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Iron, Lead, and Cobalt Absorption: Similarities and Dissimilarities (41025)[1]

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Abstract. Using isolated intestinal segments in rats, the absorption of iron, lead, and cobalt was increased in iron deficiency and decreased in iron loading. Similarly, the absorption of these metals was decreased in transfusional erythrocytosis, after intravenous iron injection and after parenteral endotoxin injection. Acute bleeding or abbreviated intervals of dietary iron deprivation resulted in increased iron absorption from isolated intestinal segments and in intact animals, while the absorption of lead and cobalt was unaffected. These results suggest that the specificity of the mucosal metal absorptive mechanism is either selectively enhanced for iron absorption by phlebotomy or brief periods of dietary iron deprivation, or that two or more mucosal pathways for iron absorption may exist.

Although the mechanism by which iron is absorbed is unknown, many factors have been identified which increase or decrease its absorption (1). Because there is evidence that other metals may share the intestinal absorptive pathway of iron, this investigation was undertaken to delineate the similarities and differences in the absorption of iron, lead, and cobalt.

Materials and Methods. Male albino rats of a pathogen-free Wistar strain (Southern Animal Farms, Inc., Prattville, Ala.) weighing 200 to 250 g at the time of absorption measurements were used in all experiments. The principles of laboratory animal care as promulgated by the National Research Council were observed. All animals were housed in polypropylene cages floored with stainless-steel grids to prevent pica for feces and absorbent bedding in a room provided with automatically controlled temperature and lighting. In experiments assessing the effects of iron deficiency, iron loading, and an iron-deficient diet, control rats were fed a powdered semisynthetic protein test diet (2) using components obtained from Teklad Test Diets, Madison, Wisconsin. The chow given to control and iron-loaded animals contained 85 mg/kg iron. Animals fed an iron-deficient diet received the same diet except that iron was not added to the components; it contained 2 mg Fe/kg diet. In all other experiments animals received standard pelleted rat food (Wayne Lab-Blox; Allied Mills, Inc., Chicago, Ill.). Calculated iron contents of all diets were verified by atomic absorption spectrometry (Perkin–Elmer Model 303; Perkin–Elmer, Norwalk, Conn.). Nutrients in the diets otherwise met or exceeded those levels proposed by the National Academy of Sciences (3). Demineralized, deionized water was used exclusively as a source of water in all experiments.

Iron, lead, and cobalt absorption studies were performed by measurement of total body radioactivity in a small animal whole-body liquid scintillation detector (Packard-ARMAC; Packard Instrument Co., Inc., Downers Grove, Ill.). Radioisotopes utilized were $^{59}Fe^{2+}$ chloride (sp act 21.9 mCi/mg of iron; New England Nuclear, Boston, Mass.), $^{210}Pb^{2+}$ nitrate (specific activity 10 mCi/mg of lead; Amersham Corp., Arlington Heights, Ill.) and $^{60}Co^{2+}$ chloride (sp act 75.5 mCi/mg of cobalt; New England Nuclear). All measurements of radioactivity were corrected for radiodecay and resolving time delay by comparison to an appropriate standard after subtraction of background radioactivity.

Absorption experiments were performed in rats fasted for 12 hr overnight from food but not water. Under intraperitoneal pen-

