

Studies on Erythropoiesis. XIV. The Relationship of Humoral Stimulation to Iron Absorption

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IT HAS LONG BEEN EVIDENT that some mechanism exists in the mammal that regulates the absorption of dietary iron.¹⁻⁴ When animals are made anemic by phlebotomy,^{5,6} or by hemolysis with acetyl phenylhydrazine,^{7,8} absorption of iron is increased. These methods of causing anemia also result in increased formation of erythropoietin,⁵ which increases the rate of erythropoiesis.⁹ The research described in this paper was undertaken to determine whether the absorption of iron is controlled by the same hormone (or hormones) that regulates the rate of erythropoiesis. This was studied by measuring the absorption of iron under conditions which we knew would elevate or depress the plasma erythropoietin titer or by introducing exogenous erythropoietin into the animals. While those conditions that elevate the amount of circulating erythropoietin increase iron absorption, and while the converse is also true, the direct test of the hypothesis by use of plasma rich in erythropoietin, or concentrated extracts of such plasma, clearly demonstrated that the erythropoietic factor as such does not directly affect the transport of iron across the gastrointestinal mucosa.

MATERIALS AND METHODS

A rapid method for the determination of iron absorption was developed. This procedure is as follows. CF No. 1 female mice, 10 weeks of age, were used. These had been kept on an "iron-free" diet¹⁰ for two to three days prior to the start of the experiment to reduce the amount of unlabeled iron in the gastrointestinal tract and thus to diminish variability that might be the result of different dilutions of added labeled iron. The mice were force-fed .4 to .6 ml. of a solution of Fe⁵⁹ as FeCl₃ with added carrier, buffered at pH 6, by means of a 1 ml. tuberculin syringe fitted with a curved, 18 gage needle. The tip of the needle was blunted and smoothed with silver-solder to prevent damage to the esophagus. Immediately after a mouse was force-fed, it was placed in a perforated, 50 ml. plastic centrifuge tube which was then tightly corked. The tube was counted in a Welch-Allyn well-type Geiger counter (type H18-20R). Repeated counts were taken at definite intervals until the total-body count per mouse was constant, which then represented the amount of iron absorbed by the animal. At the time when each series of total-body counts was done, 40 ml. of a solution containing an amount of Fe⁵⁹ equal to that given to each mouse was counted to correct for radioactive decay. The iron was diluted to 40 ml. so that the geometry of counting was as nearly comparable as possible to that of the mouse. The per cent of the original amount of Fe⁵⁹ retained by the mouse at various intervals was then calculated from the radioactivity at that interval and the corrected original count for the mouse.

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¹⁰This diet is a modification of the 4 C diet described in Wissler et al.¹⁰ with added salts containing no added iron.

RESULTS

To test the validity of the method of counting whole mice, the animals were given varying amounts of Fe^{59} , and their radioactivity was determined immediately. The data presented in figure 1 show a linear relationship between the amount of iron fed and the immediate total-body count, an indication that the counting method does measure the relative amount of isotope within the animal.

In another test of the method, we determined the way in which the total-body count was related to the amount of iron that had passed through the mucosa of the gut. Six days after iron feeding samples of blood were counted, and the results were compared with the total-body counts. As shown in figure 2, the radioiron found in the blood varied in a linear fashion with the total-body count. In addition, the complete intestinal tract of each animal was excised and counted. No detectable radioiron was found. The evidence thus indicates that the method may be used to measure the absorption of iron.

By our method of determining total-body radioactivity, we easily confirmed earlier findings^{5,6} that phlebotomy stimulates iron absorption. Mice had about one-half of their blood volume removed by cardiac puncture five days before iron feeding. This was repeated three days before iron feeding. The bled and control groups were force-fed 0.6 ml. of a 0.006 per cent solution

