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Research Article

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ON THE KINETICS OF IRON ABSORPTION IN MICE *

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In individuals with similar body stores of iron, the amount of iron absorbed from a single oral dose is not proportional to the amount of iron administered. Although a greater *amount* of iron is absorbed as the size of the oral dose increases, the percentage or *fraction* of the dose that is absorbed actually decreases (1–3). In addition, the amount of iron absorbed from a given dose is dependent upon, among other things, the iron stores of the body (1, 4–6). Thus, an individual with deficient iron stores, due solely to deficient iron intake, tends to absorb more iron from a given dose than someone with normal iron stores, while the normal person tends to absorb more iron from the same dose than the individual with excessive iron stores accumulated through a large iron intake.

Although it has been established that the size of the dose and the state of the iron stores of the body both influence the absorption of iron, the biological processes through which the absorption of iron is regulated are unknown. It has been suggested that iron absorption may be controlled either by: *1*) the degree of saturation of an intracellular iron carrier, e.g., ferritin (*7*); *2*) the degree of saturation of a plasma carrier of iron, or transferrin (8); *3*) cellular enzymatic mechanisms (9, 10); *4*) the tension of oxygen at the cellular level (11); or *5*) the degree of erythropoiesis (1). None of these hypotheses has gained wide acceptance.

It has recently been observed that the absorption of copper is related to the amount of copper ingested in a manner qualitatively similar to the relationship between dose and amount absorbed for iron (12). An analysis of this relation indicated that copper absorption is mediated through two mechanisms: *1*) a first-order process wherein the amount of copper absorbed is proportional to the amount of copper ingested, and *2*) an enzymatic or carrier process which becomes saturated or less efficient as the amount of copper ingested increases. When the intake of copper is low, the carrier process is the more important in terms of amounts of copper absorbed, while at high intakes of copper the first-order process is the more significant (12). In the study reported here, the processes regulating the absorption of iron were investigated in a similar fashion: an attempt was made to analyze the relationship between the size of the dose of iron and the amount of iron absorbed and to determine the influence of the state of the body stores of iron upon this relationship.

MATERIALS AND METHODS

Swiss albino female mice of the Webster strain were selected for study. The mice were 6 to 7 weeks of age at the onset of the investigation and had an average weight of 14 g. They were kept on zinc screens in plastic cages, the feces and urine falling through the screen to the bottom of the cage where the urine was absorbed by paper toweling.

The mice were divided into 6 groups. Group N and group N_s, mice with normal iron stores, were maintained on a Purina laboratory chow diet which contained approximately 335 mg of iron per kg of diet. Although each mouse consumed approximately 1 to 1.5 mg of dietary iron, the amount of this iron that could be utilized by the animals was unknown.

Group Fe and group Fe_s, mice with excessive iron stores, were kept on the same diet as the group N mice, but each mouse was injected with 5 mg of iron in an iron-dextran complex (Imferon) subcutaneously once a day for 5 days for a total of 25 mg of iron. The significance of the size of this dose may be judged from the observation that the injection of 10 mg of iron as iron-dextran into mice was almost uniformly fatal within 6 hours.

Group D_s, mice with deficient iron intake, were maintained for 6 weeks on a synthetic diet of purified casein, corn oil, glucose, vitamins, and essential salts with the exception of iron. The diet provided less than 2 μg of iron per mouse per day, but the mice gained weight in

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much the same way as the mice on the regular chow diet.

Group (D and Fe)$_s$, mice on synthetic diet and normal iron intake, were kept for 6 weeks on the same diet as the group D$_s$ mice but with the addition of 350 mg of iron as $FeSO_4$ per kg of diet. This provided about 1 to 1.5 mg of iron per mouse per day.

Varying amounts of $FeSO_4$ labeled with Fe^{59} were then given to the mice directly into the stomach through a polyethylene catheter. The mice of group N and group Fe received the dose of labeled iron without prior fasting, but the remaining groups, designated by the subscript s, received the labeled iron after a period of 24 hours during which food was withheld. The animals of group Fe and group Fe$_s$ were given the labeled iron 36 hours after the last injection of iron-dextran.

