

The Absorption of Nonferrous Metals in Iron Deficiency*

SIMEON POLLACK, JAMES N. GEORGE, RICHARD C. REBA, RICHARD M. KAUFMAN,
AND WILLIAM H. CROSBY †

(From the Department of Hematology, Walter Reed Army Institute of Research,
Washington, D. C.)

Iron is unique among inorganic nutriment because primary control of body homeostasis resides with absorption, not with excretion (1). However, the pathway by which iron is transported in the gut is not necessarily unique; it may be shared with cations whose absorption waxes and wanes in parallel with that of iron, but whose total body balance is maintained by ready excretion. The question of whether the transport system by which iron is absorbed is truly unique initiated the present series of experiments.

Methods

The absorption of various cations in iron deficiency was tested in groups of Walter Reed Carworth Farms strain rats rendered iron deficient by bleeding or maintained on an iron-deficient diet.¹ The rats' weights ranged from 210 to 370 g. Control and experimental animals were chosen randomly from a homogeneous group for each experiment and were prepared as follows.

In one group of rats 3.5 ml of blood was removed each of 3 successive days by bleeding from the orbital plexus. Control rats and bled rats were both maintained on standard rat biscuit diets. Rats were fasted 24 hours before study. The experiment was conducted on the seventh day following the initial bleeding.

A second group of rats was maintained on an iron-deficient diet, compounded of casein and sucrose with vitamin, mineral, and fat supplements (Table I) for 13 days before study. Control rats were maintained on a diet of the identical formula except that 93.5 mg of $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ was added to each 100 g of the iron-deficient diet (2). The diet was served in porcelain dishes. The rats were fasted 1 day before study and dosed 14 days after beginning the iron-deficient diet.

The dosing solution consisted of distilled water containing 5 μmoles and approximately 1 μc of the cation

being tested in a total volume of 0.5 ml. Dosing solutions were freshly made. The isotopes and their salts studied were as follows: Cs^{137}Cl , $\text{Mg}^{28}\text{Cl}_2 \cdot 6 \text{H}_2\text{O}$, $\text{Mn}^{54}\text{Cl}_2 \cdot 4 \text{H}_2\text{O}$, $\text{Hg}^{203}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$, $\text{Fe}^{59}\text{Cl}_3$, $\text{Co}^{60}\text{Cl}_2 \cdot 6 \text{H}_2\text{O}$, $\text{Zn}^{65}\text{Cl}_2$, $\text{Ca}^{47}\text{Cl}_2$, and $\text{Cu}^{64}(\text{NO}_3)_2 \cdot 3 \text{H}_2\text{O}$. The isotopes were obtained commercially.² The carrier salts used were the same as the isotope salts. $\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$ was dissolved by the addition of HNO_3 . The FeCl_3 was reduced by the addition of 1.6 mg ascorbic acid to each 5 μmoles of ferric salt.

The dosing solution was given through a blunt needle passed down the esophagus with the rat under light ether anesthesia. The rat was then placed in a 1-quart, vented ice cream container and kept there for 6 hours. At the end of this time the boxed rat was killed with chloroform and counted in a large, well-type liquid scintillation counter.³ After this the rat's gastrointestinal tract was carefully removed from esophagus to rectum. The rat carcass was returned to its box and a second count was obtained. Percentage absorption of the isotope was computed by dividing the second count by the first. Absorption measured in this manner includes only counts remaining in the eviscerated carcass. Isotope absorbed

TABLE I
Iron-deficient diet for rats

	%
Sucrose	72.0
Casein	20.0
Salt mix	4.0
Wesson oil mix	3.0
Vitamin mix	1.0
Salt mix	
CaCO ₃	55.350
K ₂ PO ₄	21.750
KCl	11.400
NaCl	7.050
MgCO ₃	2.550
MgSO ₄ · 7 H ₂ O	1.650
NaF	0.100
CuSO ₄	0.090
MnSO ₄	0.035
AlKSO ₄	0.017
KI	0.008

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† Address requests for reprints to Chief, Dept. of Hematology, Walter Reed Army Institute of Research, Washington, D. C. 20012.

