# The Regulation of Iron Absorption

## II. Relationship Between Iron Dosage and Iron Absorption

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In 1943 Hahn et al.[2] introduced the concept of a "mucosal block" into medicine. Studies were carried out in three dogs. When a large dose of iron was followed within 1.3 to six hours by a dose of radioactive iron, less radioactivity appeared in the erythrocytes than might have been "expected" in four of five experiments. The idea that a mucosal block existed was apparently rapidly accepted. It formed the basis for the well known suggestion that the apoferritin-ferritin system regulated the entry of iron to the body through the mucosa. Subsequently, however, controlled studies of the absorption of iron from various doses were carried out by other investigators.[3-5] Using more acceptable means of measuring iron absorption, it was found that although the percentage of iron absorbed decreased with increasing dosage, the total quantity continued to increase. In reviewing the studies of Smith and Pannacciulli[4] we noticed that the logarithm of the amount of iron absorbed bore a strikingly linear relationship to the logarithm of the amount of iron given. Because it seemed that this relationship might provide us with some insight into the mechanism of iron transport across the intestinal mucosa, the studies reported here were carried out in mice

### MATERIALS AND METHODS

Swiss male mice weighing an average of approximately 18 gm. were employed in these studies. Iron solutions were freshly prepared immediately before feeding. Sufficient carrier iron was added to $Fe^{59}SO_4$ to bring the concentration of $Fe^{++}$ to the stated level. All solutions were prepared in 0.01 N HCl.

Unless otherwise stated, 0.5 ml. of the $Fe^{59}$-labeled solution was fed to each animal using a curved blunt needle, as described previously.[6,7] All animals were fasted overnight prior to each experiment.

The total body counting technic employed has been described previously by others[6] and by ourselves,[7] and will not be detailed here It differs from the technic previously described[7] in that mice were counted at a distance of 11 cm. from a 2 by 2 inch crystal.

### EXPERIMENTAL AND RESULTS

### *The Relationship Between Iron Dosage and Iron Absorption in Normal Mice*

Eight groups of nine or ten mice each were given the following doses of iron: 0.08 μg., 0.5 μg., 5.0 μg., 50 μg., 500 μg., 1,500 μg., 5,000 μg. and 15,000 μg. All the mice that received 15,000 μg. died within thirty minutes. Three of the ten mice that received 5,000 μg. and one of the ten mice that received 1,500 μg. also died shortly after the administration of iron. The iron absorption of the survivors is recorded in Table I and has been plotted in Figure 1. It is apparent that there is a linear relationship between the logarithm of the dose and the logarithm of the amount absorbed. In a second experiment (Table II), five groups of ten mice each were investigated over a somewhat smaller range of dosages: 0.25 μg., 5.0 μg., 50 μg., 500 μg. and 1,500 μg. Although with this group of mice the equation of the line obtained differed somewhat from that

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Table I

Relationship Between Iron Dosage and Iron Absorption Over a Wide Range of Iron Dosages

| Dose (µg.) | Animals Surviving (no.) | Absorbed | |
|---|---|---|---|
| | | % | µg. ± 1 S.E. of Mean |
| 0.08 | 9 | 14.18 | 0.0114 ± 0.00217 |
| 0.5 | 10 | 9.64 | 0.0484 ± 0.00800 |
| 5 | 9 | 10.47 | 0.522 ± 0.0695 |
| 50 | 10 | 7.41 | 3.705 ± 0.489 |
| 500 | 10 | 3.25 | 16.25 ± 2.29 |
| 1,500 | 9 | 4.82 | 72.33 ± 14.20 |
| 5,000 | 7 | 4.33 | 216.43 ± 39.24 |
| 15,000 | 0 | ... | ... |

found in the first experiment, a good fit was obtained with a log-log plot (Fig. 2). In a third experiment, the possibility recently proposed by Gitlin and Cruchaud[8] that a linear relationship exists between dosage of iron and the quantity absorbed was examined critically using a relatively large number of animals at a relatively large number of dosage levels over a relatively short range. Seven groups of fourteen mice each were studied at the following dosages: 200 µg., 400 µg., 600 µg., 800 µg., 1,000 µg., 1,200 µg. and 1,400 µg. These data are presented in Table III and summarized

