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## The cadmium effect on iron absorption<sup>1-3</sup>

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**ABSTRACT** Test solutions of cadmium and labeled iron salts, soluble complexes of diferric transferrin, or hemoglobin iron were introduced orally or were injected into tied-off jejunal segments in rat. Cadmium reduced the absorption of iron salts to about half in both normal and iron-deficient rats. Hemoglobin iron absorption was enhanced, indicating that the processing of this form of iron and its release from mucosa to blood was intact. A greater reduction in iron absorption occurred in iron-deficient rats when transferrin iron was injected into gut loops. Mucosal radioiron content in animals given cadmium with either iron salts or transferrin iron was increased. The primary effect of cadmium was on intracellular processing of iron salts and transferrin iron. The major portion of cadmium taken up by the mucosa of normal animals was bound to ferritin, and the effect of cadmium within the mucosal cell may be reduced thus. *Am J Clin Nutr* 1987;45:1007-12.

**KEY WORDS** Rat, cadmium toxicity, iron absorption

### Introduction

Cadmium was reported in 1941 to cause anemia in rats (1, 2) and anemia was subsequently found in humans after cadmium exposure (3-5). The level of blood cadmium in workers exposed to cadmium dust was directly related to the degree of anemia (5). The cadmium-induced anemia had characteristics of iron deficiency with microcytosis and hypochromia of red cells, increase in circulating transferrin, and low-transferrin saturation (6-10). Further documentation was provided by the demonstration of a hemoglobin response to parenterally administered iron (7, 11). Since cadmium appeared to create iron deficiency, attention shifted to the relation between cadmium and iron in the intestinal tract. In growing rats and Japanese quail, decrease of blood hemoglobin and liver iron associated with cadmium could be reversed by added dietary iron (11-18). On the other hand, cadmium absorption was shown to be increased with iron deficiency in several animal species and in man (7, 16, 19, 20). Thus, not only did cadmium interfere with iron absorption, but its own absorption and therefore its toxicity appeared to be inversely related to the iron status of the individual (16, 20-24). This study was undertaken in an attempt to clarify the molecular events responsible for the absorptive behavior.

### Materials and methods

#### Isotopes and chemicals

Radioiron isotopes (<sup>55</sup>Fe and <sup>59</sup>Fe) were purchased from New England Nuclear (Boston, MA) as ferrous sulfate, specific activity 13-22  $\mu\text{Ci}/\mu\text{g}$  of iron, dissolved in 0.5 M HCl. Radioiodine (<sup>125</sup>I) for protein iodination (Amersham Corp, Arlington Heights, IL) had a specific activity 13-17  $\mu\text{Ci}/\mu\text{g}$  of iodine. Ferrous ammonium sulfate,  $(\text{NH}_4)_2(\text{FeSO}_4) \times 6 \text{H}_2\text{O}$ , and ferric ammonium sulfate,  $(\text{NH}_4)_2(\text{FeSO}_4) \times 12 \text{H}_2\text{O}$ , were purchased from Bio-Chemical Company (Phillipsburg, NY). Human albumin was obtained from Sigma Chemical Company (St. Louis, MO). Rat plasma transferrin was isolated from iron-saturated rat plasma as described elsewhere (24, 25). The purity of the diferric preparation was confirmed by an absorbance ratio of A465:A280 of 0.045 (25). The conversion of diferric transferrin to apotransferrin was achieved by treatment with desferrioxamine at pH 5 (26). Homogeneous labeling of the two binding sites with <sup>59</sup>Fe was achieved by adding <sup>59</sup>Fe-tagged ferrous ammonium sulfate (273  $\mu\text{g}$  Fe/mL, pH 2.0) to apotransferrin (20 mg/mL, pH 8.3, 0.05 M bicarbonate) to the point of saturation under spectrophotometric control (27, 28). <sup>125</sup>I labeling was carried

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out on purified diferric transferrin according to the procedure of McFarlane (29) followed by extensive dialysis against buffered saline.