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tobarbital anesthesia (4 mg/100 g), the urethra was tied to prevent urinary loss of absorbed metal. A laparotomy was performed, the small intestine was isolated proximally and distally with umbilical tape, and the bile duct was ligated with silk suture. Although the presence of bile in the small intestine enhances both iron (4) and lead (5) absorption, the bile duct was ligated to prevent enterohepatic circulation of metal during the experiments. One milliliter of an aqueous test solution (pH 4.0) containing radiolabeled iron (1 mg Fe as $FeCl_2$ + 1 μCi ^{59}Fe), lead (1 μg Pb as $PbCl_2$ + 1 μCi ^{210}Pb), or cobalt (1 mg Co as $CoCl_2$ + 1 μCi ^{60}Co) was injected into the isolated small intestine. Injections of the above solutions were accomplished by entering the gut lumen proximal to the proximal ligature with a 21-gauge hypodermic needle, passing it intraluminally through the ligature loop, tightening the ligature, and then injecting the test dose into the isolated ligature. The abdomen was then closed with stainless-steel clips, and the rats were placed in vented 1-qt cardboard ice cream containers. Total body radioactivity was measured in a whole-body detector and compared to a 250-ml water-filled plastic bottle containing a test dose equal to that injected into the animals. Four hours after administration of the test dose, each animal was killed by cervical dislocation. Isolated intestinal segments were excised from the carcass, and whole-body radioactivity was again measured and compared to the original whole-body radioactivity. In some experiments, metal absorption and retention in intact rats were quantified over a 7-day interval using individual metabolic cages (EconoMetabolism Unit, Maryland Plastics, Inc., Federalsburg, Md.). Animals fasted overnight from food but not water were given a standard test dose of iron, lead, or cobalt as detailed above through an olive-tipped oroesophageal needle under light ether anesthesia. The rats were placed in individual metabolic cages and allowed to eat and drink *ad libitum*. Urine and stool were collected separately. After 7 days, daily stool radioactivity in the carcass was measured. In these studies absorption was expressed as the sum of the radioactivity in the carcass and urine as a fraction of the administered dose.

Bleeding of animals was accomplished by insertion of a heparinized microhematocrit tube into the retroorbital venous sinus on the medial surface of the orbit. Intravenous injections were administered through the dorsal vein of the penis.

Groups of 8 animals were used for each category except for lead-bleeding and lead-acetylphenlhydrazine (APH) studies in which groups of 32 rats were necessary to clarify equivocal results. Within individual experiments, animals in various groups did not vary significantly in weight. Results are expressed as the mean and standard error of the mean. Single statistical comparisons were made using Student's t test for unpaired data. A modification of Tukey's test of all comparisons among means was used for the statistical evaluation of experiments involving three or more treatment groups (6).

Results. Body iron status significantly affected the absorption of all metals (Table I). In iron-deficient rats, the absorption of iron, lead, and cobalt was significantly increased by comparison to control rats ($P < 0.05$, $P < 0.05$, $P < 0.05$, respectively). Iron loading significantly decreased iron, lead, and cobalt absorption ($P < 0.05$, $P < 0.05$, $P < 0.05$, respectively).

The effects of bleeding on metal absorption are shown in Table II. When tested using an isolated small intestinal loop, iron absorption was significantly increased on the third and fifth days after bleeding by comparison to unbled controls (6.1 ± 0.6 and 6.8 ± 0.3% vs 4.2 ± 4.0% controls, $P < 0.05$, $P < 0.05$, respectively). Because preliminary experiments suggested that a slight but nonsignificant increase in lead absorption occurred following bleeding, experimental and control groups of 32 rats each were studied. The differences in lead absorption remained nonsignificant at 3 and 5 days after bleeding ($P = 0.20$, $P = 0.30$). Cobalt absorption was unchanged following bleeding. Because isolated intestinal loop experiments did not show increases in lead or cobalt absorption which paralleled augmentation of iron absorption following

TABLE I. EFFECT OF BODY IRON STATUS ON METAL ABSORPTION[a]

	Iron	Lead	Cobalt
Iron deficiency, % absorption	9.5 ± 0.5	30.1 ± 1.2	76.2 ± 2.4
Microhematocrit, %	38.2 ± 0.3	37.3 ± 0.5	39.8 ± 0.9
Iron loading, % absorption	1.4 ± 0.2	11.3 ± 0.9	29.1 ± 2.6
Microhematocrit, %	45.2 ± 0.6	45.8 ± 0.4	46.1 ± 0.5
Control, % absorption	4.5 ± 0.3	17.5 ± 1.8	48.7 ± 3.3
Microhematocrit, %	46.1 ± 0.4	45.6 ± 0.7	47.5 ± 0.3

