A Possible Humoral Regulator of Iron Absorption.* (28001)

DAVID S. FISCHER† AND DAVID C. PRICE‡ (Introduced by Eugene P. Cronkite)
Medical Research Center, Brookhaven National Laboratory, Upton, N. Y.

The classic theory of iron absorption suggested by Granick(1), has been under attack for several years(2). Recent studies have suggested that iron absorption is accelerated by many factors, including simulated high altitude hypoxia(3), active erythropoiesis and depletion of iron stores(4), but not anemia *per se*(5). In the normal human without anemia but with depleted iron stores, enhanced iron absorption has been demonstrated(6). It has been suggested that the effect may be mediated by a humoral mechanism(7), but an early attempt to demonstrate this in mice and rats was unsuccessful(8).

It seems reasonable that gastrointestinal absorption of iron may be mediated, at least in part, by such a humoral agent controlled by the body's content of storage iron. The present experiment was designed to retest this hypothesis, including the influence of body stores on iron absorption.

Materials and methods. 1. Experimental animals. A colony of 3-week-old caesarian delivered male Sprague-Dawley rats from the Charles River Breeding Laboratories was divided into 3 groups. Group I consisted of 21 rats kept in plastic cages and fed the Nutritional Biochemical Corp. "low iron" diet and distilled water *ad lib*. Group II consisted of 10 rats given the same diet, with an additional 22.7 mg of iron as ferrous ammonium sulfate per 100 g of diet (our normal laboratory rat diet contains 22.7 mg iron as ferric ammonium citrate). Group III consisted of 10 rats with 25 times this normal amount of iron added to their diet (570 mg iron/100 g diet). A second colony of adult female Sprague-Dawley rats from the same source was used as plasma recipients and as the controls for the plasma recipients.

2. Iron absorption was measured by giving 1 μc Fe^{59} as ferrous citrate with 200 μg carrier iron as ferrous sulfate to each rat through a stomach tube after a 24 hour fast. Each rat was counted in a whole body multiple plastic phosphor counter designed for this purpose at Brookhaven National Laboratory. The difference in rat Fe^{59} activity between one hour and 7 days after ingestion counted against a radioiron standard, was used to compute the percentage iron absorption after pilot studies had shown that counting was stable after 5 days.

$$\% \text{ Absorption} = \frac{\dfrac{\text{Activity day 7}}{\text{Standard day 7}}}{\dfrac{\text{Activity 1 hour}}{\text{Standard 1 hour}}} \times 100$$

3. After 8 weeks on the "low iron" diet, 14 animals from Group I were sacrificed and their heparinized plasma combined to form pools. These pools were used to inject 6 female rats of the second colony with 4-5 ml plasma each. The plasma pools had an average serum iron of 194 μg/100 ml and a total iron binding capacity (TIBC) of 694 μg/100 ml. Thirty minutes later, an iron absorption test was performed on these animals, on 13 female controls from the same colony, and on the 7 remaining rats from Group I.

4. At the time that the Group I, low iron rats used for this experiment were in apparent good health, all the rats in Group III and a few in Group II were retarded in growth and demonstrated patchy loss of large areas of fur. They were returned to the normal rat pellet diet for 2 weeks and seemed to return to normal. Then they were placed on a diet consisting of the normal pellets reduced to a powder with 22.7 mg and 570 mg of iron added to 100 g of diet as before for Groups II and III respectively. Thus, Group II was now receiving twice the normal iron ration, and Group III, 26 times as much as normal. At

* Research supported by U. S. Atomic Energy Commission.

† Present address: Dept. of Pharmacology, Yale Univ. Med. School, New Haven, Conn.

‡ Present address: Toronto General Hospital, Ontario, Canada.

TABLE I.

