Blood Cells, Molecules, and Diseases (2002) **29**(3) Nov/Dec: 336–355
doi:10.1006/bcmd.2002.0564

Pathways of Iron Absorption[1]

Submitted 09/17/02
(Communicated by E. Beutler, 09/26/02)

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ABSTRACT: Iron is vital for all living organisms but excess iron can be lethal because it facilitates free radical formation. Thus iron absorption is carefully regulated to maintain an equilibrium between absorption and body loss of iron. In countries where meat is a significant part of the diet, most body iron is derived from dietary heme because heme binds few of the dietary chelators that bind inorganic iron. Uptake of heme into enterocytes occurs as a metalloporphyrin in an endosomal process. Intracellular iron is released from heme by heme oxygenase to enter plasma as inorganic iron. Ferric iron is absorbed via a β_3 integrin and mobilferrin pathway (IMP) which is unshared with other nutritional metals. Ferrous iron uptake is facilitated by a DMT-1 pathway which is shared with manganese. In the iron deficient gut, large quantities of both mobilferrin and DMT-1 are found in goblet cells and intraluminal mucins suggesting that they are secreted with mucin into the intestinal lumen to bind iron to facilitate uptake by the cells. In the cytoplasm, IMP and DMT associate in a large protein complex called paraferritin which serves as a ferrireductase. Paraferritin solublizes iron binding proteins and reduces iron to make iron available for production of iron containing proteins such as heme. Iron uptake by intestinal absorptive cells is regulated by the iron concentration within the cell. Except in hemochromatosis it remains in equilibrium with total body stores via transferrin receptors on the basolateral membrane of absorptive cells. Increased intracellular iron either up-regulates or satiates iron binding proteins on regulatory proteins to alter their location in the intestinal mucosa.

INTRODUCTION

A newborn infant has a total body iron of about 250 mg. It derives its iron from maternal iron stores via the placenta. During years of growth iron absorption must exceed iron loss by about 0.5 mg daily in order to maintain a body iron concentration of about 60 parts per million (1, 2) An adult male (70 kg) has a total body iron of about 4 grams which remains constant throughout adult life (Fig. 1). This is maintained by a balance between absorption and body loss of iron. Persistent errors of either gain or loss result in either iron deficiency on one hand or hemosiderosis and hemochromatosis on the other.

Body iron is maintained primarily by regulation of the absorption of dietary iron in the proximal small intestine. (1–3) (Fig. 1). Humans conserve iron more efficiently than other animals. Unlike other nutritional metals, excess body iron is slowly excreted in the absence of hemorrhage or hemosiderinuria (1–3). An adult man will lose only 1 mg daily (0.025% of body iron) whereas women during childbearing years will lose twice that amount due to menses and childbirth. Other losses of iron from the body occur from desquamated epithelium of skin, intestinal cells and intestinal secretions (2–4). Usually, these sources of loss contain iron in proportion to the body stores of iron and quantitatively body loss of iron must equal absorption (Fig. 2). Excretion plays a more passive role than absorption in the regulation of body iron (5, 6). Maximal daily loss of iron without hemorrhage or hemoglobinuria is limited to about 4 mg daily (7, 8).

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[1] This was supported by Merit Award 2 R37 DK36112 from the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health, Bethesda, MD.
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1079-9796/02 $35.00

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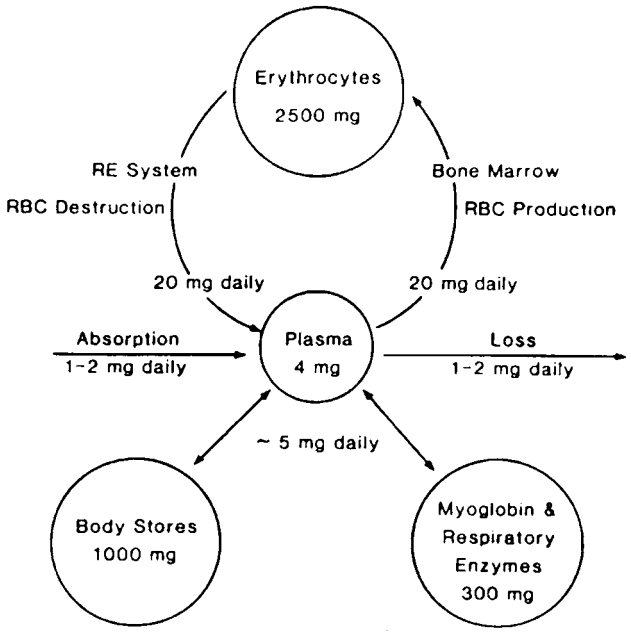


FIG. 1. An adult 70-kg man has a total body iron of about 4 g. Most of the iron is circulating as hemoglobin within the red blood cell (2.5 g). About 1 g is in body stores as ferritin and hemosiderin and approximately 0.3 g is incorporated into myoglobin and respiratory enzymes. The body absorbs only 1 mg daily from a diet containing 10–20 mg of iron in order to maintain equilibrium with body losses in iron in body secretions and exfoliates skin and gut epithelium. Most iron in the body is used for the production of hemoglobin. Since a red blood cell has a normal life span of 120 days, 0.8% of circulating red blood cells are destroyed and replaced daily.

Factors which affect iron absorption can be divided into intraluminal, mucosal, and corporeal factors (Fig. 3). This model has a luminal membrane and a basolateral membrane which permit the ingress of needed iron while limiting absorption of excessive iron. Adult males absorb about 1 mg of iron daily from a diet containing 10 to 20 mg of iron. Women eat less food than men and must absorb about 2 mg of iron daily during the childbearing years in order to avoid becoming iron deficient.

INTRALUMINAL FACTORS

Food iron occurs largely as either ferric iron or heme-iron. Pharmacological iron is usually a ferrous salt. Ferric iron is precipitated in solutions with a pH greater than 3 and must be solubilized and chelated in the stomach to be available for absorption in the less acidic proximal small intestine (Fig. 4). Prolonged achlorhydria can produce iron deficiency because chelation will not occur unless the iron is solubilized. Solubilized iron in the stomach is chelated by dietary and intestinal derived substances which keep iron in solution when it enters the less acidic duodenum. This is accomplished by intestinal mucins and dietary components to include certain amino acids, sugars, amines and amides (9, 10). Other dietary constituents cause ferric iron to precipitate and form macromolecular complexes which render the iron unavailable for absorption (phytates, carbonates, phosphates, oxalates and tanates). Some ferric iron is reduced by dietary constituents and

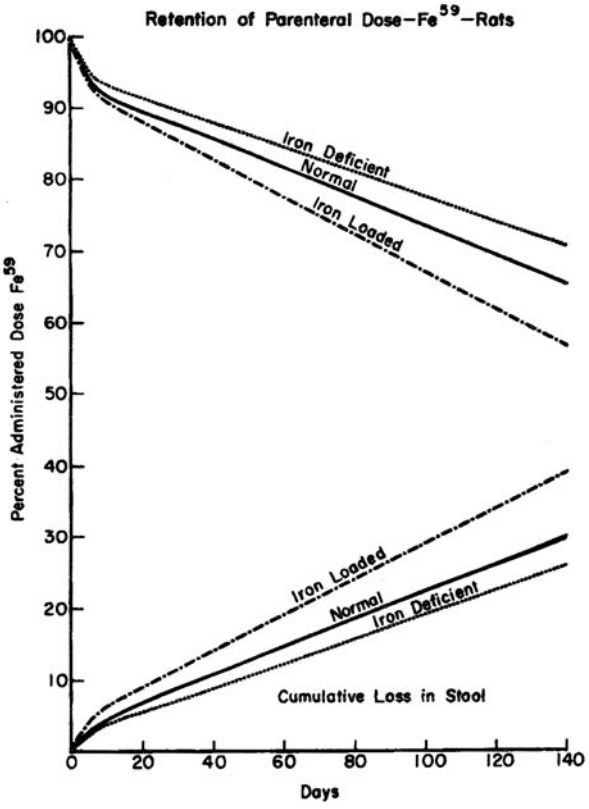
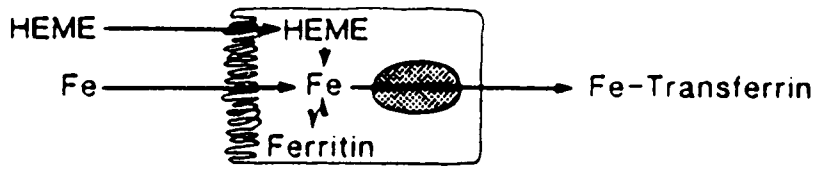


FIG. 2. Rats were made iron deficient by phlebotomy and diet and iron loaded by injection of 25 mg of dextran iron. Then each animal was injected intravenously with radio-iron and the daily loss was measured in a 4 pi small animal gamma detector. Cumulative loss of iron in stool was also measured. Significant differences were observed among the three groups of animals. Iron deficient rates retained the radio-iron while iron loaded animals lost iron at a faster rate than normal control animals. Thus there is selective loss of iron from the body depending upon the state of iron repletion.



Intraluminal Factors

Dietary Iron
Quantity
Chemical form
pH
Chelation & precipitation

Mucosal Factors

Anatomic Factors
Diminished absorptive surface
Defective epithelial cells
Mucosal life span
Intestinal Motility
Mucosal Iron
Quantity
Chemical Form
Certain Metals
Drugs affecting protein synthesis

Corporeal Factors

Body Iron Stores
Iron Turnover
Erythropoiesis
Anabolism/catabolism
Reticuloendothelial blockade
Ineffective erythropoeisis
Thalassemia,
Sideroblastic anemia
Cong. dyserythropoietic anemia
Hypoxia
Idiopathic Hemochromatosis
Neonatal hemochromatosis
Atransferrinemia
Ferrochaltase deficiency
Porphyria cutanea tarda
Ceruloplasmin deficiency
Transferrin immune complex disease
Nonalcoholic steatohepatitis
Cirrhosis
Portacaval shunt
Familial hypochromic anemia with siderosis
Sub-Saharan hemochromatosis (Bantu siderosis)

FIG. 3. Food contains both inorganic iron and heme-iron. Both forms of iron enter the absorptive cell non-competitively. Within the enterocyte, iron is released from heme by heme oxygenase so that heme-iron enters the plasma as inorganic iron. There are many factors and disorders which affect iron absorption. These can be divided into intraluminal, mucosal, and corporeal factors.

intestinal secretions to ferrous iron which is soluble at neutral pH. However, for ferrous iron to remain in that redox state requires either continuous reduction or chelation in a manner that prohibits exposure of the iron to oxygen.

