JCI The Journal of Clinical Investigation

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*J Clin Invest.* 1964;43(7):1433-1442. https://doi.org/10.1172/JCI105019.

Research Article



*Journal of Clinical Investigation*
*Vol. 43, No. 7, 1964*

# Studies on Iron Absorption. Intestinal Regulatory Mechanisms *

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Recent studies (1–3) have indicated that gastrointestinal absorption of iron is a more complicated process than was initially postulated (4, 5). For example, several investigators have proposed as the result of studies in rats (2, 3), dogs (6), and humans (7) that iron absorption involves at least 2 steps: *1*) mucosal uptake of iron from the intestinal lumen and *2*) transfer of iron from mucosal cells to plasma. Other evidence has been presented indicating that these steps are distinct, sequential processes (2). In addition, later work (3) has suggested that the second step, mucosal transfer, has two components: *1*) a rapid transport mechanism which, within 2 hours after dosing, transfers up to 80% of the iron ultimately transferred to plasma, and *2*) a much slower process which transfers the remainder over the subsequent 12 to 20 hours. It has also been shown that small intestinal mucosal cells [1] can take up and temporarily hold more iron than is ultimately transferred to plasma, resulting in loss of some of this iron as the mucosal cells are sloughed at the end of their life span (1, 3).

In view of the complex nature of the over-all process of iron absorption, our studies were made during the early, rapid phase in an attempt to gain a clearer concept of mucosal handling of iron. The experiments were designed to evaluate the rate of mucosal uptake and transfer of iron to plasma, the site of maximal absorption, and the influence of varying body iron stores on these processes.

## Methods

Male albino rats of the WRCF strain,[2] weighing 325 to 350 g, were used. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed. The basic methods used to determine iron absorption including operative technique for making duodenal loops, preparation of $Fe^{59}$-labeled test dose of iron, and radioisotope counting procedures utilizing a small animal whole body counter have been previously described in detail (3, 8, 9). In essence the procedure involved injecting a known amount of $Fe^{59}$-labeled ferrous iron into an operatively prepared closed loop of gut in anesthetized but otherwise intact rats. Formation of the closed loops was accomplished by applying a proximal and a distal ligature around the serosal surface of the segment of intestine to be studied. Care was taken not to occlude observable vessels. The color of the loops remained normal throughout the study; several loops removed 1 hour after formation showed no abnormal histologic changes by light microscopy. After a preselected time interval, absorption was terminated by removing the closed gut segment *in toto* from carcass. The segment was then opened, washed to remove unabsorbed iron, and weighed, and its radioactive iron content and that of the carcass were counted separately. Since the gut and carcass were counted separately, it was possible to measure *1*) total amount of iron absorbed from intestinal lumen (total radioiron content of gut segment plus carcass), to be referred to as "mucosal uptake," and *2*) amount of iron transferred from mucosal cells to carcass (carcass radioiron content), to be referred to as "mucosal transfer." By using the known specific activity of the test dose, results were usually converted to, and expressed as, micrograms of iron absorbed per loop of gut.

To vary body iron stores, rats were randomized into 3 groups. One group (FeD) was made iron deficient by repeated bleeding and feeding a casein base, synthetic, iron-poor diet; the last bleeding was 2 weeks before study. A second group (FeL) was loaded with 50 mg of iron dextran intramuscularly 3 weeks before

* Submitted for publication September 19, 1963; accepted March 2, 1964.

Presented in part at the American Federation for Clinical Research, April 29, 1962.

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[1] In this paper the term "mucosal cells" refers to epithelial cells of the intestinal mucosa.

[2] The Walter Reed Carworth Farm strain is derived from the Wistar strain and, as supplied, is pathogen free.

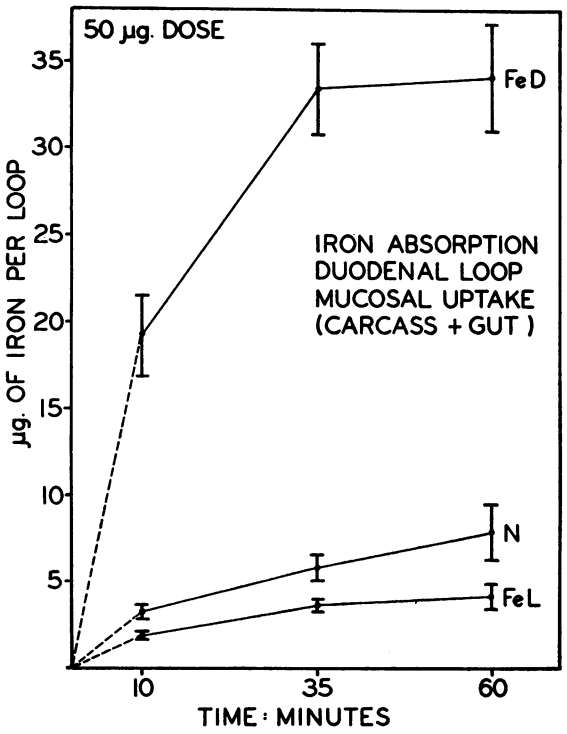


FIG. 1. RATE OF MUCOSAL UPTAKE OF IRON FROM LUMEN OF DUODENAL LOOPS AFTER A SINGLE 50-µG DOSE OF RADIOIRON. FeD = iron-deficient, N = normal, and FeL = iron-loaded rats. The brackets indicate 1 SE.

study. This amount of iron was well tolerated. A third group (N) was untreated and like the second group was maintained on regular rat chow. For all experiments, food was removed from the cages 8 to 10 hours before study.