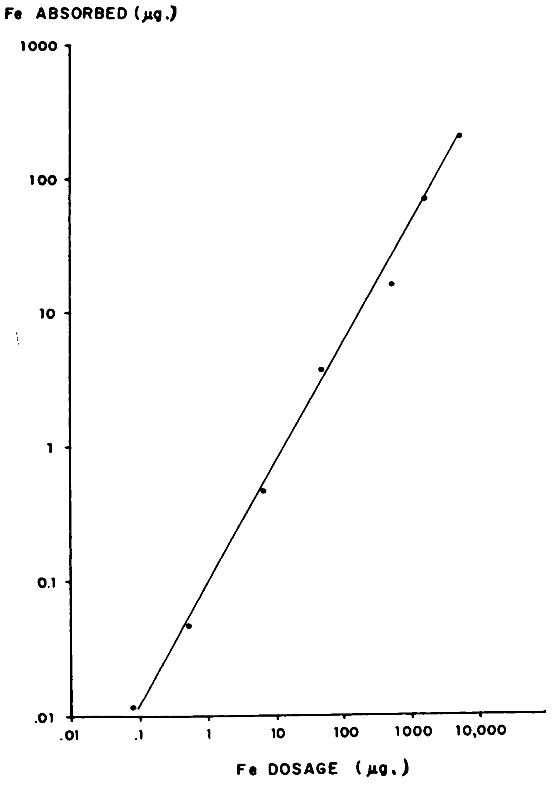

Fig. 1. The relationship between iron dosage and iron absorption in normal mice. Each point represents the arithmetic mean of all of the surviving animals at a given dose level. The standard errors of the means are given in Table I.

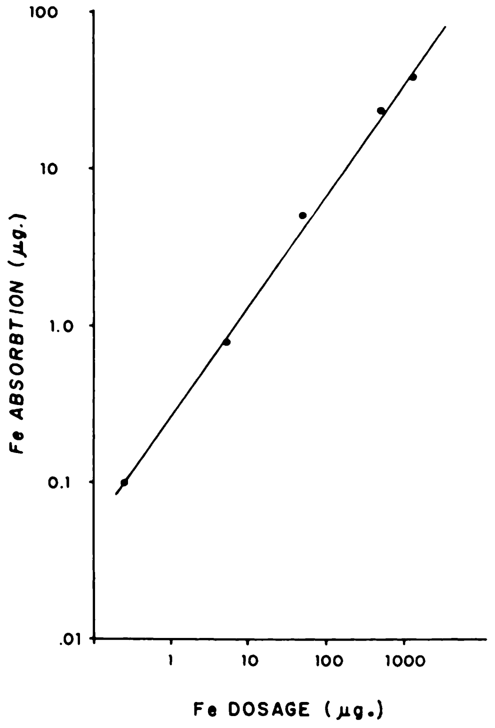

Fig. 2. The relationship between iron dosage and iron absorption in normal mice. Each point represents the arithmetic mean of all of the surviving animals studied at a given dose level. The standard errors of the means are given in Table II.

TABLE II
Relationship Between Iron Dosage and Iron Absorption Over a Wide Range of Iron Dosages

| Dose (µg.) | Animals Surviving (no.) | Absorbed | |
|---|---|---|---|
| | | % | µg. ± 1 S.E. of Mean |
| 0.25 | 10 | 38.0 | 0.0951 ± 0.0095 |
| 5 | 10 | 15.8 | 0.788 ± 0.170 |
| 50 | 10 | 10.4 | 5.20 ± 0.566 |
| 500 | 10 | 4.7 | 23.45 ± 1.87 |
| 1,500 | 10 | 2.54 | 38.10 ± 3.78 |

graphically in Figure 3. Similar results were obtained when this experiment was repeated over a somewhat larger dosage range (Table IV, Fig. 4).

