IRON METABOLISM

BY

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Ten years ago physiologists still taught that the iron metabolism of the body was regulated by a balance between absorption from the stomach and small intestine and excretion by the colon. McCance and Widdowson (1937a) first challenged this theory, arguing from facts already known that there was no evidence of excretion of iron by the body except in very small amounts, and that therefore the iron balance must be maintained by a regulation of iron absorption. In confirmation of this view McCance and Widdowson (1938) showed that when normal subjects were given iron by mouth they excreted an equal amount in the faeces, whereas when iron was given by intravenous injection the whole was retained and none excreted. The logic of this hypothesis compelled its immediate acceptance, but it could not be finally proved until a dose of iron could be "tagged" by the radio-active element so that its whole course through the body could be followed.

The principles of experiments with radio-active elements are now familiar. A minute proportion of the atoms is made radio-active by artificial means. The whereabouts and number of such atoms can be traced by measuring their radio-activity, so that they can be used to "tag" a much greater number of normal atoms, from which they do not differ either chemically or biologically. Radio-active iron is unfortunately one of the more difficult elements to work with, for its radiation is very soft and is readily absorbed by other metals. The radio-active iron must therefore be isolated in a very pure state before it can be measured, and even the metallic iron must be in a very thin layer to avoid errors from self-absorption of radiation.

Physiological Experiments with Radio-active Iron

Radio-active iron appears in the plasma soon after it is fed to a dog and reaches its maximum concentration in four to eight hours. It is, however, quickly taken up by the storage depots, and after a few hours none can be detected in the plasma (Hahn and others, 1939). The iron in the

storage depots is very mobile, for radio-active iron can be detected in the red cells within four hours of its administration by mouth. The amount of radio-active iron in the circulating red cells thereafter increases progressively to a maximum which is reached in four to seven days in anaemic dogs (Hahn and others, 1940). The level subsequently remains constant. It can therefore be presumed that all the radio-active iron absorbed has been used in haemoglobin formation, and that none remains in the storage depots.

Much work with radio-active iron has been based on this assumption that iron utilization is a measure of absorption. The supposition is certainly justified when there is iron-deficiency anaemia, in that Dubach and others (1946) have shown that even injected radio-active iron is completely utilized in six to nine days by dogs and men in this condition. In normal animals, however, there is a greater probability that some radio-active iron absorbed from the intestine may be diverted to storage depots and not appear in the circulating red cells during the course of the experiment, but it is unlikely that errors from this source have been great enough to invalidate the major conclusions from the experiments to be described below.

Iron Absorption

Iron is absorbed only in the stomach and small intestine, and not at all in the colon. When a dog is given radio-active iron by mouth the highest plasma level is reached in four to eight hours, and since this is the time when the meal is in the small intestine it is here that the maximum absorption takes place. After eighteen to twenty-four hours, when the whole meal has passed into the colon, no further absorption of radio-active iron occurs (Hahn and others, 1939a). It has also been shown that radio-active iron is absorbed into the blood stream from gastric, duodenal, and jejunal pouches in the dog (Hahn and others, 1943).

A man's daily loss of iron from the body is of the order of only 1 mg., so there must evidently

be some selective control of iron absorption—otherwise haemochromatosis would be a common complication of old age. The gap between an adequate supply and a deficiency of iron is, however, very narrow, and many factors may inhibit the absorption of iron which the body needs to maintain the haemoglobin level.

The control of iron absorption.—The body absorbs iron according to its needs, in health only enough to replace the small daily loss, but in iron deficiency as much as is offered and physiologically available. It is a common experience that a patient being treated for iron-deficiency anaemia may regenerate haemoglobin at a rate which demands the absorption of 50 mg. of iron a day, whereas McCance and Widdowson (1938) showed that when extra iron is given to a normal individual he excretes it all in his faeces. Hahn and others (1940) showed very clearly the difference in radio-active iron absorption by normal and anaemic dogs. Whereas a normal dog would absorb only very little of a test-dose given by mouth, and a plethoric dog would absorb practically none, dogs suffering from chronic iron-deficiency anaemia might utilize more than half of a small test-dose for haemoglobin production.

However, the degree of iron absorption is not determined by the haemoglobin level *per se*. Thus the intestinal iron absorption of a normal dog is not immediately increased if it is made suddenly anaemic by bleeding, but after a week, when the iron reserves are depleted by haematopoiesis, a test-dose of radio-active iron is vigorously absorbed. Nor will prolonged anoxia increase iron absorption.

Studies in man have given very similar results (Balfour and others, 1942). Normal individuals absorb very little of a test-dose of radio-active iron given by mouth, but absorption is ten times as great in patients with chronic iron-deficiency anaemia. In polycythaemia, on the other hand, only a trace of radio-active iron is absorbed, in spite of the excessive formation of red cells and haemoglobin.

Pregnant women, whether they are anaemic or not, absorb between two and ten times as much of a test-dose of radio-active iron as do normal individuals (Balfour and others, 1942). Here the degree of iron absorption evidently depends upon the demands of the foetus rather than upon any state of anaemia in the mother.

