EDITORIAL REVIEW

The Control of Iron Balance by the Intestinal Mucosa

By William H. Crosby

IN THE LATTER YEARS of the 19th Century when concepts of metabolic balance were beginning to crystallize, it was discerned that the fate of iron was different from that of most elements. Little iron was lost in the urine, but following the experimental ingestion or injection of large doses of iron, granular deposits giving a Prussian blue reaction appeared in macrophages in the intestinal villi and in epithelial cells of the small intestine.1,2 Therefore the intestine was thought to be an excretory as well as absorptive organ for iron. In 1937 McCance and Widdowson challenged this concept.4 After an analytical survey of the literature of iron metabolism they concluded: “There are indeed indications that in man and certain animals the bowel excretes practically no iron. If this is the case, the amount of iron in the body must be regulated by controlled absorption.” It is difficult to overstate the importance of this idea, for it placed the metabolic balance of iron in a unique category. If the amount of absorption is indeed a reflection of requirement, then it must follow: the intestine receives information concerning deficiency or surfeit of iron, and by means of sensory and effector mechanisms it acts in response to this information.

Experimental evidence in support of the concept began to accumulate. McCance and Widdowson injected large amounts of iron and found that loss of iron in excreta was not appreciably increased.5 Patients who received large numbers of blood transfusions were found to have heavy deposits of iron in their bodies;6 it seemed the transfused iron was in excess of requirement and, lacking an excretory mechanism, it was not unloaded. By extension of the concept, hemochromatosis was a disorder of iron absorption; the intestine absorbed unneeded iron and the body could not excrete it.

In 1943 Hahn published evidence of increased absorption of radioactive iron in humans with iron deficiency.7 By comparison, normal humans absorbed very little iron. This ability to prevent absorption of unneeded iron resided in a “mucosa block”—according to Hahn’s hypothesis—and in iron deficiency the block disappeared permitting increased absorption. To explain the mechanism of the block, Hahn postulated the existence of a “mucosal acceptor” for iron. “Physiological saturation” of the acceptor prevented further entry of iron through the mucosa into the body. He suggested that the mucosal acceptor might be apoferritin, a protein which does accept iron and which can become saturated. Granick seemed to have confirmed this hypothesis by an experiment in which he fed a large dose of iron to a number of guinea pigs, and thereafter was able to crystallize ferritin from the duodenal

From the Department of Hematology, Walter Reed Army Institute of Research, Washington, D. C.

Submitted Feb. 12, 1963; accepted for publication Apr. 23, 1963.

Blood, Vol. 22, No. 4 (October), 1963

441
tissues. Granick proposed that ferritin was a vehicle for iron absorption as well as a participant in the mucosal block. “After iron feeding, ferrous iron enters the mucosal cells, much of it temporarily stored in the form of ferritin. A point is reached at which the cells are “physiologically saturated” with respect to ferrous iron and no further iron is absorbed . . . . Only when the ferritin had decreased to a point where the mucosal cells were no longer physiologically saturated with ferrous iron would any more iron be absorbed.”

It has been generally overlooked that experiments such as Granick’s represent a failure rather than a demonstration of mucosal block. If one may assume that his guinea pigs had no requirement for iron in excess of the ordinary dietary provision, then the presence of large amounts of ferritin after ingestion of the massive dose of iron indicates that the mucosal block has been overwhelmed. Ordinarily the demands placed upon this control mechanism are limited by the amount of iron present in food; it seems incautious to form conclusions concerning a normal mechanism based upon its behavior when confronted with a massive, abnormal challenge. Much of the information concerning iron absorption has been derived from experiments with large doses of iron salts.

Reinterpretation of Granick’s experiment does not invalidate Hahn’s concept of mucosal block. The intestine can reject available dietary iron when it is not needed. Therefore the general hypothesis is sound, but it may require some revision in detail. In the light of available data the mucosal block alone seems insufficient to control the iron balance. Although the concept of McCance and Widdowson has been generally accepted—that there is no mechanism for iron excretion—evidence is to the contrary.

