The Control of Iron Absorption by the Gastrointestinal Mucosal Cell

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A B S T R A C T Gastrointestinal mucosal factors controlling rates of iron absorption were studied utilizing an in vivo closed duodenal loop technique. Cellular distribution of newly absorbed radioiron was identified by molecular sieve and iron-exchange chromatography of the mucosal cell supernate. In the normal animal, iron rapidly appeared in ferritin, and this fraction accounted for greater than 90% of mucosal supernatant radioactivity after 60 min absorption time. The nonferritin radioiron appeared to be unbound iron salts. In the presence of increased iron absorption induced by iron depletion or hemolysis, the major difference from the normal distribution pattern was an increase in the proportion and quantity of the free iron salts. Incorporation of newly absorbed iron into ferritin did not correlate with the rate of iron absorption. No evidence was found for a specific soluble iron-chelating molecule within the mucosal cell. The nonheme iron content of the mucosal supernates from iron-deficient and hemolyzing animals were significantly lower than in the normal animal.

The data are consistent with hypotheses which suggest that iron absorption rates may be controlled in part by the rate of initial iron uptake by the mucosal cell and that a membrane transport mechanism exists which is modulated by the nonheme iron content of the mucosal cell or some portion thereof.

INTRODUCTION

The primary physiologic control of iron balance is by the regulation of iron absorption since processes for enhancing iron excretion are quite limited (1). Despite

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extensive investigation, the mechanisms of control of iron absorption remain an enigma. The primary site of control of iron absorption is the upper gastrointestinal mucosa (2). The two most important physiologic factors which are related to variations in iron absorption appear to be total body iron stores and the rate of erythropoiesis (3). Since iron absorption is increased under circumstances of iron depletion and increased erythropoiesis, it can be said that the normal organism has a relative "mucosal block" to iron absorption (4). Studies of iron absorption at the mucosal level have focused upon the following two distinct steps: (*a*) mucosal uptake of iron from the lumen and (*b*) mucosal cell transfer of a portion of this iron to the carcass (5–7). The control of these phases has been related to active transport mechanisms (5, 7).

The gastrointestinal mucosal and subcellular distribution patterns of iron have been investigated (8–12) as clues to the control of iron transport. Proposed mechanisms of regulation of absorption have been related to mucosal levels of the iron-storage protein ferritin (11, 12), to the presence of a soluble cytoplasmic carrier molecule for transport across the mucosal cell (8, 9), and to the iron content of the mucosal cell itself (12).

The present studies were undertaken to investigate these possible factors by stepwise evaluation of the mucosal cell distribution of iron salts during in vivo absorption under circumstances known to be related to variations in iron absorption rates.

METHODS

In vivo absorption. Adult female Sprague-Dawley rats weighing 180–250 g and maintained on standard rat chow were used. The in vivo closed duodenal loop method of Wheby, Jones, and Crosby (7) was used since it provided a better control of the quantity and concentration of iron exposed to a given surface area of mucosa per time and

minimized the influence of intraluminal factors compared with oral administration. The iron was introduced into the duodenal loop via a fine polyethylene tube inserted through a small gastric incision. The color of the loops remained normal throughout the study period. An aqueous solution of freshly prepared ferrous sulfate was administered in a total volume of 0.5 ml to which 50 μCi of high specific activity (5.2–26.3 μCi/μg) ^{59}Fe-labeled ferrous sulfate[1] was added in order to provide satisfactory count rates.

In the standard experiments, a total dose of 40 μg of elemental iron was administered which is within the physiologic range in this preparation (7). Loops were removed at 1, 10, or 60 min after introduction of the test dose; the loops were opened and rinsed thoroughly with iced saline. The mucosa was then scraped with a glass slide and homogenized with a Teflon pestle with 5 ml of 0.01 M Tris-HCl buffer, pH 6.5. The homogenate was centrifuged at 6000 g, portions of supernate were counted, and protein concentration was determined by the method of Lowry, Rosebrough, Farr, and Randall (13). From the specific activity of the iron administered and the radioactivity and protein concentration of the supernatant samples, the concentration of newly absorbed iron was calculated and expressed as micrograms Fe/gram supernatant protein.