The iron-absorbing powers of the intestine are readily exhausted. Thus a dose of an iron salt given by mouth will diminish the absorption of radio-active iron administered one or two hours

later. The inhibition is not due to the elevation of the plasma iron level by the first dose, for an intravenous injection of iron does not reduce the absorption of radio-active iron given by mouth (Hahn and others, 1943).

Hahn has explained the above facts by postulating that the cells of the intestinal mucosa contain a protein receptor which can receive iron ions from the intestinal contents and yield them to the plasma. Thus the absorption of iron from the intestine depends on the presence of free places in the receptor, and the rate of removal of iron from the intestinal cells into the blood is determined by the level of the plasma iron and its equilibrium with the tissue reserves. The reverse process, a transfer of iron from the plasma to the intestinal cells, is not possible because the plasma iron is attached to a plasma protein, probably globulin, and cannot be yielded to the intestinal receptor. The intestinal mucosa is very readily saturated with iron—within two hours if enough iron is available in the food—but desaturation into the plasma is not complete for some days.

Granick (1946) has recently brought forward evidence that the iron receptor in the intestinal mucosa is apoferritin, the protein which binds the greater part of the tissue-storage iron (*vide infra*). Iron combines with apoferritin to form ferritin, and the amount of this compound in the intestinal mucosa increases after feeding with iron, and falls again after several days. There is evidently some local mechanism for the formation or accumulation of apoferritin in the intestinal mucosa, for the total amount of ferritin + apoferritin increases in response to iron feeding.

Very little radio-active iron appears in the red cells of patients with pernicious anaemia when they are given a "tagged" iron salt by mouth. This finding has been interpreted as meaning that the patients, in spite of their severe anaemia, absorb no more iron than do normal people because their iron stores are not depleted. Dubach and others (1946), however, have cogently argued another interpretation. They point out that the blood-level of radio-active iron after a week or so measures only the iron utilized in haemoglobin synthesis, and that much more iron might have been absorbed and stored unused. They have in fact shown this to be the case in a man who had pernicious anaemia without any iron deficiency. In this patient only about 1 per cent of a test-dose of 57 mg. of radio-active iron appeared in the circulating haemoglobin until liver treatment was started nine days later. Then, as the haemoglobin level rose the proportion of the test-dose of radio-active iron in the blood likewise increased

until more than 20 per cent of the administered dose was found in the circulating haemoglobin. Thus the initial low level of circulating radio-active iron was a sign not of small absorption but of deficient haemoglobin synthesis and iron utilization. Dubach and others (1946) have made similar criticisms of the common statement that iron absorption is not increased in haemolytic anaemias.

The theory that iron metabolism is regulated by selective absorption from the stomach and intestine and not by excretion will doubtless demand many similar modifications in detail, but its basic truth still rests on the experiments and reasoning of McCance and Widdowson (1937a, b, 1938, 1943).

Iron valency and absorption.—It is the universal experience of clinicians that in the treatment of iron-deficiency anaemia ferrous salts are effective in about half the dose of ferric salts. Table I (from Witts, 1936) shows the iron content of the average daily doses of ferrous and ferric

TABLE I

IRON CONTENT AND UTILIZATION OF THE AVERAGE EFFECTIVE DOSE OF VARIOUS THERAPEUTIC PREPARATIONS (WITTS, 1936)

Preparation	Iron content of average dose	Per cent utilization
Ferrous chloride ..	100–200 mg.	12.5–25
Ferrous sulphate ..	180 mg.	14
Ferrous carbonate (Blaud's pill)	300–400 mg.	6–8
Ferric chloride ..	400 mg.	6
Ferric citrate ..	400 mg.	6
Iron and ammonium citrate	800–1,600 mg.	1.5–3

salts necessary to raise the total blood iron by 24 mg. per day, equivalent to a haemoglobin increase of about 0.14 g. per 100 ml. It is evident that ferrous salts are absorbed and utilized at least twice as efficiently as ferric salts.

Moore and others (1939) showed that serum iron absorption curves after the administration of iron salts were higher with ferrous than with ferric salts. The difference has been more accurately demonstrated by giving the same subjects successive doses of radio-active ferrous and ferric salts. Table II summarizes some of the experiments of Moore and others (1944). Normal men utilized 1.5 to 10 times as much ferrous as ferric salt, and in hypochromic anaemia the ratio was 2 to 15. Likewise Hahn and others (1945) showed that

patients with hypochromic anaemia utilized 2 to 6 times as much ferrous as ferric salt when given successive test-doses of radio-active iron. Iron utilization can be taken as a measure of iron absorption in iron-deficient subjects if not in normal individuals, so that the superior absorption of ferrous ions is clearly demonstrated.