### *Relationship Between Iron Dosage and Iron Absorption in Iron-Loaded Mice and Mice with Hemolytic Anemia*

One hundred and eighty mice were used in these studies. Sixty animals were given 5 mg. of iron dextran subcutaneously four weeks prior to the study. Sixty animals were given 3 mg. of phenylhydrazine hydrochloride on the day prior to the feeding of iron. Sixty animals served as controls. The following iron dosages were administered to ten animals of each group: 1.45 µg., 3.0 µg., 30 µg., 100 µg.,

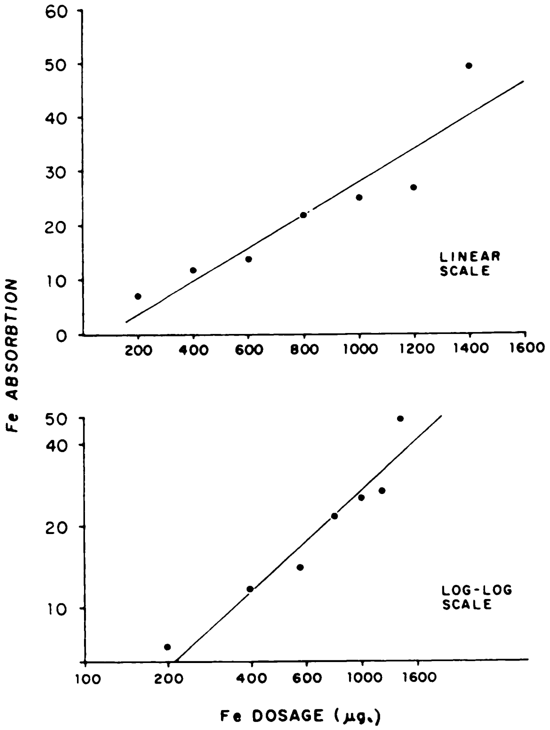

FIG. 3. The relationship between iron dosage and iron absorption over the 200 to 1,400 µg. range. The arithmetic means have been plotted both on a log-log scale and a linear scale. The standard errors are given in Table III.

TABLE III
Relationship Between Iron Dosage and Iron Absorption Over a Narrow Dosage Range

| Dose (µg.) | Animals Surviving (no.) | Absorbed | |
|---|---|---|---|
| | | % | µg. ± 1 S.E. of Mean |
| 200 | 14 | 3.63 | 7.26 ± 0.837 |
| 400 | 14 | 2.88 | 11.52 ± 1.53 |
| 600 | 13 | 2.32 | 13.92 ± 0.222 |
| 800 | 14 | 2.72 | 21.76 ± 1.332 |
| 1,000 | 13 | 2.52 | 25.20 ± 2.68 |
| 1,200 | 14 | 2.24 | 26.88 ± 1.88 |
| 1,400 | 14 | 3.50 | 49.00 ± 14.11 |

TABLE IV
Relationship Between Iron Dosage and Iron Absorption Over a Narrow Dosage Range

| Dose (µg.) | Animals Surviving (no.) | Absorbed | |
|---|---|---|---|
| | | % | µg. ± 1 S.E. of Mean |
| 200 | 12 | 7.07 | 14.14 ± 1.98 |
| 400 | 14 | 5.62 | 22.48 ± 1.53 |
| 600 | 13 | 4.11 | 24.68 ± 1.92 |
| 900 | 12 | 3.38 | 30.39 ± 3.99 |
| 1,200 | 12 | 3.82 | 45.78 ± 4.98 |
| 1,600 | 13 | 4.53 | 72.47 ± 4.81 |

