Cadmium Pathways during Gestation and Lactation in Control versus Metallothionein 1,2-Knockout Mice

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Effects of metallothionein (MT) on cadmium absorption and transfer pathways during gestation and lactation in mice were investigated. Female 129/SvJ metallothionein-knockout (MT1,2KO) and metallothionein-normal (MTN) mice received drinking water containing trace amounts of \(^{109}\)CdCl\(_2\) (0.15 ng Cd/ml; 0.074 μCi \(^{109}\)Cd/ml). \(^{109}\)Cd and MT in maternal, fetal, and pup tissues were measured on gestation days 7, 14, and 17 and lactation day 11. In dams, MT influenced both the amount of \(^{109}\)Cd transferred from intestine into body (two- to three-fold higher in MT1,2KO than MTN dams) and tissue-specific \(^{109}\)Cd distribution (higher liver/kidney ratio in MT1,2KO dams). Placental \(^{109}\)Cd concentrations in MT1,2KO dams were three- and seven-fold higher on gestation days 14 and 17, respectively, than in MTN dams. Fetal \(^{109}\)Cd levels were low in both mouse types, but at least 10-fold lower in MTN fetuses. MT had no effect on the amount of \(^{109}\)Cd transferred to pups via milk; furthermore, 85–90% of total pup \(^{109}\)Cd was recovered in gastrointestinal tracts of both types, despite high duodenal MT only in MTN pups. A relatively large percentage of milk-derived intestinal \(^{109}\)Cd was transferred to other pup tissues in both MT1,2KO and MTN pups (14 and 10%, respectively). These results demonstrate that specific sequestration of cadmium by both maternal and neonatal intestinal tract does not require MT. Although MT decreased oral cadmium transfer from intestine to body tissues at low cadmium exposure levels, MT did not play a major role in restricting transfer of cadmium from dam to fetus via placenta and to neonate via milk.

Key Words: metallothionein; cadmium; pregnancy; gestation; lactation; mice.

Metallothioneins (MTs) are low molecular weight, cysteine-rich proteins implicated in the homeostasis of essential metals such as zinc and copper (Friberg et al., 1986; Lucis et al., 1972) and in detoxification of heavy metals like cadmium (Friberg et al., 1986; Lucis et al., 1972; Templeton and Cherian, 1991). In addition to being inducible in adult mammals by heavy metals (Liu et al., 1996b), MTs are formed in response to radiation (Ono et al., 1998) and a variety of stresses (Kondo et al., 1997).

Four major isoforms of MT have been identified in mammals, with the predominant and best characterized being MT1 and MT2 (Chan and Cherian, 1993; Masters et al., 1994). The MT3 and MT4 have been found primarily in brain (Palmiter et al., 1992) and certain stratified squamous epithelia, including stomach and tongue (Quaife et al., 1994), respectively. The MT1 and MT2 proteins are considered functionally similar (Chan and Cherian, 1993). Hepatic concentrations of these latter two MT isoforms are detectable in rat fetal liver around gestation day 16 and increase into the neonatal period (Chan and Cherian, 1993).

Previous work in this and other laboratories has shown that MT gene expression and MT protein concentrations are increased in specific tissues of female mice and rats during pregnancy and lactation as a result of normal physiological changes that occur during those periods (Shimada et al., 1997; Solaiman et al., 2001). Induction of MT in the placenta occurs during gestation and has been suggested to prevent transfer of cadmium from mother to fetus during this time (Goyer et al., 1992; Itoh et al., 1996; Lau et al., 1998; Petersson and Oskarsson, 2000). Sequestration of cadmium in maternal mammary tissue also occurs during late gestation and throughout lactation (Bhattacharyya et al., 1981, 1982; Floris et al., 2000; Lucis et al., 1972; Petersson and Oskarsson, 2000), though results indicate that this cadmium is bound to high-molecular-weight proteins rather than to MT (Lucis et al., 1972).