TABLE II

UTILIZATION OF FERROUS AND FERRIC RADIO-ACTIVE IRON SALTS BY NORMAL MEN AND IN HYPOCHROMIC ANAEMIA (MOORE AND OTHERS, 1944)

Form of iron	Dose given	Normal men		Hypochromic anaemia	
		No. of cases	Per cent utilization	No. of cases	Per cent utilization
Ferrous	4 mg.	4	7	2	12.5
Ferric	4 mg.	4	2.5	2	4
Ferrous	2 mg.	3	7.5	2	27.5
Ferric	2 mg.	3	2	2	4
Ferrous	1 mg.	2	9	1	39.5
Ferric	1 mg.	2	1.5	1	17

The same rule does not apply to all animals. Thus rats absorb ferric salts as well as ferrous (Austoni and Greenberg, 1940), as do many dogs, although the majority of both normal dogs (Moore and others, 1944) and anaemic dogs (Hahn and others, 1945) absorb a greater proportion of bi-valent radio-active iron salts.

Moore and others (1944) offered three explanations for the superior absorption of ferrous salts. It is possible that only ferrous salts are capable of absorption, or that ferrous salts are much more readily absorbed than ferric, or that the ferric ions more readily form insoluble compounds in the intestine.

Relation of dosage to iron absorption.—Clinical experience has shown that the response of iron deficiency anaemia to medication depends upon the dose of iron given, and that there is often a minimum "threshold" dose below which treatment is ineffective. It is not clear to what extent this varying response depends upon the proportion of iron absorbed from the intestine, and to what extent upon iron utilization for haemoglobin synthesis. Most experiments with radio-active iron have shown that the higher the test-dose the less is the proportion which appears in the red cells, even though the total utilization may increase (see, for example, Table II). This has commonly been taken to mean that the proportion of iron

absorbed decreases with increasing dose, but it may be rather that with a larger dose a less amount of the iron absorbed is utilized. Brock and Hunter (1937), for example, showed by balance experiments that when large doses of iron were administered by mouth, twice as much iron might be retained in the body as was utilized for haemoglobin synthesis.

Dietary sources of iron.—It is easy to estimate the total iron content of a foodstuff or diet, but it is not usually possible to say what proportion of the iron is potentially available for haemoglobin synthesis. It is now known that only ionic iron can be absorbed from the intestine, but there is no rule for determining how much of the food iron can be freed in the ionic state by digestion. Iron also very readily forms insoluble compounds with various constituents of the diet, especially in a medium which is not strongly acid.

It has been amply demonstrated by both clinical and experimental studies that ionic iron is readily absorbed by the stomach and small intestine, but there is no evidence that any pre-formed iron compound can be absorbed as such and used for haemoglobin synthesis. The dietary iron is in two forms—inorganic, which readily passes into solution, and organic, chiefly porphyrin compounds which must be broken down before ionic iron is liberated. The relative proportions of the two can be distinguished by the α -dipyridyl reaction for ionizable iron, and at one time many estimates were made of the “availability” of iron in food by this reaction. Such estimates, however, bear only a very rough relation to the truly physiological availability of dietary iron. Both ferrous and ferric chloride, for example, are “100 per cent available” according to the α -dipyridyl reaction but neither is completely absorbed from the intestine even in severe iron-deficiency anaemia, and at least twice as much of the ferrous as of the ferric salt is assimilated.

Some of the “non-available” food iron which is not estimated by the α -dipyridyl reaction may also be broken down and absorbed. Thus Black and Powell (1942) found that 10 to 20 per cent of haemoglobin iron was absorbed when a litre of citrated blood was given by duodenal tube. They considered bacterial decomposition in the intestine to be important in making this iron available.

The chemical nature of iron compounds in foodstuffs is, however, probably less important for their absorption than other even less easily defined factors. Conditions in the small intestine, except in its upper part, are very unfavourable to the absorption of iron. Iron salts are scarcely

dissociated in solution at a pH above 5.0, so that few ferrous, and even fewer ferric, ions are available for absorption except in distinctly acid medium. It is not surprising, therefore, that iron-deficiency anaemia is commonly associated with gastric achlorhydria.

Iron phosphates are practically insoluble, and the ratio of phosphorus to iron in ordinary diets often exceeds 50, so that there is abundant opportunity for the formation of iron phosphates in the intestine. Phosphates may inhibit iron absorption even when no precipitate is formed, for Brock and Taylor (1934) found that the dialysis of iron salts across cellophane membranes was greatly decreased in the presence of neutral or alkaline phosphates.

Iron forms undissociated compounds with a number of amino-acids and phosphorus-containing substances such as nucleic acid, and McCance and others (1943) have emphasized that an excess of phytic acid in the diet may lead to the deviation of much iron as insoluble phytate.

An excess of calcium in the diets of animals usually inhibits iron absorption so that a mild iron-deficiency anaemia develops, but under some circumstances an excess of calcium favours iron absorption. Thus the excess phytic acid of wholemeal bread may be neutralized by calcium (McCance and others, 1943), and when the phosphate content of the diet is high a high level of calcium diminishes the formation of insoluble iron phosphates.

The vitamin content of a diet may have a direct influence on iron absorption. Ascorbic acid reduces ferric compounds to ferrous *in vivo* as well as *in vitro*, and thus facilitates absorption (Moore and others, 1939). Extra vitamin D increases haemoglobin formation and iron storage in young rats (Fuhr and Steenbock, 1943).