[a] Absorption was measured in rats 4 hr after the injection of a solution containing radiolabeled metal salt into the isolated small intestine. Iron-deficient animals were bled 3 ml and fed an iron-deficient diet for 2 weeks before the experiment. Other animals were kept on the same diet without addition of iron. Iron loading was accomplished by intramuscular injection of 50 mg of iron as iron dextran 2 weeks prior to the absorption studies.

bleeding, additional studies were performed. Absorption of the metals was quantified in bled animals and in unbled animals and in unbled control animals using individual metabolic cages. Again, a significant increase in iron absorption was noted in rats dosed 5 days after bleeding (14.0 ± 2.4 vs 7.3 ± 0.6% control, $P < 0.05$). No significant increases in lead or cobalt absorption were noted under similar circumstances.

The effects of an iron-deficient diet on metal absorption are displayed in Table III. Iron absorption was significantly increased 5 days after the feeding of an iron-deficient diet (8.1 ± 1.1 vs 4.2 ± 0.4% control, $P < 0.05$). Lesser increases in iron absorption were observed among rats fed the diet for shorter periods of time. Lead and cobalt absorption, however, were not affected by this dietary treatment.

Treatment with intraperitoneal APH resulted in moderate anemia (Table IV). This was associated with a significant increase in iron absorption from isolated intestinal loops (6.3 ± 0.2 vs 4.3 ± 0.5% control, $P < 0.01$). Because of equivocal results in preliminary studies assessing the effects of APH on lead absorption, experimental and control groups of 32 rats each were used. A

TABLE II. EFFECTS OF BLEEDING ON METAL ABSORPTION[a]

Days after bleeding	Control (not bled)	1	3	5
Iron				
Intestinal loop, % absorption	4.2 ± 0.4	5.6 ± 0.6	6.1 ± 0.6	6.8 ± 0.3
Microhematocrit, %	44.2 ± 0.5	34.0 ± 1.0	38.2 ± 0.7	41.8 ± 0.6
Metabolic cage, % absorption	7.3 ± 0.6	7.1 ± 1.2	11.8 ± 1.4	14.0 ± 2.4
Microhematocrit, %	44.6 ± 0.4	33.7 ± 0.9	37.8 ± 0.7	40.9 ± 0.8
Lead				
Intestinal loop, % absorption	15.7 ± 1.2	16.9 ± 1.4	17.8 ± 1.3	17.0 ± 1.4
Microhematocrit, %	43.8 ± 0.6	33.1 ± 0.9	37.6 ± 0.4	41.4 ± 0.7
Metabolic cage, % absorption	10.3 ± 0.9	10.1 ± 1.3	10.5 ± 0.8	10.0 ± 1.1
Microhematocrit, %	44.2 ± 0.5	33.5 ± 0.8	37.4 ± 0.8	41.1 ± 0.6
Cobalt				
Intestinal loop, absorption	55.8 ± 1.8	53.8 ± 1.6	56.7 ± 4.0	56.8 ± 1.7
Microhematocrit, %	46.2 ± 0.6	35.3 ± 0.7	38.3 ± 0.5	42.3 ± 0.5
Metabolic cage, % absorption	20.0 ± 1.9	21.1 ± 1.0	21.6 ± 2.4	23.5 ± 1.5
Microhematocrit, %	44.5 ± 0.6	33.0 ± 1.0	36.9 ± 1.2	40.1 ± 0.6

[a] Absorption of radiolabelled metal at intervals after blood loss of 3 ml is shown above and compared to unbled animals. In half of the animals, absorption was measured from the small intestine 4 hr after injection of the test dose. In the remainder, absorption was measured 7 days after an oral dose. The latter animals were kept in metabolic cages for total urine collection. In these animals absorption was calculated from the sum of the radioactivity in the carcass plus that which was excreted in urine.