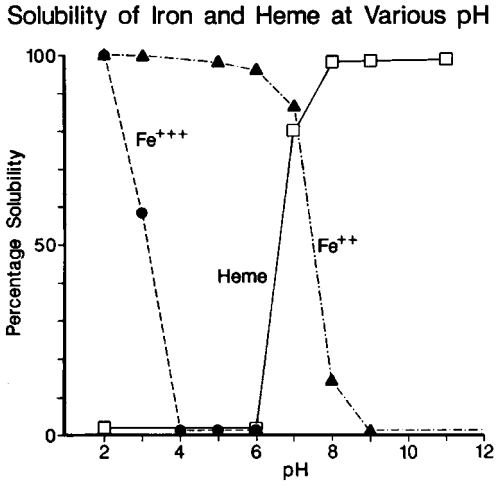


FIG. 4. In the absence of iron chelators, ferric iron is precipitated at a pH greater than 3. Ferrous iron is not rendered unavailable for absorption until near neutral pH. In contrast, heme is soluble at alkaline pH and precipitated in acid solutions. Certain chelators permit each form of iron to remain soluble over a wide range of pH.

In meat eating countries, heme-iron constitutes about one-third of dietary iron but is the source of two-thirds of body iron (11). Preferential absorption of heme-iron occurs because heme is soluble at the pH of the small intestine and its uptake by absorptive enterocytes is not influenced by the dietary constituents that adversely affect the absorption of inorganic iron (12, 13) (Fig. 5).

MUCOSAL FACTORS

Iron absorption occurs and is regulated in the duodenum and jejunum (1–3). Sufficient iron must be absorbed to compensate for body loss of iron while available unneeded iron is rejected.

In 1943, Hahn *et al.* postulated that iron absorption was the primary method of maintaining body iron homeostasis and that the was regulated by a mucosal receptor that blocked iron absorption when it became satiated with iron (14). For two decades, it was believed that ferritin was the

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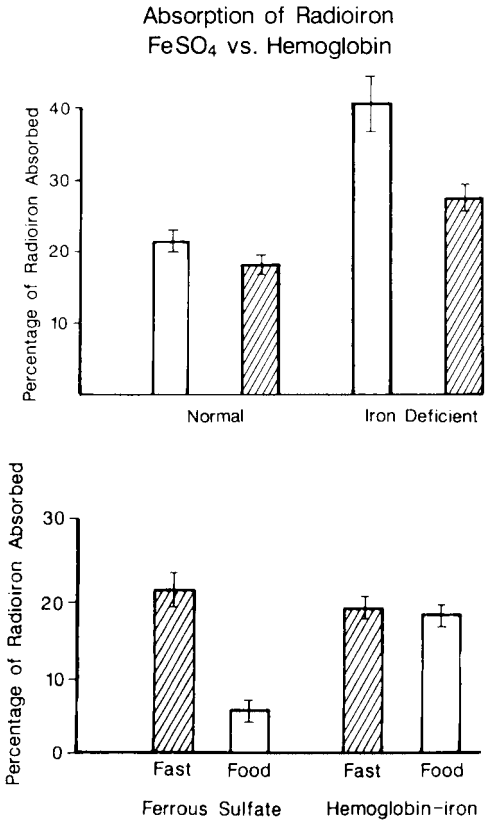

FIG. 5. Comparison of the absorption of iron from ferrous sulfate and rabbit hemoglobin in human using a 4 pi whole body gamma detectors. Values are the mean of four subjects. Absorption of both ferrous iron (open bars) and hemoglobin iron (crosshatched) was increased by deficiency induced by phlebotomy. However, a lesser increase in iron absorption was observed with hemoglobin iron. In the lower graph, the effect of adding the test dose to a meal is shown. Absorption while fasting is shown in the crosshatched bars. Administration of the test dose with food significantly diminished iron absorption from ferrous sulfate but had little effect upon the absorption of iron from radio-labeled hemoglobin.

receptor that regulated iron absorption and that apoferritin enhanced iron absorption while holoferritin blocked iron uptake. This hypothesis was disproved by immunological studies showing that there was little or no apoferritin in the absorptive cells of iron deficient animals (15, 16).

In 1983, it was postulated that transferrin was secreted into the intestinal lumen to bind iron and enter the absorptive cell as a transferrin iron complex in a manner similar to non- intestinal cells (17). The inability to demonstrate transferrin receptors on the intestinal microvilli and observations that patient with atransferrinemia became iron-overloaded made this less likely (18, 19). This led to a search for novel iron transport proteins over the next two decades. (Figs. 6–8).[7] Several candidate proteins were identified which appeared to be involved in the transmembrane transport of iron (20–32). These identifications were predicated upon demonstrations that the protein either bound radio-iron in vivo or were upregulated in iron deficiency or both. Each of these proteins were shown to exist in non-intestinal cells as well as enterocytes despite the fact that iron is largely transported into most cells via transferrin mediated pathways (32). Thus the physiologic role of non-transferrin pathways in non-intestinal cells is unclear in the absence of iron overloaded states. The pathway for inorganic iron transport independent of transferrin into nucleated non-intestinal cells was shown to be the same as that used by intestinal cells (32). This suggests that the proteins of these pathways have intracellular functions in addition to the transport of iron across the microvillous membrane of the enterocyte. Identified pathways for uptake of non-transferrin bound iron into enterocytes are the Integrin–Mobilferrin pathway (IMP) and the Divalent Metal Transporter-1 pathway (DMT-1, Nramp 2).

The IMP pathway solely transports ferric iron and not other metals of nutritional importance (32) (Fig. 9). It was discovered by biochemical isolation and identification of radio-labeled proteins after an intraluminal dose of radioactive ferric chloride. Proteins associated with the IMP pathway include (1) mobilferrin, a homologue of calreticulin which probably lacks the KDEL carboxy-terminal sequence that binds calreticulin to endoplasmic reticulin and (2) a β3 integrin—a known adhesion protein. These proteins associate with each other in the cytosol bound to flavin monooxygenase. This macromolecular complex (520 kDa) was named paraferritin because of its apparent mass on sizing chromatography columns. Paraferritin had ferrireductase activity (23). Recent data suggest that DMT-1 is also a component of this complex (33). Since ferrochelatase requires ferrous iron for synthesis of heme the intracellular purpose of this complex may be delivery of reduced iron to the mitochondria for the

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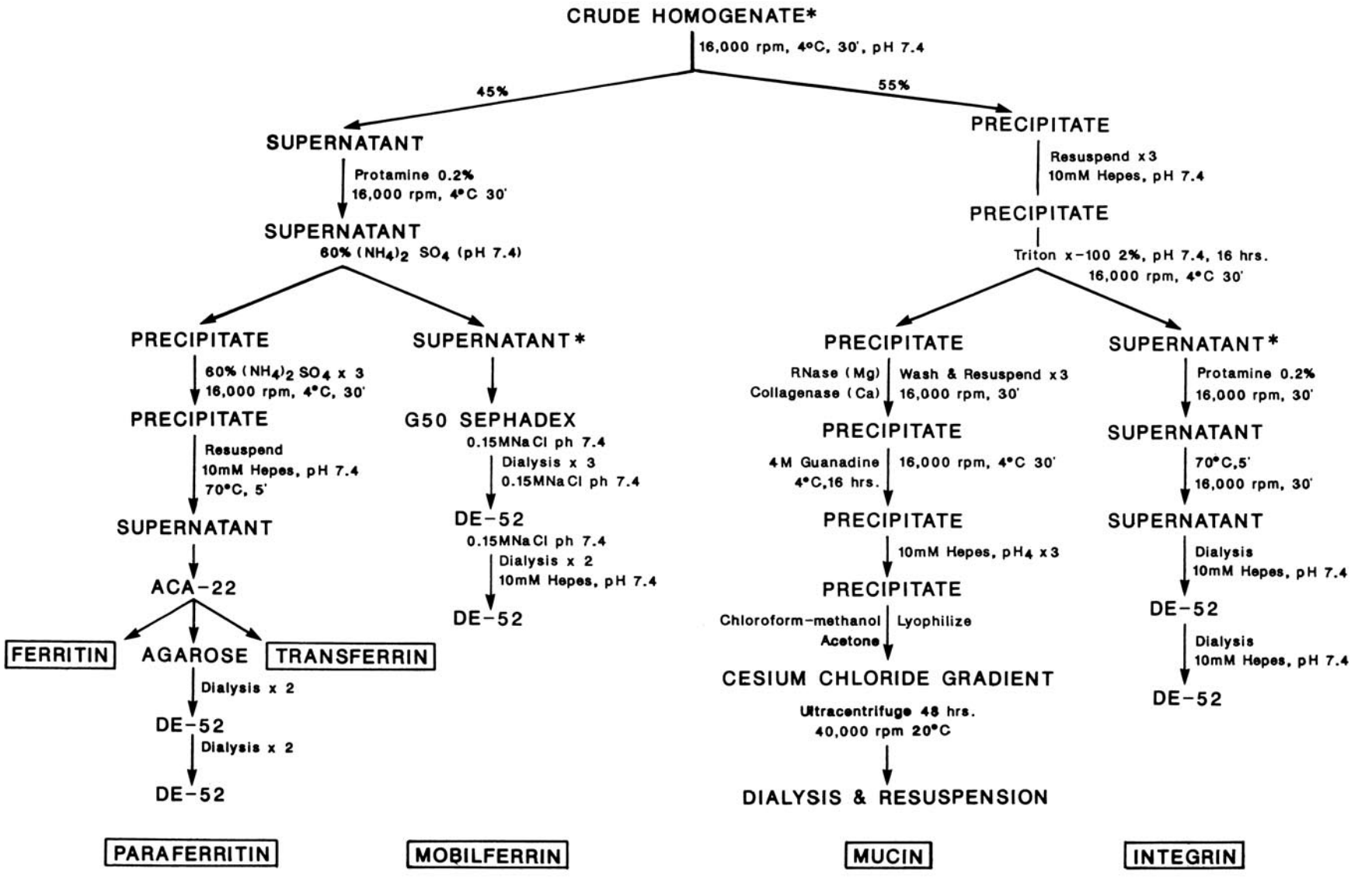
PURIFICATION OF RADIOIRON LABELED SUBSTANCES FROM RAT DUODENAL MUCOSA



