a public health problem in Latin America and the Caribbean—a fact that was reiterated at the first and second meetings of the PAHO Technical Advisory Committee on Nutrition in 1962 and 1968, respectively (*5*). Moreover, the FAO/WHO Joint Committee at its various meetings, beginning in 1949 (*7*), has stressed the urgency for investigation of the etiology and prevention of anemias, in view of the widespread prevalence of this condition in the developing countries of the tropical and subtropical zones throughout the world.

Although deficiency of several essential nutrients may result in anemia, those deserving primary consideration are iron, folate, and vitamin B_{12}. It is the general consensus of opinion that dietary deficiency, poor absorption and utilization, physiological requirements, or increased losses of these nutrients are the underlying causes of most of the anemias in the world. Associated parasitism may enhance the significance of one or more of these factors.

In the prevention of iron-deficiency anemia, several approaches may be considered. Long-term goals can be achieved through programs designed to improve the dietary intake of iron by means of education and increased availability of iron-rich foods, and through environmental sanitation campaigns to control parasitism associated with blood loss. Immediate and short-term measures may consist of iron supplementation for vulnerable groups, such as school-children and pregnant and lactating women, as well as enrichment and fortification of foods consumed by infants and preschoolers and by all segments of the population. Long-term programs such as sanitation, education, and food production must be supplemented by more immediate methods, and, undoubtedly, along with the distribution of prophylactic iron to the vulnerable groups, the fortification of foods with iron is an urgent need in most problem areas.

Long-term programs

Dietary measures

The optimum dietary iron requirement may be defined as the amount and kind of food iron that will make it possible for absorption to cover physiological losses and demands in all subjects under all physiological conditions, including growth and pregnancy.

In areas where iron-deficiency anemia is common, an increase in iron intake through natural foods is desirable. This alone will not overcome anemia, but it will help to maintain normal hemoglobin levels once other measures have corrected the established disease. It is difficult to change the dietary habits of people, but nutrition education programs carried out in maternal and child health centers and schools and among other groups over many years have been shown to produce permanent improvement. Increasing the availability of iron-rich foods by expanding their production and marketing and by reducing their price will help to induce the people to consume them in greater quantity.

Data already presented at this meeting have shown that the absorbability of iron in different foods varies considerably (*3*). In assessing the nutritional sufficiency of dietary iron, it is necessary to know not only the total amount of iron in the diet but also the relative contribution of iron from different foodstuffs and the possible effect of one food on the absorption of iron from another. Attention should also be drawn to unsatisfactory cooking practices, which may result in iron loss and/or decreased availability.

In many countries in which iron deficiency is common the diets are also deficient in other nutrients. Action programs should therefore be directed toward an over-all improvement of the quality of these diets, particularly with respect to protein.

Environmental sanitation

Control or elimination of an underlying disease process such as hookworm, trichiuris infection, or schistosomiasis is highly desirable. In this respect, emphasis should be placed on the interruption of transmission of parasites by improvement of environmental sanitation and personal hygiene practices, as well as by effective treatment of the infected persons. These measures should be intensified, particularly in rural areas where there is a high prevalence of hookworm infection. Past experience has shown, however, that this is not an easy task; it takes a long time and results cannot be expected to be spectacular. In general, the effectiveness of any sanitation program is intimately linked to raising the standard of living of the population.

Short-term measures

Iron supplementation for pregnant and lactating women

There is a high incidence of iron deficiency among pregnant and lactating women throughout the world, and from consideration of iron balance it is clear that the requirements of pregnancy cannot be met by diet alone or made available from stores. Iron intake during the nonpregnant state should be adequate to allow an accumulation of iron stores that can be drawn upon in the last trimester of pregnancy and during lactation. Pregnant women will derive great benefit from a routine supplement of a ferrous salt containing at least 60 mg of elemental iron, given once daily during the second and third trimesters of pregnancy and the first six months of lactation (*8*). Such treatment will supply iron for both the mother and the infant, thus providing for the needs of the two groups that in any community will have increased requirements for iron.

Iron supplementation for schoolchildren

Studies have shown that improvement in hemoglobin levels can be obtained in children heavily infected with parasites by the daily administration of small amounts of iron for a specified period of time. Once the regimen is discontinued, however, their hemoglobin levels return to pretreatment status within several months.