Iron absorption. Carcass iron absorption during the period of study was determined. After instillation of the test dose of iron, the animal, in a 1000 ml plastic beaker, was placed on a constant rotating table[2] at 20 rpm. Total radioactivity was measured using a 36° angle–collimated probe with a 1.5 in. crystal at a constant distance of 25 cm from the center of the rotating table. The 50 μCi dose of ^{59}FeSO$_4$ produced count rates by this method of approximately 80,000 cpm above background. After removal and processing of the isolated loop, the animal carcass was counted in a similar manner. The ratio of net counts per minute of the carcass after the loop was removed to the net counts per minute of the animal with the loop intact was taken as an estimate of carcass iron absorption during the period of study and expressed as percentage carcass absorption.

Chromatographic studies. The 6000 g supernates were examined by chromatographic separation, both by molecular sieve and ion-exchange methods. The primary media used was Sephadex G-200[3] from which ferritin emerged just past the void volume. In addition, other media were utilized which have different molecular weight exclusion ranges, i.e., Sephadex G-10, G-25, and G-75, polyacrylamide gel (Biogel P-60)[4] and agarose gel (Biogel A-5m).[4]

A measured quantity, usually 0.5 ml, of the supernatant solution was placed on a freshly prepared Sephadex G-200, 55 × 0.9 cm, column. The sample was eluted with 0.01 M Tris-HCl, pH 6.5, containing 0.15 M NaCl, and 2.4-ml fractions were collected. Radioactivity of the samples from one bed volume were counted in a well-type scintillation counter. Values were expressed as percentage of total radioactivity applied to the column.

Ion-exchange chromatography of the supernate was also performed using DEAE-cellulose and stepwise or linear gradients of increasing ionic strength.

Antiferritin antibody studies. Whole supernatant and selected chromatographic fractions were studied for the presence of ferritin utilizing either rabbit anti-horse spleen ferritin antiserum[5] or goat anti-rat ferritin antibody (salt fractionated).[5] The antibody was added to the test sample in excess. In some experiments coprecipitation was carried out using carrier horse spleen ferritin (crystalized two times).[6] A portion of the sample-antibody mixture was removed immediately after mixing, and the remainder was incubated at 37°C for 1 hr and then refrigerated for 2–10 days at 4°C. The precipitate was then centrifuged at 6000 g, and a portion of the resulting supernate was removed. Whole normal serum was used in control tubes in place of the antiserum. The differences in supernatant radioactivity before and after precipitation were used to calculate the ferritin radioactivity precipitated.

Physiologic alterations. Iron depletion was achieved by raising weanlings on an iron-deficient chow[7] and serial tail vein bleeding. They were utilized for absorption studies after they reached the age of 60 days. Iron deficiency (FeD)[8] was confirmed by the presence of anemia, hypochromic microcytic erythrocytes, decreased serum iron (14), increased total iron-binding capacity (14), and a significant increase in iron absorption as compared with the normal animals. Hemolysis (PHZ) was induced with 1 ml subcutaneous dose of 10 mg of phenylhydrazine and confirmed by evidence of anemia and reticulocytosis. Absorption studies were performed on the 4–6 day after injection.

Mucosal nonheme iron content. The protein concentration of portions of mucosal homogenates and the 6000 g supernates was measured, and the nonheme iron was extracted by the method of Brückmann and Zondek (15). Iron concentration was measured by the method of Bothwell and Mallett (16). Results were expressed as micrograms Fe/milligram of homogenate or supernatant protein.

In vitro studies. ^{59}FeSO$_4$ was added to the 6000 g supernate preparation, and the mixture was incubated at 37°C for 10 or 60 min. A portion was chromatographed on Sephadex G-200.

Miscellaneous methods. Paper electrophoresis was performed using barbital buffer pH 8.6, ionic strength 0.075. Radioautographs of the paper strips were made on X-ray film exposed for 3–14 days. Hematologic measurements were made by routine methods (14).