* Proteolytic Inhibitors

LXXVIII PREPARATION

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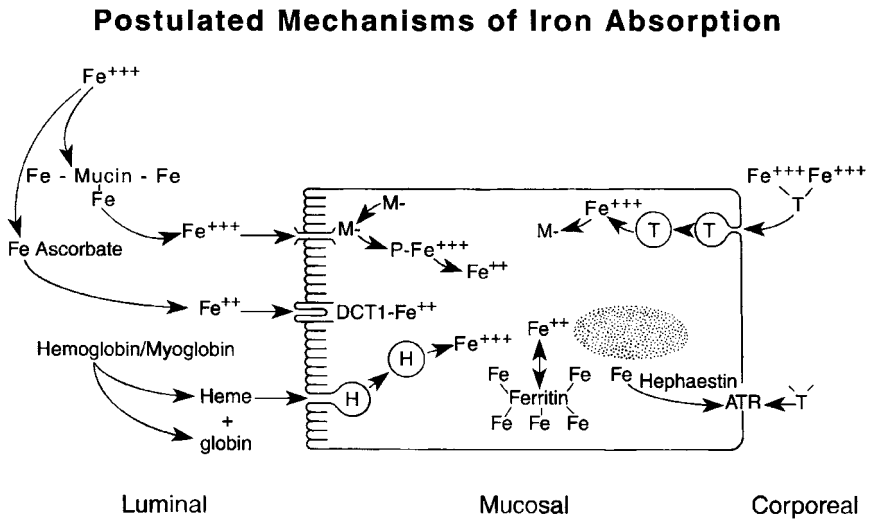

FIG. 7. Three pathways exist in the small intestine for uptake of food iron. These are separate uptake pathways for heme, ferric, and ferrous iron. Most dietary iron is in the ferric state. It is mobilized from food in the acid stomach for chelation with mucins, histidine, fructose, etc. Chelation keeps ferric iron soluble when it enters the less acidic duodenum. Ferric iron uptake is facilitated by mobilferrin and a β_3 integrin. A large protein complex is formed named paraferritin which contains iron, mobilferrin, β_3 integrin, flavin monooxygenase and serves as a ferrireductase so that ferrous iron is made available for incorporation into compounds such as ferritin and heme. Recently, DMT-1 was shown to participate in the paraferritin complex. Ferrous iron enters cells via a DMT-1 facilitated pathway. Heme is freed from hemoglobin by luminal enzymes so that heme can enter the cell as a metalloporphyrin. In the cell, iron is freed from heme by heme oxygenase so that it enters the plasma as inorganic iron. Iron enters the cell from the plasma via the classical holotransferrin receptor pathway to inform the cell of the iron status of the body. We postulate, from limited evidence, that an apotransferrin receptor is present on the basolateral membranes of enterocytes to permit preferential docking of apotransferrin on this receptor to facilitate transfer of iron from the cell to the plasma.

synthesis of iron containing proteins such a hemoglobin (Fig. 8).

DMT-1 is a protein which was discovered by expression cloning of proteins up-regulated in iron deficiency (27–30). It was found to be defective in Belgrade rats and mk mice which phenotypicaly have iron deficient anemia. DMT-1 facilitates the uptake of ferrous iron and certain other metals to include manganese (Fig. 9) (27, 32). DMT-1 can be isolated from mucins in guanidine-soluble, water, and Triton X-100 insoluble fractions of duodenal mucosa homogenates (32).

Immunofluorescent microscopy of rodent duodenum from iron deficient animals showed DMT-1 was intensely concentrated in close association with the intestinal microvilli. This was not observed in gut from normal animals (35). We confirmed this finding using anti-DMT-1 antibody and iron deficient rats (38). Similar findings were observed in experiments using anti-mobilferrin antibody suggesting that iron deficiency altered the location of mobilferrin in the cells such that it was proximate to the microvilli rather than being bound to endoplasmic reticulum (38). These experiments were repeated except that tissues were prepared for examination by electron microscopy (Fig. 10). We observed that much of the reactive proteins was outside the cell in mucins layering the microvilli. This was shown by both electron microscopy and by identification of both proteins

FIG. 6. Iron binding proteins were identified in the homogenates of duodenal mucosa from iron deficient rats after radio-labeled ferric chloride was injected into duodenal loops. Water soluble fractions containing mobilferrin and paraferritin were separated and isolated to near homogeneity with 60% ammonium sulfate, sizing, and ion exchange chromatography. Water insoluble proteins were purified using Triton X-100, solubility in guanadine and urea, and chromatography. This separated β_3 integrin from mucin. Later we discovered the DMT-1 was recovered in a water and Triton X-100 insoluble, guanadine soluble faction.

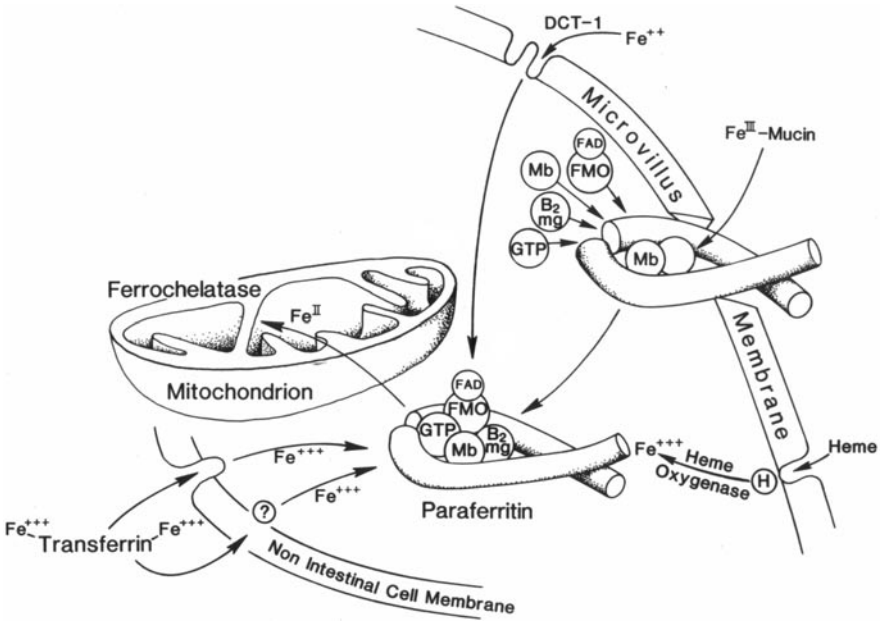

FIG. 8. Paraferritin is a 520-kDa protein complex isolated from the cytosol of duodenal mucosal cells. The complex was initially shown to contain a β_3 integrin, mobilferrin, and flavin monooxygenase. Subsequently, β_2 microglobulin and DMT-1 were identified on Western blots of paraferritin isolates from sizing chromatography. Data indicate that mobilferrin and DCT-1 enter cells by different pathways. The association of DMT-1 with paraferritin may be to transport iron reduced by flavin monooxygenase and FAD to make it available within mitochondria for production of hemoglobin.

immunologically in intraluminal mucin.goblet cells from iron deficient animals contained a plethora of anti-mobilferrin and anti-DMT-1 reactive constituents admixed with the mucins. There was relatively little reactive protein in the goblet cells of normal iron replete rat duodenum. This suggested that mobilferrin and DMT-1 may be concentrated in the goblet cells of iron deficient animals for secretion into the gut mucosa by the goblet cells where they can bind iron to facilitate absorption of both ferrous and ferric iron (Figs. 11 and 12).

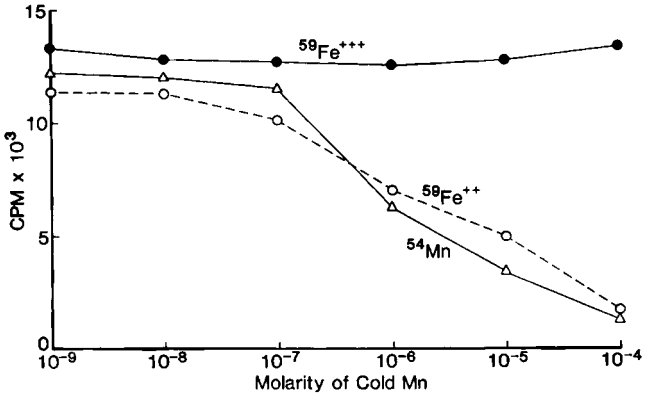

FIG. 9. Competitive inhibition studies in K562 erythroleukemia cells showed that increasing concentrations of manganese chloride diminished the uptake of ferrous iron but not ferric iron. This indicated that ferric and ferrous iron enter cells by different pathways and that the ferrous pathway was shared with manganese. Likewise, manganese can be used as a surrogate for ferrous iron in studies of cellular uptake of nontransferrin-bound iron. The existence of two pathways was also shown by a combination of biochemical, immunological, and genetic methods. Each method demonstrated the existence of separate pathways for uptake of ferric and ferrous iron (32).