## Experimental Procedures and Results

*Effect of common bile duct ligation on the absorption of radioiron.* Since bile accumulated in the closed duodenal loops during the absorption period, the effect of common bile duct ligation on iron absorption was determined by preparing duodenal loops in the usual manner in 8 normal rats. Immediately preceding preparation of the loop in 4 of the rats, the bile duct was ligated just above its entry into the duodenum. Fifty µg of labeled iron was injected into each loop, and an absorption time of 60 minutes was allowed.

*Results.* Ligation of the common bile duct did not affect iron absorption significantly as shown in Table I. As a result of this finding, the bile duct was not ligated for any of the other studies.

TABLE I

*Effect of common bile duct ligation on radioiron absorption 60 minutes after injection of 50-µg test dose into duodenal loops in normal rats*

| | Mucosal uptake from lumen* | Mucosal transfer to carcass* |
|---|---|---|
| | µg Fe/loop | |
| Control | 8.75 ± 0.7† | 4.72 ± 0.92 |
| Bile duct ligated | 9.89 ± 0.99† | 4.64 ± 1.7 |

* Mean ± SE.
† Difference not significant at 10% level with *t* test (10).

*Onset of iron absorption.* An attempt was made to determine how rapidly iron absorption would begin after the dose was administered. A duodenal loop was prepared in each of 4 iron-deficient rats, and 2.5 µg of $Fe^{59}$-labeled ferrous iron was injected. Fifteen to 26 seconds after injection the loops were removed to terminate absorption, and the carcass and gut were counted in the usual manner.

*Results.* The onset of iron absorption was extremely rapid. Radioiron was detected in the carcass of iron-deficient rats as early as 15 seconds after the test dose was put into the duode-

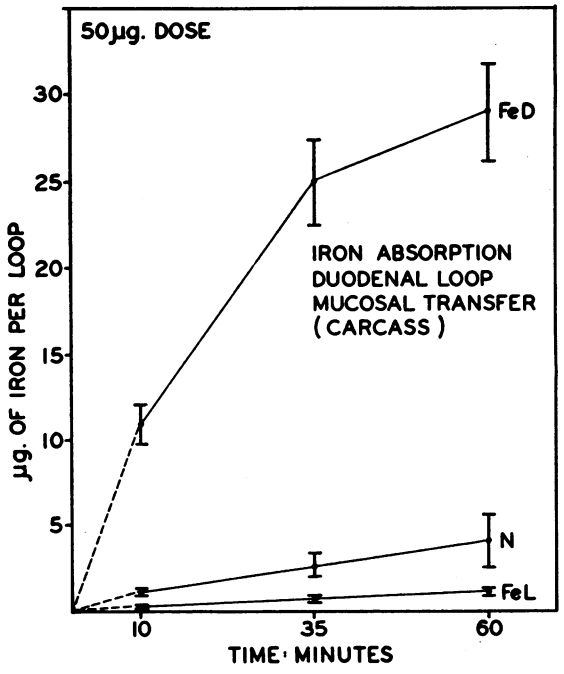


FIG. 2. RATE OF MUCOSAL TRANSFER OF IRON TO CARCASS AFTER A SINGLE 50-µG DOSE OF RADIOIRON. The brackets indicate 1 SE.

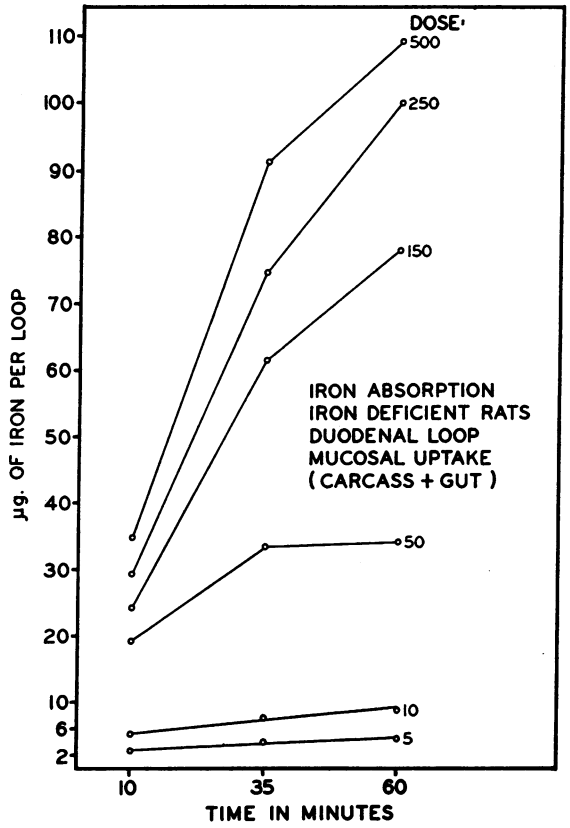


FIG. 3. RATE OF MUCOSAL UPTAKE OF IRON FROM LUMEN OF DUODENAL LOOPS IN IRON-DEFICIENT RATS AFTER A SINGLE DOSE OF VARYING AMOUNTS OF RADIOIRON.