Pregnant and lactating female animals (also called by the animal breeder’s term, “dams”) absorb and retain substantially more dietary cadmium than do their nonpregnant counterparts (Bhattacharyya et al., 1982, 1986, Floris et al., 2000). However, only a small fraction of the cadmium is passed from dams to offspring: in cadmium-transfer studies in which pregnant...
mice were chronically exposed to tracer levels of $^{109}$Cd in drinking water, fetal cadmium concentration was much lower than maternal levels, and only about 0.01% of the $^{109}$Cd dose ingested by the dam was transferred to each 21-day-old pup during lactation (Whelton et al., 1993). Cadmium pathways in maternal and neonatal animals have been hypothesized to reflect their changing MT concentrations, but this hypothesis has not been directly tested.

The current investigation was designed to determine the effects of maternal and neonatal MT on (1) the tissue distribution of orally absorbed cadmium during pregnancy and lactation and (2) the transfer of cadmium to offspring via milk. Concentrations of Cd and MT were determined in both generations of MT1- and MT2-normal (MTN) and MT1- and MT2-knockout (MT1,2KO) mice (Masters et al., 1994; Michalska and Choo, 1993), following $^{109}$Cd exposure via drinking water. By using tracer amounts of $^{109}$Cd, cadmium pathways were studied in the absence of exogenous MT-inducing agents, making this study as relevant as possible to humans exposed to environmental levels of cadmium. These results demonstrate that some of the pathways previously proposed to involve binding of Cd to MT in a particular tissue are in fact MT-independent.

**MATERIALS AND METHODS**

*Animals.* Both MTN and MT1,2KO mice of strain 129/SvJ (Jackson Laboratories, Bar Harbor, ME) were maintained on a Purina basal diet 5755 (Purina Mills Inc., St. Louis, MO) containing 0.6% calcium and 0.4% phosphorus. Mice were maintained on a 12/12 h light/dark cycle (light 1800–0600 h; dark 0600–1800 h) with food and water provided ad libitum. Protocols for animal use were approved by the Argonne National Laboratory Animal Care and Use Committee. Animals were maintained in the Argonne animal care facility, which is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

**Experimental protocol.** Female MTN and MT1,2KO mice, 36 of each strain, aged between 55 and 62 days, and weighing 19.7 ± 0.2 g (MTN) and 19.0 ± 0.2 g (MT1,2KO; mean ± SE), were bred with male mice of the same age and strain (MTN or MT1,2KO). One male was housed with three females beginning at 2 h into the animals’ dark period on the first day of mating. Females were checked for vaginal plugs 8 h later on the day of mating, and at daily intervals thereafter. On the day each mouse showed a vaginal plug (day of conception), it was placed individually in a plastic cage.

Each dam was provided deionized water containing $^{109}$CdCl$_2$ at a concentration of 0.15 ng Cd/ml (74 nCi/10$^9$Cd/ml; specific activity, 490 nCi/10$^9$Cd; Amersham, Burlington, MA). This very low cadmium concentration was avoided influencing the natural pattern of maternal cadmium in the MTN dams during gestation and lactation (Sauer et al., 1998; Solaiman et al., 2001). Water and $^{109}$Cd consumption were separately determined for each dam by weighing each water bottle and measuring $^{109}$Cd concentration when it was full and again when it was replaced with a fresh $^{109}$Cd-water bottle, thus avoiding dosage errors due to adherence of Cd ions to glass water bottles and rubber stoppers. Radiation safety protocols were supervised by the Environmental Safety and Health Division of Argonne National Laboratory.

There were four experimental groups of pregnant dams for each MT gene type (MTN and MT1,2KO), with 8–9 plug-positive females/group. In addition, six MTN and six MT1,2KO female mice of the same age, weight, and strain were kept out of the mating protocol to serve as nonpregnant (NP) controls.