The relative physiological importance of the IMP and DMT-1 pathways in the absorption of iron in humans is not known. However, the following information is available: (1) DMT-1 missense mutations were associated with a disease in rodents (Belgrade rates and mk mice) (29) and the calreticulum knockout mouse has not been viable (34). (2) DMT-1 protein was reported to be dramatically increased in intestinal cells of severely iron deficient mice (35) and the activity and cellular location of IMP was altered in iron deficient rats. (36, 37). (3) The DMT-1 protein was immunologically undetectable in microvilli of freshly obtained normal human duodenum at Whipple surgery (unpublished data) and the IMP pathway was active in normal human duodenum (21). (4)

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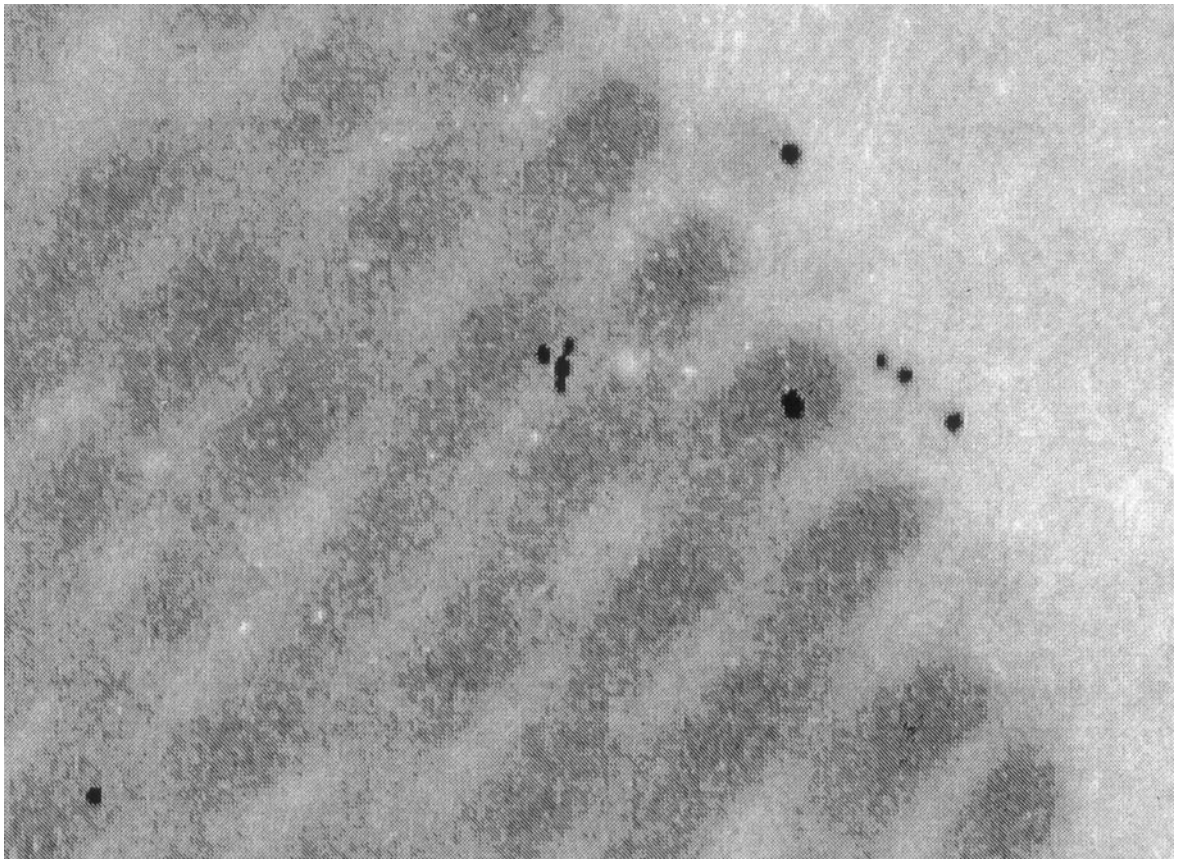

FIG. 10. Electron micrographs of duodena of normal and iron deficient rats were prepared. The specimens was incubated with anti-DMT-1 antibody and then with a gold conjugated secondary antibody. Markedly increased DMT-1 was seen in mucin on the surface of the microvilli and in goblet cells of iron deficient rats by comparison to intestinal specimens from normal animals. Similar findings were seen using anti-mobilferrin antibodies. This suggests that mobilferrin and DMT-1 are secreted with mucin into the lumen of the gut where they associate with dietary iron to enhance iron absorption.

The DMT-1 protein appeared to transport some non-ferrous metals of nutritional importance whereas the IMP pathway was specific for transport of ferric iron into the cell. (32) (5) A defect in the IMP pathway was identified in a family with sideroblastic anemias, a genetic disorder with iron overloading (39). (6) DMT-1 was demonstrated in close association with the high molecular weight paraferritin complex on chromatographic sizing columns suggesting an intracellular interaction between the IMP and DMT pathways. (33). (7) The quantity of both mobilferrin and DMT-1 was increased in goblet cells from iron deficient animals suggesting they bind mucins to facilitate iron absorption from the gut lumen.

The importance of the iron concentration in enterocytes has been known for forty years (Fig. 13). Mucosal uptake of iron from the intestinal lumen appears to be regulated by the quantity of iron within the absorptive cell (40). The absorptive cell receives iron from both the diet and via the holotransferrin receptors on the basolateral surfaces of the cell. The holotransferrin receptor operates in the absorptive cells as it does in non-absorptive cells by transporting iron into the cell. This receptor has a 500-fold greater affinity for holotransferrin than for apotransferrin. The iron delivered to the enterocyte from the plasma by the transferrin receptor keeps the intestinal cell informed of the status of body-iron stores. Iron deficiency is associated with little stainable iron within the intestinal cells whereas iron overloaded animals have excess iron in the enterocytes (41). The enterocytes of untreated hemochromototics contain little stainable iron similar to the iron deficient gut and absorb excessive amounts of dietary iron (Fig. 14). The iron visualized by electron microscopy in the cytosol of absorptive

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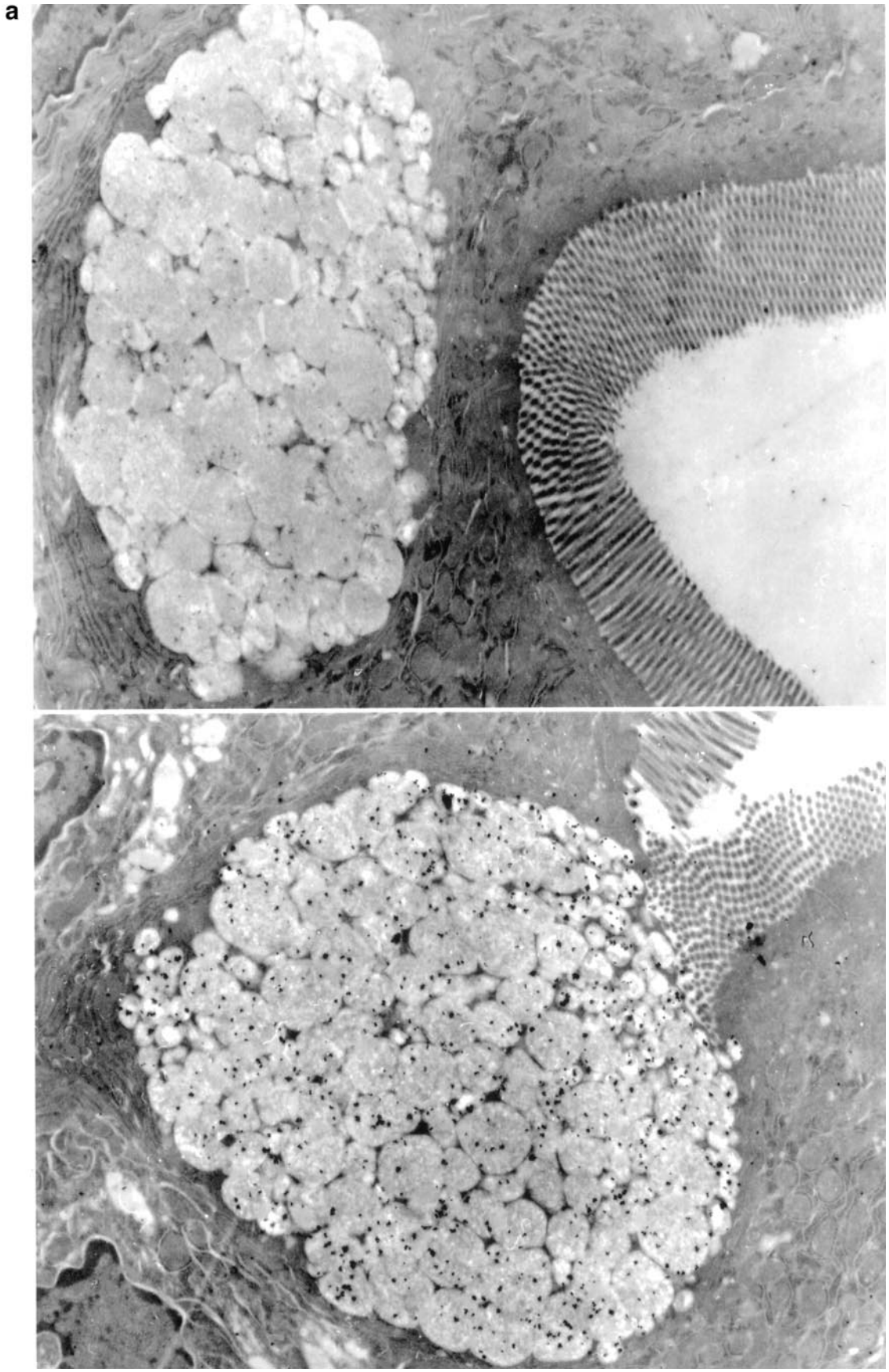

FIG. 11. Electron micrographs of goblet cells in duodenal mucosa from normal (upper) and iron deficient rats (lower) which had been incubated with either anti-mobilferrin (left) or anti-DMT-1 antibodies (right) showed a marked increase in the concentrations of both mobilferrin and DMT-1 in the mucin of the goblet cells from iron deficient animals (EM by M. Simovich).