TABLE V
Relationship Between Iron Dosage and Iron Absorption In Iron-Loaded, Phenylhydrazine-Treated and Control Animals

| Dose (µg.) | Animals Surviving (no.) | Absorbed | |
|---|---|---|---|
| | | % | µg. ± 1 S.E. of Means |
| *A. Iron Loaded* | | | |
| 1.45 | 8 | 7.97 | 0.116 ± 0.02 |
| 3.0 | 9 | 6.32 | 0.189 ± 0.05 |
| 30 | 9 | 2.43 | 0.73 ± 0.10 |
| 100 | 8 | 3.00 | 3.0 ± 0.32 |
| 1,000 | 8 | 2.00 | .20 ± 4.24 |
| 3,000 | 8 | 2.39 | 71.6 ± 11.19 |
| *B. Phenylhydrazine Hydrochloride* | | | |
| 1.45 | 7 | 33.7 | 0.4886 ± 0.115 |
| 3.0 | 7 | 46.8 | 1.407 ± 0.053 |
| 30 | 9 | 15.3 | 4.60 ± 1.25 |
| 100 | 6 | 18.9 | 18.9 ± 5.57 |
| 1,000 | 7 | 6.66 | 66.6 ± 12.92 |
| 3,000 | 2 | 3.75 | 112.5 ± 16.50 |
| *C. Control Animals* | | | |
| 1.45 | 10 | 15.2 | 0.221 ± 0.0707 |
| 3.0 | 10 | 21.5 | 0.644 ± 0.151 |
| 30 | 10 | 8.30 | 2.49 ± 0.533 |
| 100 | 10 | 3.75 | 3.75 ± 0.881 |
| 1,000 | 10 | 1.75 | 17.46 ± 1.96 |
| 3,000 | 7 | 2.13 | 63.9 ± 8.45 |

1,000 µg. and 3,000 µg. The results of this study are given in Table V and Figure 5.

### *Analysis of Variability*

It is apparent from Tables I through V that considerable variability was encountered in the amount of iron absorbed within each group. It appears that this variability is due to individual variations among the animals, rather than variability introduced by the method. Fourteen animals were each given 10 µg. of iron. Two independent determinations of iron absorption were made in each mouse by removing the animal from its container, replacing it and recounting. Figure 6 presents these data. It is apparent that there is little variability between duplicate determinations carried out in the same animal. The possibility that iron absorption might be influenced by the volume which is injected into the stomach of the mouse has also been investigated. It was found to have little or no influence either on the amount of iron absorbed or on the variability (Fig. 7).

## COMMENTS

We have found that the logarithm of the dose of iron administered to a mouse bears a linear relationship to the logarithm of the amount absorbed over a range of doses extending from doses of 0.08 µg. (representing high specific activity radioiron without added carrier iron) to doses which are lethal to considerable proportion of the animals. The slope of the curve was consistently less than one. Only at a slope of one would a linear relationship be observed by dose and absorption without the log transformation. Analysis of data which have