RESULTS

Radioiron distribution in normal mucosa

Fig. 1 shows a typical elution pattern from Sephadex G-200 of the 6000 g supernate of the mucosal homogenate from a normal rat after a 10 minute absorption period with a dose of 40 μg of elemental iron. Two distinct peaks of radioactivity were seen. A large, sharp peak appeared just past the void volume (peak I), and a smaller, broader peak (peak II) occurred at the total volume of the column. It was also found that a proportion of the radioactivity applied to the column was nonelutable.

The percentage of the radioactivity applied to the column appearing in each peak at 1, 10, and 60 min of
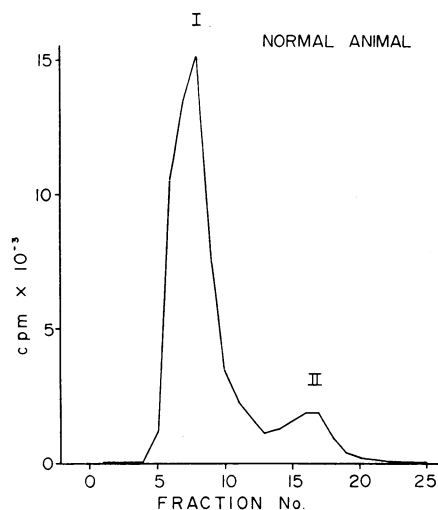

FIGURE 1 Typical normal elution pattern. Distribution of radioactivity eluted from Sephadex G-200 of the 6000 *g* supernate of the mucosal homogenate from a normal rat after 10 min absorption time for a 40 μg dose. 17% (13,000 cpm) of the radioactivity applied was not eluted.

FIGURE 2 Changes in radioiron distribution in 6000 *g* mucosal supernate of normal animals with time. (A) Peak I. (B) Nonelutable fraction. Values represent concentration of the radioiron in each fraction expressed in terms of supernatant protein.

absorption is shown in Table I. With time, in the normal animal with a 40 μg dose, there was a rapid increase in peak I and a concomitant decrease of peak II and nonelutable radioactivity.

The concentration of radiolabeled iron in the mucosal cell supernates was calculated as micrograms of newly absorbed iron per gram of supernatant protein. The amount of radiolabeled iron in each peak was calcu-
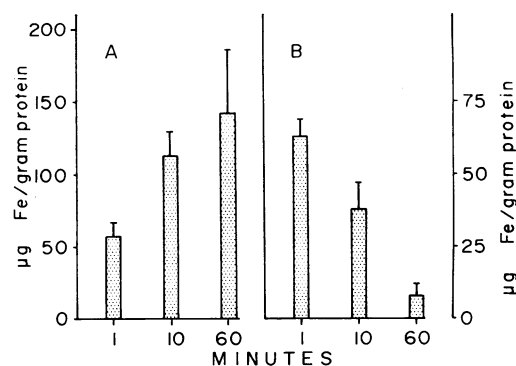
lated from the percentage radioactivity per peak. There was no statistically significant change in the concentration of total newly absorbed iron at 1, 10, and 60 min (135.9, SE 10.8, 148.6, SE 34.3, and 175.1, SE 40.9 μg Fe/g supernatant protein, respectively). Thus, the increase in peak I and the decrease in nonelutable radioactivity were both absolute changes (Fig. 2).

Attempts were made to identify other radioactive fractions utilizing chromatographic media with different ranges of molecular weight separation. With polyacrylamide gel (P-60) and agarose (5M), only a single peak of radioactivity was identified. With Sephadex