num. With only 26 seconds or less for absorption, mucosal uptake varied from 1.2 to 4.1% of the 2.5-µg dose, and mucosal transfer was 0.46 to 2.5%.

*Rate of iron absorption and the effect of varying body iron stores.* Studies on rate of iron absorption and the influence of body iron stores were done by preparing loops of duodenum in groups N, FeL, and FeD. Mucosal uptake and mucosal transfer to carcass were determined 10, 35, and 60 minutes after injecting a dose ranging from 5 to 500 µg of $Fe^{59}$-labeled carrier iron into groups N and FeD and from 5 to 125 µg in group FeL. Four to 7 rats from each group were studied to obtain a mean absorption for each time and each dose; a total of 260 experiments were performed.

*Results.* Figures 1 and 2 show a comparison of the rate of absorption from duodenal loops in normal (N), iron-deficient (FeD), and iron-loaded rats (FeL) after a dose of 50 µg of labeled iron. Figure 1 shows the comparison of mucosal uptake of iron from intestinal lumen, i.e., the sum of the iron transferred to carcass plus the iron retained by the washed gut segment. At each time, group FeD mucosal uptake was approximately 9 times greater than group FeL and 5 times greater than group N. The differences observed between the means for groups FeL and N were slight but statistically significant; values for p obtained with the *t* test (10) were $< 0.05$, $< 0.05$, and $< 0.1$ for 10, 35, and 60 minutes, respectively. Shown also in Figure 1 is the cessation of mucosal uptake after 35 minutes in group FeD. That this was due principally to a decreasing and thus limiting amount of iron in the lumen is shown in Figure 3. When larger amounts of iron (150 to 500 µg) were used, mucosal uptake continued after 35 minutes although at a somewhat decreased rate.

Figure 2 illustrates the comparison of mucosal transfer of iron to carcass for groups N, FeD, and FeL. A more marked difference between groups was found with this comparison than when mucosal uptake was compared (Figure 1). At each time, transfer by group FeD was approximately 30 to 40 times greater than group FeL and 9 times greater than group N. The differ-

TABLE II

*Mucosal uptake and mucosal transfer to carcass from duodenal loops by 35 minutes for each test dose in rats with varying body iron stores*

| Group | Dosage | Mucosal uptake* | Mucosal transfer* |
|---|---|---|---|
| | µg/Fe | µg Fe/loop | |
| Normal (N) | 5 | 2.9 ± 0.79 | 1.9 ± 0.65 |
| | 10 | 3.3 ± 0.4 | 2.1 ± 0.4 |
| | 50 | 5.8 ± 0.75 | 2.7 ± 0.7 |
| | 150 | 13.6 ± 2.2 | 4.0 ± 1.5 |
| | 250 | 17.7 ± 1.8 | 6.8 ± 1.6 |
| | 500 | 34.8 ± 8.3 | 10.7 ± 2.7 |
| Iron loaded (FeL) | 5 | 1.6 ± .25 | 0.35 ± 0.17 |
| | 10 | 2.5 ± 1.0 | 0.5 ± 0.09 |
| | 50 | 3.6 ± 0.38 | 0.67 ± 0.28 |
| | 125 | 13.6 ± 3.0 | 2.8 ± 0.28 |
| Iron deficient (FeD) | 5 | 3.9 ± 0.14 | 3.6 ± 0.13 |
| | 10 | 7.5 ± 0.38 | 6.9 ± 0.32 |
| | 50 | 33.4 ± 2.7 | 25.0 ± 2.4 |
| | 150 | 61.6 ± 3.9 | 34.3 ± 4.0 |
| | 250 | 74.7 ± 8 | 41.7 ± 1.4 |
| | 500 | 91.4 ± 7.4 | 39.6 ± 2.2 |

* Mean ± SE.