#### Animals

Male Wistar rats weighing 180–200 g were studied. The National Research Council's guide for the care and use of laboratory animals was followed. Normal rats were fed regular diet (F-6 Rodent Blox, Wayne Pet Food Division, Continental Grain Co, Chicago, IL) that contained 380 mg of iron/kg. In order to produce iron deficiency, rats at age 4 wk were placed on a low-iron diet (8 mg/kg) and 1.5 mL of blood was periodically bled to accelerate iron depletion (30). The rats were studied 18 h after the 4th bleeding after an overnight fast. Mean values for hematocrit, plasma iron, and total-iron binding capacity in normal rats were  $42 \pm 6\%$ ,  $180 \pm 58$ , and  $510 \pm 40 \mu\text{g/dL}$  plasma, respectively. The corresponding values for iron-deficient animals were  $20 \pm 2\%$ ,  $43 \pm 16$ , and  $810 \pm 16 \mu\text{g/dL}$  plasma, respectively.

#### Absorptive studies

Test solutions of cadmium and  $^{59}\text{Fe}$  were administered by gastric intubation and absorption of the radioiron, which has been shown to accurately reflect carcass activity (30), was determined at day 5 from the combined activity of blood, skeleton, liver, spleen, and kidneys.

A standardized gut loop technique was also used to measure absorption (31). Animals were anesthetized with ether and the abdomen was opened by a 3–4 cm cut along the linear alba. A 20 cm segment of upper jejunum was isolated. The proximal end was ligated first and the distal end was ligated after a 1.5 mL test solution was introduced. Either ionized iron at pH 2 or radioiron bound to transferrin at pH 7 with or without cadmium was introduced. Isotope dosages employed in the different studies included  $0.1\text{--}1 \mu\text{Ci } ^{59}\text{Fe}$ ,  $0.6\text{--}2 \mu\text{Ci } ^{55}\text{Fe}$ ,  $1\text{--}2 \mu\text{Ci } ^{109}\text{Cd}$ , and  $0.2\text{--}2 \mu\text{Ci } ^{125}\text{I}$ . The amount of carrier iron was  $12.8\text{--}44.7 \mu\text{g}$  and the amount of cadmium was  $25\text{--}50 \mu\text{g}$ . The composition of the individual solutions is given with the tables. Metal salts were injected at a pH of 2 and albumin and transferrin iron complexes containing  $9\text{--}32 \text{ mg}$  of protein were adjusted to a pH of 7 with  $1 \text{ M}$  bicarbonate solution. After materials were introduced into the gut loop, the wound was closed by clips and the animal was kept in a temperature-controlled environment until being killed 60 min later.

At the time of death, 7–8 mL blood was removed through the abdominal aorta. Blood samples were placed on ice and centrifuged at  $4^\circ\text{C}$ . Meanwhile, the animal was perfused with 2–3 blood volumes of 0.9% saline warmed to  $37^\circ\text{C}$ . Total activity of the blood, perfusate, and carcass exclusive of the gut was measured. The gut loop was washed and then counted. Results were expressed as 1) gut uptake, ie, activity in the washed gut loop segment, 2) carcass uptake, ie, radioactivity in the carcass including blood after gut was removed, and 3) total activity, ie, activity of washed-gut segment and carcass. Absorption was considered identical with carcass activity (30).

#### Mucosal analyses

The gut loop, removed from the animal, was opened without delay and perfused with 50 mL ice-cold saline.

$^{59}\text{Fe}$  and  $^{125}\text{I}$  radioactivity of the washed gut loop was determined and the mucosa was scraped with a glass slide. After addition of 5 parts 0.9% saline, a mucosal homogenate was prepared using a glass-terflon homogenizer. From this homogenate a nonparticulate fraction was obtained by centrifugation at  $100\,000 \times g$  for 1 h (Spinco L50, Beckman Instruments, Palo Alto, CA). The distribution of the radionuclides between the particulate and nonparticulate fraction was determined. Analysis of the  $^{125}\text{I}$  and  $^{59}\text{Fe}$ -tagged components in the nonparticulate fraction was done using chromatography on LKB-ultragel AcA 34 (column size  $5 \times 65 \text{ cm}$ ), LKB Instruments, Gaithersburg, MD), equilibrated with  $1 \text{ mM}$  phosphate buffered saline (pH 7.4). The column was eluted with the same buffer at  $60 \text{ mL/h}$  and fractions of  $20 \text{ mL}$  were collected. UV absorption in the eluate was monitored at  $280 \text{ nm}$  using a UV II detector unit (Pharmacia, Piscataway, NJ).