In areas where surveys on schoolchildren show a high prevalence of anemia and parasitic infection, a combined program of deworming and treatment, with distribution of 30 mg of elemental iron per day in ferrous form throughout the school year, may provide good results as an emergency action (*4*). An evaluation of the cost/effectiveness of this type of program should always be carried out in a pilot area, however, before it is implemented on a national scale.

Enrichment and fortification of foodstuffs

In the opinion of the PAHO/WHO expert groups (*3*), enrichment of food with iron is one potentially effective method of preventing iron deficiency. Iron enrichment of wheat flour as well as food for infants is currently practiced in some countries, but as yet little information is available concerning the effectiveness of this procedure.

Before a food fortification program is undertaken, it is essential that the problem of anemia be carefully investigated. The level of existing iron intake should be assessed, and the foods most suitable for enrichment should be selected,

taking into consideration their current use by the population of the region or country in question. The selection of a suitable iron compound as an enriching agent will involve studies on the absorbability of the enriched foods, the stability of the enriched product, and the cost and economics of production and marketing, as well as its acceptability. Pilot trails should be conducted to determine its effectiveness before it is applied on a national scale.

The choice of iron enrichment level will depend on the quantity of the food ordinarily consumed. The level should be adjusted so as to afford at least partial protection for vulnerable groups without exposing the others to the risk of excessive iron stores. While an enrichment program is being carried out, the hematological survey must be continued in order to check on the program's effectiveness, as indicated by the attainment and stabilization of normal hemoglobin levels by the majority of the population at risk.

This stepwise development is essential to ensure a program that can be recommended with confidence for adoption by governments.

In general, food-enrichment programs should be directed toward all segments of the population and tailored to the existing levels of deficiency. Special attention should be given to reaching economically deprived groups and children, both by the fortification of infant formulas and by the development of high-protein foods for preschoolers.

The recent (1968) recommendation of the U.S. National Research Council (*16*) and INCAP (*2*) for iron intake is 15 mg a day during the first year of life. This amount is difficult to achieve with any diet that is not fortified with iron, and many authorities in the field have suggested routine prophylaxis of some kind.

The most simple and economic method, as well as the most effective, is the incorporation of iron into formulas and/or special high-protein foods for infants and preschoolers, especially in underprivileged groups. Several studies on the use of iron-fortified formulas have shown satisfactory results, and good utilization of iron has been demonstrated even in the earliest months of life. In a recent study it was demonstrated that the administration of 12 mg of iron per quart of reconstituted formula, beginning at birth or at three months of age, reduced the incidence of anemia from 76 to 9 per cent (*1*).

Conclusion

The prevention of iron-deficiency anemia remains a continuing challenge to the public health worker in the Western Hemisphere until such time as simpler and more effective techniques are developed to improve population iron stores and to meet the special requirements of vulnerable groups.

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GENERAL DISCUSSION

Moderator: We shall now have a discussion of the papers we just heard.

Dr. Cohen: I would like to ask my clinical friends here what is "clinically" wrong with an adult male who has 12 grams of hemoglobin and four million red cells.

Moderator: My impression is that little is known about the influence of anemia on behavior, particularly in borderline anemias. In fact, I remember a patient that walked into the hospital with 1.5 grams of hemoglobin. We asked him what was wrong with him and he said he didn't feel particularly bad. I think a great deal of old-fashioned human physiology and psychology come into the picture, and further research needs to be done in this area.

Dr. Cohen: I am concerned that we may have created the appearance of a widespread anemia by fixing on a level of hemoglobin and red cell count that probably was established initially by using the population of medical students, physicians, nurses, and so forth as a standard.

Returning to my previous question, I would like to know if you can diagnose a man with 12 grams of hemoglobin and four million red cells as having "anemia" without knowing this information from the laboratory. Aside from the fact that he may have lost a little color, what is wrong with such a person, and are we really talking about anemia as a significant clinical factor when we are dealing with levels as high as this?

Dr. Moore: It is certainly conceivable that for a given individual such values would be normal. The only way to make reasonably certain would be to correct any suspected deficiency or to treat successfully any disease discovered, and then determine whether erythrocyte values rise.

Your question asks what harm really results to an individual who has a hemoglobin level of 12 grams when the normal level for that person might be 15 grams per 100 ml. There is no convincing evidence that his health or performance is impaired by a difference of that magnitude.

Dr. Finch: I think we get into a problem if we approach deficiency states through anemia. On the one hand, you can raise the question of what is adequate or optimum iron nutrition and deal with that, but if you have to interpose anemia, which is difficult to diagnose in any one individual, you raise another question that obscures the first. I would make a plea for separating these two and considering that anemia is only an indication of the intensity of a deficiency state.