It is evidently difficult to decide whether a given diet will supply enough iron to maintain the haemoglobin level, and in practice the question is decided by experience rather than by any scientific knowledge of the physiological availability of iron in various foods. An ordinary mixed diet of the type eaten in England will provide enough iron for a normal man if its total iron content exceeds 5 mg. daily, and a total of 15 mg. daily will suffice a normal woman or growing child. The average English diet to-day probably gives 15–20 mg. of iron daily, an amount which in theory is rather more than adequate for women and children, and generous for men. The margin for women is small, however, and it requires relatively little disturbance of iron absorption from such causes as

gastric achlorhydria, or relatively little increased iron loss from menorrhagia or frequent child-bearing, to produce an iron deficiency.

The value of foodstuffs as iron sources is similarly a matter of empirical rather than scientific knowledge. Liver, eggs, dried fruits, chocolate, sardines, and green vegetables are all good sources, but nowadays are not freely available. Oatmeal, peas, beans, and whole-meal bread are also good sources, but white bread contains less than one-third of the original iron of the grain. Such staple foods as meat, fish, chicken, milk products, and beer are relatively poor iron sources.

Iron Transport

The plasma is the chief medium for the transport of iron, which is carried firmly bound to a plasma protein, probably globulin. It is, however, uncertain whether iron for haemoglobin synthesis is thus carried to the bone marrow as ferric ions, or as some pre-formed compound.

Iron is absorbed chiefly, and perhaps entirely, as ferrous ions, but the oxygen tension of the plasma is sufficient for the immediate conversion of the ions to the ferric state. The plasma iron is probably bound to the serum globulin as a ferric hydroxide complex.

Powell (1944) found the mean plasma iron of normal men to be 143 γ ($S=24 \gamma$), whereas the level in women was significantly lower, with a mean of 117 γ ($S=27 \gamma$). There was a significant reduction in the plasma iron of women during menstruation.

The plasma iron rises sharply during the absorption of a dose of iron from the intestine. The height of the increase depends upon the rate of deposition of iron in the tissues as well as upon the rate and amount of intestinal absorption, but plasma iron curves can be used to demonstrate whether iron is in fact being absorbed, and to obtain a rough idea of the relative absorption of different compounds. Moore and others (1939) have shown that the rise in the iron content of the plasma during absorption is not preceded by an increase in the iron content of the thoracic duct lymph, so that iron must pass directly from the intestinal mucosa into the blood stream.

The plasma iron is reduced below normal in patients with iron deficiency, and also during periods of active erythropoiesis such as occur after acute haemorrhage or during the cure of pernicious anaemia. Conversely, the plasma iron is usually high when erythropoiesis is depressed, as in untreated pernicious anaemia or hypoplastic anaemia. The level is very variable in haemolytic

anaemias, depending upon the balance between red cell destruction and haemoglobin synthesis.

Easily split-off iron.—About 5 per cent of the blood iron can be easily dissolved by digestion with decinormal hydrochloric acid, and it was believed until recently that this "easily split-off iron" might be of importance in iron transport. Sixty to seventy per cent of the easily detached iron is derived from the red blood cells, but if the haemoglobin is converted into carboxyhaemoglobin this iron can no longer be removed by acid digestion. Legge and Lemberg (1941) have shown that this fraction is an artefact—when red blood cells are digested with acid, oxygen is liberated from its combination with haemoglobin and oxidizes a variable amount of the corpuscular haemoglobin into open-ring compounds similar to the iron-containing bile pigments, from which iron is easily removed by acid digestion. The remainder of the easily detached iron is derived from bile pigment-haemoglobin, blood catalase, and the plasma iron.

Iron Storage

It is evident from clinical observation that the body normally holds a considerable reserve of iron readily available for haemoglobin synthesis. A patient who, through acute haemorrhage, has lost a third or more of his circulating red cells may rapidly regain a normal haemoglobin level without any assistance from iron therapy, although the loss of 700 mg. or more of iron could scarcely be replaced from the diet within six months. We have little knowledge of the size of this available iron reserve. The greater part of the body iron is normally circulating as haemoglobin, and perhaps half of the balance is in the form of myohaemoglobin and cell-enzymes such as cytochrome, and is not available for haemoglobin synthesis. Table III shows Hahn's (1937) estimate of the iron

TABLE III
PERCENTAGE DISTRIBUTION OF THE TOTAL BODY IRON OF
A NORMAL DOG (FROM THE DATA OF HAHN, 1937)

Circulating iron	Available reserves	Non-available iron
Blood haemoglobin.....57%	Liver, spleen, and marrow15% Elsewhere ...5%	Myohaemoglobin 7% Parenchyma iron.....16% (cell enzymes, etc.)
Total 57%	20%	23%

distribution in a normal dog. On a similar basis a normal man would have an available iron reserve of about 850 mg., sufficient to replace about one-third of his circulating haemoglobin.

