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Cadmium Metabolism in *cdm/cdm* Mice

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The uptake and metabolism of cadmium by cadmium-susceptible (+/+) and cadmium-resistant (cdm/cdm) *strains of mice have been compared. These strains did not differ with respect to the quantitative uptake of cadmium into liver, kidney, or testis. After intraperitoneal administration of a nontoxic dose, more than 80% of the cadmium in liver and testis of both strains is bound to low molecular weight proteins. The chromatographic behavior of these cadmium-binding proteins is not affected by* cdm *genotype.*

KEY WORDS: cadmium; metallothionein; testis; genetic susceptibility.

INTRODUCTION

Cadmium is an environmental pollutant with toxic effects on mammalian testis (Chiquoine and Suntzeff, 1965) and other tissues (Kolonel, 1976; Adams *et al.*, 1969). Inbred strains of mice are known to differ in their susceptibility to cadmium-induced testicular necrosis (Gunn *et al.*, 1965; Chiquoine and Suntzeff, 1965). Taylor *et al.* (1973) demonstrated that a single genetic locus on mouse chromosome 12, designated cadmium resistance *(cdm)*, is responsible for the difference in cadmium sensitivity between the resistant strain C57BL/6J, designated *cdm/cdm*, and the sensitive (+/+) strain DBA/2J. In sensitive strains, testicular necrosis is visible 48 hr after the intraperitoneal administration of 50 μmol/kg of cadmium chloride. In resistant strains, even the lethal dose will not produce testicular necrosis (Gunn *et al.*, 1965). The biochemical basis for differential cadmium sensitivity is not known.

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The major portion of cadmium taken up by mammalian tissues becomes associated with low molecular weight, cysteine-rich proteins referred to as metallothioneins or cadmium-binding proteins (Kagi *et al.*, 1974; Nordberg *et al.*, 1971; Tsunoo *et al.*, 1978). Since these binding proteins are important in cadmium metabolism, we have compared their characteristics in *cdm/cdm* and +/+ mice.

MATERIALS AND METHODS

Male mice of strains C57BL/6J and DBA/2J were obtained from the Jackson Laboratory. [^{109}Cd] Cadmium chloride (1–5 Ci/g) was purchased from New England Nuclear. Animals were injected intraperitoneally with 2–30 μCi of ^{109}Cd. The total dose of cadmium varied from 0.4 to 4 μmol/kg. (the minimum dose required to produce testicular necrosis in susceptible mice is approximately 50 μmol/kg.) Tissue samples were counted in a Packard Autogamma scintillation spectrometer 5230.

Tissues were homogenized by hand with a ground glass homogenizer in 9 vol of 0.25 M sucrose, 0.01 M tris, *p*H 8.6. After centrifugation at 20,000*g* for 20 min, an 0.5-ml aliquot of the supernatant was applied to a Sephadex G-50 column. An aliquot (5–50 μl) of each fraction was combined with 3 ml of Aquasol (New England Nuclear) and counted in a Beckman LS100 scintillation counter (gain 300, window 350–500).

The peak fractions containing the low molecular weight cadmium-binding proteins were pooled and subjected to ion-exchange chromatography on DEAE-Sephadex (Sephadex A-25, Pharmacia). The column (0.6 by 13 cm) was first washed with 30 ml of 0.001 M tris, *p*H 8.6, and then eluted with a 130-ml linear gradient of increasing tris concentration (0.001–0.4 M) (Shaikh and Lucis, 1971). Fractions (1.3 ml) were collected, and 0.5-ml aliquots were counted as above.

RESULTS

Uptake of Cadmium into Tissues of Resistant and Susceptible Strains

The distributions of [^{109}Cd] cadmium chloride in three tissues of C57BL/6J and DBA/2J mice were compared. Twenty-four hours after a single injection of isotope, the two strains did not differ with respect to ^{109}Cd content of liver, kidney, or testis (Table I, Experiment 1). We did not observe the previously reported fourfold interstrain difference in testicular cadmium uptake (Lucis and Lucis, 1969). In the previous work, zinc had been administered with the cadmium. Therefore, we repeated the ^{109}Cd uptake experiment with simultaneous administration of zinc (Table I, Experiment 2). Again, the uptake of ^{109}Cd was independent of *cdm* genotype.

Table I. Distribution of ^{109}Cadmium in Tissues of Sensitive (+/+) and Resistant (*cdm/cdm*) Mice[a]

	N	Genotype[b]	Body weight (g)	Tissue weight (mg)			Tissue radioactivity (cpm/mg tissue)		
				Testis	Liver	Kidney	Testis	Liver	Kidney
Experiment 1[c]									
C57BL/6J	4	*cdm/cdm*	29.2 ± 0.6	215 ± 14	538 ± 5[d]	298 ± 4	221 ± 21	4150 ± 292	2840 ± 260
DBA/2J	5	+/+	32.1 ± 1.2	225 ± 7	457 ± 40[d]	403 ± 16	280 ± 15	4470 ± 274	2060 ± 241
Experiment 2[e]									
C57BL/6J	15	*cdm/cdm*	22.5 ± 0.5	227 ± 3	1420 ± 56	314 ± 15	11.5 ± 1.4	154 ± 11	45.0 ± 4.0
DBA/2J	18	+/+	24.3 ± 0.7	241 ± 7	1250 ± 39	362 ± 20	14.4 ± 1.5	148 ± 8	40.8 ± 2.5

[a] Values represent means ± SEM.