A given dose of labeled iron was administered to each of four mice of each group. The amount of iron in each dose given to all groups except group N$_s$ and group (D and Fe)$_s$ was approximately: 0.11 or 0.65, 5, 10, 25, 50, 100, 500, and 1,000 μg; groups of four mice in group N also received 2,000 and 3,000 μg of labeled iron. The mice of group N$_s$ received only doses of 100, 500, and 1,000 μg and those of group (D and Fe)$_s$ received doses of 50, 100, 500, and 1,000 μg. It should be noted that the LD_{50} for iron given as a solution of $FeSO_4$ into the stomach in these mice was 3,000 μg, which corresponded to an LD_{50} of about 200 mg of iron per kg of body weight. Each dose of labeled iron was 0.2 ml in volume and contained 0.7 to 1.0 μc of Fe^{59}. The amount of iron actually received by each animal was determined from the amount of radioactivity present in the animal soon after the dose was given and from the specific activity of the labeled iron given. Beginning about 2 hours after the dose, the animals were allowed access to the regular chow diet.

The specificity of the influence of body stores of iron upon iron absorption was examined by studying copper absorption in two other groups: group N$_{Cu}$, normal mice, maintained on the chow diet, and group Fe$_{Cu}$, iron-loaded mice, also chow-fed but which received in addition 25 mg of iron subcutaneously, as did group Fe. Varying amounts of copper acetate labeled with 0.5 μc of Cu^{64} were then placed directly into the stomach through a polyethylene catheter in order to compare the absorption of copper in these two groups. Three mice were used for each dose in each group and the doses used were 1.2, 12, 27, 37, and 112 μg of copper.

Each mouse was assayed for radioactivity by counting the whole mouse in a well measuring 1.63 inches in diameter and 2.63 inches in depth in a 3 × 3 inch NaI crystal scintillator. When assayed for Fe^{59}, the mouse was counted within 10 minutes after administration of the dose, 3 and 6 hours later, at daily intervals for 4 to 5 days, and then on alternate days. When Cu^{64} was assayed, the mice were counted within 5 minutes after administration of the dose, 3, 6, 9, and 12 hours later, and then at 12-hour intervals for a total of 3 days. The amount of iron or copper remaining in an animal at a given time from a given dose was determined from the amount of radioactivity in the animal at that time and from the specific activity of the dose given.

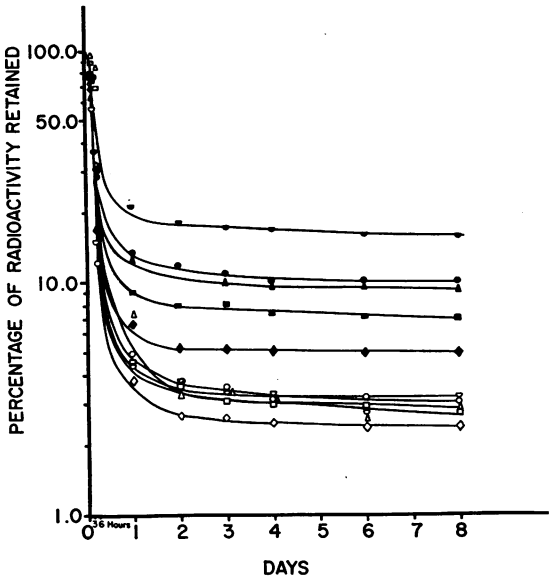

FIG. 1. DISAPPEARANCE OF RADIOIRON FROM MICE OF GROUP N GIVEN VARIOUS AMOUNTS OF THE LABELED IRON AS $FeSO_4$ ORALLY AS A SINGLE DOSE. Each curve is the average of four mice and the doses of iron administered were: ◡ 0.6, ● 5, ▲ 10, ■ 25, ◆ 50, ○ 100, ◡ 500, ◇ 1,000, □ 2,000, and △ 3,000 μg.

RESULTS

Disappearance of labeled iron from the body after a single oral dose. The disappearance of labeled iron from normal mice (group N) after oral administration is shown in Figure 1. It will be noted that the general pattern of the disappearance curves was qualitatively the same regardless of the size of the dose; the amount of labeled iron in the animal from a given dose fell rapidly soon after administration and then declined much more slowly after 24 hours. The labeled iron lost was completely recovered in the feces of the animals. After 4 to 6 days, the amount of iron lost per day was from 0.5 to 3 per cent of the amount of labeled iron remaining in the animal; this rate of excretion is equivalent to the turnover of body iron in the mouse (13). Less than 1 per cent of the labeled iron retained in the animal by this time was present in the gastrointestinal lumen. Therefore, the amount of labeled iron remaining in an animal between 4 and 6 days after administration of a given dose was considered to be the minimum amount of

iron absorbed from that dose. The general pattern of disappearance of radioactivity from mice of the remaining groups given labeled iron per os was qualitatively similar to that observed for group N mice (Figure 2), and in these animals also, the amount of labeled iron retained after 4 to 6 days was a measure of the minimum amount of iron absorbed from a given dose.