TABLE III. EFFECT OF IRON-DEFICIENT DIET ON METAL ABSORPTION[a]

Days of diet	0	1	2	3	5
Iron absorption, %	4.2 ± 0.4	5.7 ± 0.6	5.9 ± 0.1	6.2 ± 0.5	8.1 ± 1.1
Lead absorption, %	16.4 ± 1.1	17.0 ± 1.7	16.0 ± 1.5	16.3 ± 1.3	17.1 ± 1.3
Cobalt absorption, %	50.5 ± 3.9	54.2 ± 2.6	55.3 ± 3.3	52.5 ± 2.6	53.9 ± 3.2

[a] Absorption of radiolabelled metallic salts was measured in rats at intervals after initiation of an iron-deficient diet. Absorption from isolated small intestine was measured 4 hr after injection of the radioactive solution into the small intestine.

small increase in lead absorption produced by APH was documented (28.9 ± 2.0 vs 22.6 ± 1.8% control, $P < 0.05$). No increase in cobalt absorption occurred following APH infections. Intraperitoneal transfusion resulted in a significant increase in the microhematocrits of all animals studied by comparison to controls (Table IV). Absorption of iron, lead, and cobalt was significantly decreased by the transfusion maneuver. Intravenous iron given 24 hr prior to metal absorption tests significantly diminished iron, lead, and cobalt absorption ($P < 0.0001$, $P < 0.01$, $P < 0.001$, respectively) (Table V). Similarly, the intraperitoneal injection of endotoxin 18 hr prior to quantification of iron, lead, and cobalt absorption reduced absorption of the metals by comparison to control rats given saline ($P < 0.005$, $P < 0.01$, $P < 0.05$, respectively) (Table V).

Discussion. Changes in iron absorption have been related to alterations in the body iron content and to the rate of erythropoiesis (1). The quantity of iron absorbed is inversely proportional to the amount of iron in body stores such that increased iron absorption occurs in iron deficiency and diminished absorption of iron is observed with iron overloading. In addition, iron absorption is stimulated by bleeding or acute hemolysis and diminished when erythropoiesis is inhibited by hypertransfusion, starvation, descent from high altitudes and the administration of endotoxin (7–11). These factors seem to initiate an unidentified signal to the gut which alters either the quantity of iron or its chemical form in the intestinal mucosa to increase or decrease iron absorption (12–13). Changes in the diet seem to be an additional controlling factor because the ingestion of an

TABLE IV. EFFECT OF APH HEMOLYSIS AND TRANSFUSION ERYTHROCYTOSIS ON METAL ABSORPTION[a]

	Iron	Lead	Cobalt
APH, % absorption	6.3 ± 0.2	28.9 ± 2.0	46.4 ± 3.8
Microhematocrit, %	31.3 ± 0.6	29.2 ± 0.8	30.1 ± 0.7
Control, % absorption	4.3 ± 0.5	22.6 ± 1.8	47.5 ± 3.5
Microhematocrit, %	45.2 ± 0.7	44.3 ± 0.6	44.8 ± 1.0
Transfusion, % absorption	1.9 ± 0.2	15.9 ± 1.8	29.0 ± 1.9
Microhematocrit, %	61.3 ± 1.7	60.8 ± 3.6	59.9 ± 1.1
Control, % absorption	6.1 ± 0.4	21.0 ± 2.7	49.8 ± 3.5
Microhematocrit, %	45.2 ± 0.3	44.1 ± 2.0	45.3 ± 0.5

[a] The percentage of a test dose of a radiolabelled metal detected in the carcass of rats 4 hr after injection into the isolated small intestine is reported above. Hemolysis was induced in one group of animals by daily intraperitoneal injections of acetylphenylhydrazine (2 mg/100 g/d in 1 ml of 0.9% NaCl) 5 days prior to measurement of absorption. Another group of rats received two transfusions of rat erythrocytes and absorption was measured 1 week later. Each intraperitoneal transfusion consisted of 4 ml/100 g of 70% erythrocytes suspended in 0.9% NaCl, pH 7.4. The donor blood was collected under sterile conditions from other rats. Erythrocytes were washed three times with 0.9% sterile saline and the buffy coat was removed prior to transfusion. The nontransfused control animals for the transfusion experiment received an amount of iron as iron–dextran equivalent to the transfused hemoglobin iron as an intramuscular injection (about 6.3 mg iron).