[b] Reference: Taylor (1973).

[c] Male mice, between 96 and 104 days of age, were injected i.p. with 4 μmol/kg cadmium chloride, containing 30 μCi ^{109}Cd, in 0.25 ml of phosphate-buffered saline. Twenty-four hours later, tissues were removed, weighed, and counted.

[d] Analysis on partial liver sample.

[e] Male mice, 58 days of age, were injected i.p. with 0.4 μmol/kg cadmium chloride, containing 2 μCi ^{109}Cd, plus 0.07 μmol/kg zinc chloride in 0.25 ml of saline. After 24 hr, tissues were removed, weighed, and homogenized with a Polytron in 10 ml of 0.25 M sucrose, 0.01 M tris, *p*H 8.6. Triplicate 3-ml aliquots were counted.

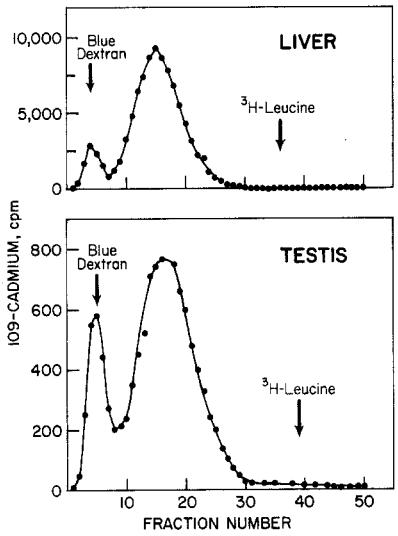

Fig. 1. Gel filtration of tissue extracts on Sephadex G-50. Mice were injected intraperitoneally with 30 μCi of [^{109}Cd] cadmium chloride. Two weeks later, liver and testis were removed and homogenized as described in the text. Aliquots of the soluble fraction (75 μl from liver or 200 μl from testis) were applied to an analytical column (0.6 by 8 cm) of Sephadex G-50 Fine. The column was eluted with 0.05 M tris, *p*H 7.5. Fractions (0.25 ml) were collected, and radioactivity was measured by scintillation counting. A: Liver from C57BL/6J male. B: Testis from DBA/2J male.

During the 6-week period after injection of isotope there was little loss of ^{109}Cd from liver and testis, and no difference in ^{109}Cd retention was observed between these strains.

Association of Cadmium with Low Molecular Weight Proteins

Tissue extracts were analyzed by gel filtration on Sephadex G-50. Typical elution patterns for liver and testis extracts are presented in Figure 1. The major portion of the cadmium is eluted as if it were associated with small proteins of molecular weight between 5000 and 10,000. The percentage of total tissue cadmium associated with these proteins did not differ between susceptible and resistant strains: $81\% \pm 2\%$ for DBA/2J testis and $79\% \pm 5\%$ for C57BL/6J testis (Mean $\pm$ SD, $n=6$). Free (unbound) ^{109}Cd was not present in tissues of either strain.

Ion-Exchange Chromatography of Cadmium-Binding Proteins

The low molecular weight cadmium-binding proteins from liver and testis of both strains were prepared by gel filtration and compared by chromatography on DEAE-Sephadex. The elution patterns revealed one major component and two minor peaks. Eighty to ninety percent of the radioactivity applied to the columns was recovered in the peak fractions. The elution volumes and proportions of ^{109}Cd-labeled proteins were identical in both tissues. No differences between C57BL/6J and DBA/2J tissues were observed.

DISCUSSION

We have investigated the effect of the *cadmium resistance* locus on the tissue uptake and protein-binding of cadmium in strains C57BL/6J and DBA/2J. We found that the amount of ^{109}Cd in testis was approximately 3% of that in liver plus kidney, in both *cdm/cdm* and +/+ mice. This observation does not agree with the earlier report of Lucis and Lucis (1969), who found that testis of the susceptible DBA/1J strain accumulated $1.9\% \pm 0.5\%$ (SE, $n=7$) of a cadmium dose per gram tissue, while the resistant C57BL/6J strain accumulated only $0.38\% \pm 0.08$ (SE, $n=7$). Lucis and Lucis administered 0.002 μmol cadmium per animal (0.1 μmol/kg); this is close to our dose in Experiment 2. The time allowed for metabolism and the observed uptake into liver and kidney were similar to those in the earlier work. Administration of zinc with the cadmium, as in the previous work, did not influence our result. In our hands, tissues of *cdm/cdm* and +/+ mice accumulate comparable quantities of an exogenous nontoxic dose of cadmium.

We have also compared the intracellular fate of exogenous cadmium in susceptible and resistant strains. Approximately 80% of cadmium in testis and liver was found in association with low molecular weight proteins during the period between 1 day and 2 weeks after administration. There was no accumulation of unbound cadmium in tissues of the susceptible strain. The cadmium-binding proteins prepared from +/+ and *cdm/cdm* tissues did not differ in their elution profiles from DEAE-Sephadex, indicating that *cdm/cdm* mice do not produce an altered cadmium-binding protein.

We have failed to observe any qualitative or quantitative abnormality in cadmium-binding proteins in susceptible mice. One remaining possibility is that deficient induction in response to toxic doses of cadmium might account for the greater sensitivity of DBA mice. Our observations make it more likely that the *cdm* locus affects the sensitivity of some unrelated cell component. For example, there is histological evidence that the testicular capillaries may be the primary site of damage (Chiquoine, 1965).

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