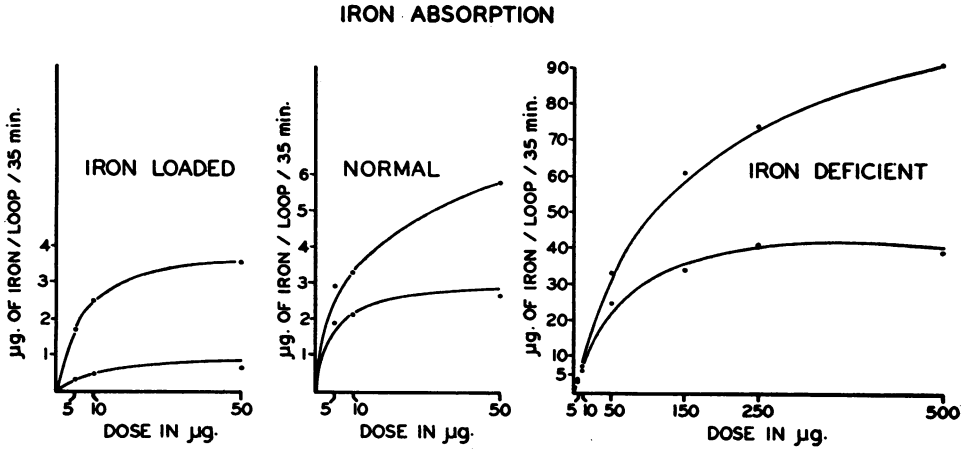


FIG. 4. RATE OF MUCOSAL UPTAKE OF IRON FROM LUMEN (UPPER CURVES) AND RATE OF MUCOSAL TRANSFER TO CARCASS (LOWER CURVES) AFTER A SINGLE DOSE OF VARYING AMOUNTS OF RADIOIRON. Status of body iron stores is indicated. Note difference in units of coordinates for iron-deficient group.

ences between the means for groups FeL and N were small but significant; values for p were $< 0.01$, $< 0.05$, and $< 0.1$ for 10, 35, and 60 minutes, respectively.

Table II contains the values obtained for mucosal uptake and mucosal transfer at 35 minutes for each animal group and test dose of iron. A portion of these data is shown in Figure 4. In all 3 groups, as the dose was increased, both mucosal uptake and transfer increased in a curvilinear fashion, and both appeared to be approaching a maximal transport rate. When these data were plotted in the double reciprocal manner of Lineweaver and Burk, a linear relationship characteristic of enzymatic processes (11) was observed (Figure 5). In group FeD, this relationship was maintained to the 500-µg dose, indicating that under these conditions the absorptive mechanism in iron-deficient rats has kinetics consistent with an enzymatic process. In groups FeL and N, the linear relationship between the reciprocal of the absorption rate and the reciprocal of the dose no longer held when the dose exceeded 50 µg of iron. Above this dose there was a greater increase in mucosal uptake and mucosal transfer than if the previous relationship had been maintained. The new relationship in group N, shown in an arithmetic plot in Figure 6, indicates that as the test dose of iron was increased from 50 to 500 µg, both mucosal uptake and mucosal transfer increased linearly. Both lines in Figure 6 are least squares regression lines (10). They show that a statistically significant [3] ($p < 0.01$) linear relationship exists between dose of iron and both mucosal uptake and mucosal transfer. This type of relationship is very suggestive of passive diffusion of iron across the intestinal mucosa. These results indicate that in group N,

TABLE III

*Relationship between mucosal uptake and mucosal transfer to carcass for duodenal loops at each test dose and absorption time in rats with varying body iron stores*

| | Dose | Ratio mucosal transfer: mucosal uptake Absorption time | | |
|---|---|---|---|---|
| | | 10 minutes | 35 minutes | 60 minutes |
| | µg Fe | | | |
| Normal (N) | 5 | 0.47 | 0.66 | 0.59 |
| | 10 | 0.41 | 0.63 | 0.63 |
| | 50 | 0.35 | 0.46 | 0.52 |
| | 150 | 0.18 | 0.30 | 0.34 |
| | 250 | 0.25 | 0.39 | 0.49 |
| | 500 | 0.15 | 0.31 | 0.14 |
| Iron loaded (FeL) | 5 | 0.16 | 0.20 | 0.42 |
| | 10 | 0.18 | 0.20 | 0.41 |
| | 50 | 0.13 | 0.18 | 0.26 |
| | 125 | 0.19 | 0.20 | 0.20 |
| Iron deficient (FeD) | 5 | 0.83 | 0.91 | 0.92 |
| | 10 | 0.81 | 0.92 | 0.90 |
| | 50 | 0.57 | 0.75 | 0.85 |
| | 150 | 0.41 | 0.56 | 0.69 |
| | 250 | 0.36 | 0.56 | 0.58 |
| | 500 | 0.32 | 0.43 | 0.45 |

[3] Tested by analysis of variance (10).