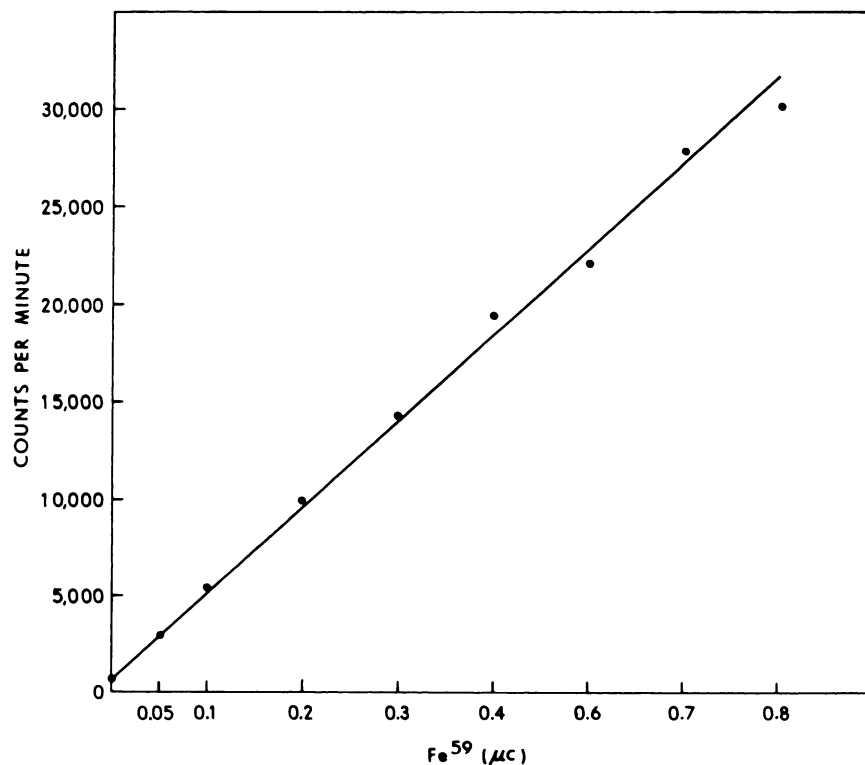


FIG. 1.—Effect of dose of Fe^{59} on total-body count rate of mice.

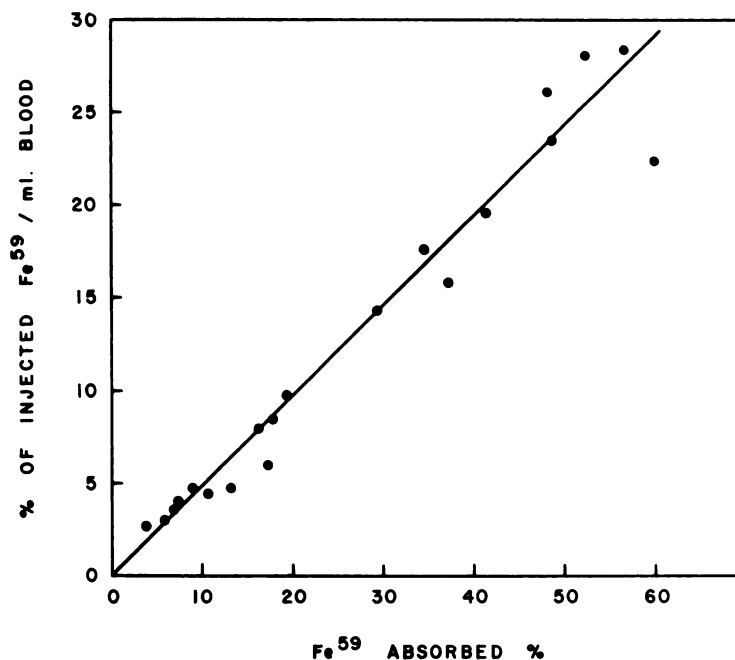


FIG. 2.—Relationship between blood count rate and total-body count rate.

of FeCl_3 containing $0.33 \mu\text{C. Fe}^{59}$. The results of this experiment are illustrated in figure 3. The initial 5 hour period represents the time before excretion begins. The unabsorbed iron was eliminated quite rapidly, and the leveling of the curve at a constant value represents absorbed iron. It is apparent from the curves that the bled mice retained approximately twice as much iron as did their controls.

Mice treated with 0.8 mg. of phenylhydrazine for three consecutive days and then with 1.2 mg. on the day preceding administration of Fe^{59} also showed a marked increase in absorption (fig. 4). The control values are substantially lower than those shown in figure 3 since an amount of carrier iron (0.4 ml. of 0.17 per cent; $0.37 \mu\text{C.}$) larger by about 20 was used.

Atmospheric hypoxia has also been reported to increase iron absorption,¹¹ and we have found this to be true with our assay system. Ten mice were placed in an atmosphere containing 8 to 10 per cent oxygen for 21 hours, and 10 others were used as controls. At the end of this period both groups were given the Fe^{59} orally. The results in table 1 demonstrate an increased absorption by the animals made hypoxic.