Data related to the control of iron balance have been divided below into eight groups. The first three describe phenomena relating to iron balance; the next three are indications of cellular control of iron; the last two describe the evidence for iron excretion.

1. Iron absorption is a discontinuous phenomenon, occurring for short periods during and after meals which contain more or less dietary iron. In spite of the erratic presentation of iron for absorption the iron balance of the body is carefully controlled. Normal humans do not accumulate iron and given an adequate diet they do not become iron deficient.

2. Not all available dietary iron is absorbed by normal humans. However, the mucosal block against absorption of unneeded iron is not complete.
   a. The mucosal block can be overwhelmed: irrespective of need, large amounts of iron are absorbed when large amounts are ingested.
   b. Even when the body is loaded with an excess of iron, as in dietary or therapeutic siderosis, a portion of any small dose of ingested iron is absorbed.
   c. The absorption of food iron by normal humans is increased when excess ascorbic acid is added to the food. It is unlikely that the requirement for iron is increased by ascorbic acid.

   Absorption of unneeded iron requires a compensatory system to prevent accumulation of such iron.
3. The intestine is not quickly responsive to changes in the state of the body's iron stores. After acute bleeding the requirement for iron is temporarily increased to replace that lost as hemoglobin. Absorption of iron from the diet increases. However, the increase of absorption does not begin until approximately 3 days after the bleeding has occurred.14,53

4. The duodenal mucosa of iron-loaded rats accepts less iron than does the mucosa of normal rats; most of the "accepted" dose does not proceed into the animal's body but is soon lost in the feces, presumably by the sloughing of the deciduous cells which accepted it.15 This indicates that the mucosal absorptive epithelium of the iron-loaded rat is different from the normal in its ability to resist the incursion of iron from the lumen.

5. Villous epithelial cells may retain some of the iron they absorb.
   a. A lag is noted in the clearance of radioactive iron from the gut of normal humans following oral administration of a small test dose, suggesting that a portion of the dose is temporarily sequestered. In iron deficient humans this lag is not observed.16,17
   b. Radioautographic experiments on intestinal mucosa of rats indicate that the sequestration occurs in the villous epithelium of the duodenum and jejunum.17,18 The iron is accepted by these absorptive cells, but not all of it is allowed to pass into the animal's body. A portion is retained and when the villous cells are desquamated that iron is lost into the gut. Villous epithelium of iron-loaded and iron-deficient rats does not show a radioautographic tag after ingesting radioiron.18 Furthermore, in iron-loaded and in normally iron-replete animals, iron20 given parenterally finds its way into duodenal epithelial cells as they are being formed in the crypts of Lieberkühn, and the radioactivity remains in these cells as they ascend during their life cycle.18 Thus newly generating absorptive cells are partially loaded with "intrinsic" iron from the animal's body, and this may modify their ability to absorb dietary iron.
   c. These results and interpretation are substantiated by experiments in which the radioactivity of the intestine was measured by counting rather than radioautography. Two hours following ingestion of radioactive iron the intestine had accepted more iron than was ultimately absorbed into the animal's body.15 A portion of the iron was accepted and then lost.

6. The absorptive cells of the duodenal villi possess demonstrable mechanisms that may impede the movement of iron through the cell.
   a. Electron microscopy reveals small accumulations of ferritin in the epithelial cells of villi of normal human duodenum.19 These accumulations seem to be less frequently encountered in the duodenum of humans with induced iron deficiency and of patients with hemochromatosis.20
   b. Following ingestion of a large dose of iron, accumulations of Prussian blue are found by light microscopy in areas of the villous epithelial cells where ferritin bodies are not observed.19,21 This is the phenomenon of acervation, the intracellular piling up of an absorbed noxious substance in combination with nucleic acid or some other cytoplasmic component. The acervation of iron in the villous epithelial cells is evidence of the cell's accepting iron at
a faster rate or in greater quantity than it dispenses of it. It may pertain only to absorption of iron salts since stainable iron is not seen in the villous epithelium when only dietary iron has been ingested.

