Haemopoietic factors

Iron absorption

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Physiological control over iron balance is normally exercised through the regulation of iron absorption. Although it is known that the intestinal epithelium can reject unwanted dietary iron or absorb increased amounts when stores are low it is not known how iron crosses the epithelial cell or how this regulation is mediated. The principal factors which affect absorption are those acting in the lumen of the gastrointestinal tract which determine availability of iron for mucosal uptake, intraepithelial mechanisms controlling iron transit across the mucosa, and changes within the body which signal iron requirements to the intestinal cells.

Intraluminal Factors

Much work on iron absorption is related to the behaviour of simple inorganic iron salts and although the data obtained are relevant to normal physiological mechanisms the absorption of iron from food introduces some complications. Probably the most important factor affecting total food iron absorption is the iron content of the diet itself. A recent survey in Cardiff showed that a mean daily iron intake of 17.5 mg in men and 14.1 mg in women, though the range of dietary iron was from 6 to 27 mg a day. The amount of food iron which is available for absorption depends primarily on the amount released by the digestive process. Less than half the total iron in food is released by peptic digestion in the stomach and this is largely in the form of ionizable inorganic iron except in the case of meat where iron is released as the haem complex. The amount released is considerably reduced in subjects with gastric atrophy (Jacobs and Miles, 1969a). Direct measurements of iron absorption from foodstuffs, using foods labelled biologically with radioactive tracers, show considerable variation, mean absorption being about 10%. Iron availability is not only related to the particular food source but also to the interaction with other foods and with gastrointestinal secretions. For example, iron absorption from veal is inhibited by the presence of corn or beans whereas iron incor-

porated in corn or black beans is better absorbed when eaten with either veal or fish (Layrisse, Martínez-Torres, and Roche, 1968). The stimulating effect of fish on iron absorption from black beans appears to be due to the presence of cysteine in the digestive products of the fish (Martínez-Torres and Layrisse, 1970).

The fate of ionizable iron released from food in the gut lumen is largely determined by its chemical reactions. Ferric ions undergo increasing polymerization forming colloidal gels as the pH rises towards neutrality and finally form a precipitate of ferric hydroxide. Ferrous ions do not undergo such marked polymerization and their solubility is greater than ferric ions at any given pH which accounts, at least partly, for their greater availability. The unpolymerized ions of both species found at low pH are chemically reactive and when the gastric contents pass into the jejunum and neutralization occurs they combine with available ligands to form complexes. Iron binding in this situation may result in the formation of insoluble complexes such as those with phytate or phosphate, or it may produce complexes such as ferrous ascorbate which are available for absorption. Under normal circumstances the largest part of the iron-binding capacity of gastric contents is provided by the gastric secretions themselves.

Haem iron released from food in the stomach appears to enter the intestinal epithelial cells unchanged. When neutralization of gastric contents by pancreatic juice occurs the polymerization of haem is prevented by the presence of other protein degradation products and these help to maintain its availability to the mucosal cells (Conrad, Benjamin, Williams, and Foy, 1967). The availability of haem iron is uninfluenced by the various intraluminal factors which affect ionizable iron.

GASTRIC JUICE

There is much conflicting evidence regarding the role of gastric juice in iron absorption but there seems little doubt that hydrochloric acid itself plays an important part (Jacobs, Bothwell, and Charlton,

1964). Hydrochloric acid secretion maintains the low pH necessary for the peptic digestion of food and ensures that released iron remains in a chemically reactive form which can produce soluble complexes.

A number of workers have suggested that gastric secretions contain an intrinsic factor for iron (Koepeke and Stewart, 1964; Murray and Stein, 1967). This factor was thought to promote the absorption of iron by the small intestine and variations in its secretion were thought to be responsible for the physiological control of iron absorption in iron-deficient and iron-overload states. Davis, Luke, and Deller (1966) demonstrated in gastric juice an iron-binding substance with a high molecular weight, which they called gastroferrin, and they suggested that this was a physiological inhibitor of iron absorption. Haemochromatosis was considered to be an inborn error of metabolism in which there was a failure to secrete gastroferrin and in iron-deficiency anaemia there was said to be a similar decrease of secretion allowing an increased absorption of dietary iron. Neither the intrinsic factor nor the gastroferrin hypothesis has been supported by the observations of other investigators (Jacobs, Rhodes, and Eakins, 1967).