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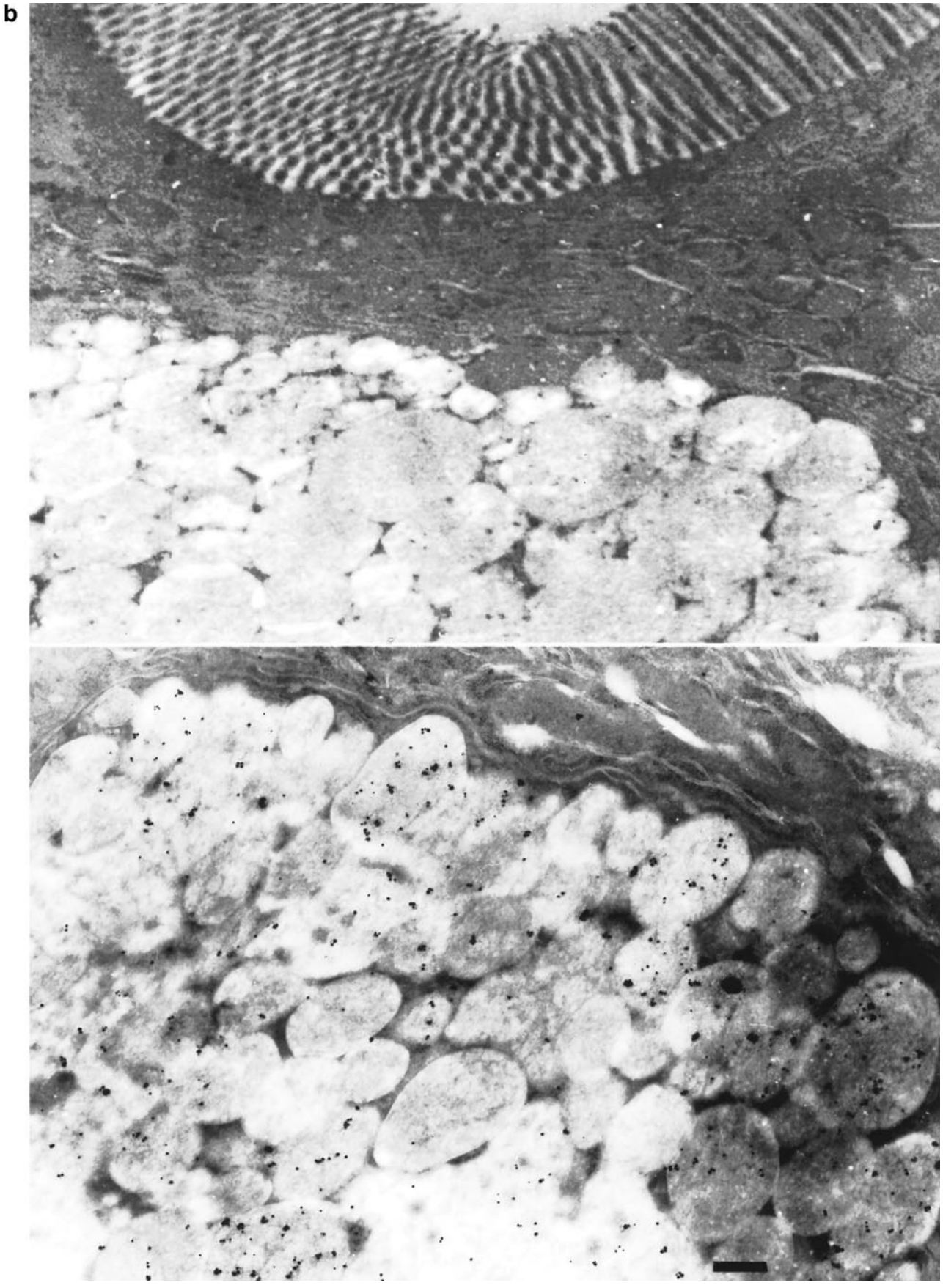

FIG. 11—*Continued*

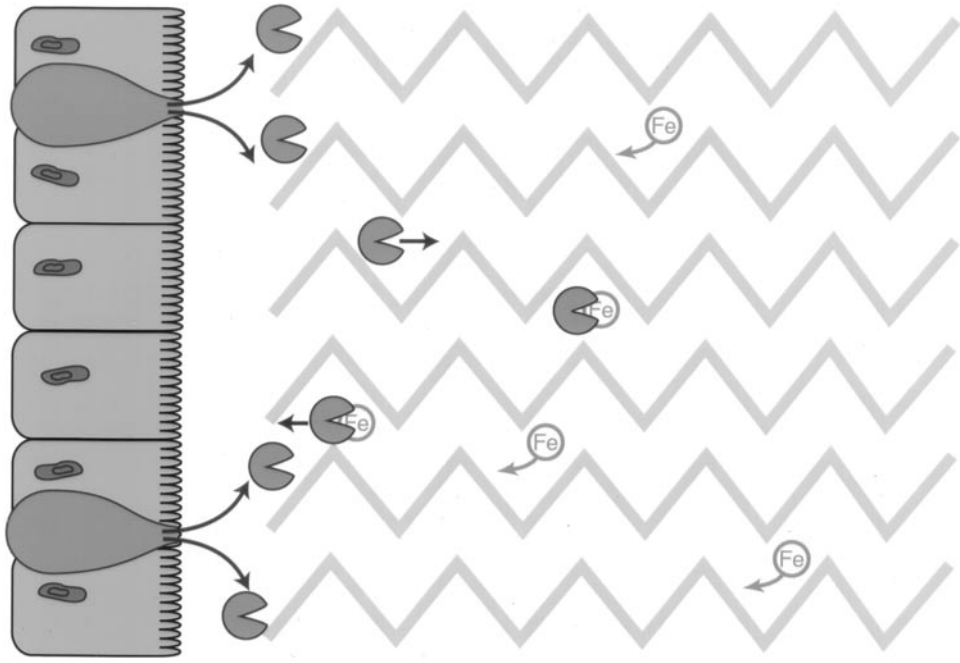

FIG. 12. Cartoon depicting a possible role for mucins in the uptake of inorganic iron from the intestinal lumen. Mobilferrin and DMT-1 are found in significantly increased concentrations in association with the mucin within goblet cells. Mobilferrin and DMT-1 are secreted with mucins into the intestinal lumen where they bind either ferric or ferrous iron to maintain the iron available for absorption by the enterocytes.

cells is predominantly ferric iron because it stains with acid ferrocyanide and not ferricyanide (41). Pretreatment of tissues with iron nitrilotriloacetic acid (Fe-NTA) showed similar quantities of iron within the cells in each of the states of iron repletion suggesting that the differences observed were not primarily caused by changes in the concentration of cytosolic iron binding proteins. On the basis of these observations, we postulated that iron uptake in absorptive cells may be modified by satiation of iron binding sites by iron on either iron responsive elements or iron binding proteins or both. In addition to providing a mechanism to control iron absorption, this mechanism serves as a method for preferential loss of iron from the body (Fig. 15).

In North America and Europe, two-thirds of dietary iron is present as ferric iron and one-third as heme-iron (11). However, most body iron is derived from heme because of much of the non-heme iron is bound in the lumen of the gut to chelators that polymerize and precipitate the inorganic iron rendering it unavailable for absorption (Fig. 15). Countries where meat is scarce usually have a high incidence of iron deficiency even though the diet may contain a similar quantity of total iron. Unlike inorganic iron, heme is soluble at alkaline pH and precipitated under acid conditions. Heme is freed from myoglobin and hemoglobin largely by pancreatic enzymes (12, 13). Globin degradation products are important for maintaining heme in a de-polymerized state so that it is available for absorption. Heme enters the enterocyte as an intact metalloporphyrin. Heme uptake is not competitive with non-heme iron (Fig. 16). Ultrastructural studies suggest heme-iron uptake occurs, at least in part, as an endocytic process similar to transferrin-iron (42, 43). Once within the absorptive cells, the heme porphyrin ring is split with the release of inorganic iron which then must compete with other inorganic iron within the cell for transit to transferrin (44). It must be these latter steps which foster absorption of increased amounts of iron from dietary heme in both iron deficient and hemochromatotic humans. The proteins(s) involved in the uptake of heme-iron from the intestinal lumen is unidentified. While there is a plethora of literature describing the absorption of inorganic iron, there are few articles devoted to heme-iron absorption. In part, this may be because rodents are poor models for the absorption of heme-iron and uptake in ro-