¹ The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

² Oak Ridge National Laboratories, Oak Ridge, Tenn.; Isoserve, Cambridge, Mass.

³ Armac counter, Packard Instrument Co., La Grange, Ill.

and returned to the gut does not contribute to the final count. Isotope absorbed and excreted in the urine remains in the counting box and is included in the final estimate of absorption.

The intestinal transit time of the various salts as used was tested with carmine red as a marker; in no instance was the dye found to have passed beyond the cecum at 6 hours.

Correction for decay was obviated by the negligible time lapse between first and second counts of each animal. The shortest lived of the isotopes used (Cu^{64} , $t_{1/2}$ 12.8 hours) was associated with a maximum of 2% decay in the interval between the first and second counts (10 minutes). Ca^{47} decay yields a low energy gamma-emitting isotope (Sc^{47}); the counts from Sc^{47} were excluded by adjusting the lower window of the scintillation counter.

Results and Discussion

The percentage of absorption of cesium, magnesium, mercury, zinc, calcium, manganese, cobalt, iron, and copper in normal rats and rats rendered iron deficient by bleeding is shown in Table II. Only the absorption of manganese, cobalt, and iron was increased in the iron-deficient rats. Bled animals absorbed almost twice as much manganese and almost three times as much cobalt as their normal counterparts. The absorption of iron was increased approximately fivefold.

It is apparent that the iron-deficient gut, which has an increased ability to absorb iron, does not undergo a nonspecific change rendering it more readily penetrable to all cations. It is also apparent that the percentage of absorption of the cations tested bears no simple relation to ionic size (3). In testing these cations we suspected

TABLE II
Absorption of 5 μmoles of cation

	Bled rats	Control rats	p*
	%		
Cs^{137}	74.6 \pm 0.9† (10)‡	74.2 \pm 1.3 (10)	NS
Mg^{28}	28.9 \pm 5.2 (10)	28.8 \pm 7.4 (10)	NS
Hg^{203}	6.8 \pm 1.0 (9)	6.2 \pm 1.3 (9)	NS
Zn^{65}	10.6 \pm 6.8 (9)	8.8 \pm 3.1 (10)	NS
	5.9 \pm 4.9 (8)	7.8 \pm 5.1 (12)	NS
Ca^{47}	44.0 \pm 18.6 (12)	39.9 \pm 12.1 (13)	NS
Cu^{64}	6.5 \pm 5.7 (13)	7.8 \pm 7.1 (15)	NS
Mn^{54}	4.7 \pm 2.5 (8)	2.5 \pm 1.4 (9)	<0.05
Co^{60}	28.7 \pm 11.3 (9)	10.4 \pm 3.0 (8)	<0.001
Fe^{59}	37.7 \pm 11.1 (3)	7.9 \pm 4.9 (9)	<0.001

* Statistical comparison is made by the two-tailed t test. NS means not significant at the 0.05 level.

† Mean \pm 1 SD.

‡ The numbers in parentheses are the number of rats in the group tested.

TABLE III
Absorption of 5 μmoles of cation

	Deficient diet rats	Control diet rats	p*
	%		
Cs^{137}	78.4 \pm 4.6† (12)‡	76.7 \pm 2.6 (11)	NS
Mg^{28}	45.2 \pm 8.1 (11)	42.1 \pm 5.3 (12)	NS
Hg^{203}	8.4 \pm 5.0 (12)	11.2 \pm 7.0 (12)	NS
Zn^{65}	20.6 \pm 5.9 (10)	14.5 \pm 6.7 (10)	<.05
Ca^{47}	34.3 \pm 7.2 (12)	35.0 \pm 7.7 (12)	NS
Cu^{64}	5.8 \pm 2.2 (15)	6.2 \pm 1.8 (14)	NS
Mn^{54}	5.7 \pm 2.6 (12)	3.5 \pm 1.9 (14)	<0.02
Co^{60}	34.8 \pm 7.8 (12)	18.4 \pm 5.8 (10)	<0.001
Fe^{59}	27.8 \pm 12.8 (12)	9.2 \pm 7.3 (11)	<0.001

* Statistical comparison is made by the two-tailed t test. NS means not significant at the 0.05 level.