Dr. Cohen: All the information we have from the surveys and the recommendations for improvement of "insufficiency" are based on the fact that a large group of individuals have 12 grams of hemoglobin as opposed to a questionable standard of 15 grams. I am not minimizing the possibility that the observation might be important, but I would like to know what evidence there is to establish this.

Colonel Conrad: I would like to answer your question by an experiment. If you take an individual and make him methemoglobinemic by giving him oxidant drugs and you produce 25 per cent methemoglobinemia, you have an individual who is very close to 12 grams of hemoglobin in terms of functional oxyhemoglobin. He is symptomatic at this level. You now take that same individual and give him methylene blue; the methylene blue will convert his hemoglobin over a period of several hours back to oxyhemoglobin, but his symptoms disappear almost immediately, indicating that something other than the conversion of methemoglobin to oxyhemoglobin was responsible for his symptoms. It may be cytochrome or catalase that is more important in terms of the symptoms which some of these people certainly claim they have.

Dr. Cohen: I am sorry to persist in this. I am not sure that an erythrocyte with 25 per

cent methemoglobin will function adequately. The presence of this amount of methemoglobin could have a very profound effect on the function of hemoglobin. But I am worried that we are fixed on a set of numbers. I insist that we must establish whether the value of 15 grams of hemoglobin is either valid or not valid before we go making the world "rustier" than it is by giving iron to everybody. I am only raising the question of whether or not this number really has the meaning that has been read into it in the traditional view of what constitutes anemia.

Moderator: Probably one of the answers for the case with four million red cells would be to give the man iron and see whether his hemoglobin goes up, and whether he feels better. In the long run, the old-fashioned clinic will ask patients how they feel and what symptoms they have.

Dr. Cook: On the positive side, if you increase the number of subjects in the population with a hemoglobin of 12, then you are going to increase proportionately the number with a hemoglobin of 6. I think in this sense your example may be important as a reflection of the severity of nutritional anemia in the population as a whole. I also think it is unfair to choose the male for your example, because the consequences of iron-deficiency anemia may be much less important than in the female.

Dr. Waterlow: I think Dr. Sánchez-Medal gave in his tables 10 grams as the level below which infants were considered to be anemic; it seems to me that we really know extremely little about what one might call the normal or expected levels in infants—even less than in adults. Since this is one of the groups with which we are particularly concerned, it is rather an important point.

I wanted to ask another question, too. Dr. Gandra's tables showed that hemoglobin levels tended to be elevated among people living at high altitudes in Bolivia and Ecuador. Are their diets better, or could one say that if they need the iron they absorb it better—just as we find that infants recovering from malnutrition absorb 50 to 70 per cent of the iron in their diet?

Dr. Gandra: What we know from studies carried out in Peru is that when people go from low to high altitudes the rate of iron absorption increases. However, the amount of iron in the food at high altitudes is not necessarily different from that in the diet at low altitudes. Of course, this varies according to the data you are considering.

Dr. Waterlow: This is very interesting, because you could argue from this that if somebody has a hemoglobin of 12, that is all he needs; he would have absorbed more if the stress or need had been present.

Dr. Sánchez-Medal: Dr. Waterlow, what figure would you like to fix for speaking of anemia in infants? I selected 10 grams because it has been used most widely.

Dr. Waterlow: I don't know. I am asking for information, really.

Moderator: Since most statistics on so-called "normal" groups include a good number of people with iron deficiency and low hemoglobin, it is probable that the "cut-off" levels are rather too low than too high.

Dr. Finch: I think this is true. The purpose of defining anemia has been to get a level below which the clinician might find disease. Any point that is taken will have to include, from a physiologic sense, certain people below the line of separation who are quite normal and others above the line who are really anemic. We are looking at a system of oxygen transport that has great reserve in regard to cardiac output, hemoglobin level, and oxygen availabilty. It has recently been shown that there is an inverse correlation between 2+3 diphosphoglycerate which can shift the oxygen dissociation curve and the hemoglobin level. So I think we have to presume that a number of factors are interacting.

Dr. Gandra: What is normal and what is not is a pretty difficult question to answer. I just want to say that we have often worked with schoolchildren having less than 10 or 9 grams of

hemoglobin, and we have used different treatment schemes. Sometimes the general average of hemoglobin concentration did not reach 11 grams per 100 ml. Perhaps part of the answer has to do with the relationship of hemoglobin concentration to metabolic activity in these children.