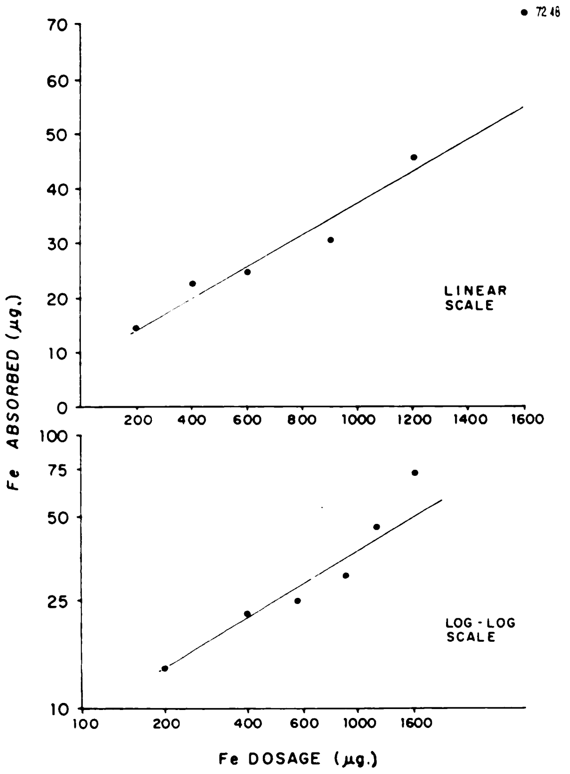

FIG. 4. The relationship of iron dosage and iron absorption over the 200 to 1,600 μg. range. The arithmetic means have been plotted both on a log-log scale and a linear scale. The standard errors are given in Table IV.

been reported by Smith and Pannaccuilli[4] and by Bonnet et al.[5] suggests that the same thing is true in human subjects (Fig. 8 and 9). We have found no other data in the literature which appear to contradict this relationship except for that recently reported by Gitlin and Cruchaud.[8] Since these authors have not given the standard error of their measurements, it is difficult for us to determine whether their data differ significantly from the findings which we have reported. The inherent variability of iron absorption in a group of mice seems to be large. This is true with all data using this technic which have been reported. Thus, in the first study of this series,[7] we found the following means and sample standard deviations in normal mice given 100 μg. of iron: 7.9 ± 2.5, 6.0 ± 4.5, 4.1 ± 1.2 and 9.6 ± 2.0. At the 10 μg. level, iron absorption of 20.4 ± 11.1 per cent was reported. Mendel[9] fed doses of about 7 μg. of iron to normal mice

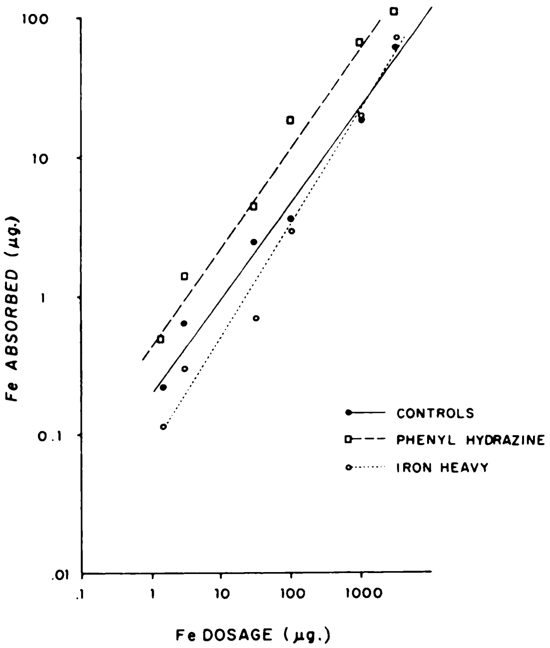

FIG. 5. The effect of iron loading with iron dextran and of phenylhydrazine on iron absorption in mice. Phenylhydrazine increased iron absorption at all dosage levels. The effect of iron loading was most evident at the lower dosage levels.