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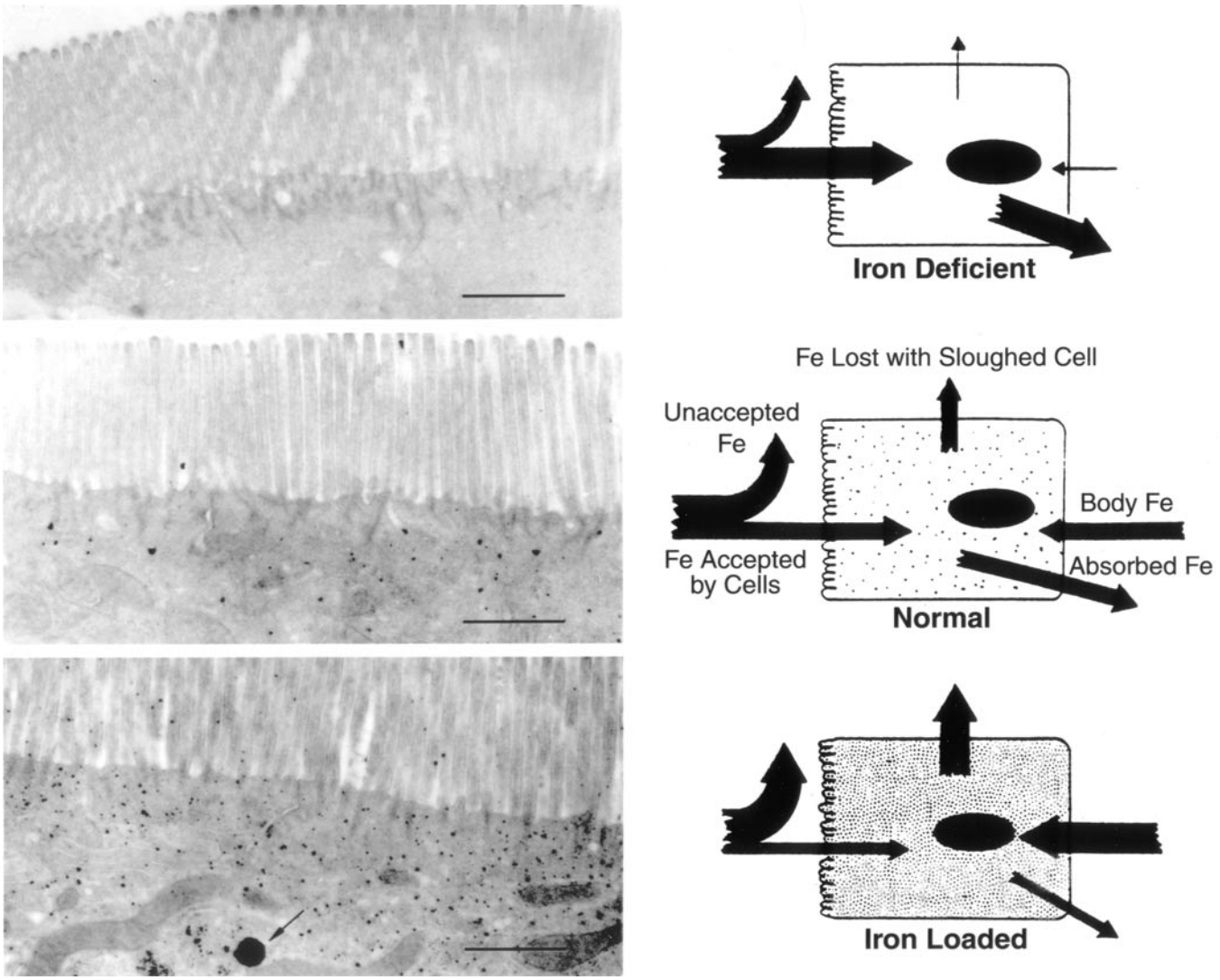

FIG. 13. Iron was identified in enterocytes of iron deficient (top), normal (middle) and iron loaded rats (bottom). Iron was stained with acid ferrocyanide and can be identified by small dark deposits in the cytoplasm. There is little iron in the iron deficient guts and surplus in the intestine of iron loaded rats. The potential consequences of these findings are shown in the cartoons to the right of the electron micrographs. Similar examinations were performed using acid ferricyanide and showed no reactive areas. This indicates that the majority of iron within the enterocytes is ferric iron (EM by R. J. Parmley).

dents is not enhanced by iron deficiency (12). It may be possible to learn more from bacteria which require chocolate agar plates because they utilize heme as a source of iron (45, 46).

There are other proteins which were described in recent years that appear to be important in the cellular transport of iron. Examples are SFT (47, 48) (stimulator of iron transport) and Haephestin (49). They seem to be related to either the egress of iron from the absorptive cells or intracellular handling of iron or both. They are not discussed in this article because less is known about their role in absorption than IMP and DCT-1.

CORPOREAL FACTORS

The most important known stimuli to iron absorption are tissue iron stores, the rate of erythropoiesis, and hypoxia (1, 2). Accelerated red blood cell production and rapid plasma iron turnover seem related to enhanced iron absorption whether the cause be hemorrhage, hemolysis or hypoxia. Conversely, diminished erythropoiesis such as occurs with blood transfusion, return to sea level from high altitudes and starvation decrease iron absorption. It is tempting to postulate an erythropoietin-like factor which informs the

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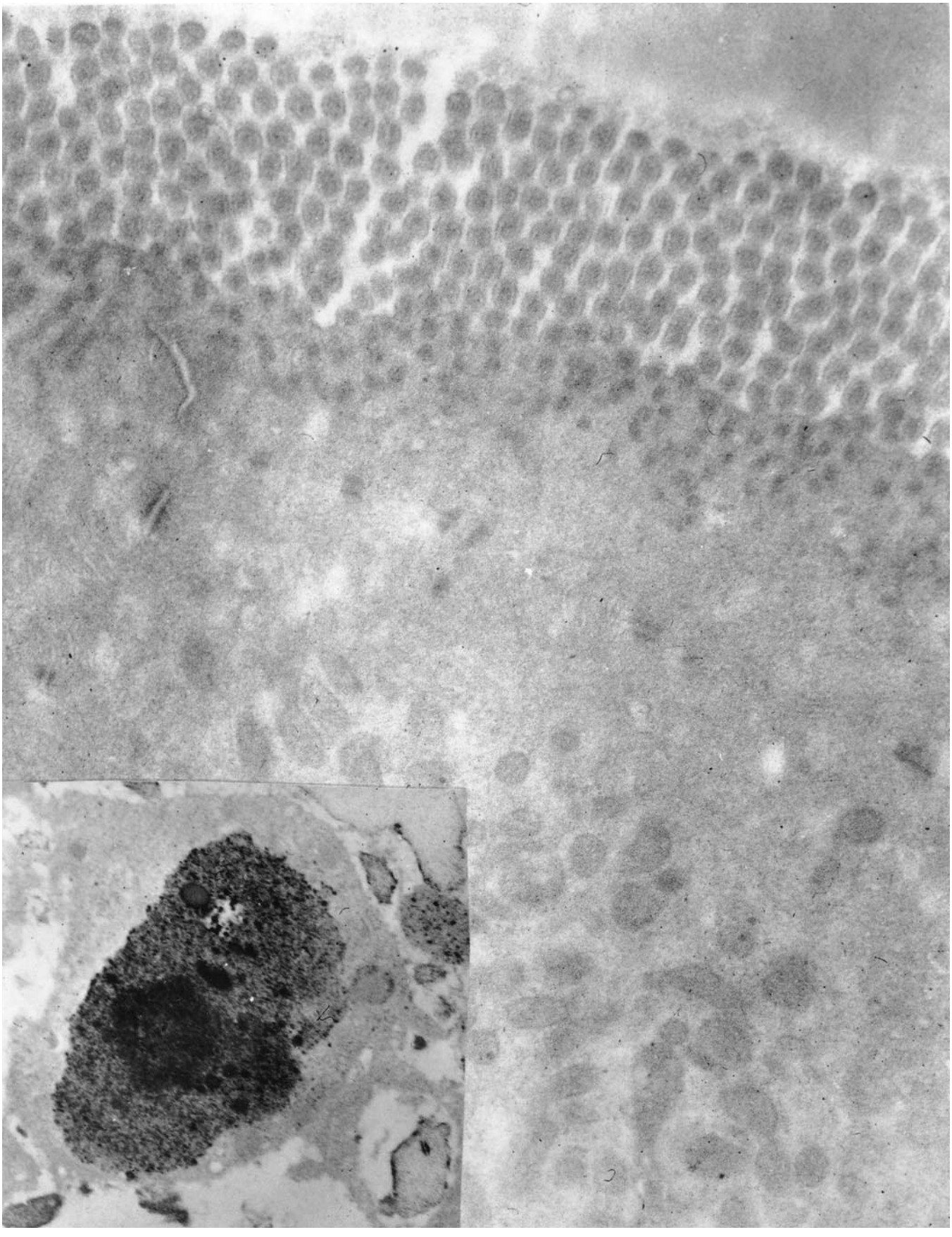