TABLE V. EFFECTS OF INTRAVENOUS IRON AND PARENTERAL ENDOTOXIN ON METAL ABSORPTION[a]

	Iron	Lead	Cobalt
Intravenous iron treatment, % absorption	2.6 ± 0.3	10.9 ± 1.7	23.1 ± 1.5
Control, % absorption	4.5 ± 0.2	22.2 ± 0.9	40.5 ± 3.8
Endotoxin treatment, % absorption	2.6 ± 0.2	14.5 ± 1.9	39.2 ± 2.8
Control, % absorption	5.9 ± 0.7	22.6 ± 1.3	47.8 ± 2.5

[a] Absorption of radiolabelled metallic salts was measured from isolated small intestine 4 hr after injection of the solution into the small intestine. One group of animals were injected intravenously with 1 mg of iron as $FeCl_2$ in 0.5 ml of 0.9% NaCl (pH 7.4) 24 hr prior to absorption studies. Another group of animals were given 0.04 mg/100 g of endotoxin (*Escherichia* coli Serotype 0127B8, phenol extract Lot 128C-0099, Sigma Chemical Co., St. Louis, Mo.) as an intraperitoneal injection 18 hr prior to measurements of absorption. Control animals received injections of 0.9% NaCl in each experiment. The intervals which were selected for study were chosen because maximal suppression of iron absorption following infusion of iron and injection of endotoxin are observed at that time (11, 12).

iron-deficient diet for a few days causes significant increase in iron absorption that is out of proportion to changes in body stores and unassociated with alterations in iron kinetic studies (14). It is postulated that an iron-deficient diet results in a depletion of iron in the intestinal mucosa and that this may be responsible for the increase in iron absorption.

Certain nonferrous metals seem to share the absorptive pathway with iron. Iron deficiency produced by the combination of phlebotomy and an iron-deficient diet results in the increased absorption of cobalt, nickel, manganese, zinc, cadmium, and lead (15–19). Contrariwise, iron loading is associated with decreased absorption of cobalt, manganese, and lead (19–21). The addition of iron to test doses of these metals diminishes their absorption probably by competing for shared mucosal receptors (19). It is unlikely that this is a nonspecific change rendering the gut more or less penetrable to cations because the absorption of other metals remains unchanged and there seems to be no single relationship to their ionic size (15). While portions of the absorptive pathway are shared, unshared portions also seem to exist. Zinc absorption is increased in rats consuming an iron-deficient diet but is unchanged in bled animals (15, 19). Intestinal mucosal ferritin seems to bind iron in a relatively specific manner but does not bind appreciable quantities of other metals (20). In this study we investigated factors known to affect iron absorption to ascertain whether they also affected the absorption of cobalt and lead. It is postulated that the similarities and differences can be exploited to provide a more basic understanding of the absorption of metal cations.

Iron deficiency produced by the combination of phlebotomy and iron-deficient diet resulted in increased absorption of iron as well as lead and cobalt. Contrariwise, iron loading was associated with decreased absorptions of these metals. These data confirm observations in previous studies which postulate that the absorptive pathway for iron is shared by other metals (15–19). Other treatments such as hypertransfusion, endotoxin administration, and intravenous injection of iron which are known to decrease iron absorption (7–11) similarly affect the absorption of lead and cobalt. APH-induced hemolysis produced increased iron absorption in rats (22) but large numbers of animals were needed to show a similar effect upon lead absorption. Hemolysis produced no significant increase in cobalt absorption. Further, phlebotomy or the consumption of an iron-deficient diet enhanced the absorption of iron (7, 14) but had no significant effect upon the absorption of cobalt or lead.

These data suggest that acute bleeding, abbreviated intervals of dietary iron deprivation and perhaps APH-induced hemolysis exert an effect which is specific for iron absorption whereas the other factors studied were less selective. An explanation for these differences is that there are two absorptive pathways for iron: one

which is shared with certain other metal cations and another which is not. Exploitation of these differences using a combination of biochemical and radioisotopic techniques could prove useful in delineating the biologic role of the metal-binding proteins which have been identified in intestinal mucosa and probably act to regulate the absorption of iron and other metal cations (1, 19).

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