Since phenylhydrazine, bleeding, and atmosphere hypoxia are all followed by an increased plasma erythropoietin titer,^{9,12} and since it has been shown that cobaltous ion can also induce a rise in plasma erythropoietin,¹³ we tested the effect of cobalt on iron absorption. Nine mice were injected subcutaneously for three days with $5.3 \mu\text{M}$ of CoCl_2 in 0.1 ml. saline per day, while control mice were given saline. On the fourth day, the animals were force-fed Fe^{59} and the per cent absorption was determined. As indicated in table 1, cobaltous ion appreciably increased the absorption of iron.

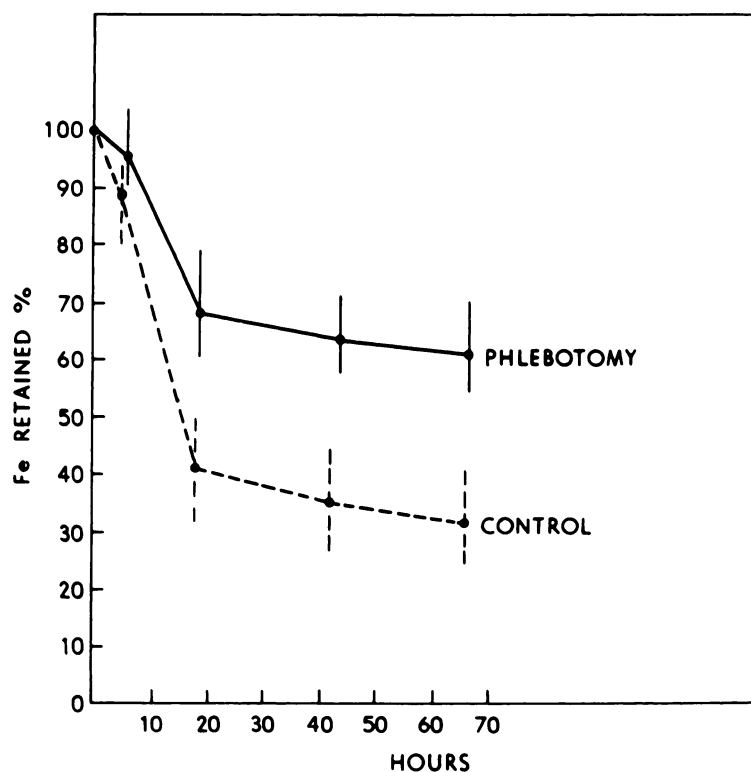


FIG. 3.—Effect of phlebotomy on Fe absorption.

Another parallelism between iron absorption and the rate of erythropoiesis is seen in table 2. Mice were made polycythemic by 11 injections of 0.5 ml. of an 80 to 90 per cent suspension of homologous red cells in saline over a period of three weeks, at the end of which their hematocrits were 70 to 80 per cent. Erythropoiesis was completely depressed in such mice.⁹ One group of polycythemic mice was given cobalt ion as in the previous experiment. The other group was given saline, and the results from these two groups were compared with those from normal mice given cobalt ion and saline.

Polycythemia reduced drastically the absorption of iron, and whereas cobalt had a pronounced effect on the normal mouse, it had a very slight net effect on the polycythemic mice. In these cobalt-treated, polycythemic mice, erythropoiesis was mildly accelerated as shown by a rise in reticulocytes from zero in the untreated animals to 0.1 to 0.4 per cent in the cobalt-treated groups.

The very dramatic effect of cobalt ion seen in the groups of normal mice is obviously not due to the toxic action of the metal ion on the gastrointestinal mucosa, which might increase the transport of iron. If toxicity were involved, the magnitude of response would be expected to be about the same in the polycythemic group as in the normal group. Similar results have been reported by Bothwell et al.¹⁴

The effect of iron loading on the mice after absorption of dietary iron was determined after injection of iron-dextran (Imferon). It is readily seen that