† Mean \pm 1 SD.

‡ The numbers in parentheses are the number of rats in the group tested.

that copper and iron absorption might be linked because the copper content of the liver in hemochromatosis is markedly elevated (4), but copper absorption was not significantly increased in bled rats.

Bleeding depletes total body iron, but it does more than this, reducing total body protein and the various trace elements contained in whole blood as well. Consequently, there is no guarantee that bled animals behave as they do solely because of iron deficiency. However, iron absorption can be increased in rats by placing them on iron-deficient diets. In so doing there is no reason to suspect depletion of any nutriment except iron. The iron-deficient diet (Table I) we use causes rats to increase their iron absorption significantly after only 4 days and markedly after 14 days (2).

The experimental group of rats was maintained on this diet. The control group of rats was maintained on the identical diet except that iron was added. The absorption of the various cations tested is listed in Table III. After 2 weeks on the iron-deficient diet the rats absorbed twice as much cobalt and about half again as much manganese as their counterpart controls. In both the bleeding and feeding experiments the increased absorption of cobalt and manganese was statistically significant. The other cations' absorption was unchanged, with the exception of zinc, the absorption of which was increased in animals consuming the synthetic iron-deficient diet, but not in bled animals. The reason for this discrepancy is not apparent.

TABLE IV
Absorption of Co^{60} and Mn^{54} given with Fe

	%	p^*
Co^{60} (5 μ moles)	$21.2 \pm 10.7 \dagger$ (16)‡	
Co^{60} (5 μ moles) + iron (40 μ moles)	15.5 ± 6.5 (15)	$0.1 > p > 0.05$
Mn^{54} (5 μ moles)	2.0 ± 1.2 (10)	
Mn^{54} (5 μ moles) + iron (40 μ moles)	2.6 ± 2.1 (10)	$p < 0.5$

* Statistical comparison is made by the two-tailed t test.

† Mean \pm 1 SD.

‡ The numbers in parentheses are the number of rats in the group tested.

It is apparent that iron deficiency is associated with increased intestinal absorption of cobalt and manganese. This effect could derive from a shared pathway of transport. Competitive inhibition of cobalt and manganese absorption by iron was sought to support this supposition. It was not found in the dose range investigated (Table IV). These data are of limited significance, since competitive inhibition would not be demonstrable in any system till the system had been taxed to near its capacity (5). Since large doses of inorganic iron have gross toxic effects on the gastrointestinal tract (6), any effect on intestinal absorption by amounts of iron larger than those already used would be ambiguous. Cobalt and manganese in large doses are also toxic (7, 8). For these reasons further tests of competition between these cations were not made.

The chemistry of cobalt, manganese, and iron is similar. Their ionic radii are similar, and they tend to coordinate with six ligands and form octahedral complexes (3). These similarities are possibly responsible for their similar behavior during absorption from the intestine.

The biological significance of increased cobalt and manganese absorption when iron absorption is increased is uncertain. Hemochromatosis is a disease in which this overlap could be of importance. The cirrhosis of hemochromatosis has not been explained; large doses of parenteral iron given to animals do not lead to cirrhosis (9). Manganese or cobalt can produce cirrhosis when given parenterally (10–13), but the possibility of their involvement in the cirrhosis of hemochromatosis has received scant attention (4).

The present study adds to these other observations showing interrelationships between cobalt, manganese, and iron metabolism: the erythro-

poietic effect of cobalt (14), manganese incorporation into hemoglobin (15), the complementary relationship between manganese and iron in subcellular fractions of rat liver (16), and the interference of large amounts of dietary manganese with iron absorption (17).

Summary

1. The intestinal absorption of cobalt and manganese was increased in rats rendered iron deficient by bleeding and diet.
2. The intestinal absorption of cesium, magnesium, mercury, calcium, and copper was not increased in iron-deficient rats. Zinc absorption was increased in rats consuming an iron-deficient diet but was unchanged in bled rats.

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