Experiments *in vitro* with human gastric juice show that it has the ability to form a complex with ionized iron which remains soluble at neutral pH. In order for this complex to be formed the iron and gastric juice must mix at acid pH and be followed by neutralization, the normal sequence of events. The iron-binding fraction of human gastric juice has a molecular weight in excess of 200,000 and is probably a mucopolysaccharide (Jacobs and Miles, 1969b). The amount of iron-binding substance secreted by the stomach does not differ from normal in either iron-deficiency anaemia or haemochromatosis.

Under normal circumstances most of the ionizable iron released from food in the stomach either binds to the mucopolysaccharide in the gastric secretions or becomes unavailable on neutralization (Fig. 1). Only in exceptional circumstances, when a meal contains large amounts of a chelating agent, such as ascorbic acid or citric acid, do other low molecular weight complexes form.

PANCREATIC AND BILIARY SECRETIONS

There is no clear evidence that pancreatic extracts or juice have any special effect on iron absorption (Murray and Stein, 1966). Bicarbonate is known to promote the formation of macromolecular iron complexes when free iron is present but no consistent effect of bicarbonate on iron absorption has been found experimentally (Benjamin, Cortell, and

Fig. 1 Possible reactions of inorganic iron in the gastrointestinal lumen. In normal circumstances it is probable that most iron follows the lowest pathway.

Conrad, 1967; Kavin, Charlton, Jacobs, Green, Torrance, and Bothwell, 1967). Balcerzak, Peternel, and Heinle (1967) have suggested that the increase in iron absorption sometimes found in chronic pancreatitis is due to a number of possible artifacts the most important of which is coexistent iron deficiency. Olatunbosun, Ludwig, Corbett, Simon, and Valberg (1970) have similarly shown that the increased iron absorption found in portal sclerosis is usually associated with decreased iron stores.

The importance of pancreatic secretions in iron absorption is an indirect one. The breakdown of polypeptide chains by pancreatic enzymes results in the appearance of amino acid and dipeptide molecules in the upper jejunum. Conrad *et al* (1967) have shown that the absorption of pure haem in iron is relatively poor unless protein degradation products are also present to prevent polymerization of the haem complex. It is possible that amino acids may also be important in removing ionizable iron from the high molecular weight mucopolysaccharide complex which forms on the neutralization of gastric contents to form low molecular weight complexes which are available to the intestinal mucosa (Fig. 1). Studies *in vivo* and *in vitro* suggest that this is certainly the case for cysteine (Jacobs and Miles, 1969a; Martinez-Torres and Layrisse, 1970). Bile may play a small part in promoting iron absorption but the suggestion that this is due to its ascorbic acid content has not been supported by our own observations (Jacobs and Miles, 1970).

The Mucosal Cell

Intraluminal factors in the gastrointestinal tract affect the availability of ionizable iron for uptake by epithelial cells but they play no part in the physiological regulation of iron absorption. Iron which is destined to pass across the small-intestinal mucosa and enter the circulating plasma does so very rapidly

after its uptake by the epithelial cells. Halberg and Solvell (1960) have shown that after a single oral dose of iron most of the absorbed portion had entered the blood stream within two hours. Wheby and Crosby (1963) have shown a similar rapidity of absorption after the intragastric administration of iron in rats. When labelled iron is injected directly into a loop of small intestine there is virtually no time lag between the injection and the appearance of labelled iron in the circulating blood (Charley, Stitt, Shore, and Saltman, 1963).

IRON AT THE MUCOSAL SURFACE

Investigation of the absorption mechanism has been hampered by ignorance of the chemical form in which iron enters the mucosal cell. Ferric and ferrous ions are unlikely to exist in the free state either in the intestinal lumen or in the tissues. The ionic iron released from food by peptic digestion binds to gastric mucopolysaccharide on neutralization and much of the iron present in the jejunum is in a macromolecular form. Cell fractionation carried out during the absorption process in rats shows no evidence of iron uptake by pinocytosis (Worwood and Jacobs, 1971) and it seems likely that iron bound to the gastric carrier in the lumen is detached by suitable ligands to form low molecular weight complexes which cross the brush border. When avid chelators, such as cysteine or ascorbic acid, are present this process may occur in the lumen. Examination of jejunal contents one to two hours after the ingestion of a standard meal of shepherd's pie shows that 30-40% of the ionizable iron present is dialysable and on gel filtration with Sephadex G-25 appears in the bed volume, indicating a molecular weight below 2,000. Sugars and amino acids which act as iron chelators may also be formed in the brush border as part of the digestive process and in these circumstances the high local concentration between the microvilli will give the optimal conditions for chelation and absorption. A third source of potential iron chelators is from the epithelial cells themselves which are known to secrete amino acids into the lumen and presumably these also will be present in a relatively high concentration in the brush border. If iron enters the mucosal cell as a chelate then subsequent reactions will depend on its stability. Unstable amino acid complexes may transfer their iron to endogenous ligands in the cell.