#### Measurements

Carcass activity was measured in a small-animal counter.  $^{59}\text{Fe}$  and  $^{109}\text{Cd}$  activities of tissue aliquots were measured independently in a gamma counter. In some studies,  $^{59}\text{Fe}$  and  $^{125}\text{I}$  were measured simultaneously with a scintillation spectrometer (Packard, Model 5330, Packard Instrument Co, Inc, Downers Grove, IL) with correction (of  $\sim 12\%$ ) for the  $^{59}\text{Fe}$  cross-count into the  $^{125}\text{I}$  channel (30). In other studies,  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  were measured simultaneously (32). Such samples were prepared for counting by the method of Eakins and Brown (33) and were counted in a Packard Model 4530 Tri-Carb liquid scintillation spectrometer (Packard Instruments Co, Inc, Downers Grove, IL). Appropriate corrections were made for cross-counting. Plasma iron determination was carried out as described by the International Standardization Committee (34). Total iron-binding capacity was determined by the method of Cook et al (35). Hematocrits were determined by standard microhematocrit technique. Protein determinations were carried out using the Biorad protein assay kit (Biorad Laboratories, Richmond, CA) with bovine plasma gamma globulin and/or bovine serum albumin (BSA) as standards.

Data were analyzed using "Student's"  $t$  tests. Significance was assumed at  $p < 0.05$ . Variations of multiple determinations were expressed as  $\pm 1 \text{ SD}$  when not otherwise indicated.

#### Results

Absorption studies were carried out over a 5-d period by use of either iron salts or hemoglobin administered orally by gastric tube (Table 1). At that time most of the activity of radioiron was in the carcass. While equal molar amounts of cadmium had little effect on iron absorption (data not shown), the administration of a 2-fold molar excess of cadmium given by stomach tube was sufficient to reduce absorption to about half that observed in control animals. On the other hand, hemoglobin iron showed a greater absorption ( $p \leq 0.05$ )

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TABLE 1  
Cadmium effect on absorption of radioiron at 5 d by iron-deficient rats\*

Luminal contents	Uptake (% of administered dose)		
	Total	Gut	Carcass
Fe <sup>2+</sup>	77.5 ± 22.8	3.2 ± 0.1	74.3 ± 22.0
Fe <sup>2+</sup> + Cd	44.9 ± 5.4†	2.8 ± 0.1	42.1 ± 4.7†
Hb Fe	18.6 ± 10.5	2.0 ± 1.3	16.6 ± 9.4
Hb Fe + Cd	37.9 ± 12.5	5.8 ± 1.4†	32.1 ± 12.8†

\* Rats were given 100 µg iron as ferrous ammonium sulfate or 60 µg iron as hemoglobin by gastric intubation. The cadmium dose of 150 µg Cd<sup>2+</sup> was given by stomach tube 90 min before the <sup>59</sup>Fe test dose. Results on groups of six animals were expressed as percent of administered <sup>59</sup>Fe.

† p < 0.05.

when given in combination with cadmium than when given without cadmium. Iron-deficient animals were used in this study since absorption of hemoglobin iron in normal animals is negligible (36).

In order for the mucosal events to be observed in more detail, cadmium and iron were introduced into jejunal loops of normal and iron-deficient animals as summarized in Table 2. When ferrous and ferric ammonium sulfate (pH 2) were injected into the gut lumen with cadmium, absorption in both normal and iron-deficient animals was reduced to approximately half. Although mucosal iron content was increased, this may have in part represented adsorption. A further study was carried out employing diferric transferrin, which would be unlikely to be adsorbed and which is known to be taken up and processed by the

mucosal cell. In this instance total uptake in the presence of cadmium was somewhat decreased in normal rats and absorption was again decreased. More conspicuous was the effect of cadmium on transferrin iron absorption in iron-deficient rats with a reduction to 3.8% from the 39.9% value of controls.