7. It has been repeatedly observed that iron-loaded animals\textsuperscript{13,21,23} and siderotic patients\textsuperscript{24-26} have a heavy concentration of iron-containing macrophages in the lamina propria of the intestinal villi. After iron has been fed, some of these macrophages are found in the mesenteric lymph nodes, and it has been suggested that such cells participate in the movement of iron into the body.\textsuperscript{2} Some appear to be crossing the epithelium at the tips of the villi and it has been suggested that such cells have an excretory function.\textsuperscript{3,22,26} These cells have been observed in the duodenal villi of normal guinea pigs killed while not fasting; they are absent in similar animals after fasting.\textsuperscript{2} Thus the villous macrophages appear to participate in the reception of normal dietary iron, perhaps to assist in assimilation or rejection of iron which has passed the epithelium.

8. Iron contained within decidual cells is lost when the cells are desquamated.
   a. All cells contain small amounts of iron as hematin enzymes. Loss of this functional iron in dead, desquamating cells is unavoidable and may be regarded as an obligatory loss.
   b. In iron storage diseases certain types of decidual cells become heavily loaded with storage iron.\textsuperscript{25,27} Loss of this nonfunctional iron via desquamation of these cells may be regarded as a compensatory loss.
   c. Absorbtive iron in absorptive intestinal epithelial cells (6b above) is lost if the cells are shed before the iron can be removed.
   d. Moore demonstrated that normal humans lost in their feces a small but significant increment of an injected dose of radioiron; iron-deficient patients lost even less but they did lose some; and a single patient with transfusion siderosis lost considerably more.\textsuperscript{26} This variability favors the concept of obligatory loss (iron deficiency) and compensatory loss (iron storage disease). Another patient with transfusion siderosis, studied after his anemia had been cured, was found to have lost 3.5 mg. per day of excess iron over a period of 12 years.\textsuperscript{26}
   e. In hemochromatosis and other iron storage diseases there is evidence of unloading of iron by incorporating it as nonfunctional accumulations within decidual cells.\textsuperscript{25,27} This iron can be recognized histologically by the Prussian blue reaction. The gastric and duodenal mucosa contains much iron concentrated in the glandular epithelium. In contrast there is none in the absorptive epithelium nor in the cells of the crypts of Lieberkühn. Epithelial cells of mucous glands throughout the body are laden with iron. Iron is also picked up by tissue macrophages in the intestinal villi, in the submucosa of the renal pelvis, the alveolar septa of the lungs and in the skin. Iron-laden Kupffer cells are found free in the hepatic venules, and sometimes there are iron-laden mononuclear cells in the peripheral blood. Also in the urine and sputum are cells, probably macrophages, which contain hemosiderin.
   f. Although this excretory mechanism functions in hemochromatosis (c. above), iron does accumulate indicating that the excretory capacity is less
EDITORIAL REVIEW

than the amount of iron absorbed from the diet. Nevertheless the rate of accumulation is slower because of the excretion. In families of patients with hemochromatosis there are people with moderately high plasma iron or a slight increase of iron stores in the liver. In these mildly affected subjects the active excretory mechanisms may prevent an accumulation of sufficient iron to cause clinical disease.