The greater part of the iron reserve is in the liver, spleen, and bone marrow. Much of the iron in the marrow is present as haemoglobin in formed or immature red blood cells, whereas the reserve iron in other tissues is probably held in the reticulo-endothelial cells.

Many attempts have been made to gain further knowledge of the iron reserves by injecting colloidal iron intravenously into animals, but it is at least possible that the animal treats iron in this form as a foreign particle rather than as a normal dissolved metabolite.

The chemical form of the iron reserves is still not completely understood. Haemosiderin, which histologically is so prominent a product of red-cell destruction, is certainly not the major iron reservoir. It is probably an intermediate stage or side product in the conversion of haemoglobin into bile pigment, but little is known of the chemical reactions involved.

Ferritin.—Iron-storing tissues contain a protein, apoferritin, capable of combining with as much as 23 per cent of its weight of iron. The iron is firmly held to the surface of the apoferritin molecule as small micelles of ferric hydroxide—ferric phosphate to make a protein, ferritin, of variable iron content. Both ferritin and apoferritin can easily be crystallized as the cadmium salt, and minute quantities of either can be detected by a precipitin reaction with an anti-apoferritin serum.

Studies with radio-active iron have shown that at least a part of iron which is injected is quickly converted into ferritin, and that when "tagged" red cells are lysed the liberated iron can be detected in ferritin. It seems probable that ferritin functions as the most important, though not the only, store of iron readily available for haemoglobin synthesis. It may also be the intestinal iron receptor (*vide supra*). The chemical properties and functions of ferritin have been well reviewed by Granick (1946).

Some of the earliest studies with radio-active iron showed that the iron liberated by destruction of effete red blood cells was immediately re-used for haemoglobin synthesis in preference to the reserve iron stores (Hahn and others, 1942; Cruz and others, 1942). Greenberg and Wintrobe (1946) have pointed out that when a small quantity (about 5 mg.) of radio-active iron is injected into a normal individual, about 15 per cent of the dose is built into haemoglobin each day. If the injected

iron merged with the whole available iron reserve the utilization should be only 2 to 3 per cent daily, whereas if it were used in proportion to the daily liberation of iron from effete red cells the daily utilization should be about 83 per cent. It therefore appears that about one-quarter of the iron reserves form a "labile iron pool" used for normal haemoglobin synthesis. Dubach and others (1946) have also shown that injected iron, and presumably, therefore, iron from recently lysed red cells, is used for haemoglobin synthesis in preference to the iron reserves.

Iron Excretion

McCance and Widdowson (1937a, 1938) first demonstrated that iron excretion in man is too small to play any significant part in the control of iron metabolism. They pointed out that the faecal iron output varies with and nearly equals the food iron, and that when iron is injected practically none is excreted. In one dramatic experiment (McCance and Widdowson, 1937b) they treated a polycythaemic patient with acetyl-phenylhydrazine and reduced his haemoglobin level from 168 to 47 per cent Haldane. They calculated that over 6 g. of iron was liberated from the lysed red cells, yet less than 0.5 per cent of this amount was excreted. More recently (1943) the same authors transfused 28 pints of blood into a patient with haemolytic anaemia in a hundred days. All this blood was haemolysed, liberating about 80 mg. of iron a day, yet the total daily iron excretion (5.2 mg.) was slightly less than the dietary intake (5.6 mg. per day), so that none of the iron freed by haemolysis can have been excreted.

Hahn and others (1939b) followed the fate of radio-active iron injected as gluconate into dogs. The urinary excretion was negligible, apart from a "spill-over" immediately after the injection, and the faecal excretion was as small in plethoric as in anaemic dogs. Any considerable haemolysis of red cells increased the faecal iron excretion, and the authors received the impression that most of the iron thus excreted was derived from the bile.

Biliary iron excretion.—Hawkins and Hahn (1944) have recently studied the biliary excretion of iron by establishing biliary fistulae in dogs. After determining the basal daily iron and bile pigment excretion they produced severe haemolysis with acetyl-phenylhydrazine. The biliary iron and pigment excretion rose in parallel, sometimes as much as tenfold, so that it was reasonable to conclude that the biliary iron, like the pigments, was derived from the breakdown of haemoglobin. The iron was probably a part of the pigments, for

iron injected intravenously was not excreted in the bile.

Hawkins and Hahn concluded from their experiments that about 3 per cent of the iron freed by haemolysis was excreted in the bile, and that the remainder was stored or re-used for haemoglobin synthesis.

Urinary iron excretion.—The importance of the urinary iron excretion is still uncertain. The careful analyses of Barer and Fowler (1937) suggest that the normal daily excretion of iron in the urine of men is 0.4 mg. and in women 0.5 mg.—a substantial proportion of the total daily loss. Other workers have regarded the urinary iron excretion as negligible, and derived from cell debris rather than from ionic transfer across the glomerular membrane.

Daily Iron Requirements

The minimum daily iron requirements are evidently equal to the total loss from the body by excretion in the bile and urine, desquamation of cells, and haemorrhage. The necessary dietary intake, in order to allow for unabsorbed iron, must of course exceed these physiological requirements, but a calculation of the latter provides a basis for comparing the needs of different classes of individuals.