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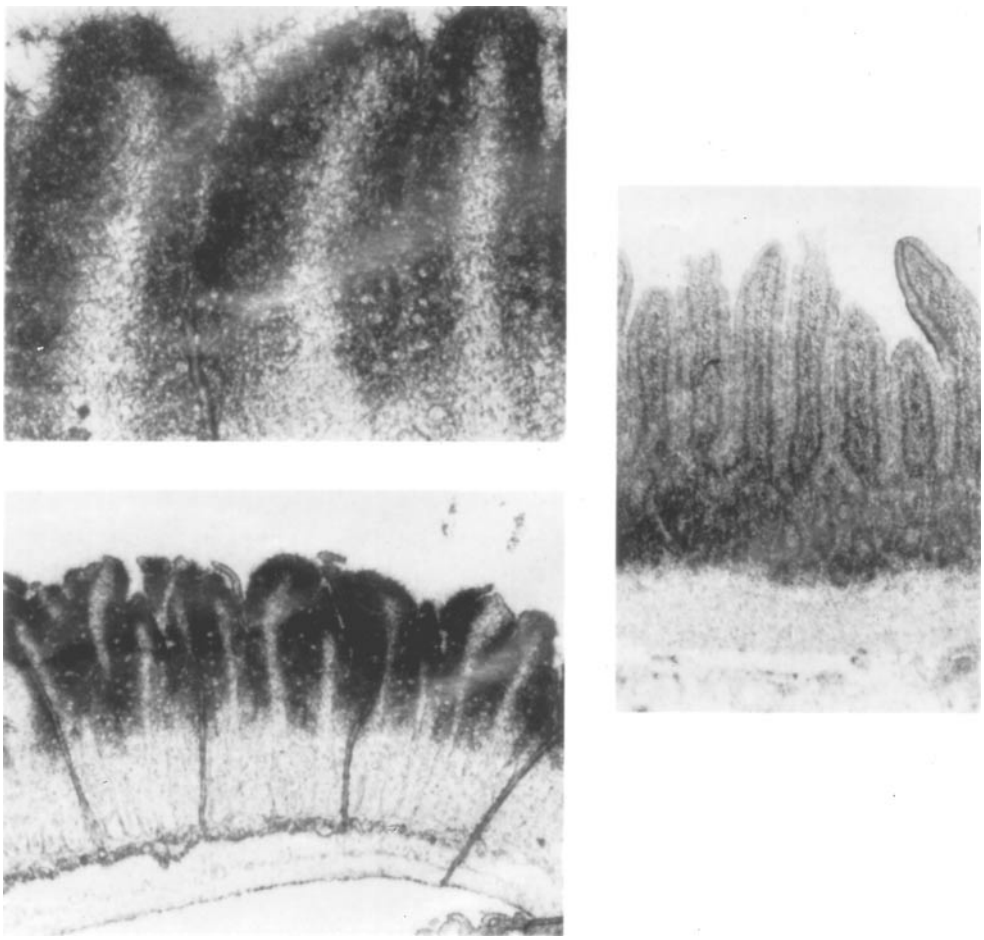
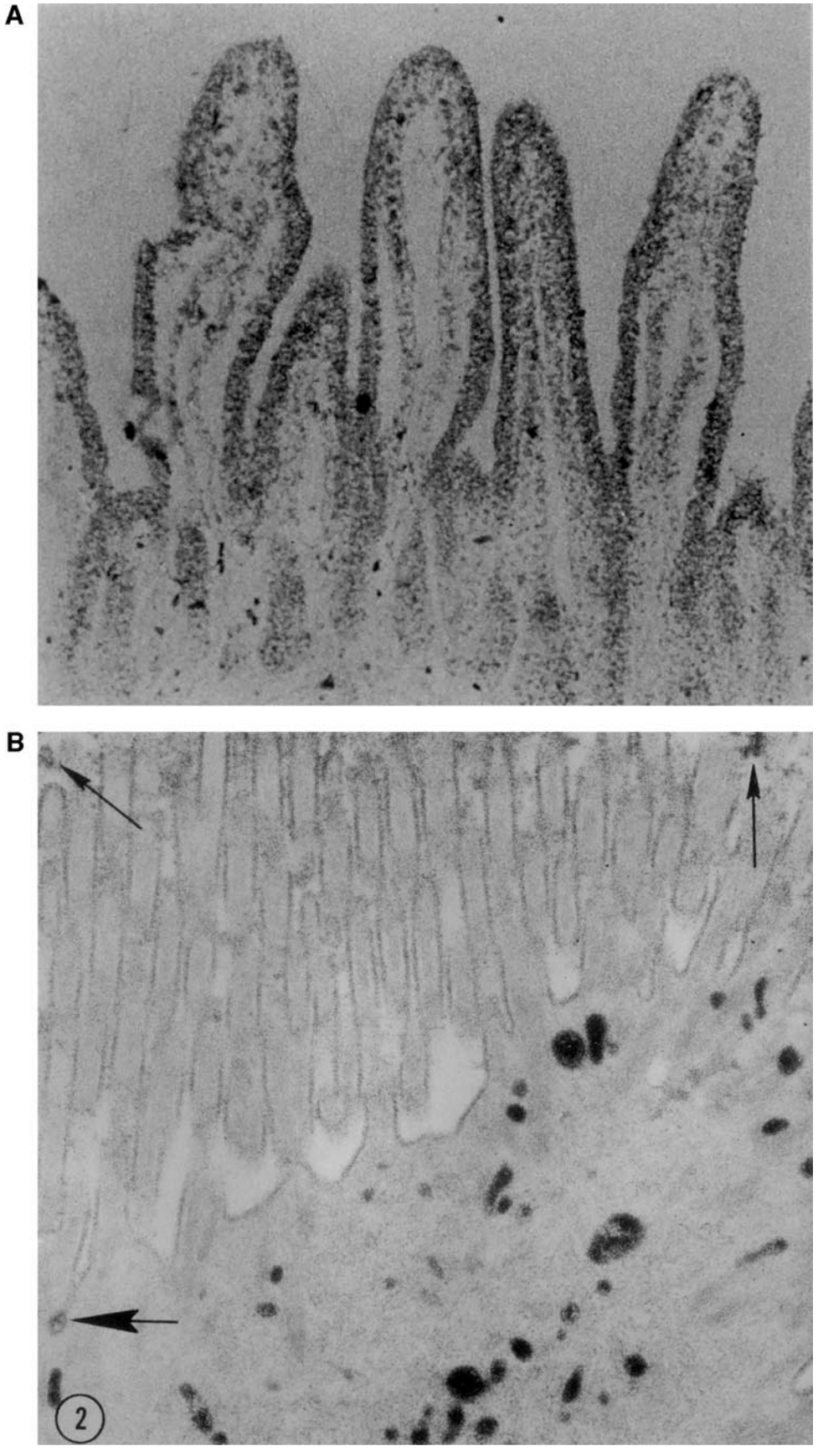


FIG. 15. Duodena were removed from rats at intervals after an intraluminal dose of radio- iron (left). Autoradiographs were made from sections. Two hours following dosing with radio-iron, most of the enterocytes were radio-labeled (upper left) At 24 h after dosing, only the cells on the distal half of the villi were radio-labeled (lower left). In contrast, if the dose of radio-iron was administered intravenously, the cells in the crypts of Leiberkuhn were preferentially labeled 16 h after dosing and the enterocytes toward the tip of the villi were spared (right) (EM by R. J. Parmley).

gut of body requirements for iron. However, enhanced iron absorption continues in iron deficient subjects long after the red cell mass returns to normal and persists until iron stores are repleted (50). Transfusion of reticulocytes which increases the number of circulating transferrin receptors increases plasma iron turnover and enhances iron absorption (51). Since accelerated hemoglobin production depletes iron stores, the level of labile iron in body stores may be a more important basic regulator. If one postulates the existence of tissue iron receptors, increased iron absorption would be expected to continue until the receptors are satiated. Surplus iron would then become available for incorporation into intestinal cells via transferrin receptors to inhibit iron absorption and enhance body losses of iron in exfoliated intestinal absorptive cells. Then hemochromatosis would be either a disorder of tissue iron receptors or an inability to use them properly.

Many investigators have searched for a blood factor that signals the gut to either enhance or

FIG. 14. Duodenal biopsy specimens were obtained from untreated iron overloaded hemochromatotics. Acid ferrocyanide incubation showed no stainable iron within the enterocytes similar to findings in iron deficient humans and animals. However, macrophages in the laminal propria containing ferritin (inset) stained intensely with acid ferrocyanide. Since heme-iron absorption is increased in hemochromatosis and heme and inorganic iron are absorbed non-competitively, this suggests that the basic defect in hemochromatosis does not involve the microvilli. (EM by R. J. Parmley).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diminish iron absorption. Clinical observations indicate that the hemoglobin concentration and plasma levels of iron and transferrin did not perform these functions acutely (50). Multiple studies of the effect of transferrin receptors have been performed and failed to show the changes in the quantity of iron absorbed (52). Further, it is difficult to assign the regulation of iron absorption to transferrin saturation in conditions in which there is increased absorption of iron but only a small amount of unsaturated transferrin such as is observed in iron overloading disorders and atransferrinemia. Similar experiments with ferritin have failed to show that quantity of circulating ferritin affected iron absorption (53). A criticism of these experiments is that they were performed acutely over a short period of time.

Most corporeal factors that alter iron absorption do not exhibit an effect for several days (4, 14). This delay has been attributed to the two to three day life span of the enterocyte (41) (Fig. 17). It was postulated that iron was deposited in newly formed cells in the crypts of Lieberkuhn in quantities inversely proportional to the existent requirement for iron. Subsequently, the cell migrates towards the villus tip maintaining most of its iron until it is sloughed into the intestinal lumen at the end of its three day life span. Another possibility is that it requires several days delay to affect erythropoiesis and the production of transferrin receptors. In that model, most of the intestinal cells on the villus would remain in equilibrium with body requirements and preferential radiolabeling of crypt cells would be detected in autoradiographs only because they contained little intracellular iron at the time of dosing and would accept a larger proportion of radio-iron than more mature enterocytes which had received their quota of iron prior to the availability of the radio-iron. The latter hypothesis is more in accord with the

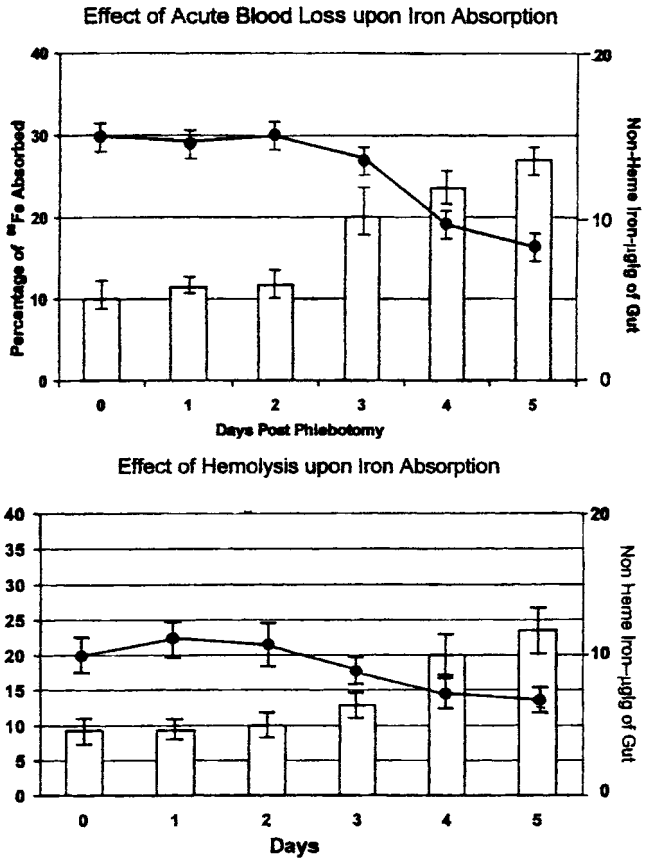

FIG. 17. Following either phlebotomy or production of hemolysis by administration of acetylphenylhydrazine to rats, there was a 3-day delay before iron absorption was increased (bars) and the non-heme iron concentration in duodenal specimens was decreased (line).