The distribution of <sup>125</sup>I-tagged transferrin and <sup>59</sup>Fe iron in the mucosa of normal and iron-deficient animals was then examined (Table 3). In normal cadmium-treated animals at 60 min, both radioiron and transferrin content of the gut were increased over control animals, the latter more than the former. In iron-deficient animals, there was little change at 15 min, but at 60 min the same phenomenon occurred and was enhanced by administration of cadmium prior to the injection of iron.

The distribution of <sup>109</sup>Cd in normal and iron-deficient rat mucosa is shown in Figure 1. A major portion of the radiocadmium was localized in the ferritin fraction of the normal animals mucosa, but in the iron-deficient animal little or no ferritin localization was seen.

The distribution of radioiron within the mucosa was examined by fractionation and chromatography. The pooled particle fraction of the mucosal homogenate of six normal animals contained 66% of the mucosal <sup>59</sup>Fe in the particle-free fraction. The amount was the same (63%) in the mucosal fraction of five normal animals treated with cadmium. In five iron-deficient animals, the respective values were 43 ± 5 and 61 ± 6 (p < 0.05). The corresponding <sup>125</sup>I transferrin activity in the supernate was 85% ± 5 in both normal and iron-deficient animals.

TABLE 2  
Effect of cadmium on iron absorption from a gut loop\*

Luminal contents	Uptake in normal rats			Uptake in iron-deficient rats		
	Total	Gut	Carcass	Total	Gut	Carcass
Fe <sup>3+</sup>	35.7 ± 16.6	22.2 ± 15.8	13.5 ± 8.1	50.6 ± 17.4	21.5 ± 13.9	29.1 ± 10.6
Fe <sup>3+</sup> + Cd	54.8 ± 11.6†	48.5 ± 23.1†	6.2 ± 3.4†	66.2 ± 7.9	54.8 ± 8.6	14.4 ± 3.6†
Fe <sup>2+</sup>	52.3 ± 24.8	32.1 ± 19.0	20.2 ± 6.3	77.4 ± 12.7	22.1 ± 6.1	55.3 ± 7.2
Fe <sup>2+</sup> + Cd	46.3 ± 8.3	34.6 ± 8.8	11.7 ± 2.3†	65.4 ± 9.8	30.4 ± 7.1	35.0 ± 6.9†
Fe <sup>3+</sup> Tf	26.1 ± 11.1	11.3 ± 6.0	14.8 ± 6.4	50.5 ± 23.7	10.6 ± 4.0	39.9 ± 20.6
Fe <sup>3+</sup> Tf + Cd	22.8 ± 4.8	14.1 ± 4.2	8.7 ± 3.1	12.9 ± 3.6†	9.2 ± 3.0	3.8 ± 1.2†

\* Absorption expressed as percentage of administered dose. Cd dose 50 µg; Fe dose 45 µg. Mean values ± 1 SD of groups of six animals except for Fe<sup>3+</sup>Tf where eight normal animals and 16 iron-deficient animals were studied. Tf is rat diferric transferrin.

† Significant difference between control and cadmium-treated rats (p ≤ 0.05).

TABLE 3  
Effect of cadmium on absorption of  $^{59}\text{Fe}$  and mucosal uptake of  $^{125}\text{I}$  transferrin from a gut loop\*

	Luminal contents	Radioiron Uptake (% of administered dose)			Transferrin Uptake (% of administered dose) Gut	
		Total	Gut	Carcass		
1. Normal (Absorption at 60 min)	FeTf	33.0 ± 9.5	15.5 ± 5.8	17.5 ± 5.4	5.2 ± 3.6	
	FeTf + Cd	28.4 ± 9.3	19.8 ± 4.2	8.6 ± 7.3	10.9 ± 4.3	
2. Iron deficient (Absorption at 15 min)	FeTf	25.0 ± 5.6	11.9 ± 2.3	13.1 ± 2.5	2.0 ± 0.7	
	FeTf + Cd	17.6 ± 8.8	9.0 ± 2.9	8.6 ± 6.4	1.6 ± 0.9	
	(Absorption at 60 min)	FeTf	52.4 ± 25.7	11.4 ± 3.8	41.0 ± 22.4	2.6 ± 1.6
		FeTf + Cd	31.7 ± 12.9†	15.4 ± 4.2	16.2 ± 8.9‡	8.8 ± 2.4‡
	FeTf + Cd†	28.7 ± 20.9	17.8 ± 7.0	10.4 ± 5.9‡	10.4 ± 5.9‡	

\* The dose of transferrin was 32 mg, of iron 45  $\mu\text{g}$ , and of cadmium 25  $\mu\text{g}$ . Transferrin iron and cadmium were introduced into the gut loop at the same time.