Normal men.—An average man has 6 litres of blood with a haemoglobin content of 15.5 g. per 100 ml., so that his total circulating haemoglobin is 930 g. If we take the average life of the red cell to be 120 days, the daily breakdown of haemoglobin is $930/120=7.75$ g., containing 26 mg. of iron (1 g. of haemoglobin contains 3.34 mg. of iron). If we assume that man, like the dogs of Hawkins and Hahn (1944), excretes 3 per cent of the liberated iron in the bile, the daily loss by this route is 0.8 mg. Urinary iron excretion, according to Barer and Fowler (1937), amounts to 0.4 mg. daily, giving a total iron excretion of 1.2 mg. per day (Table IV).

TABLE IV

DAILY IRON LOSSES OF NORMAL MEN AND WOMEN

	Men (mg.)	Women (mg.)
Biliary excretion	0.8	0.6
Urinary excretion	0.4	0.5
Menstruation	—	1.0
Total	1.2	2.1

Normal women.—A similar calculation can be made for normal women, but the iron loss of menstruation must be added. McCance and Widdowson (1937a) found by direct analysis that normal women lost 10–40 mg. of iron in each menstrual period, so that the average loss can be expressed as about 1 mg. per day over the whole menstrual cycle. A woman must therefore replace nearly twice as much iron each day as a man (Table IV), and even moderate menorrhagia may raise the requirements to 5 or 6 mg. daily.

Child-bearing.—Davidson and Fullerton (1938) calculated that a pregnant woman contributes 400 mg. of iron to the foetus and 150 mg. to the placenta and uterus. A blood loss in parturition of about 500 ml. accounts for a further 175 mg. of iron, making a total of 725 mg. The greater part of this iron drain occurs towards the end of pregnancy when the foetus is laying down most of its iron stores, but for convenience of comparison the iron loss may be expressed as an average of 2.7 mg. per day throughout the pregnancy. The total iron demands of a pregnant woman are thus about 3.8 mg. daily—nearly twice as much as a non-pregnant woman needs.

The iron drain of lactation is comparatively light, about 1.5 mg. a day, but when, as often happens, menstruation recommences during lactation the total iron loss is almost as great as in pregnancy.

Children and adolescents.—The increasing blood volume and tissue iron of children makes a considerable addition to their iron needs. Heath and Patek (1937) calculated the annual iron requirements for the increase in blood volume and parenchyma iron in each year of life; their figures make no allowance for any increase in the available iron stores. The additional iron thus laid down in the first year of life amounts to 200 mg., but the figure falls to 100 mg. during the third year and does not rise again appreciably until the ninth year, when the growth rate begins to increase. The amount of iron laid down annually then begins to rise to a maximum of 350 mg. during the seventeenth year, and then falls to zero as growth stops at about the twenty-first year. Thus the extra iron demands of growth never exceed 1 mg. a day, but even this is a substantial addition to the needs of a menstruating girl.

Clinically it appears that the iron needs of children are greater than these calculations admit, and it may be that their greater metabolic rate increases their iron needs.

Iron Losses from Haemorrhage

The reserve powers of the bone marrow are great enough to cope with haemorrhage at a rate of at least 150 ml. of blood a day, provided enough iron is available. A normal man replaces the red cells of 50 ml. or so of blood every day and only uses about one-quarter of his total marrow capacity, but his daily iron loss is only of the order of 1 ml. a day—the iron content of 2 ml. of blood. Even if the amount of iron absorbed from an ordinary diet were 20 mg. a day, a very improbable figure, the marrow would not receive enough iron to double its output. It is not, therefore, the loss of blood which prolongs the anaemia of haemorrhage, but the loss of iron.

Acute haemorrhage.—The blood loss of acute haemorrhage not exceeding one-third of the blood volume is usually quickly made good with the aid of the body's iron reserves, but it is well to remember how enormous is the iron loss in relation to the potential dietary intake. A pint of blood contains about 90 g. of haemoglobin and 300 mg. of iron, and even if the diet were capable of yielding an extra 5 mg. of iron a day in excess of a woman's normal requirements (2 or 3 mg. daily), it would take two months to replenish the iron reserves.

When repeated acute haemorrhages reduce the haemoglobin to below 50 per cent of its normal value the iron reserves will certainly be exhausted before recovery is complete, and unless extra iron is given the final rate of recovery will be limited by the available iron of the diet to something less than 2 per cent per week.

It is evident that iron therapy may be given with advantage during convalescence from acute haemorrhage, for with adequate dosage the body can absorb and utilize more than 50 mg. of iron a day. The idea is familiar to physicians, who most often deal with acute haemorrhage from peptic ulcer, often complicated by previous iron deficiency from chronic haemorrhage, but surgical and obstetrical patients also would probably benefit from iron therapy.