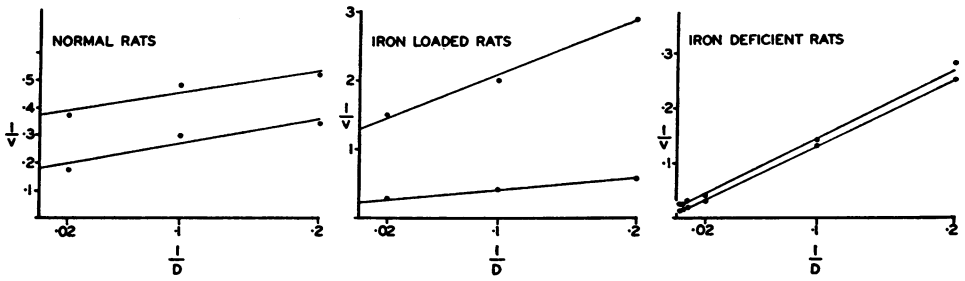


FIG. 5. PLOT OF THE RECIPROCAL OF MUCOSAL UPTAKE (LOWER LINE) AND MUCOSAL TRANSFER (UPPER LINE) AT 35 MINUTES AGAINST THE RECIPROCAL OF THE DOSE OF IRON USED. Status of body iron stores is indicated. Note difference in units for 1/v in each plot.

with small doses of iron (5 to 50 μg), the predominant absorptive mechanism has kinetics consistent with an enzymatic process, but with larger doses (50 to 500 μg), another process with the kinetics of passive diffusion supervenes.

The comparisons shown in Figure 4 clearly illustrate that the rate of mucosal uptake of iron from lumen exceeded the rate of mucosal transfer of iron to carcass. This difference in rate was reflected by the persistence of a ratio of less than 1 for the relationship of mucosal transfer: mucosal uptake. This ratio is shown in Table III for each group at each point of time and test dose. In general in all groups, as the test dose was raised, the difference in rates was increased as indicated by the ratio. As the absorption time was increased from 10 to 60 minutes, the ratio indicates that the difference between the rates was decreasing. In group FeD the ratio was close to 1 until test doses of 50 μg or greater were used, indicating little difference in rate with the lower doses. In groups N and FeL a difference in rate was apparent even with the 5-μg dose, as indicated by the low ratios of 0.59 in group N and 0.42 in group FeL at 60 minutes. This rather marked difference in rates meant that more iron was entering mucosal cells than could be transferred to carcass even by 60 minutes.

*Effect of prefeeding iron.* The effect of prefeeding iron on subsequent duodenal mucosal uptake and mucosal transfer was evaluated. Thirty normal rats were randomized into 3 groups of 10 each. Each group was divided equally into a control and prefed subgroup. After a period of 10 hours without food, one group was prefed 1 hour, another group 6 hours, and the third group 24 hours preceding the absorption study. The prefeeding consisted of 250 μg of nonradioactive

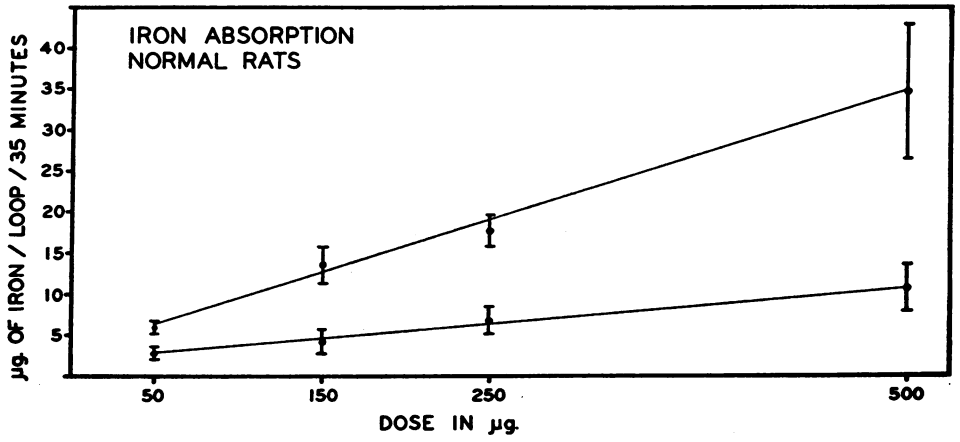


FIG. 6. RATE OF MUCOSAL UPTAKE OF IRON FROM LUMEN (UPPER LINE) AND RATE OF MUCOSAL TRANSFER TO CARCASS (LOWER LINE) AFTER A SINGLE DOSE OF VARYING AMOUNTS OF RADIOIRON IN NORMAL RATS. The brackets indicate 1 SE.

TABLE IV

*Effect of prefeeding iron on subsequent radioiron absorption 30 minutes after injection of 12.5-μg test dose into duodenal loops in normal rats*

| Time after prefeeding until study | Mucosal uptake from lumen* | | p | Mucosal transfer to carcass* | | p |
|---|---|---|---|---|---|---|
| | Control | Prefed | | Control | Prefed | |
| | *μg Fe/loop* | | | *μg Fe/loop* | | |
| 1 hour | 1.54 ± 0.08 | 1.01 ± 0.19 | <0.05 | 0.96 ± 0.1 | 0.38 ± 0.18 | <0.05 |
| 6 hours | 1.37 ± 0.25 | 1.51 ± 0.12 | NS† | 0.68 ± 0.2 | 0.63 ± 0.1 | NS† |
| 24 hours | 1.35 ± 0.19 | 1.73 ± 0.28 | NS† | 0.66 ± 0.16 | 0.63 ± 0.11 | NS† |

* Mean ± SE.
† Not significant at 10% level with *t* test (10).

ferrous iron as ferrous sulfate given intragastrically by gavage. Each control subgroup was treated in the same manner except that 0.9% saline was given instead of iron. Only water was allowed from the time of prefeeding until study. For the determination of mucosal uptake and transfer, two changes were made in the usual technique for preparing and dosing duodenal loops. First, a polyethylene cannula was placed in the distal end of the loop, and second, before the test dose of radioiron was injected, the loop was gently flushed with 5 ml of warm saline to remove any iron remaining in the lumen. Control subgroups were treated similarly. The test dose was 12.5 μg of $Fe^{59}$-labeled ferrous iron, and the absorption time was 30 minutes.