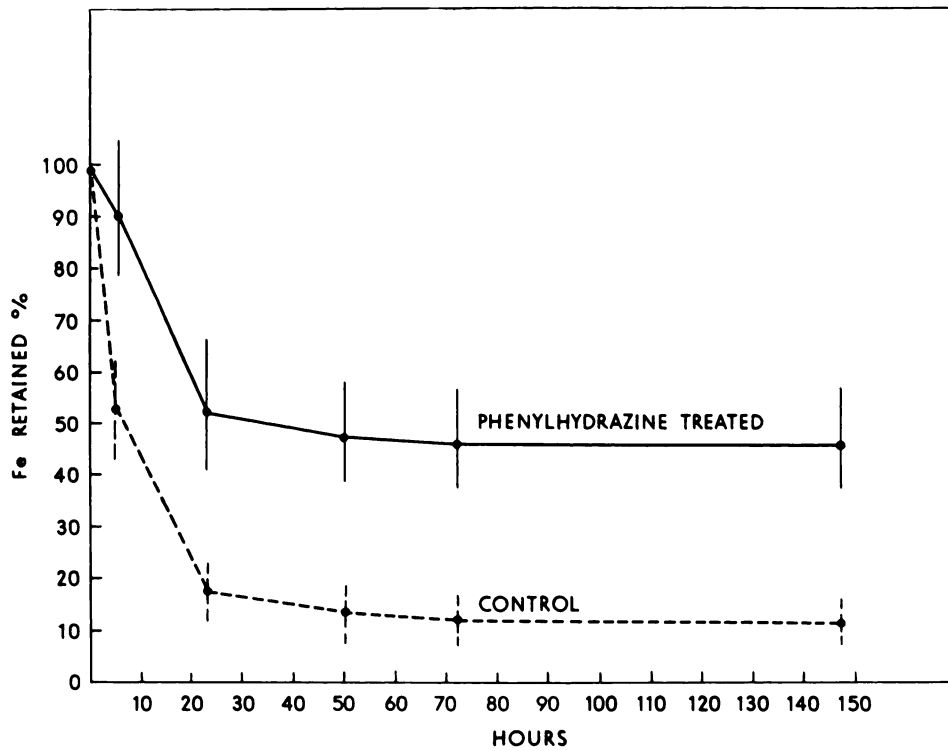


FIG. 4.—Effect of intravenous hemolysis on Fe absorption.

TABLE 1.—Effect of Erythropoietic Stimulation on Iron Absorption

| Mice (no.) | Time (hrs.) | Treatment | Fe ⁵⁹ Absorbed (%) |
|------------|-------------|--------------------|-------------------------------|
| 10 | 96 | Control | 7.5 ± 1.4* |
| 10 | | Hypoxia | 17.9 ± 6.0 |
| 9 | 168 | Control—NaCl | 9.7 ± 5.2 |
| 9 | | Cobaltous Chloride | 31.1 ± 11.6 |

*Standard deviation of the mean.

excess body iron drastically reduces the absorption of iron by the gastrointestinal mucosa.

An attempt was also made to dissociate erythropoiesis from iron absorption by x-irradiation. We hoped to depress marrow function and to observe the effect upon absorption. In a preliminary experiment, mice that had been exposed to 200 r 36 hours before being force-fed Fe⁵⁹ showed about a twofold increase in iron absorption (controls, 13 per cent; irradiated, 34 per cent). However, subsequent experiments have shown a wide scatter of effect, including a depression of iron absorption induced by x-irradiation. These re-

TABLE 2.—*Effect of Induced Polycythemic and Cobaltous Ion on Iron Absorption*

| Mice (no.) | Time (hrs.) | Treatment | Fe ⁵⁹ Absorbed (%) |
|------------|-------------|-------------------------------|-------------------------------|
| 5 | | Normal Control—NaCl | 8.4 ± 3.5° |
| 6 | | Polycythemic Control—NaCl | 0.7 ± 0.3 |
| | 96 | | |
| 5 | | Normal Co ⁺⁺ | 21.8 ± 4.8 |
| 6 | | Polycythemic Co ⁺⁺ | 3.5 ± 3.3 |

°Standard deviation of the mean.

TABLE 3.—*Effect of Iron-Loading on Iron Absorption*

| Mice (no.) | Time (hrs.) | Treatment | Fe ⁵⁹ Absorbed (%) |
|------------|-------------|-----------------|-------------------------------|
| 8 | | Control—NaCl | 7.7 ± 2.2° |
| 10 | | Imferon—11 Days | 1.7 ± 0.6 |
| | 96 | | |
| 14 | | Control—NaCl | 14.2 ± 4.9 |
| 15 | | Imferon—4 Days | 2.5 ± 1.0 |

Mice given injection of Imferon (0.5 mg. iron equivalent) for times indicated. Control mice injected with saline at same times. After last injection, tracer amount given by stomach tube and per cent absorption measured.

°Standard deviation of the mean.

sults may be the consequence of radiation-induced mucosal damage and have dissuaded us from further efforts along this line.