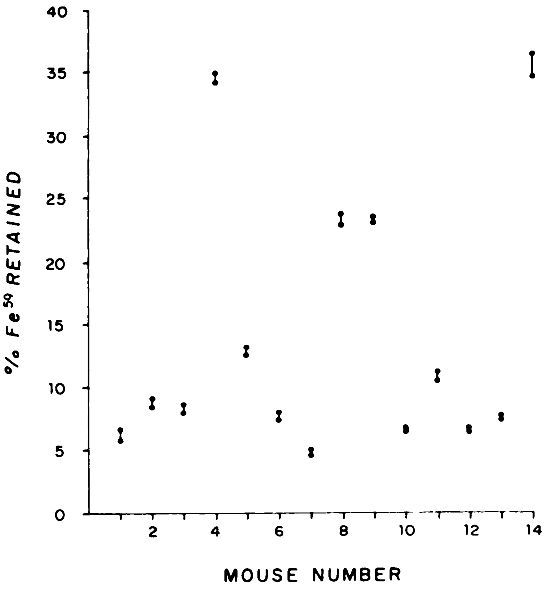

FIG. 6. Duplicate iron absorption determinations carried out on fourteen mice given 10 μg. of iron. The duplicate values are connected by a vertical bar. It is evident that although the amount of iron absorbed by the animals varies from less than 5 to more than 35 per cent, remarkably consistent results were obtained on each individual animal.

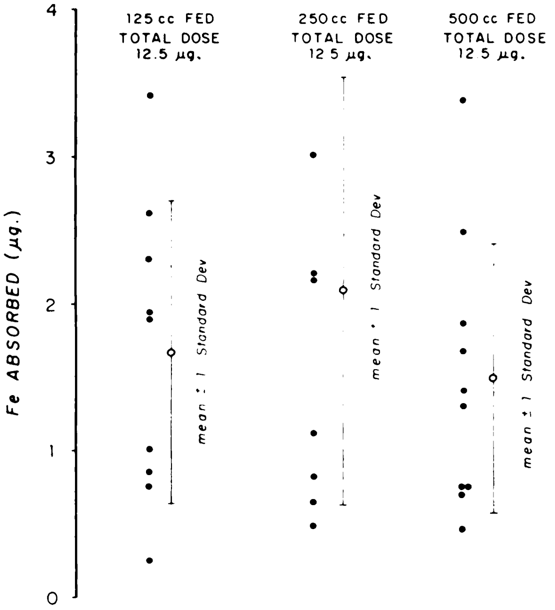

FIG. 7. The effect of the volume of iron solution fed on the absorption of iron and variability of absorption. Each animal was fed 12.5 µg. of iron, but the volume of the solution varied from 0.125 to 0.500 cc. Varying the volume appeared to influence neither quantity of iron absorbed nor the variability.

and obtained an iron absorption of 18.8 per cent with the sample standard deviation of approximately 15 per cent. In another experiment, an iron absorption of 15.3 per cent was associated with a standard deviation of 7.6 per cent. Krantz et al.[6] reported even larger variability. It is apparent, therefore, that variability in iron absorption of laboratory mice is marked. Unless mean values obtained from a relatively large number of animals are studied it is impossible to draw valid conclusions regarding iron absorption. Gitlin and Cruchaud[8] studied only four mice at each dosage level. Although no estimate of variability is presented in their paper, it seems reasonable to suppose that it was of the same order of magnitude as has been observed by previous investigators using the same system. If this is the case, the sample standard deviation would be approximately 50 per cent of the mean value and the standard error of the mean with a sample of four would be approximately 25 per cent. Thus, 95 per cent confidence limits of each point obtained would be approximately ±50 per cent of the stated value. These authors reported that a linear relationship existed between iron dosage and iron absorption in iron dosage levels greater than 100 µg. They based a theory of iron absorption upon this relationship in which it was proposed that the carrier mechanism was active when smaller amounts of iron were given, but that iron passed through the bowel with first order kinetics at higher dosage ranges. In examining the higher range of iron dosages, using a large number of animals, we are unable to differentiate over the short range available between a linear relationship and a log-log relationship.

There is considerable risk in attempting to draw conclusions about the mechanism of transport from the mathematical relationship between dose and the quantity absorbed. It is possible, however, to look at certain models and to determine whether the data obtained are compatible with these models. It is clear, for example, that it is impossible to explain the type of relationship observed between dosage and quantity of iron absorbed on the basis of simple diffusion of iron across a gradient. If this were the case, one would expect a decreasing percentage of iron to be absorbed as the difference between the intralumenal iron concentration and the plasma iron concentration became smaller. Thus, as the iron dosage decreased, the percentage of iron absorbed should approach zero as the plasma iron concentration is approached. A far more attractive model might be one in which the limiting step in iron absorption is the complexing of iron with a receptor. As the receptor became saturated with iron less and less sites would be available for iron-binding and, therefore, less and less iron would be absorbed. It can be shown mathematically (see Appendix) that this simplified model does not fit the log-log relationship found to exist between dosage of iron and absorption.