TABLE I

Distribution of Mucosal Supernatant Radioactivity

Group	Absorption time	Dose	Mean carcass absorption	Per cent radioactivity per fraction*			
				I	II	III	NE
	min	*μg*	*%*				
N (3)‡	1	40	0.7	44.7 ±3.3	20.4 ±1.7	0	34.9 ±1.6
FeD (4)	1	40	—	14.1 ±1.2	21.8 ±2.8	12.5 ±1.8	51.6 ±4.0
N (6)	10	40	1.5	69.3 ±2.1	9.1 ±0.8	0	21.6 ±1.9
FeD (6)	10	40	8.6	18.2 ±2.7	15.9 ±1.4	8.3 ±0.8	57.6 ±2.8
PHZ (4)	10	40	8.6	52.0 ±9.6	8.4 ±2.3	0	39.6 ±7.5
N (4)	60	40	2.8	94.0 ±2.0	0	0	6.0 ±2.0
FeD (5)	60	40	23.3	61.4 ±6.5	10.9 ±2.1	13.0 ±1.7	14.7 ±5.0
PHZ (3)	60	40	23.4	69.3 ±4.4	11.5 ±3.4	0	19.2 ±2.0
N (3)	10	4	2.6	78.2 ±4.1	1.7 ±0.7	0	20.1 ±4.1
FeD (4)	10	4	32.6	21.8 ±3.3	10.2 ±2.9	20.4 ±1.3	47.6 ±1.5
N (3)	60	4	5.3	91.5 ±2.0	0	0	8.5 ±2.0
FeD (3)	60	4	49.5	24.5 ±3.6	16.0 ±1.2	39.0 ±1.3	20.5 ±3.6

N, normal; FeD, iron deficient; PHZ, phenylhydrazine-treated animals; NE, nonelutable radioactivity.
* Mean ±1 SE.
‡ Numerals in parentheses represent number of animals studied.

G-10 and G-25, a single peak of radioactivity was eluted at the void volume. Using Sephadex G-75, a major peak of radioactivity appeared at the void volume followed by a minor peak. With all of these media, a proportion of radioactivity, comparable with that found using Sephadex G-200, was nonelutable.

Characteristics of the fractions

Peak I. Since ferritin is known to be a major iron-containing protein in gastrointestinal mucosal cells (11, 17, 18), peak I was investigated for the possibility that it represented radioiron bound to ferritin. When the mucosal homogenates were centrifuged at 105,000 g, radioactivity in peak I was reduced by 80% as compared with the same homogenate spun at 6000 g before chromatography. 82% of crystallized horse spleen ferritin sedimented at this speed, as expected (11). Paper electrophoresis and radioautography of a fraction of peak I showed that the radioactivity had an identical mobility to crystallized horse spleen ferritin. The fractions from peak I, when rechromatographed on Sephadex G-200, eluted at the same position with approximately 100% recovery and, in addition, had identical elution points to crystallized horse spleen ferritin on Sephadex G-200, polyacrylamide gel, agarose, and by gradient elution from DEAE-cellulose.

In nine experiments a mean of 69.1% (SE 4.2) of the radioactivity in peak I was precipitated by anti-horse spleen and anti-rat ferritin antibody. 65% of crystallized ferritin, eluted from a Sephadex column, was precipitated by these antibodies. The radioactivity precipitated from peak I could be increased to 82% by coprecipitation with carrier crystallized ferrin. In addition, 69% of whole supernatant radioactivity from a normal animal, after 60 min absorption time, was precipitated by antibody. If carrier ferritin was added to the whole supernate-antibody mixture, peak I was no longer identifiable when the resulting supernate was chromatographed. Thus, peak I had the characteristics of ferritin under all conditions tested.

Nonelutable fraction. A reproducible amount of radioactivity was nonelutable from all media used. A similar quantity was also nonelutable from DEAE-cellulose with an ionic gradient to 1 M NaCl. This radioactivity could be eluted from the DEAE with 0.1 N HCl.

The nonelutable radioactivity was chelated by EDTA or transferrin when these substances were added to the whole supernate before chromatography.

When ^{59}Fe-labeled ferrous sulfate was applied to a Sephadex G-200 column, a variable proportion of the radioactivity was eluted at the point of the total column volume. The percentage eluted correlated with the quantity of salt applied but never exceeded 30% (0.25 μg

resulted in less than 2% recovery and 2.5 μg resulted in recovery of 27.8%). When the elutable radioactivity was then rechromatographed, 0–10% was eluted. When ferrous sulfate was applied to a DEAE-cellulose column, none was eluted at pH 6.5 and 1 M NaCl. However, 65% or more of the radioactivity was then eluted with 0.1 N HCl. Thus, the nonelutable fraction behaved in a manner essentially identical with iron salts under these chromatographic conditions.