Absorption of labeled iron at different dose levels in normal mice. In Figure 3 is shown the relationship between the size of the dose and the minimum amount of iron absorbed from that dose in the mice of group N. As the size of the dose increased the amount of iron absorbed increased, even when toxic doses were attained. Above doses of approximately 100 μg, it will be noted that the amount of labeled iron absorbed increased in linear fashion with an increase in the size of the dose and the *linear portion* of the relationship could be expressed by:

$$a_n = \frac{a_2 - a_1}{D_2 - D_1} D_n + A \qquad [1]$$

where a_n is the amount of iron absorbed from a dose D_n which is greater than 100 μg; a_1 and a_2 are amounts absorbed at doses D_1 and D_2, respectively, which are also greater than 100 μg; and A is the intercept of the line with the axis at zero dose. When A was set at zero, a line parallel to the linear portion of the absorption curve was obtained (Figure 3); subtraction of this line from the *total* absorption curve yielded another curve which, as indicated in Figure 3, reached a maximum value equivalent to A at a dose of 100 μg and remained at this maximum value at higher dose levels. The latter curve had at least a superficial resemblance to those encountered in enzymatic or carrier reactions, and an attempt was made to test this impression. A simplified reaction for the absorption of iron by means of an enzymatic or carrier mechanism similar to other enzyme-catalyzed reactions (14) was considered:

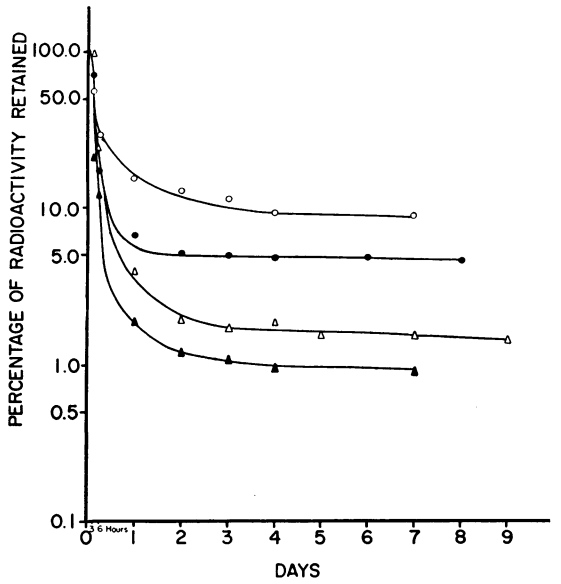

FIG. 2. DISAPPEARANCE OF RADIOIRON FROM MICE GIVEN 100 μG OF THE LABELED IRON AS $FeSO_4$ ORALLY. ○ = Group D_s, △ = group Fe_s, ● = group N, and ▲ = group Fe.

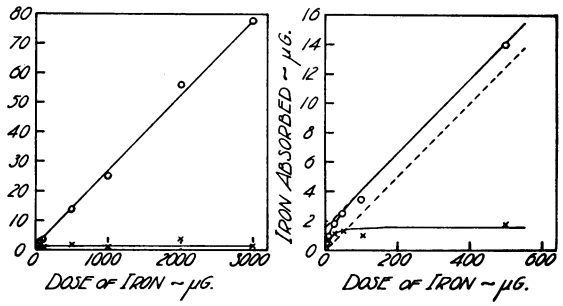

FIG. 3. THE AMOUNT OF IRON ABSORBED BY NORMAL MICE OF GROUP N FROM A GIVEN DOSE OF IRON ADMINISTERED ORALLY. Each point is the average of 4 mice. Figure on right is enlargement of area about the origin of the figure on the left. ○ = Amount of iron absorbed. Dashed line is first-order relation derived from Equation 1 when A = zero and X is the total absorption curve less the first-order process.