*Results.* The effect of prefeeding iron on subsequent mucosal uptake and mucosal transfer is shown in Table IV. Giving 250 μg of "cold" ferrous iron to normal rats 1 hour before the absorption study with radioiron depressed mucosal uptake 34% and mucosal transfer 60%. Six hours after feeding, the depressive effect was no longer noted.

TABLE V

*Comparison of radioiron absorption 35 minutes after injection of 50-μg test dose into loops of duodenum, jejunum, and ileum in rats with varying body iron stores*

| | Mucosal uptake from lumen* (a) | Mucosal transfer to carcass* (b) | b:a |
|---|---|---|---|
| | *μg Fe/loop* | | |
| Iron loaded (FeL) | | | |
| Duodenum | 3.82 ± 0.31 | 0.88 ± 0.6 | 0.23 |
| Proximal jejunum | 4.44 ± 0.36 | 0.22 ± 0.1 | 0.05 |
| Distal ileum | 2.09 ± 0.6 | 0.08 ± 0.07 | 0.04 |
| Normal (N) | | | |
| Duodenum | 6.85 ± 1.26 | 3.04 ± 0.35 | 0.44 |
| Proximal jejunum | 2.9 ± 2.4 | 0.20 ± 0.16 | 0.07 |
| Distal ileum | 1.73 ± 0.53 | 0.07 ± 0.06 | 0.04 |
| Iron deficient (FeD) | | | |
| Duodenum | 28.00 ± 0.04 | 20.01 ± 0.8 | 0.72 |
| Proximal jejunum | 10.63 ± 1.23 | 3.76 ± 0.73 | 0.35 |
| Distal ileum | 3.88 ± 0.23 | 0.05 ± 0.06 | 0.01 |

* Mean ± SE.

*Site of iron absorption and the effect of varying body iron stores.* Mucosal uptake and mucosal transfer of radioiron to carcass by loops of duodenum, jejunum, ileum, colon, and stomach were compared in groups N, FeL, and FeD. The duodenal loops measured approximately 4 to 5 cm in length. The other intestinal sites were studied with the same operative technique for preparing loops and for injecting the labeled iron. Proximal jejunal loops approximately 7.5 cm in length were made. Distal ileal loops were prepared by placing a distal ligature just above the ileocecal valve and an upper ligature approximately 12 cm proximal. The different lengths were used to compensate for variation in surface area (12). Colonic loops approximately 10 cm in length were made just distal to the cecum. Since ileum and colon usually contained feces, the test sites were washed clean with a 2-ml injection of warm 0.9% saline before the distal suture was applied and the test dose injected. For preparing the closed stomach one ligature was placed around the esophagus just below the diaphragm and another at the pylorus. The test dose of iron was injected into the pyloric end of the stomach as the ligature placed there was drawn tight. The test dose in all of these studies was 50 μg of $Fe^{59}$-labeled ferrous iron. In comparing absorption from duodenum, jejunum, and ileum, 4 rats from each group were used for studying each site. The absorption time allowed was 35 minutes. For the stomach and colon experiments, 3 rats from each group were used for each site, and the absorption time

was 1 hour. All results were expressed as micrograms of iron absorbed per loop.

*Results.* A comparison of mucosal uptake and mucosal transfer by loops of duodenum, proximal jejunum, and distal ileum in rats with varying body iron stores is shown in Table V, and a similar comparison of stomach and colon is shown in Table VI. Values are listed for mucosal uptake, mucosal transfer, and the ratio of mucosal transfer to mucosal uptake. Table V shows that with one exception (FeL, mucosal uptake) both mucosal uptake and mucosal transfer were greatest in duodenum, less in jejunum, and least in ileum. The difference was more marked for mucosal transfer, which showed a rather abrupt decrease below the duodenum. This was reflected by the low ratio of mucosal transfer to mucosal uptake for jejunum and ileum. A similar low ratio was found for stomach and colon (Table VI).

The effect of varying body iron stores was most evident in duodenum, where iron deficiency (FeD) affected an increase, and iron loading (FeL) a decrease, in both mucosal uptake and mucosal transfer of iron as compared to normal animals (N). The most marked effect was on mucosal transfer (Table V). The iron-deficiency state resulted in a change in the proximal jejunum, where an increase in both steps was found, and as a consequence, absorption was approximately as efficient as in normal duodenum. Mucosal transfer to carcass was the same for jejunum in groups N and FeL, and similarly, there was no difference in the 3 groups when ileum (Table V) or stomach and colon (Table VI) were compared. Except for a slight increase in mucosal uptake by colon in group FeL, mucosal uptake and mucosal transfer by stomach and colon were the same regardless of body iron stores (Table VI).