SITE OF ABSORPTION

Dowdle, Schachter, and Shenker (1960) in experiments with rats showed that serosal transfer of iron was maximal in the region 5-10 cm immediately distal to the pylorus. Later experiments showed that mucosal uptake of iron decreased very little from the

pylorus to the terminal ileum but serosal transfer was largely limited to the duodenum. Wheby and Crosby (1963) showed that after a gastric dose of labelled iron the maximal uptake was in the first quarter of the small intestine. Uptake was greatest in iron-deficient animals and least in iron loaded animals. Isolated brush borders obtained from rats can take up iron from their suspending medium (Greenberger, Balcerzak, and Ackerman, 1969). Jejunal brush borders are more active than those from the ileum. Prior iron loading of the animals decreases brush border uptake while iron depletion leads to an increased uptake, suggesting that the brush border itself may play some part in the regulation of iron absorption.

INTRACELLULAR MECHANISMS

The major mechanism determining the amount of iron rapidly transported to the plasma is probably located within the mucosal cell and is extremely sensitive to the iron requirements of the body. Unwanted iron which enters the mucosal cell is sequestered there in the form of ferritin from which only a relatively small amount of iron is eventually transferred to plasma. Most of the mucosal ferritin is ultimately lost from the gut wall with the exfoliation of the villous cells at the end of the normal life span. It has been assumed that the main regulator of the amount of iron absorbed into the body via the rapid transport pathway is the ability of the cell to sequester iron as ferritin. Numerous attempts have been made to identify the way in which the body's iron requirements are signalled to the mucosa. Conrad, Weintraub, and Crosby (1964) have shown that parenterally administered iron enters the intestinal mucosal cells from the serosal pole at the time of their emergence from the crypts of Lieberkuhn. They suggested that iron entering the cells in this way provides information of the body's iron requirements. In iron-deficient states relatively little iron enters the cells and in cases of iron overload the cells take up increased amounts of iron. A number of experimental observations support this hypothesis. When there is an acute change in the body's iron requirements there is a short lag period before the small intestine adjusts its level of absorption. This lag period corresponds to the time taken for newly formed cells from the crypts of Lieberkuhn to migrate to the functional part of the villus. The parenteral administration of iron stimulates ferritin synthesis in small intestinal cells (Smith *et al*, 1968) and it has been suggested that newly formed epithelial cells contain a greater or lesser amount of ferritin and apoferritin which serves to trap incoming iron. This iron is then excreted into the lumen as ferritin. In iron-deficiency anaemia the absence of a

'ferritin curtain' allows the unobstructed passage of iron through the cell. On the other hand, in iron overload a considerable amount of iron present within the cell results in increased ferritin synthesis with consequent trapping of incoming iron preventing its transfer to the plasma. If this interpretation is correct then it would be expected that the mucosal iron content would be inversely related to the amount of iron absorbed from a given dose and earlier evidence suggested that this was the case (Conrad *et al*, 1964). More recently, however, it has been shown that the apparent inverse relationship between mucosal iron concentration and iron absorption is due to the inclusion of subepithelial iron in the estimation. When epithelial cells are separated off from the mucosa and the non-haem iron content is measured then there is no difference between control cells and those from iron-deficient or iron-loaded animals, even though there are considerable differences in iron absorption in these groups (Balcerzak and Greenberger, 1968). Brittin and Raval (1970) have measured ferritin protein synthesis in small-intestinal epithelial cells of the rat. They showed that when an oral dose of iron was administered the duodenal mucosa of iron-deficient rats synthesized as much ferritin protein as that of iron-replete rats, suggesting that these cells contain as efficient a 'ferritin apparatus' as normal cells. Their results imply that ferritin is not of prime importance as a regulator of iron absorption but that its formation is a secondary result of deviation of iron from the rapid transit pathway by some other mechanism.