We are unable to propose a single simple model which explains the relationship we have found between iron dosage and the quantity of iron absorbed. It is likely that no simple model will fit iron absorption data in the intact mouse. One of the components of the iron

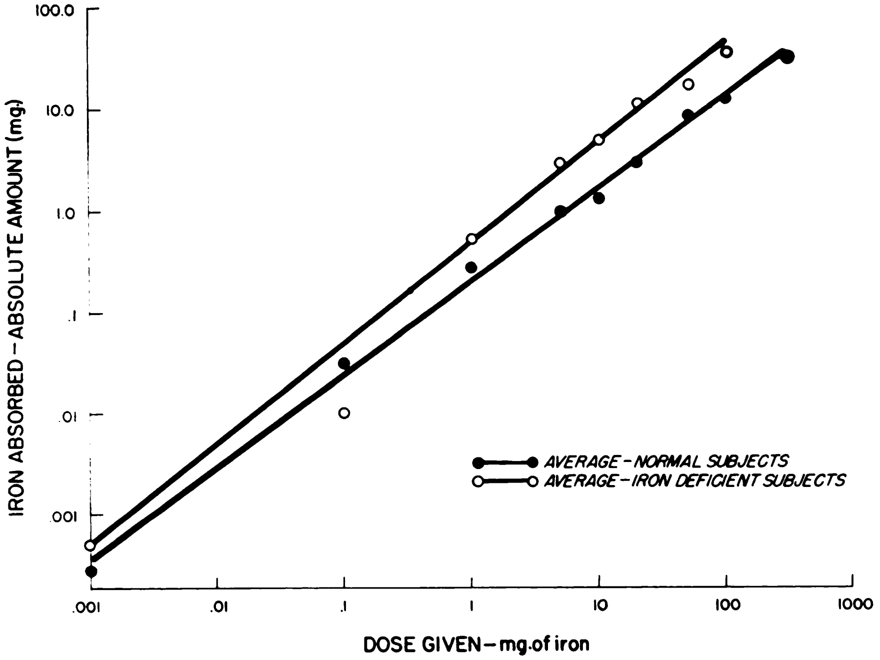

FIG. 8. The relationship between iron dosage and quantity of iron absorbed in man (based on the data of Smith and Pannacciulli[4]).

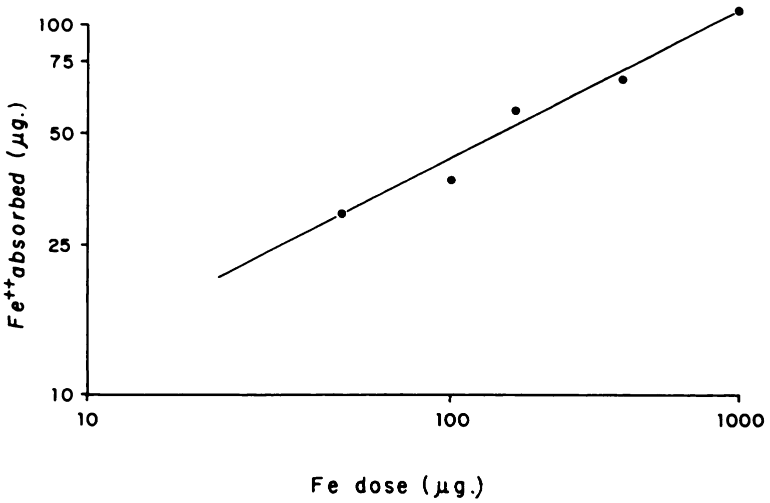

FIG. 9. The relationship between iron dosage and quantity of iron absorbed in man (based on the data of Bonnet et al.[5]).