Date	Animal group	No. in group	% Fe^{59} absorption Range	Mean	IS. D.	IS. E.
5/ 8/62	Control	13	4.8-28.6	18.1	5.3	1.5
5/ 8/62	Plasma transfused	6	20.2-30.9	25.6	4.9	2.0
5/ 8/62	Iron deficient	7	36.9-65.4	50.1	9.7	3.7
6/ 7/62	Repeat control	11	6.6-17.9	13.4	3.7	1.1
6/ 7/62	Previously transfused	5	14.9-22.8	19.0.	3.4	1.5
6/14/62	Twice normal dietary iron	9	3.4-11.3	6.6	2.4	.8
6/14/62	26 times normal dietary iron	9	1.7- 5.7	3.5	1.6	.5
7/31/62	2nd repeat controls	5	2.7-10.5	6.3	2.7	1.6
	" " transfused	5	3.6- 9.9	6.0	1.7	.4

time of sacrifice, Groups II and III had an average serum iron of 399 and 359 μg/100 ml and a TIBC of 666 and 674 μg/100 ml respectively.

5. After 3 weeks on the latter increased iron diet, and 13 weeks after beginning the experiment, an iron absorption test was performed on Groups II and III.

6. Four weeks and 12 weeks after their first iron absorption test, the plasma transfused rats and their controls had a second and third iron absorption test.

Results. A summary of the absorption studies is given in Table I. The iron deficient rats had the greatest absorption, averaging 50.1%, and the iron heavy Group III rats the least, averaging 3.5%. The original control group had an average absorption of 18.1% and the plasma transfused group had 25.6% absorption. The difference between these 2 groups is significant at $P<0.05$. The Group II rats with twice the normal iron in their diet absorbed only 6.6%.

A repeat absorption test performed one month later showed that the controls absorbed 13.4% in contrast to 19.0% absorption of the previous plasma-transfused rats. The difference is still significant ($P<0.01$). A final absorption test, after an additional 8 weeks, showed no difference in absorption between the two groups.

Conclusions. Previous reports that iron absorption is directly related to the level of iron stores are confirmed. These non-definitive experiments strongly suggest the existence of a humoral agent influenced by the body's iron stores, which controls intestinal iron absorption. Previous reports indicate that the humoral agent is not erythropoietin (9). Although some studies have suggested that it is not unsaturated transferrin(2,10), there is some evidence to the contrary(11). The present studies do not resolve this question, which requires further investigation.

Summary. $Iron^{59}$ absorption was significantly increased in rats transfused with plasma from iron deficient rats. Rats fed a high iron diet absorbed decreased amounts of Fe^{59}. It is suggested that a humoral mechanism may reflect iron storage, and regulate, at least in part, gastrointestinal iron absorption.

1. Granick, S., *Bull. N. Y. Acad. Med.,* 1949, v25, 403.

2. Brown, E. B., Dubach, R., Moore, C. B., *J. Lab. & Clin. Med.,* 1958, v52, 335.

3. Reynafarje, C., Ramos, J., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 868.

4. Bothwell, T. H., Pirzio-Biroli, G., Finch, C. A., *J. Lab. & Clin. Med.,* 1958, v51, 24.

5. Pirzio-Biroli, G., Bothwell, T. H., Finch, C. A., *ibid.,* 1958, v51, 37.

6. Pirzio-Biroli, G., Finch, C. A., *ibid.,* 1960, v55, 216.

7. Bothwell, T. H., Finch, C. A., *Am. J. Dig. Dis.,* 1957, v2, 145.

8. Beutler, E., Buttenwieser, E., *J. Lab. & Clin. Med.,* 1960, v55, 274.

9. Mendel, G. A., *Blood,* 1961, v18, 727.

10. Yuile, C. L., Hayden, I. W., Bush, J. A., Tesluk, H., Stewart, W. B., *J. Exp. Med.,* 1950, v92, 367.

11. Hallberg, L., Solvell, L., Brise, H., *Proc. VIIth Internat. Cong. Hemat.,* v2, Sept. 7-13, 1958, Rome.

Received October 12, 1962. P.S.E.B.M., 1963, v112.