Peak II. As noted in Table I, peak II was variable in its quantity but roughly paralleled the proportion of the nonelutable fraction, in that it was highest when the nonelutable proportion was highest and essentially disappeared when little nonelutable iron remained. It appeared at the total column volume. When a fraction from peak II was rechromatographed on Sephadex G-200, little or none was eluted (< 10%).

A 6000 g supernate was prepared from FeD animals and chromatographed on Sephadex G-200. ^{59}Fe was added to the pooled fractions eluted at the total bed volume and rechromatographed. No radioactivity was eluted, suggesting that there was little or no iron-chelating ability of the low molecular weight compounds which were eluted at the point of peak II under these conditions.

Peak II radioactivity and the small proportion of elutable ^{59}Fe-labeled ferrous sulfate appeared in the same fractions on Sephadex G-10, G-25, G-75, and G-200. Thus, peak II chromatographed identically to the small amount of free iron salts that were eluted from all media studied when a solution of ferrous sulfate was applied.

Effects of iron depletion and hemolysis on mucosal radioiron patterns

Two physiologically different circumstances which are associated with increased iron absorption were studied in a fashion identical with the normal animals to determine whether the increased iron absorption could be correlated with any change in the normal mucosal distribution of newly absorbed ferrous sulfate.

Effect of iron depletion. The iron-deficient (FeD) animals showed a different elution profile from the normal mucosal supernate (Table I and Fig. 3). There was a significantly smaller proportion of the newly absorbed iron in peak I at all times. Conversely, the nonelutable fraction predominated in the iron-deficient animal early and still accounted for 15% of the total supernatant radioiron at 1 hr. The third major difference in the FeD animals was the presence of another radioactive peak (peak III) intermediate between peaks I and II.

Effect of hemolysis. In animals who were actively hemolyzing (PHZ), a third pattern of radioiron frac-
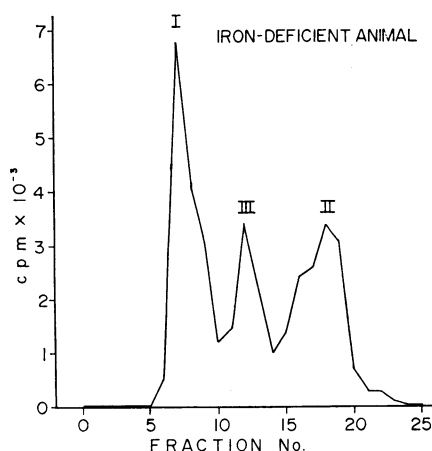
FIGURE 3 Typical elution pattern from an iron-deficient animal. Distribution of radioactivity eluted from Sephadex G-200 of the 6000 g supernate of the mucosal homogenate from an iron-deficient rat after a 10 min absorption time for a 40 μg dose. 62% (62,000 cpm) of the applied radioactivity was not eluted.

tions was seen (Table I). At 10 min peak I contained approximately 50% of the radioactivity, but 40% was also nonelutable. In these animals, no definite peak III was identifiable. It was notable that the per cent carcass iron absorption in the PHZ animals was quite similar to that of the FeD animals.

The relative differences in the distribution of radioiron in the three groups (Table I) also represented absolute differences in the quantities of radioiron in the fractions. Table II lists the concentration of total radioiron in the mucosal cell supernate at 10 min of absorption in the three groups. The quantity of newly absorbed iron in each peak was calculated from the percentage distribution of radioactivity per peak. The results for peak I and the nonelutable fraction are shown in Fig. 4. There is no correlation between the quantity of newly absorbed iron in peak I and rates of iron absorption. In contrast, the nonelutable fraction

TABLE II

Concentration of Radiolabeled Iron in 6000 g Supernate after 10 min Absorption of a 40 μg Dose

Group	No. of studies	Concentration of iron	P value
		μg Fe/g supernatant protein*	
N	8	148.6 ±34.3	—
FeD	13	258.2 ±44.5	0.05
PHZ	4	330.5 ±53.3	<0.05

N, normal; FeD, iron deficient; PHZ, phenylhydrazine-treated animals.
* Mean ±1 SE.