Chronic haemorrhage.—Relatively slight chronic haemorrhage greatly increases the iron losses of the body. Normal blood contains about 50 mg. of iron per 100 ml., so that the loss of only 10 ml. of blood a day increases the iron demands of a man fivefold. The drain of iron may to some extent be diminished by the development of anaemia, for the haemoglobin level and iron content of the blood fall in parallel.

It is uncertain how much haemoglobin iron is reabsorbed when chronic bleeding occurs into the small intestine. The question is of great importance in tropical countries, where iron deficiency from hookworm infestation is a major cause of ill-health. A load of 100 hookworms is only moderate, and will not always cause anaemia, yet, if we accept the usual estimate, each worm withdraws 0.5 ml. of blood a day, making a total iron loss of 25 mg. daily. With really heavy infestations of 1,000 or more worms anaemia is almost inevitable, but even if the blood haemoglobin level is reduced to 3 g. per 100 ml. the blood withdrawn by the worms will contain 50 mg. of iron each day. It is scarcely possible for the whole of this deficiency to be replenished from the diet, so that unless the worms are less voracious than is commonly believed a great part of the haemoglobin iron must be reabsorbed from the intestine.

If, on the other hand, enough iron is supplied the reserve function of the bone marrow is sufficient to maintain the haemoglobin level in spite of chronic haemorrhage, unless prolonged anaemia has led to marrow hypoplasia.

Iron Therapy in Anaemia

It is possible to estimate the actual utilization of iron during recovery from anaemia if the changes in blood volume as well as in haemoglobin level are known. Some examples of such calculations are given below in order to emphasize how great is the amount of iron used in raising the haemoglobin to normal levels.

Iron-deficiency anaemias.—Table V shows the blood volume and haemoglobin level of a young woman suffering from a simple iron-deficiency anaemia. During the first nine days of treatment the total circulating haemoglobin increased by 90 g., corresponding to 300 mg. of iron, an increase of 50 per cent. The blood count did not change during the first four days of treatment, so from the fifth to the ninth day 60 mg. of iron were

TABLE V
TOTAL HAEMOGLOBIN VALUES IN A WOMAN
WITH IRON-DEFICIENCY ANAEMIA

Day of treatment	0	9	23	49
Haemoglobin g. per 100 ml.	6.7	8.5	10.5	13.3
Plasma volume ml. . .	2,120	2,250	2,410	2,100
Blood volume ml. . .	2,830	3,310	3,700	3,690
Total haemoglobin g.	190	280	390	490

Patient's weight: 50 kilogrammes.

being utilized for haemoglobin synthesis each day. Since the anaemia was due to a complete exhaustion of the patient's iron reserves, all of this iron must have been absorbed from the intestine. Exsiccated ferrous sulphate was being given in a dose of 1.8 g. (27 gr.) daily, containing 600 mg. of iron, 10 per cent of which was being absorbed and utilized. At the end of seven weeks the haemoglobin had still not quite reached a normal level, but the total amount in circulation had increased by 300 g., corresponding to 1,000 mg. of iron. Over the whole period of treatment the patient had utilized iron at an average rate of 20 mg. a day.

Before this patient was treated she had a normal plasma volume and, consequently, a low blood volume, so that her haemoglobin level underestimated her total iron deficiency. Her cure demanded not only a return of the haemoglobin level to normal, but also an increase of the total red cell volume to give a normal blood volume. Thus whereas during the course of treatment her haemoglobin level rose to 100 per cent above its original level, the total circulating haemoglobin increased by 160 per cent. These figures illustrate how great may be the discrepancy between the true iron utilization and the apparent utilization calculated simply from the haemoglobin level without regard to the changing blood volume.

Pernicious anaemia.—Theoretically, iron should not be necessary in the treatment of pernicious anaemia, for the haemoglobin level was once normal, and nearly all the iron from the missing red cells is retained in the body. In practice, however, iron deficiency, as shown by a mean corpuscular haemoglobin concentration below 32 per cent, is often present when the anaemia is first diagnosed, or else develops during treatment. Iron deficiency is so common in women that in them it must often precede pernicious anaemia, but the frequent development of iron deficiency during the treatment of pernicious anaemia in men is not so readily explained. Dubach and others (1946) have shown that recently injected radioactive iron is more readily utilized than the tissue iron reserves during the cure of pernicious anaemia, and it may be that much of the great excess of iron in the tissues is not readily available for haemoglobin synthesis.

Table VI indicates the great amount of iron which may be utilized during the cure of a case of pernicious anaemia. The patient, an old woman, had a total blood volume which was low for her weight, though the plasma volume was definitely above the normal. Her total circulating haemo-

TABLE VI
TOTAL HAEMOGLOBIN VALUES IN A WOMAN
WITH PERNICIOUS ANAEMIA

Day of treatment	0	50
Haemoglobin g. per 100 ml. . .	3.9	14.0
Plasma volume ml.	3,070	2,300
Blood volume ml.	3,470	3,800
Total haemoglobin g.	130	530

Patient's weight: 45 kilogrammes.

globin rose during treatment from 130 to 530 g., an increase utilizing 1.34 g. of iron. The patient was a very small woman, weighing only 45 kilos, and on the same basis a man of 70 kilos with pernicious anaemia of the same severity would utilize about 2 g. of iron in raising his haemoglobin to normal.