† In this study 50  $\mu\text{g}$  of cadmium dissolved in 1 mL of 0.01 N HCl was given by stomach tube 90 min before the gut loop was isolated and the dose of iron transferrin introduced.

‡  $p < 0.05$ .

Chromatography of the supernate from normal animals revealed two peaks of radioiron, the first representing mucosal ferritin and the second representing transferrin (Fig 2). In cadmium-treated animals, radioiron in mucosal ferritin was reduced with no significant change in the activity in the transferrin frac-

tion. The  $^{125}\text{I}$  profile showed two peaks, one in the position of the intact  $^{125}\text{I}$  transferrin area and the other representing a smaller molecular fraction, possibly transferrin degradation products. In both normal and iron-deficient animals, the proportion of transferrin and transferrin iron was increased.

## Discussion

There are two aspects of cadmium toxicity, both of which are well documented clinically. One is the interference with iron absorption by cadmium, the other is the effect of iron status on cadmium toxicity. The former is illustrated by the development of hypochromic microcytic anemia as a result of cadmium exposure. The latter is best portrayed by the illness, ITAI-ITAI (bone lesions and anemia), described in Japan in individuals drinking water of high-cadmium content (5). Interactions between iron and cadmium appear to be implicated in both these effects.

In these studies the depressing effect of cadmium on iron absorption was demonstrated when given by mouth and when introduced into jejunal loops with an intact blood supply. The latter technique permitted standardization of luminal conditions and differentiated between mucosal uptake and transfer into the body with a precision not usually achieved when iron is administered by mouth (30, 37). Initial measurements with ferrous and ferric salts in normal rats exposed to cadmium showed an initial increase in mucosal uptake.

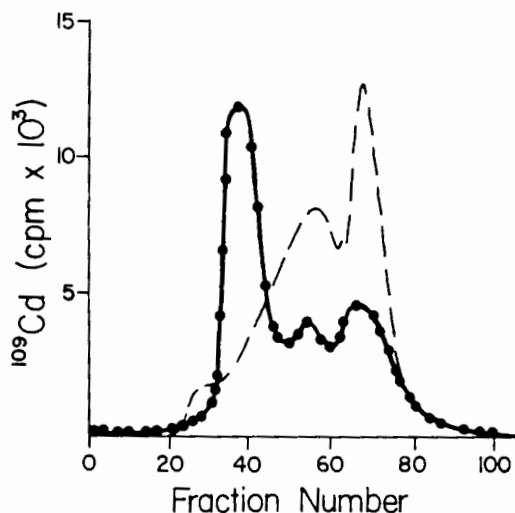


FIG 1. Gel chromatographic profile of the mucosal supernate obtained 1 h after the injection of a mixture of 50  $\mu\text{g}$   $\text{CdCl}_2$  and 45  $\mu\text{g}$   $\text{FeCl}_3$ -albumin. The elution position of mucosal ferritin was at about fraction number 40, that of albumin 55, and that of metallothionein 70. These three peaks are clearly visible in the normal control treated with cadmium (solid line). The dotted line indicates the corresponding profile in iron deficient animals where the ferritin peak is virtually absent.