In another attempt to depress marrow function without deleterious effects to the gastrointestinal mucosa, we treated mice with Myleran or Ca⁴⁵. Myleran generally made the animals too sick to be useful, and Ca⁴⁵ was of such low specific activity that by the use of physiologically tolerated amounts, the rate of erythropoiesis, as determined by reticulocyte count, was never greatly diminished until the animals died.

The hypothesis that erythropoietin may exert a direct effect upon iron absorption was tested by using a concentrated extract of plasma from rabbits made severely anemic by phenylhydrazine. This extract, made by perchloric acid precipitation of the plasma¹³ when assayed at 12 mg. per rat per injection in starved animals by the iron incorporation method,¹⁶ induced a Fe⁵⁹ incorporation value of 10.6 per cent, while the control value (saline or normal plasma) was 3.7 per cent. This material could also stimulate reticulocyte formation in polycythemic mice. Polycythemic mice given 51 mg. per mouse of the anemic plasma extract over a 3 day period before iron feeding had a 2.6 per cent absorption as compared with 2.1 per cent for control mice (table 4). In addition, whole plasma from mice treated with cobaltous ion, a method known to increase erythropoietin titers,¹³ failed to significantly

TABLE 4.—*Effect of Erythropoietin on Iron Absorption*

| Mice (no.) | Time (hrs.) | Treatment | Fe ⁵⁹ Absorbed (%) |
|------------|-------------|--|-------------------------------|
| 3 | 72 | Polycythemia—NaCl | 2.1 ± 0.1* |
| 8 | | Polycythemia + anemic plasma extract | 2.6 ± 1.8 |
| 10 | 144 | Polycythemia + normal plasma | 3.7 ± 1.5 |
| 9 | | Polycythemia + Co ⁺⁺ plasma | 4.5 ± 2.7 |

*Standard deviation of the mean.

increase iron absorption in polycythemic mice. Mice given 5 cc. per mouse of "cobalt plasma" over a period of 5 days had an iron absorption of 4.5 per cent, while those given normal plasma absorbed 3.7 per cent (table 4). Mice with transfusion-induced polycythemia were used in these experiments to determine whether there was a direct effect on the mucosa independent of active erythropoiesis. In view of the magnitude of error in these measurements, the slightly higher values seen with both of the above experimental series cannot be considered adequate evidence of stimulated absorption.

The idea expressed by Bothwell et al.¹⁴ and Moore¹⁵ that iron absorption appears to parallel the rate of erythropoiesis seems to be valid in the light of our findings. However, the question still remains as to how the intestinal mucosa is stimulated by increased erythropoiesis. The possibility that the absorbing mucosal surface responds to a simple displacement of the equilibrium between tissue cells and plasma by a fall in plasma iron would require a separate compartment of iron in the plasma to which the mucosa would respond. This would have to be the case because the total iron in the plasma of animals made anemic by hemolysis is greater than that in normal animals.* Data presented by Colehour et al.¹⁷ indicate that siderophilin does increase in the hypoxic animal. We have, so far, been unable to demonstrate any humoral factor that affects the intestine as a consequence of alterations in the marrow, nor any humoral factor that affects both concomitantly. If such a humoral factor exists, it does not appear to be erythropoietin. The possibility remains that the intestine is acted on directly by the same external conditions that alter the rate of erythropoiesis, the most apparent of which is hypoxia.

SUMMARY AND CONCLUSIONS

A new method for the study of gastrointestinal absorption of iron in mice has been described. Phlebotomy, intravenous hemolysis, hypoxia and cobaltous ion increase iron absorption. Transfusion-induced polycythemia depresses iron absorption in mice. Iron-loading by means of Imferon also depresses iron absorption. Under the conditions described for these experiments, exogenous plasma erythropoietin has no direct effect on iron absorption.

*Unpublished data from our laboratory.

SUMMARIO E CONCLUSIONES IN INTERLINGUA

Es describe un nove methodo pro le studio del absorption gastrointestinal de ferro in muses. Phlebotomia, hemolyse intravenose, hypoxia e ion de cobalt augmenta le absorption de ferro. Polycythemia inducite per transfusiones deprime le absorption de ferro in muses. Le injection de un excesso de ferro per medio de ferro-dextrano (Imferon) etiam deprime le absorption de ferro. Sub le conditiones describe pro iste experimentos, le erythropietina ab plasma exogene ha nulle effecto directe super le absorption de ferro.

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