These figures emphasize the necessity for watching for the development of iron deficiency during the treatment of pernicious anaemia, or, if frequent haematocrit investigations are not possible, of supplementing the liver therapy with adequate doses of iron.

The Anaemia of Infection

Infections in man are commonly complicated by a normocytic or slightly microcytic anaemia which is very resistant to treatment until the infection is cured. The frequency of hypochromia in this type of anaemia suggests that it is at least in part due to some disturbance of iron metabolism, and Cartwright and others (1946a, b) have recently done much to confirm this view. They found that the plasma iron was markedly diminished during infections, and that the hypoferraemia persisted until the infection was cured, even though there was no anaemia. The fall in the plasma iron might be evident within two or three days of the onset of an acute infection. When 1 g. of ferrous sulphate was given by mouth the plasma iron level did not rise, although in normal subjects such a dose would increase the level twofold. Even an intravenous injection of iron disappeared from the blood with abnormal rapidity, so it seemed that the anaemia was due at least in part to a deviation of iron from the bone marrow to some other site—probably the infected tissues.

Hypoferraemia followed by anaemia was found when staphylococcal or sterile turpentine abscesses were produced in dogs, but neither staphylococcal toxin nor killed typhoid vaccine produced these changes.

Hahn and others (1946) have also shown that anaemic dogs utilize radio-active iron much more

slowly after the induction of a sterile turpentine abscess than before.

We still do not know whether this deviation of iron from blood formation to infected tissues is a feature of the defence mechanism or simply a toxic reaction.

Summary

There is no evidence of excretion of iron by the body except in very small amounts, and the iron balance must therefore be maintained by a regulation of iron absorption. The greater part of the iron freed from broken-down red cells is re-used for haemoglobin synthesis, and only about 1 mg. a day is excreted in the bile and urine. Even the iron losses of normal menstruation, pregnancy, and lactation do not raise the average daily loss above 4 mg.

Iron absorption from the intestine varies, in accordance with the body's needs, from the 1 mg. or so a day necessary to maintain the iron balance of a normal man, to the 50 mg. or more a day which may be absorbed and utilized during intensive treatment of iron-deficiency anaemia. The amount of iron absorbed is determined by the iron level in the cells of the intestinal mucosa, where a protein receptor, probably ferritin, can receive iron from the intestinal contents and yield it to the plasma. Iron is transported by the plasma to the tissue stores, and the equilibrium between the iron level in the stores, the plasma, and the intestinal receptor determines the saturation of the last and the degree of iron absorption from the food. Anaemia *per se* has no influence on iron absorption.

Iron can be absorbed from the stomach and small intestine so long as the reaction is acid. Only ions are absorbed, so that the non-ionizable porphyrin iron of the food must be broken down by digestion or bacterial decomposition before it can be assimilated. It is possible that only ferrous ions can be absorbed, and they are certainly much more readily absorbed than ferric ions, but the latter are also more apt to form insoluble compounds in the intestine. The value of a diet as an iron source is largely determined by the amount of substances, such as phosphates, certain protein derivatives, and phytic acid, which form undissociated compounds with iron. As a general rule, however, an ordinary mixed diet suffices for a man if it contains a total of 5 mg. of iron, or for a woman or growing child if it contains 15 mg.

Some 60 per cent of the total body iron is circulating as haemoglobin in the blood, and a further 20 per cent is held in the tissues in a form potentially available for haemoglobin

synthesis. The greater part of this storage iron is present as ferritin. Iron recently absorbed or liberated from destroyed red cells is used for erythropoiesis in preference to the iron reserves, and about one-quarter of the latter seems to be more immediately available than the rest.

Of the remaining body iron, about one-third is in myohaemoglobin and about two-thirds in such cell enzymes as cytochrome. None of this iron is available for haemoglobin synthesis even in extreme iron depletion, but beyond this scarcely anything is known of its metabolism.

The iron losses of even moderate haemorrhage are very heavy in relation to the body's usual needs. A man normally absorbs only a milligramme or so of new iron a day, whereas 100 ml. of his blood contains about 50 mg. of iron. A blood loss up to about one-third of the total blood volume can be replaced from the iron reserves if these are saturated, but any greater loss must be replaced from the diet, which if unsupplemented will allow a haemoglobin increase of only some 2 per cent per week. The iron loss of chronic haemorrhage can easily exceed the capacity of an ordinary diet, but if the bleeding is into the stomach or duodenum a part of the haemoglobin iron can be re-absorbed.

Recovery from severe anaemia may utilize 2 g. of iron, a variable amount of which can be taken from the body's reserves. At the one extreme, in iron-deficiency anaemia all the iron must be absorbed; at the other, in pernicious anaemia the iron reserves are theoretically adequate to raise the haemoglobin to normal, although in practice the whole reserves are apparently not always readily available.

The anaemia of infection is associated with a low plasma iron, and there is evidence that iron is deviated from blood formation to the site of infection.

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