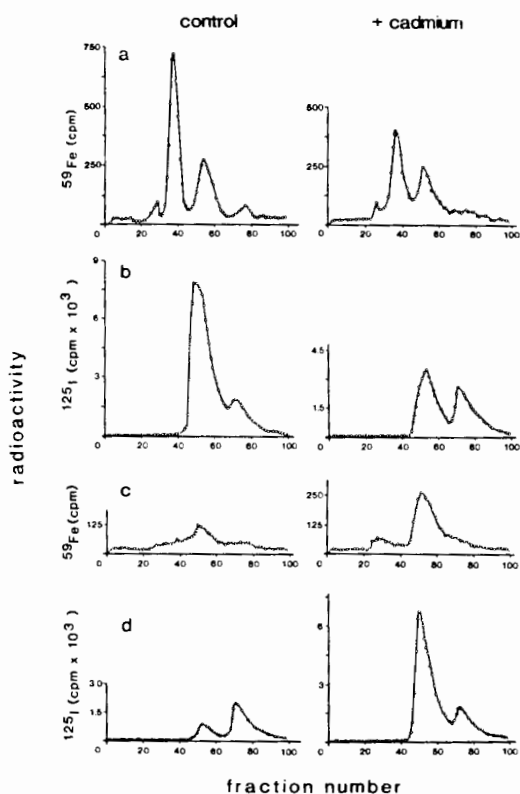


FIG 2. Gel chromatographic separation of  $^{59}\text{Fe}$ ,  $^{125}\text{I}$ -tagged transferrin in the non-particulate mucosal fraction on AcA34. Control profiles are shown on the left side and cadmium treatment on the right. (a) shows the iron profiles of normal rats with and without cadmium. The first large peak (about fraction number 38) is ferritin while the second peak at about 55 is transferrin. (b) shows the distribution of the  $^{125}\text{I}$  transferrin tag, which is reduced with cadmium treatment. The second peak at about 74 is presumed to represent degraded transferrin. (c) shows the iron profile in iron deficient rats where there is virtually no ferritin iron. (d) shows the corresponding  $^{125}\text{I}$  transferrin profile.

particularly of ferric iron, which may have been due to adsorption to the mucosal surface. To circumvent this possibility, transferrin-bound iron was employed for subsequent studies. Transferrin is believed to provide an auxiliary pathway of nonheme iron absorption (30). The transferrin-iron complex within the lumen is taken up by the mucosal cell, iron is released, and transferrin is returned to the gut lumen. When introduced into gut loops in the rat, transferrin iron is absorbed almost as well as is ferrous sulfate. The mucosal content of transferrin iron increased in the presence of cadmium, but absorption (carcass uptake) was significantly depressed ( $p < 0.05$ ). The cad-

mium block was greater in iron-deficient animals, and the effect was most pronounced when cadmium was introduced 90 min before iron was injected. It appears that cadmium in some way blocks the transferrin cycle within the cell. On the other hand, the absorption of hemoglobin iron in iron-deficient animals exposed to cadmium was enhanced, indicating that the internal release of iron into the plasma was unimpaired once iron was processed by the mucosal cell. The enhancement might be due to the induction of heme oxygenase system by cadmium (38) and perhaps also due to the slower processing time of heme, which may have allowed the cadmium concentration in the cell to decline before iron was freed.

The other aspect of cadmium interaction was its binding to mucosal ferritin. Not only was the cadmium immobilized, but its presence on the ferritin molecule was shown to interfere with the subsequent deposition of iron within the molecule as has been described elsewhere (39, 40). It has been suggested that cadmium binds to catalytic sites necessary for oxidation and deposition of ferritin iron (41).

The results of these studies may be described with respect to interacting cadmium and iron blockades. In the iron-replete animal a portion of absorbed cadmium is irreversibly bound to mucosal ferritin. It may be this effect that accounts for the inverse relationship between cadmium toxicity and iron status. On the other hand, cadmium uptake within the mucosal cell interferes with the absorption of iron. Since the iron content of the mucosal cell during the cadmium effect is if anything increased, there must be an impairment in the processing of iron by the mucosal cell. Most pronounced is the effect of cadmium on iron release from transferrin in the iron-deficient rat. In reticulocytes the cadmium effect is shown to be due to impaired intracellular release of iron from the transferrin-iron-receptor complex (42). Interference with the cycle of transferrin uptake in the mucosa also appears affected by the presence of cadmium. Indeed, the quantitative effect on this pathway is more severe than the effect on the absorption of iron salt in iron-deficient animals.  $\blacksquare$

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