Intestinal lumen	Gastrointestinal mucosa	Blood plasma

$$Fe^L + X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} FeX \underset{k_4}{\overset{k_3}{\rightleftharpoons}} X + Fe^A$$

where Fe^L is the absorbable iron in the gastrointestinal lumen, X is the free enzyme or carrier, FeX is the product or products resulting from combination of iron from Fe^L or Fe^A with X, Fe^A is the iron derived from Fe^L that has been absorbed and is in a form which can react with X; k_1, k_2, k_3, and k_4 are total rate constants for the reaction in the given direction and may involve more than one rate constant in that direction. With an excess of iron in the gastrointestinal lumen, the net rate of formation of FeX will equal the net rate of decomposition of FeX:

$$k_1[Fe^L][X] - k_2[FeX] = k_3[FeX] - k_4[X][Fe^A] \qquad [2]$$

Since, in the initial stages of absorption, when Fe^L is large, Fe^A will be infinitesimally small and $k_4\,[X][Fe^A]$ is virtually zero, then

$$k_1[Fe^L][X] = k_2 + k_3[FeX] \text{ or}$$
$$\frac{[X]}{[FeX]} = \frac{k_2 + k_3}{k_1[Fe^L]} \qquad [3]$$

If $[X]_t$ is the concentration of total enzyme or carrier in the system, $[X] = [X]_t - [FeX]$. Substituting for X in Equation 3 and transposing:

$$\frac{[X]_t}{[FeX]} = \frac{k_2 + k_3}{k_1[Fe^L]} + 1 \qquad [4]$$

If a carrier or enzymatic mechanism is responsible for absorption of iron, the maximum rate of absorption or the maximum amount absorbed, A, per unit time will be proportional to $[X] + [FeX]$ or $[X]_t$, and the actual rate of absorption or the actual amount absorbed, a, per unit time will be proportional to $[FeX]$ (15). These rates may be expressed, however, as amounts absorbed, A and a, if it is considered that within each group of mice the time of exposure of the dose to the intestinal mucosa is the same for each mouse. In support of this assumption was the observation that in a given group of mice the transit time of the administered oral dose through the gastrointestinal tract appeared to be the same for the different doses of iron, although the transit time of the different doses of iron through the duodenum and jejunum, where the greatest absorption of iron would be expected to occur, was not known:

$$\frac{[X]_t}{[FeX]} = \frac{A}{a} = \frac{k_2 + k_3}{k_1[Fe^L]} \qquad [5]$$

Substituting $[D]$ for $[Fe^L]$ and K for $(k_2 + k_3)/k_1$ in Equation 5 and dividing both sides of the equation by A, the result is, as expected, a form of the Michaelis-Menten equation:

$$\frac{1}{a} = \frac{K}{A}\left(\frac{1}{[D]}\right) + \frac{1}{A} \qquad [6]$$

Plotting $1/a$ vs $1/[D]$ should, therefore, yield a straight line.

Plotting $1/a$ vs $1/D$, the curve obtained from the data for the group N mice was indeed linear (Figure 4). The slope of this line, however, was not K/A but $(K/A)\cdot(1/C)$ where C was the volume of the gastrointestinal contents into which D was distributed. This was a consequence of plotting $1/D$ rather than $1/[D]$:

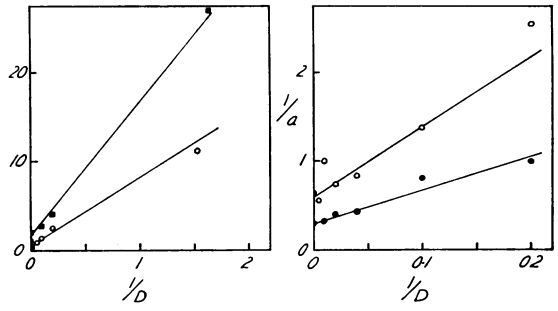

FIG. 4. PLOT OF THE RECIPROCAL OF THE AMOUNT OF IRON ABSORBED ($1/a$) VS THE RECIPROCAL OF THE AMOUNT OF IRON IN THE DOSE ($1/D$). Note that there is a difference in the coordinates in each figure; the figure on the right includes an expansion of the origin of the figure on the left for the normal mice, group N. ■ = Group Fe_s, ○ = group N, and ● = group D_s.