TABLE III

Concentration of NonHeme Iron in 6000 g Supernate

Group	No. of studies	μg Fe/mg super-natant protein*	P value
N	9	0.327 ±0.038	—
FeD	7	0.065 ±0.008	<0.001
PHZ	8	0.169 ±0.014	<0.005

N, normal; FeD, iron deficient; PHZ, phenylhydrazine-treated animals.
* Mean ±1 SE.

in both the FeD and PHZ animals was significantly greater than in the normal animals.

Characteristics of the fractions. In both the FeD and PHZ animals, peaks I and II and the nonelutable fraction had the same qualitative characteristics as those described in the normal animals.

Peak III was seen only in the iron-deficient animals. On Sephadex G-200 and DEAE-cellulose, this peak eluted at a point identical with rat transferrin.

Since peak III had chromatographic characteristics identical with transferrin, the possibility was investigated that this represented contamination with blood at the time of preparation of the homogenate. An homogenate was prepared from an adjacent segment of small bowel removed from an iron-deficient animal after 60 min absorption at the same time as the isolated loop was removed. A sample of serum was obtained simultaneously. When the radioactivity of the supernate from the adjacent loop was calculated in terms of counts per minute/milligram protein, it was < 1% of the value for the loop which had been in contact with the iron. In addition, based on the counts per minute/milliliter of radioactivity in the serum sample, 1 ml of serum (1.4
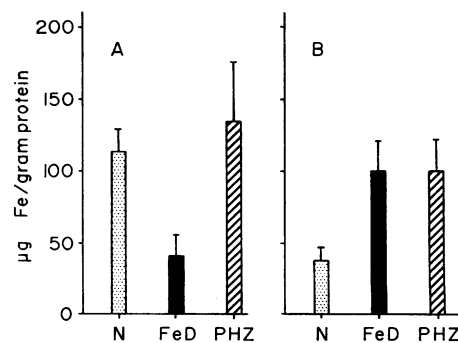

FIGURE 4 Difference in radioiron distribution in 6000 g mucosal supernate of normal (N), iron-deficient (FeD), and phenylhydrazine-treated (PHZ) animals. (A) Peak I. (B) Nonelutable fraction. Data are for 10 min absorption time for a 40 μg dose. Values represent concentration of the radioiron in each fraction expressed in terms of supernatant protein.

ml of whole blood) would have had to contaminate the isolated loop homogenate to account for the amount of radioactivity recovered as peak III. This was unlikely since no gross blood was detectable and the calculated volume was greater than the entire volume of mucosal scrapings. Thus, although peak III had the chromatographic and electrophoretic behavior of transferrin, its presence did not appear to result from whole blood contamination at the time of scraping and preparing the mucosal homogenate.

Effect of varying the dose of ferrous sulfate

Since the presence of a rapidly exchanging cytoplasmic carrier molecule could have been obscured by the carrier iron used in the 40 μg dose study, experiments were carried out in which only a 4 μg dose of iron was administered (30–70 μCi of radioiron without additional carrier). In both groups of animals, the elution patterns were qualitatively similar to those with the 40 μg dose, and no additional peaks of radioactivity were identified (Table I).

Mucosal distribution of intravenously administered ferrous sulfate

Conrad, Weintraub, and Crosby (12) had demonstrated that a proportion of parenterally administered iron appeared in the gastrointestinal mucosal cells and later disappeared, presumably due to the sloughing of those mucosal cells. To determine the mucosal distribution of parenteral iron by chromatographic separation, 100 μCi of ^{59}Fe-labeled ferrous sulfate (8 μg of elemental iron) was injected into the tail vein of normal rats, the animals sacrificed at 2 and 6 days, mucosal homogenates prepared and chromatographed on Sephadex G-200. At 2 days, 88% of recoverable radioactivity (10,000 cpm/ml supernate) eluted as peak I. The remaining 12% was nonelutable. At 6 days less than 400 cpm/ml of radioiron was present in the supernate.