*Effect of bypassing duodenum.* In an effort to determine what effect bypassing duodenum would have on total amount of iron absorbed, the following two studies were done. Instead of preparing closed loops, when the abdomen was opened, the test dose of iron was injected directly into the intestinal lumen by puncture with a 27-gauge needle. Careful checks revealed no leakage of radioactivity. Puncture for duodenal injection was made at the pylorus, for jejunal injection aproximately 4 to 6 cm distal to the pylorus, and for ileal injection approximately 15 cm proximal to the ileocecal valve. After the dose had been injected, the abdominal wall was closed, and the rats were allowed to recover.

TABLE VI

*Comparison of radioiron absorption 60 minutes after injection of 50-μg test dose into the closed stomach and colonic loops in rats with varying body iron stores*

| | Mucosal uptake from lumen* (a) | Mucosal transfer to carcass* (b) | b:a |
|---|---|---|---|
| | *μg Fe/loop* | | |
| Iron loaded (FeL) | | | |
| Stomach | 1.6 ± 0.2 | 0.15 ± 0.04 | 0.09 |
| Colon | 3.2 ± 0.56 | 0.11 ± 0.01 | 0.03 |
| Normal (N) | | | |
| Stomach | 1.3 ± 0.2 | 0.12 ± 0.02 | 0.09 |
| Colon | 1.5 ± 0.2 | 0.14 ± 0.02 | 0.09 |
| Iron deficient (FeD) | | | |
| Stomach | 2.4 ± 0.46 | 0.12 ± 0.02 | 0.05 |
| Colon | 1.7 ± 0.4 | 0.16 ± 0.02 | 0.09 |

* Mean ± SE.

In the first study involving 8 normal rats, 25 μg of labeled iron was put into duodenum in 4, and into jejunum in the other 4. When the rats were killed, the entire gastrointestinal tract was removed, opened, washed to remove unabsorbed iron, and counted separately from carcass as previously described (3).

In the second study 12.5 μg of labeled iron was injected into each of 15 normal rats, 5 into duodenum, 5 into jejunum, and 5 into ileum. Final absorption was determined by counting the whole rat 8 days later, at a time when all unabsorbed iron had been excreted (9).

*Results.* The effect of bypassing duodenum on iron absorption is shown in Tables VII and VIII. As would be expected from the results of the loop studies, bypassing the duodenum decreased mu-

TABLE VII

*Comparison of radioiron absorption 3 hours after direct injection of 25-μg test dose into intestinal lumen of normal rats*

| Site of injection | Mucosal uptake from lumen* (a) | Mucosal transfer to carcass* (b) | Decrease a | Decrease b |
|---|---|---|---|---|
| | *μg Fe* | *μg Fe* | % | % |
| Duodenum | 12.3 ± 2.3 | 8.4 ± 2.1 | | |
| Proximal jejunum | 3.9 ± 1.3 | 1.3 ± 0.5 | 68 | 85 |

* Mean ± SE.

TABLE VIII

*Comparison of radioiron absorption 8 days after direct injection of 12.5-μg test dose into intestinal lumen of normal rats*

| Site of injection | Total absorption* | Decrease in absorption |
|---|---|---|
| | *μg Fe* | % |
| Duodenum | 8.8 ± 0.5 | |
| Proximal jejunum | 3.2 ± 0.7 | 64 |
| Distal ileum | 0.12 ± 0.02 | 98 |

* Mean ± SE.

cosal transfer to a greater extent than mucosal uptake. In the 3-hour absorption study mucosal uptake was decreased 68% and mucosal transfer 85% (Table VII).

Table VIII shows the marked effect of bypassing the proximal segments of small intestine on over-all iron absorption. Bypassing only 4 to 6 cm of proximal small intestine (duodenum) decreased absorption 64%. This result represents a conservative estimate of the true effect. Some reflux into duodenum probably occurred when the test dose was injected into the jejunum. When all but approximately 15 cm of distal small intestine was bypassed, absorption was decreased 98% compared to that found after injection of the dose into duodenum. This suggested that distal ileum and colon contributed very little to total iron absorption.