$$\frac{1}{a} = \frac{K}{A}\cdot\frac{1}{C}\left(\frac{1}{D}\right) + \frac{1}{A} \qquad [7]$$

It would appear then that the absorption of iron in the group N mice could be described by two processes operating simultaneously. *1*) A first-order process indicated by the linear relationship between the size of the dose and the amount absorbed as described by Equation 1 with A equal to zero. In this system, the amount of iron absorbed per unit time would be limited by the amount of absorbable iron in the gastrointestinal lumen that is presented to the absorbing surface and the upper limit would be determined by the size of the lethal dose. *2*) A process which appears to fit the kinetics of an enzymatic or carrier process in which the amount of enzyme or carrier in the system would be the limiting factor for the maximum amount of iron absorbed by this mechanism.

Effect of variations in body iron stores upon absorption of labeled iron. In mice of group Fe, the expansion of body iron stores with subcutaneously injected iron resulted in two differences in iron absorption compared with that in group N mice: *1*) the amount of iron absorbed was proportional to the size of the dose so that a carrier or enzymatic process could not be detected with certainty; and *2*) the fraction of the dose that was absorbed with first-order kinetics was

TABLE I

Constants for iron absorption in normal mice, in mice on iron-deficient diet, and in mice given iron subcutaneously

Group	Enzyme- or carrier-limited process		First-order process Per cent of dose‡
	A*	K_a†	
D_s	3.5	18.6	3.2
N	1.6	11.0	2.5
Fe_s	0.45	7.3	1.6
Fe	0		0.9

* Obtained graphically as the intercept on the ordinate when the linear portions of the absorption curves in Figure 3 and 5 were extrapolated to zero iron dose (cf Equation 1).

† $K_a = K/C$. As indicated in Equation 7, the slope of the straight lines in Figure 4 obtained by plotting $1/a$ vs $1/D$, was the constant K/AC. Multiplying K/AC, obtained from Figure 4, by A yielded K/C or Ka.

‡ The slope of the linear portions of the absorption curves in Figures 3 and 5 multiplied by 100 per cent.

somewhat decreased (Table I and Figure 5). Depriving mice of food for 24 hours prior to oral administration of labeled iron, as in group Fe_s, did increase the amount of iron absorbed by both processes (Table I and Figure 5) compared with group Fe.

In those mice kept on an iron-deficient diet (group D_s), the fraction of each dose of iron absorbed by the first-order process was somewhat increased over that for the group N mice (Table I and Figure 5), but the amount of iron absorbed by the enzymatic or carrier process was increased to a greater extent.

The mice in groups N_s and $(D + Fe)_s$ absorbed the same amount of iron at the doses tested, and the amount of iron absorbed from a given dose was only slightly greater than that

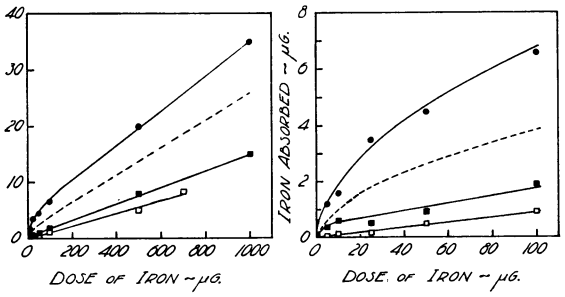

FIG. 5. THE AMOUNT OF IRON ABSORBED FROM A GIVEN DOSE OF IRON ADMINISTERED ORALLY. Figure on right is enlargement of area about the origin of the figure on the left. ● = Mice on iron-deficient diet, group D_s; – – = mice on normal diet, group N; ■ = iron-loaded mice, group Fe_s; and □ = iron-loaded mice, group Fe.

absorbed by the mice of the group Fe_s for the same iron dose.

Effect of variation in body iron stores upon absorption of labeled copper. While increasing the body stores of iron drastically reduced iron absorption, the subcutaneous injection of 25 mg of iron did not affect the absorption of copper. The relationship between the amount of copper absorbed and the size of the copper dose was the same for the mice in both group N_{Cu} and group Fe_{Cu} (Figure 6). Neither the first-order absorption of copper nor the enzymatic process for copper absorption was influenced by the expansion of body iron stores.