transport process, siderophilin, is circulating and is thus brought to the bowel in varying states of saturation during the absorption process. It is possible that if a receptor in the bowel represents a limiting factor in iron absorption that it changes in concentration when iron is presented to the bowel and/or that its dissociation, as well as its formation, limits the rate of iron transport. Either the model is not correct, or the apparent log-log relationship between dosage and absorption, which we have found, is not rigidly correct, or additional modifying factors influence the actual amount of iron absorbed. However, we would be inclined to interpret our data as indicating that the same absorptive mechanisms are active when small doses of iron are given as when massive amounts of iron are fed. This would suggest that acute iron poisoning may not be due to destruction of the intestinal mucosa with subsequent "leakage" of toxic inorganic iron across the membrane in lethal amounts but rather to an extension of the normal absorptive process into the lethal range.

### APPENDIX

The following calculations examine the consistency between a model system for iron absorption and the data obtained in this study. The model postulates that n molecules of free iron (F) combine with m molecules of a free chelating compound (G) in the reaction

$$nF + mG \rightleftharpoons F_nG_m \qquad (1)$$

and that this single step is the limiting one in regulating iron absorption. From the data presented earlier in this study, one deduces that

$$D = K_1x^c \qquad (2)$$

in which x denotes the total number of iron molecules present, D is the number of $F_nG_m$ molecules, and $K_1$ and c are positive constants (independent of iron concentration over a wide range), with $0 < c < 1$.

If (1) is true, it is known from the theory of reaction kinetics for dilute solutions that there is an equilibrium constant K (independent of the concentrations of iron or chelating compound) satisfying

$$K = \frac{(x - nD)^n (K_2 - mD)^m}{D} \qquad (3)$$

where $K_2$, another constant, is the total number of molecules of (G). It will be demonstrated, however, that (3) fails to hold over even a small range of iron concentrations, regardless of any possible choice of c,m,n,K,$K_1$, and $K_2$. Hence, (1) cannot be a valid model for the mechanism of iron absorption.

To express (3) directly in terms of iron concentration, one substitutes $D = K_1x^c$ (see (2)) into (3). If (3) also has both sides divided by K, there results

$$\frac{(x - nK_1x^c)^n\ (K_2 - mK_1x^c)^m}{KK_1x^c} = 1 \qquad (4)$$

which must be satisfied for all positive x if (3) is to hold. By taking logarithms of both sides of (4), it is seen that (4) is, in turn, equivalent to

$$-\log KK_1 + n \log x + n \log (1 - nK_1x^{c-1}) + m \log K_2 + m \log \left(1 - \frac{mK_1}{K_2} x^c\right) - c \log x = 0 \qquad (5)$$

Now call the left side of (5) f(x); then (3) is true if and only if $f(x) = 0$ for all positive x. The latter implies that the derivative $f'(x) = 0$. But

$$f'(x) = \frac{n - c}{x} - \frac{n^2(x - 1)K_1x^{c-2}}{1 - nK_1x^{c-1}} - \frac{(m^2cK_1/K_2)\ x^{c-1}}{1 - (mK_1/K_2)\ x^c} \qquad (6)$$

which we show to be zero at no more than one value of x. Indeed,

$$xf'(x) = (n - c) + \frac{n^2\ (1 - c)K_1x^{c-1}}{1 - nK_1x^{c-1}} - \frac{(m^2cK_1/K_2)x^c}{1 - (mK_1/K_2)\ x^c} \qquad (7)$$

since $0 < c < 1$, the second term is strictly decreasing with x and the third strictly increasing. Hence, $f'(x)$ is strictly decreasing, and can be zero only at one point.

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