DISCUSSION

Based upon these data a concept of the control of iron balance is proposed which includes a limited capability for iron excretion. The iron-excretory mechanisms compensate for the imperfections of the mucosal block (2, above) and provide a means for the unloading of iron in the iron storage diseases (8, above). Leading to a modification of Hahn’s hypothesis were the demonstration of ferritin actually within the villous epithelial cells (6a, above) and radioautographic evidence of “permanent” sequestration of ingested iron in these cells (5, above). It is proposed that iron which is incorporated in epithelial ferritin is not there as a stage of absorption but is permanently detained to prevent its absorption. In iron deficiency the mucosal block disappears to a considerable extent. The absence of epithelial ferritin in this condition (6a, above) suggests that the villous epithelial cells have been constructed without the ability to form ferritin and thereby the entry of dietary iron into the body is relatively unimpeded. In case of systemic iron overload the ferritin capacity of the absorptive cells may be saturated by iron from the excess already in the body: The “loading from the rear” of these deciduous cells would serve to carry some unneeded iron out of the system. Also by saturating the ferritin and the iron-carrier mechanisms of the absorptive cells it may induce a “refractory state” and thereby inhibit the acceptance of unneeded dietary iron (4 and 5, above). Within this concept, hemochromatosis may represent a genetically determined lack of the ability to form ferritin in the intestinal epithelial cells (6a, above); in the absence of a functional ferritin apparatus the entry of iron into the body would be relatively unimpeded.

In the normal state of iron repletion some iron is required for replacement of obligatory loss (8a, above), but much of the available dietary iron is rejected. It is predicated that in the normal state of iron repletion the iron acceptor mechanism of the absorptive epithelial cells is only partially saturated by iron from the animal’s body (5b, above), the degree of unsaturation de-

The life span of the villous epithelial cells in the human intestine may be about 3 days. The entire epithelial covering is renewed within this time. If it is predicated that the response to iron deficiency requires the production of modified epithelial cells to permit increased absorption, then we cannot expect a prompt response to removal of a large amount of iron (by bleeding) or to acute changes in plasma iron-binding capacity (by injection). After the intestine receives information concerning changes in the body’s iron, the production of modified epithelial cells would begin, and as they gradually replace the former cells a change in intestinal absorptive capacity would develop. This hypothesis can explain the lag in iron absorption which is observed following acute blood loss (see 3, above) and the failure of the gut to react in acute experiments involving changes of plasma iron and unsaturated iron-binding capacity.
pending upon the size of the requirement for new iron. Thus the "refractory state" is partially relaxed permitting acceptance of some of the dietary iron. A portion of this accepted iron proceeds into the animal's body, but some of it is retained by the epithelial cell to complete the saturation of the acceptor mechanism. This iron is lost when the cells are lost at the end of their life cycle (5b, above). During the postprandial periods, when the dietary iron is presented to the gut, absorption may exceed requirement. This excess iron would then be incorporated into the iron acceptor system of the next generation of cells, thereby increasing refractoriness to absorption. The iron in the acceptor system would also be lost when epithelial cells are sloughed at the end of their life cycle.

The ability of some kinds of cells to accept large amounts of excess iron evidently provides a safeguard for prevention or correction of iron overload (8b and 8e, above). The unneeded iron is loaded into deciduous cells as stainable, nonfunctional accumulations and when these cells are sloughed the iron is lost. In the iron-storage diseases tissue macrophages also accumulate stainable iron and, by diapedesis into the excretory passages for feces, urine and sputum, these cells carry at least some of the unneeded iron out of the body.

Summary

In this hypothetical model of intestinal function the ferritin apparatus of the absorptive epithelial cells provides the most important mechanism for day-to-day control of iron balance. Iron incorporated into epithelial ferritin cannot be released and therefore is lost when the cell is lost at the end of the life cycle. Information concerning the state of the body's iron stores is conveyed to the intestine, perhaps by the concentration of plasma iron or iron-binding capacity. When the iron stores are replete the newly forming epithelial cells are constructed to contain a functional ferritin apparatus capable of synthesizing apoferitin and incorporating iron into ferritin, thus preventing its entering the body. However, this mechanism is not completely adequate to intercept all unneeded iron. When too much iron has bypassed the ferritin apparatus it may be intercepted by tissue macrophages in the villus and lost when these macrophages work their way into the lumen; or it may be loaded into newly forming villous epithelial cells thereby saturating a part of their ferritin capacity. Cells thus loaded would develop increased resistance to the incursion of dietary iron; they would also carry the ferritin iron away with them at the end of their life span. In iron deficiency the absorptive cells are constructed with a relative lack of ferritin apparatus so that dietary iron can pass freely into the cell and thence freely into the body.