Moderator: Going back just for a minute to the effect of anemia on the individual, particularly his work performance, I think very few studies have been done on this subject so far. There is an old paper by Colonel Lane filled with letters that he received from the heads of coolie estates. These people wrote that they were very thankful to him because since the worms had been removed from the coolies they could carry many more bags of tea in a day; the actual number of bags of tea was mentioned. This is about the only study I know of, but I think it is significant because it deals with actual work performed *in situ*.

I am wondering why there is so little motivation to do something about these anemias. People don't really complain too much when they have them. Perhaps this explains why so little is done. Here in Dr. Chopra's abstract something is written that amazes me. It says, "Iron enrichment of certain foodstuffs poses few if any technical problems; thus, the means to prevent the nutritional deficiency exist." I rather doubt whether the matter is that easy. If this is true, why isn't food being enriched and distributed with larger amounts of iron? Is it because the problem does not appear to be very important?

Dr. Cohen: Does a nutritional deficiency exist in someone who has a hemoglobin level of 12 grams? If it doesn't, is the international effort warranted to try to boost everybody up to 15 grams of hemoglobin?

Moderator: In our countries there is no problem, because there are many people well below that level.

Dr. Kevany: Just to answer your specific question about whether or not the means exist, the technical aspects of food processing are such that there are no serious problems presented to enriching cereals with iron anywhere in the hemisphere. The major problem is that around 50 per cent of the people live in rural areas on a subsistence type of economy, where the food supply is at no stage processed outside of the household itself or the local village mill.

Moderator: These are the very people who need it most.

Dr. Kevany: Iron can be added to salt. There is no technical problem that I am aware of, provided you can get a stable iron compound and reasonably high-quality salt. A lot of salt has chemical impurities and a very high moisture content, which makes it more difficult to stabilize as an enrichment vehicle.

Dr. Zubirán: Undoubtedly the problems of deficiency are very important, especially in rural areas, but the deficiencies in protein are much more important. In Mexico half our population eats corn. This corn contains sufficient quantities of iron. We would not need to enrich it in any way. It would be fine if only we could achieve the absorption of this iron. In order to provide better nutrition to the rural population in Mexico, we would have to enrich their diet with ascorbic acid and soybean, which we feel are the most appropriate elements.

I would like to ask Dr. Layrisse for his opinion on the following question: In a population that consumes 400 grams of corn per person a day and which has much more than 10 mg of iron in the diet, would the problem of iron absorption be solved by adding or supplementing this corn-and-black-bean fare with soybean? Would we improve nutrition by this addition?

Dr. Layrisse: Is this a course, dry material, or is it cooked?

Dr. Zubirán: It is dry.

Dr. Layrisse: We know now that inorganic iron absorption is reduced about 80 per cent when mixed with corn. For instance, if you give, let us say, 1.0 mg of supplemented iron with 100 grams of corn, you will get something like 0.03 mg of absorption from inorganic

iron. I think we have to know more about the interaction between inorganic iron and various foods before we start programs on food iron fortification.

Moderator: Do the people in Asia eat much soybean?

Colonel Conrad: The diet in Asia varies considerably from one country to another. Soybean is eaten in some countries in fairly large amounts because it is imported or grown. In seacoast countries, fish is eaten because it is available. Inland, less fish is available, and I believe there is a greater incidence of iron deficiency.

Moderator: I think we have looked at the iron problem rather thoroughly. One of the things that impresses me in the whole matter is the fact that iron, unlike many of the metabolites in food, is vulnerable and seems to be always on a borderline of sorts. Even those people who absorb enough iron have very little in the way of reserve, so that the least loss over what is normal can cause important changes.

Another thing that is impressive—I think Dr. Finch showed this—is the slowness of iron metabolism and the fact that the effect of an event that takes place today may show itself many months later. For example, in the case of phlebotomy, the effect on iron absorption still appears one year later. This explains also —I failed to mention it when I discussed the hookworm situation—why the impact of a marked, short-lived infection may show itself many months later, especially when iron absorption is very low.

I am still puzzled as to why something more drastic or aggressive is not being done about the iron situation. I think that at least the school population could be treated routinely with prophylactic quantities of iron.

One major area in which work should be done is that related to the effects of anemia and what it means from the socioeconomic point of view.