Mucosal cell iron content

To test the proposed hypothesis (12) that the mucosal cell content of iron may relate to the control of iron absorption, the total nonheme iron content of mucosal cell homogenates from normal, iron-deficient, and PHZ-treated animals was measured. No significant differences were noted between these groups. However, when the nonheme iron content of the supernatant fraction after centrifugation at 6000 g was measured, significant differences were noted (Table III). Both of the groups with increased iron absorption (FeD and PHZ) had statistically significant lower nonheme iron concentrations per milligram of supernatant protein than the normal animals.

In vitro addition of iron to mucosal supernate

Experiments were performed to determine whether or not the distribution of ferrous sulfate in the mucosal supernate differed when the iron was added in vitro as opposed to the in vivo uptake and distribution. The chromatographic patterns, after incubation of ^{59}Fe with supernates from normal and FeD animals, were similar to the in vivo distribution in that peak I predominated in the normal animal, peak III was present only in the iron-deficient animal, and the major portion of radioactivity from the iron-deficient supernate was nonelutable.

DISCUSSION

These studies indicate that during the early phase of active iron absorption, the newly absorbed iron appears in distinct separable fractions within the duodenal mucosal cells. In the normal animal, the predominant incorporation is into ferritin, even as early as 10 min, and accounts for more than 90% of the radioactivity at 1 hr. This differs from the findings of Brown and Rother (8, 9) and Manis and Schacter (10), who were unable to identify significant amounts of new ferritin iron in the mucosal homogenate. However, Brown and Rother studied the 105,000 g supernate where little ferritin remains because of its high density. Manis and Schacter were using an in vitro system, which may not be representative of the in vivo physiologic mechanisms. These results are similar to those of Charlton, Jacobs, Torrance, and Bothwell (11) who also demonstrated that ferritin radioactivity was the major fraction in the normal animal. In the iron-deficient animal, we found only minimal incorporation into ferritin until the major proportion of iron transfer to the carcass had occurred, at which time (60 min) newly absorbed iron in ferritin was approaching that in the normal animal. In vitro addition of iron to the mucosal supernate from FeD animals showed only a minimal capacity to bind iron to ferritin as compared with the normal animals. These findings could be interpreted to mean that iron absorption is increased in the iron-depleted animal because little apoferritin is present in the mucosal cell until exposure to absorbable iron at which time apoferritin synthesis is induced, and as ferritin is formed, further iron transfer is inhibited (11). The observation of increased iron absorption in the hemolyzing animal despite iron incorporation into ferritin, equivalent to or greater than the normal animal, would suggest, however, that ferritin binding of iron does not significantly limit the rate of iron absorption during the early phase. Recently Brittin and Raval (18) have shown that, indeed, ferritin synthesis is induced in the iron-depleted animal, but their studies also indicated

that this did not significantly limit subsequent iron absorption in these animals.

The major difference between the normal animal and the two circumstances associated with enhanced iron absorption was the finding of a large fraction of the radiolabeled iron in the latter two groups in a form which had the characteristics of free iron salts. It could be suggested that this ionic iron was present because of adsorption to the luminal surface of the mucosal cell and did not truly represent an intracellular pool. This is unlikely because in the normal animal supernate at 60 min, little or none of this fraction is seen despite the fact that the greatest proportion of the administered radioiron is still present in the luminal fluid. It would appear, therefore, that the increased rate of iron absorption in both the iron-depleted state and in the presence of hemolysis is most closely correlated with a significant increase in the amount of free ionic iron in the mucosal cell. Brown and Rother (9) felt that a similar major fraction in the iron-depleted animal was bound to small molecular weight chelates, particularly amino acids. However, as pointed out by Charlton et al. (11), the EDTA utilized for washing the mucosal cells probably was the source of the low molecular weight chelate in these studies. With the elimination of EDTA from our preparative procedure, there is no evidence for the presence of low molecular weight compounds which specifically chelate iron. A small proportion of the radioiron does elute from Sephadex G-200 at the total bed volume. The characterization of the fraction suggested that it is not a specific separate chelated iron pool, but represents a small proportion of the free ionic iron which can be eluted from the column.