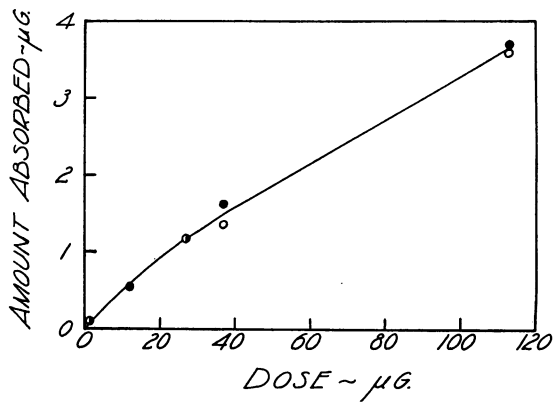

FIG. 6. ABSORPTION OF COPPER AFTER ORAL ADMINISTRATION. ○ = Normal mice, group N_{Cu}; and ● = iron-loaded mice, group Fe_{Cu}.

DISCUSSION

In the mouse, as in man, an increase in the size of the oral dose of iron results in an increase in the *amount* of iron absorbed but a decrease in the *fraction* of the dose absorbed. The data in this study suggest that iron absorption in the mouse is mediated by two different mechanisms which operate simultaneously: *1*) a process with enzymatic or carrier characteristics in which the limiting factor appears to be the amount of enzyme or carrier available, and *2*) a first-order process in which the limiting factor appears to be the amount of absorbable iron in the gastrointestinal lumen that is presented to the absorbing surface. In the mouse, the process with enzyme- or carrier-limited kinetics would appear to have the major role in the absorption of iron at dose levels of iron comparable to those in a

normal diet. With increasing doses of iron, even up to LD_{50} doses, the absorption of iron appears to be dependent to an increasing degree upon the first-order mechanism. While the data suggest that both processes are affected by the state of the body's iron stores, the enzyme-limited process appears to be affected to a greater extent than the other. Even when the stores of iron in the body are markedly increased, the amount of iron that is apparently absorbed by the first-order process, while diminished, is still at least one-third that of normal, despite the fact that the enzyme-limited process appears to be markedly or almost completely inhibited. The inhibitory effect of increased body iron stores upon absorption appears to be fairly specific, since increased stores of body iron do not inhibit the absorption of copper; the absorption of other metals in iron-loaded animals was not studied.

The nature of the process, which has first-order kinetics, is entirely unknown, but a number of physical or biochemical processes may be likely. Diffusion, for example, is a first-order process, but it would be difficult to explain: *1*) how increased body iron stores could inhibit the *simple* diffusion of iron across the gastrointestinal mucosa, since this process would be virtually independent of the amount of iron in the mucosal cells; and *2*) how such inhibition would be specific, since copper absorption is not affected by increased body iron stores. This should *not* be construed to suggest that iron diffusion across cell membranes is unlikely, but rather that diffusion alone probably is not the controlling factor in the first-order process. On the other hand, the absorption of iron based on a carrier system in which the carrier is present in large excess would also have first-order kinetics. Such a carrier system would be quite different and separate from the enzyme-limited or carrier-limited process in which the amount of iron absorbed appears to be limited by the amount of available enzyme or carrier present. In this first-order process, the concentration of absorbable iron in the gastrointestinal lumen which is presented to the absorbing surface appears to be the limiting factor, at least up to LD_{50} doses of iron. If a carrier is involved in the first-order process, it should be noted that the carrier could be a relatively small ion or it could be a macromolecule, but other mechanisms might, of course, also account for the first-order kinetics. In any event, it appears that iron absorption does not result from a single process but rather from at least two processes, each of which may be influenced to a different degree by a given stimulus or body state.

SUMMARY

Iron absorption was studied in mice using single doses of iron labeled with Fe^{59}. Iron absorption appears to be mediated by at least two different mechanisms which operate simultaneously: *1*) a process with kinetics that suggest an enzymatic or carrier reaction in which the amount of enzyme or carrier present is the limiting factor for the maximum amount of iron absorbed by this mechanism, and *2*) a process with first-order kinetics in which the concentration of absorbable iron in the gastrointestinal lumen which is presented to the absorbing surface is the limiting factor to the amount of iron absorbed by this mechanism, and the maximum amount absorbed is determined by the size of the lethal dose. Each of these two processes is influenced to a different degree by the state of the body iron stores, absorption being inhibited by an increase or enhanced by a decrease in body iron.

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