In iron storage disease other populations of cells are used for excretion of iron. In general these are the glandular epithelial cells and tissue macrophages. Both varieties of cells are able to accept and store relatively large amounts of nonfunctional iron. They are deciduous cells which are shed from the internal surfaces of the body, and when they go their iron goes with them. The capacity of the excretory mechanism is not great, perhaps about 5 mg. per day. When the rate of introduction of iron into the body exceeds this
capacity, siderosis of parenchymal tissues develops due to accumulation of excess iron.

**Summario in Interlingua**

In le hic-presentate modello del function intestinal, le apparato de ferritina del absorptive cellulas epitheliali provide le plus importante mechanismo pro le regulation de-die-in-die del balancia de ferro. Ferro incorporate in ferritina epitheliali non pote esser liberate e per consequente es perdite quando le cellula es perdite al fin de su ciclo vital. Information concernente le stato del reservas de ferro in le corpore es transmitite al intestino, possiblemente in le forma del concentration de ferro in le plasma o del capacitace del plasma de ligar ferro. Quando le reservas de ferro es replete, le cellulas epitheliali nove-mente in formation es construite de maniera que illos contine un apparato functional de ferritina capace de synthetisar apoferritina e de incorporar ferro ad in ferritina e assi de prevenire le entrata del ferro ad in le corpore. Tamen, iste mechanismo non es completamente sufficiente pro interciper omne le non-requistite ferro. Quando troppo ferro ha essite lassate passar le apparato de ferritina, illo pote esser intercipite per macrophagos tissular in le villos e esser perdite quando iste macrophagos trova lor cammino ad in le lumine; o illo pote esser cargate in villose cellulas epitheliali nove-mente in formation, con le resultato que un parte de lor capacitace pro ferritina es saturate. Cellulas assi cargate disvelopparea un augmentate resistentia contra le incursion de ferro dietari. Illos etiam effererea con se le ferro de ferritina al fin de lor ciclo vital. In carentia de ferro, le cellulas absorptive es construite con un manco relative del apparato de ferritina de maniera que le ferro dietari pote entrar liberemente ad in le cellula e ulteriormente etiam ad in le corpore.

In morbo de thesaurisation de ferro, altere populationes de cellulas es usate pro le excretion de ferro. A generalmente parlar, istos es le cellulas de epithelio glandular e le macrophagos de tissu. Ambe iste typos de cellulas ha le capacitace de acceptar e thesaurisar relativamente grande quantitates de ferro non-functional. Illos es cellulas decidue que es disjicite per le superficies interne del corpore, e quando illos dispare, lor ferro dispare con illos. Le capacitace del mechanismo excretori non es grande, forsanz 5 mg per die. Quando le introduction de ferro ad in le corpore excede iste ration, siderosis de tissu parenchymal se disveloppa in consequentia del accumulation de excessos de ferro.

**REFERENCES**

5. —, and —: The absorption and excretion of iron following oral and intravenous administration. J. Physiol. 94: 148, 1938.
6. Kark, R. M.: Two cases of aplastic anaemia, one with secondary haemo-
chromatosis following 290 transfusions in nine years, the other with secondary carcinoma of the stomach. Guy's Hosp. Rep. 87:343, 1937.

William H. Crosby, Colonel, MC, Director, Division of Medicine, and Chief, Department of Hematology, Walter Reed Army Institute of Research, Washington, D. C.
Editorial Review: The Control of Iron Balance by the Intestinal Mucosa

WILLIAM H. CROSBY, Colonel