Although it is apparent that most of the absorbed iron does not follow the ferritin pathway there is only little evidence regarding the nature of non-ferritin iron in the epithelial cells. Worwood, Edwards and Jacobs (1970) have studied the uptake of iron by rat intestinal cells during the early phase of absorption and have shown that a high molecular weight non-ferritin compound appears at this time in the non-particulate cell fraction. The transfer of iron from the intestinal cell to the plasma is closely related to the disappearance of this compound from the cells and it is suggested that it is an intracellular iron carrier involved in iron transfer during the rapid phase of absorption.

The transport of iron from the serosal surface of the cell to plasma has been studied largely by the use of everted sacs and closed loops of small bowel. Serosal transfer appears to be an active metabolic process inhibited by a lack of oxygen and by a number of poisons including iodoacetate and 2,4-dinitrophenol (Manis and Schachter, 1962) but there is little direct information regarding the kinetics of iron absorption.

Fig. 2 A possible route for inorganic iron crossing the small-intestinal cell. C = carrier; F = ferritin, i, ii, and iii (see text) are sites where the passage of iron may be regulated.

No clear picture of intracellular mechanisms has yet emerged but it appears that regulation of iron transfer across the epithelial cell may occur at three points (Fig. 2). Uptake of inorganic iron by the cell (i) may be related to chelation at the cell surface, metabolic activity at the brush border, or the availability of endogenous ligands. It is assumed that only iron attached to the intracellular carrier is available for serosal transfer and the main factor determining the proportion following this pathway (ii) may well be the amount of unsaturated carrier present. Regulation of iron leaving the cell (iii) may depend on metabolic activity at the serosal pole but the availability of plasma receptors is possibly of importance.

HAEM IRON

A significant proportion of dietary iron reaches the small intestine as a haem complex and probably enters the epithelial cells in this form. Conrad *et al* (1966) showed that when radioactive iron incorporated into haemoglobin is given orally to guinea pigs it appears in the intestinal epithelial cells still in the haem form. The absorption of haemoglobin iron in man is not affected by the simultaneous administration of phytate, desferrioxamine, EDTA, or ascorbic acid. When haemoglobin is given at the same time as a large dose of ferrous iron then haem iron absorption is decreased (Hallberg and Solvell, 1967). Similarly, haemoglobin had an inhibitory effect on the absorption of a tracer dose of ferric chloride suggesting that the absorption pathways for the two forms of iron are related at least in part. When ferric chloride and haemoglobin labelled with different isotopes of iron are given simultaneously there is a delay of about 30 minutes in the appearance of the peak level of haemoglobin iron in the plasma. This lag may result from the existence of a haem-splitting mechanism, probably of enzymatic nature, in the epithelial cells (Weintraub, Weinstein, Huser, and Rafal, 1968). From the point where iron is released

from the porphyrin ring it follows the same metabolic pathway as iron which has entered the cell in an ionizable form.

Humoral Factors Affecting Iron Absorption

The method by which the body influences the absorbing cells of the small intestine is not simple. The level of absorption is certainly affected by the iron status of the body and it is probable that even in healthy non-anaemic subjects variations in iron stores are a major cause of variation in absorption (Kuhn *et al*, 1968). Information regarding body iron stores could be conveyed to the epithelium through the incorporation of messenger iron from the plasma into the newly formed cells in crypts of Lieberkuhn. In some circumstances, however, there is an alteration of absorption inappropriate to the iron status of the individual. This can occur as a response to haemorrhage or in haemolytic anaemias and is usually associated with an increased rate of erythropoiesis. It has been suggested that the incorporation of messenger iron in epithelial cells is determined not simply by serum iron levels but by the plasma iron turnover rate. *In vitro* studies have suggested that the delivery of messenger iron to the epithelial cells depends on the manner in which iron is attached to transferrin molecules (Fletcher and Huehns, 1968) but confirmation *in vivo* is still awaited.

The association of increased iron absorption with a high rate of erythropoiesis has suggested that a circulating erythropoietin-like factor may act as a messenger to the small intestine. Erythropoietin could of course act by stimulating marrow proliferation and thereby increasing plasma iron turnover; however, there is some evidence that anaemia itself may act as a stimulus to iron absorption, possibly through the effect of anoxia. In both mice and in patients with absent erythropoiesis iron absorption is increased at low haemoglobin levels (Schiffer, Price, and Cronkite, 1965). The search for humoral factors which could regulate iron absorption has yielded a variety of results. Some workers have reported plasma factors which either stimulate or inhibit absorption but there is no substantial evidence on this point at the present time (Conrad, 1969).

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