rapid diminution of iron absorption observed following either an injection of endotoxin or removal of a hypoxic stimulus and the rapid increase of iron absorption that occurs when reticulocytes are transfused into the circulation (54). Thus factors that have a delayed effect on upon iron absorption such as erythropoietin, thyroid and pituitary extracts indirectly affect iron uptake by the gut by increasing body iron requirements and not by a direct effect upon enterocytes (56, 57). Similarly,

FIG. 16. Rabbit hemoglobin was injected into isolated duodenal loops of beagle dogs. Then biopsy specimens were obtained and washed with 0.15 M saline. The specimens were examined by both light and electron microscopy after staining with 3,3′-diaminobenzidine. Intestinal villi showed benzidine reacting material within the surface epithelium indicating that heme entered the absorptive cells as an intact metalloporhyrin (top). Electron microscopy of enterocytes (bottom) identified heme on the surface of cells (small arrows) and in endocytic caveolae (large arrows) and in tubovesicular structures. These data support the hypothesis that heme enters intestinal enterocytes as a metalloporhyrin and that uptake was at least partially an endocytic process (EM by R. J. Parmley).

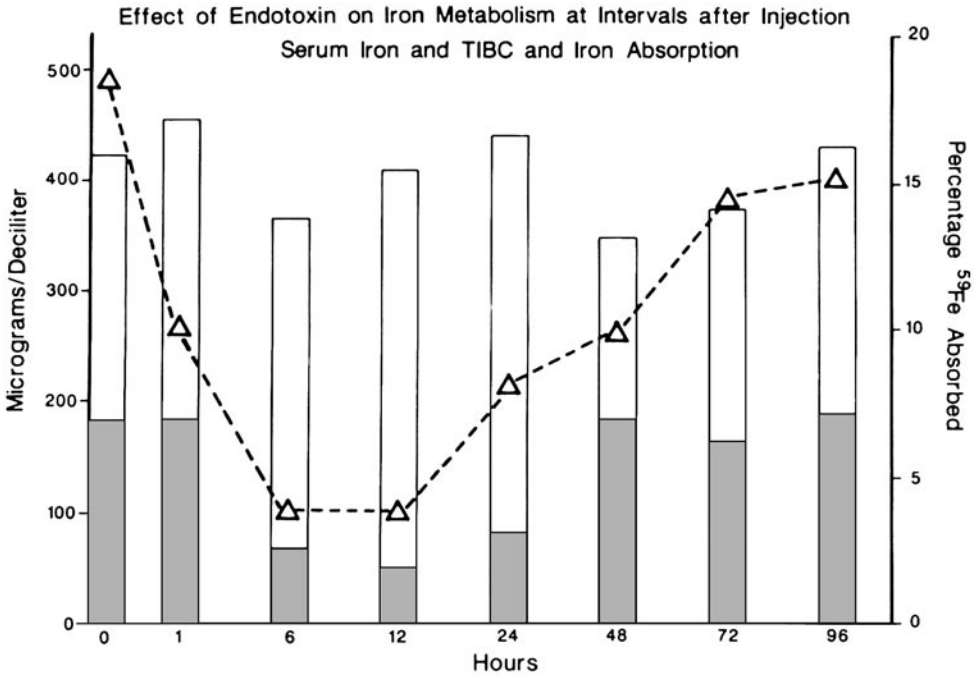

FIG. 18. Unlike most other factors which change iron absorption, the effects of a dose of endotoxin were observed within 1 h after injection (dotted line). This occurred before changes in either the serum iron concentration or the iron binding capacity of serum (bars). Unlike other factors which alter iron absorption, there was no change in the non-heme concentration of duodena in the endotoxin treated animals. This suggests that endotoxin alters iron absorption in a different manner than is observed elsewhere.

evidence that erythropoietin is not a direct regulator of iron absorption was shown by the lack of effect of erythropoietin administration in animals when bone marrow was depressed by irradiation. (58). These latter experiments suggest that hypoxia acts a regulator independent of body iron stores and iron turnover because it increased iron absorption following marked bone marrow suppression by radioactive strontium.

Several days after phlebotomy, humans develop a decrease in serum iron concentration (Fig. 17). Simultaneously, there is an increase in the rate of plasma iron turnover and an increase in iron absorption. Rapid iron turnover persists so long as iron absorption is enhanced and after the serum iron concentration normalized (59,60). Animal studies show a similar relationship between increased absorption of iron and rapid plasma turnover of iron in many physiologic experiments (60). In contrast, the injection of endotoxin causes the reverse relationship (Fig. 18). There is a decreased absorption of iron within hours after endotoxin administration with a rapid plasma iron clearance and a diminished iron turnover (54). This suggests that endotoxin affects iron absorption by a different mechanism of action than other factors. Enhanced iron absorption following transfusion of reticulocytes supports the hypothesis that the number of available iron receptors influences iron absorption (51). However, the manner in which either plasma iron clearance and turnover or the number of available iron receptors could affect absorption by the intestine independent of changes in the serum iron concentration or the quantity of apotransferrin is unknown. Further, aged individuals with a relatively hypoplastic bone marrow absorb as much iron as young adults (61). Thus a messenger which affects iron absorption has never been convincingly demonstrated. However, parabiont mice which were either normal or hypoxic caused changes in the iron absorbed by the other animal (62).

Transferrin accepts iron from intestinal absorptive cells and serves as the mechanism for delivering iron to other organs. We postulate this is facilitated by an apotransferrin receptor located

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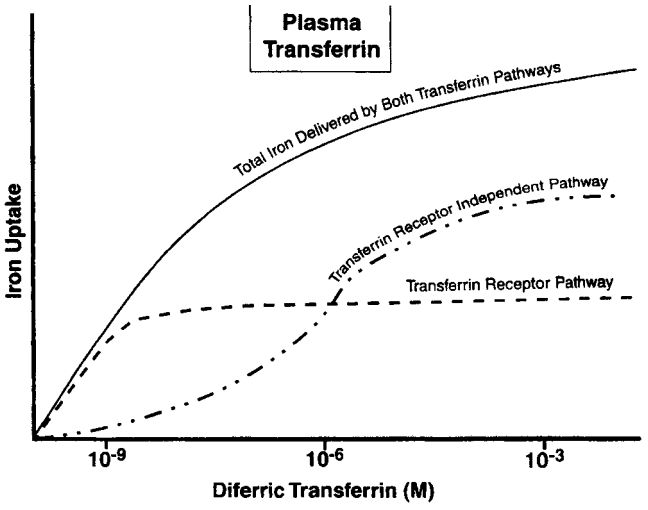

FIG. 19. Iron uptake from transferrin involves two separate pathways. This explains the nonsaturability of transferrin binding to cells.The classical transferrin-transferrin receptor pathway explains uptake of iron from transferrin at low diferric transferrin concentrations (high affinity, low capacity system). In this pathway holotransferrin is bound by transferrin receptors on the surface of the cell. Then the surface invaginates to form a vesicle that becomes acidic to release iron into the cell cytosol and return to the cell surface to deliver the transferrin into the plasma for reutilization. This pathway fails to elucidate what occurs with increasing concentrations of holotransferrin. A low affinity, high capacity system has been described that is independent of the transferrin receptor. It has been named the transferrin receptor independent pathway (TRIP). Blocking β_3 integrin antibodies inhibit uptake of transferrin-iron via the TRIP pathway and have little effect upon iron uptake via the classical pathway. Conversely, only the classical pathway can be inhibited by ammonium sulfate which prevents acidification of the endosome for the release of iron. The TRIP pathway is probably a functional pathway because radioiron delivered to the cell via this pathway is incorporated into heme synthesized by the cell. The physiological concentration of diferric transferrin is depicted by the rectangle and includes the most substrate sensitive portion of the TRIP curve.

in the basolateral surface of the enterocyte. The apotransferrin receptor differs from the well described holotransferrin receptor in that it facilitates the egress of iron from the cell and preferentially binds apotransferrin. In contrast, the classical holotransferrin receptor preferentially binds transferrin–iron complexes and delivers iron to the absorptive cell to keep it informed of the status of body iron stores. This mechanism is disrupted in both hemochromatosis and atransferrinemia. One of the major roles of transferrin is to bind iron so that it is unavailable to facilitate free radical formation. The transferrin pathways (63) (Fig. 19) not only facilitate iron uptake by cells but also assure that it is delivered to cells at a slowed rate that probably minimizes free radical formation; plasma iron turnover of iron unbound to transferrin is rapid and probably occurs via the IMP pathway because it is ferric iron. Cellular uptake of iron is slow in normal humans and is significantly increased in untreated hemochromatosis; this may account for much of the tissue damage seen in the iron overloading disorders and the delayed occurrence of tissue damage from this genetic abnormality until there is sufficient iron overload to saturate circulating transferrin.

ACKNOWLEDGMENTS

Presented at the Symposium on “Molecular and Clinical Aspects of Human Iron Metabolism,” September 28–October 3, 2002, Lake Chiemsee, Bavaria, Germany.

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