Another fraction is found when the supernate from FeD animals is chromatographed. Preliminary chromatographic and electrophoretic studies suggest that this is similar to or actually transferrin. None of this peak occurs in the normal animal nor is it consistently found in the PHZ animals. It does not seem that it is merely serum contamination of the mucosal scrapings. Although this could be intracellular in origin, it may also be membrane bound as suggested by Levine and Weintraub (19), who found increased binding of transferrin to the mucosal cells of FeD animals. The fact that we were unable to identify similar amounts of this fraction in the PHZ supernate, despite Fe absorption rates similar to the FeD animals, suggests that its role as a specific cytoplasmic carrier for the control of iron absorption is doubtful.

These studies are in keeping with some of the hypotheses regarding the mucosal cell control of iron absorption rates. They indicate that one feature in common in two disparate physiological states associated with increased iron absorption is the initial rate of

mucosal cell uptake of iron. This is particularly evident after the 10 min absorption time. Conrad et al. (12) have suggested that the mucosal cell iron content may be a factor in regulating the uptake and, as a consequence, the transfer of iron to the body. Previous studies (20–22) have failed to show significant differences in the nonheme iron content of whole homogenates of mucosal cell preparations from normal and iron-deficient animals and man. Our studies confirmed these observations. However, when the nonheme iron content of the 6000 g supernate was measured, significant differences were demonstrated. Of added interest was the finding that the mucosal supernatant iron content was also significantly reduced in the PHZ animals. Conrad et al. (12) have also shown that after acute blood loss in the iron-overloaded animal, the mucosal iron adsorption increased in conjunction with a drop in the total nonheme iron content of the mucosal cells. These observations are consistent with the postulate that the soluble mucosal cell nonheme iron concentration, or some portion thereof, regulates an uptake or transport system in the mucosal cell wall at the luminal surface. Greenberger, Balcerzak, and Ackerman (23) were unable to demonstrate a difference in binding of iron to proximal mucosal cell brush border preparations from normal and iron-depleted rats, but this proposed transport system may require an intact metabolically active cell for its function.

Once the iron has been transferred into the mucosal cell cytoplasm, we were unable to identify any specific chelating molecule for its solubilization or transport to the serosal side. Our data also argue against the thesis that with physiologic doses of iron, ferritin binding of iron significantly inhibits mucosal transfer of iron. It would appear from both the in vivo and in vitro studies that the distribution of newly absorbed iron in the mucosal cell during the early phase of absorption is merely a function of the initial rate of uptake and the amount of apoferritin or partially saturated ferritin molecules present in the cell at the time of exposure to iron. Circulating transferrin-bound iron also appears to be distributed in the mucosal cell in a similar manner. Because of the rapid turnover of the gastrointestinal mucosa, the state of its iron content could be quickly varied depending upon the conditions existent at the time of production of a new cell population. If, in turn, the mucosal cell represents one of the readily available labile iron pools, then its labile iron content or some fraction would reflect the immediate needs of the organism for iron. This model would explain our observations as well as those regarding the transient impairment of further iron uptake by the iron-deficient animal after an initial exposure to iron (24–26) and the early appearance of increased iron absorption after acute

blood loss (12) or an hemolytic process despite the preceding status of the total body and even total mucosal iron content.

Our studies do not provide data regarding the mechanism of subsequent transfer of the newly absorbed iron to the carcass.

Thus, our observations, in conjunction with previously published studies, are consistent with the possibility that an apical surface membrane, carrier-mediated transport mechanism, either facilitated or active, may exist for iron uptake. This process may be modulated by cytoplasmic iron content. This hypothesis is presently being investigated.

ACKNOWLEDGMENTS

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