## Discussion

Our data support those previously reported (2, 13, 14) which indicate that iron is absorbed by an active transport mechanism comprised of at least two steps: *1*) mucosal uptake of iron from the intestinal lumen and *2*) mucosal transfer of iron to carcass. Differences were observed between the two steps. For example, mucosal transfer was restricted more specifically to the proximal small intestine; the rate of mucosal transfer was less than the rate of mucosal uptake and was thus more limiting to over-all absorption of iron. There were also similarities, in that both steps varied inversely with changes in body iron stores and both were decreased by prefeeding iron; however, mucosal transfer was influenced by these factors to a greater extent than mucosal uptake. These observations are in close agreement with those described by Manis and Schachter (2, 14). Our studies show that during the early, rapid phase of iron absorption, when up to 80% of the total amount absorbed was accomplished (3, 7), the kinetics of the absorptive mechanism were consistent with those shown by enzymatic processes. This mechanism has previously been shown not to be dependent on availability of unsaturated transferrin to maintain a favorable gradient across mucosal cells (8, 15). In iron-deficient animals, the kinetics consistent with an enzyme-mediated process applied up to the largest dose of iron used (500 μg), where attainment of a maximal rate of transport appeared imminent. This is further suggestive evidence supporting an active transport mechanism (16). Since in normal and iron-loaded rats similar kinetics were also found, the same type of absorptive mechanism was apparently operative as in iron-deficient rats, although with a greatly decreased capacity. In contrast to iron-deficient animals, however, when the test dose of iron exceeded 50 μg, the kinetics of the absorptive mechanism changed to those consistent with passive diffusion. We interpret this change to mean that the regulating effect of a limited mucosal transport and storage capacity for iron ("mucosal block") was exceeded to such an extent that another process, passive diffusion of iron across the intestinal mucosa, began to occur. Whether diffusion occurred or not appeared to depend on the relationship between dose of iron given and transport capacity of the mucosa. Although this phenomenon was not observed in iron-deficient animals, it very likely would have occurred if greater amounts of iron had been given to exceed further the transport capacity. From our studies with duodenal loops, we cannot predict accurately what dose of iron given orally, or intragastrically, would be required to achieve a "critical" level for diffusion to occur. Factors avoided by the use of closed intestinal loops, as for example, gastric emptying and intestinal transit time, would affect significantly both the amount of iron reaching an intestinal site and also the time during which the intestinal site is in contact with the iron. Gitlin and Cruchaud (17), in studies of over-all iron absorption in mice, found a change from a nonlinear to a linear relationship when doses greater than approximately 100 μg of iron were given. Their interpretation of the significance of this

change is similar to ours, i.e., when a critical amount of iron is exceeded, a change in the predominant mechanism of absorption occurs.

In addition to the rapidly acting transport mechanism, previous studies have shown evidence for a second, slower absorptive pathway (3, 7, 18) involving a process putting iron into a more stable, slowly released form. In our studies, iron seemingly became available for intracellular storage as a result of the rate of mucosal uptake exceeding the rate of mucosal transfer. The amount of iron put into the slowly released form is important, since we have found previously in normal (1, 3) and iron-loaded rats (3) that a relatively large portion of the amount temporarily stored was not subsequently transferred to carcass but was lost with the sloughed mucosal cells.

The present experiments indicate that mucosal cells possess an absorptive mechanism which is responsive to body iron requirements and which within limits is capable of regulating iron absorption. In addition to this, another finding should be emphasized, i.e., the rather marked restriction of the most effective iron absorbing area to the proximal 6 cm of small intestine in normal rats. Iron-deficient rats showed an increase in the area of effective iron absorbing mucosa. The proximal jejunum, even after a brief period of iron deficiency, came to resemble the normal duodenum in its effectiveness of iron absorption. These adaptive changes in rate and area are very important in determining total amount absorbed from a single dose of iron, since the time during which iron is available for absorption is limited by formation of nonabsorbable complexes (6) and by movement of iron distally in the intestinal lumen.

A basic problem yet to be solved is the manner in which these changes are mediated in response to body iron requirements. Recent work from this laboratory has postulated (1, 19) that the concentration of plasma iron or the iron binding capacity might influence or determine the intrinsic iron absorptive capacity built into newly formed cells. Whether control of iron absorption is truly an intrinsic property of mucosal cells may be established more definitely when biochemical characterization of the mucosal transport and storage mechanisms is accomplished.

## Summary

Studies on rate and site of iron absorption were performed during the early, rapid phase of iron absorption by the use of closed intestinal loops in anesthetized but otherwise intact rats with varying body iron stores. With the use of $Fe^{59}$ and whole body counting, it was possible to determine total iron absorbed from intestinal lumen and iron transferred to carcass during accurately timed absorption periods. The findings suggest that iron is absorbed by an active transport mechanism comprised of at least two steps: *1*) mucosal uptake of iron from lumen and *2*) mucosal transfer of iron to carcass. Both steps varied inversely with the state of body iron stores. Compared to mucosal uptake, mucosal transfer was more restricted to duodenum, more affected by changes in body iron stores and prefeeding iron, and regardless of body iron stores showed a lower rate. In addition to the rapid transport system, mucosal cells have a mechanism for putting iron into temporary storage. The transport and storage capacity appeared to regulate the amount absorbed into carcass until a critical dose of iron was given to normal and iron-loaded rats. When this dose was exceeded, a process suggestive of passive diffusion of iron was seen.

The site studies confirm the duodenum as the most efficient site of iron absorption and emphasize the rather marked decrease in absorption found when the test dose of iron was injected only 4 to 6 cm distal to the pylorus. Extension into proximal jejunum of the more effective area for iron absorption was observed in iron-deficient animals.

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