**$^{109}$Cd determination in mouse tissues.** Tissues were homogenized with a glass homogenizer and Teflon pestle in 5 volumes of 5% sulfosalicylic acid. The sulfosalicylate acidified the tissues, allowing for release of $^{109}$Cd from cellular components. Aliquots of the tissue homogenates were suspended in Ready Safe liquid scintillation cocktail (Beckman Instruments, Inc., Fullerton, CA) and counted in a Packard Model 2200CA Liquid Scintillation Analyzer (Packard Instrument Company, Downers Grove, IL). The $^{109}$Cd was measured by the activity of its 0.16 MeV Auger electron emission. All $^{109}$Cd values were corrected for decay back to the first day of the experiment. Samples with low amounts of $^{109}$Cd were counted for long times to acquire a total of at least 1000 counts (< 3% RSD). Because of differences in amounts of $^{109}$Cd ingested by the dams (Table 1), the values of $^{109}$Cd radioactivity in the dam and pup tissues reported in Figures 1 and 2 and Table 7 were adjusted to represent equivalent cadmium intake levels. For example, the $^{109}$Cd values for the MT1,2KO pup samples were divided by 1.2 to account for the 1.2-fold greater consumption of $^{109}$Cd by the L11 MT1,2KO versus MTN dams (11.8 versus 10.0 µCi/mouse). This approach corrected for any increase in dam or pup $^{109}$Cd content due solely to increased $^{109}$Cd intake by the dam.

**MT determination.** Metallothionein protein concentration in tissues was determined using the cadmium-hemoglobin affinity assay of Eaton and Cherian (1991) with a slight modification. Frozen and thawed tissue samples were homogenized in 4 volumes of cold buffer (10 mM Tris–HCl, pH 7.4) with a glass homogenizer and Teflon pestle, homogenates were centrifuged at 10,000 g for 10 min at 4°C, and the supernatant fractions (the sample) used for the assay.

To perform the assay, 100 µl of each prepared sample was assayed for MT, using the method of Eaton and Cherian (1991). The contribution of $^{109}$Cd to the MT analysis and thus did not significantly affect the MT assay.
Table 1: Cumulative Water and 109Cd Consumption by MTN and MT1,2KO Mouse Dams during Pregnancy and Lactation

<table>
<thead>
<tr>
<th>Exposure group</th>
<th>Total water consumption (ml/mouse)</th>
<th>Total 109Cd consumption (µCi/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTN</td>
<td>MT1,2KO</td>
</tr>
<tr>
<td>G7</td>
<td>23 ± 1 (6)</td>
<td>20 ± 3 (3)</td>
</tr>
<tr>
<td>G14</td>
<td>55 ± 1 (4)</td>
<td>70 ± 4 (6)</td>
</tr>
<tr>
<td>G17</td>
<td>75 ± 7 (4)</td>
<td>81 ± 4 (6)</td>
</tr>
<tr>
<td>L11</td>
<td>191 ± 11 (8)</td>
<td>200 ± 12 (5)</td>
</tr>
<tr>
<td>NP20</td>
<td>96 ± 4 (6)</td>
<td>77 ± 7 (6)</td>
</tr>
</tbody>
</table>

Note. Mice received 109Cd in drinking water from day of observation of vaginal plug to day indicated by group name. Values are mean ± SE; number of mice shown in parentheses. MT, metallothionein; MT1,2KO, mouse strain in which MT1 and MT2 genes are inactivated (“knockout” strain); MTN, mouse strain of mice shown in parentheses. MT, metallothionein; MT1,2KO, mouse strain exposed to 109Cd for 20 days before sacrifice (similar to GD 17).

Validation of the MT determination. Because preliminary measurements indicated low MT concentrations in the experimental tissues, additional MT determinations were made to validate the assay procedure and to demonstrate the ability of this strain of MTN mice to produce MT in response to Cd exposure. Four days after gavage administration of 200 μg CdCl2/mouse, the same MT assay was performed upon livers, kidneys, and duodena from NP female mice of the same age and strain as the MTN mice in this study.

Statistics and data analysis. Comparisons involving more than two groups were made by ANOVA and Fischer’s least significant difference test (LSD). Comparisons between two groups of direct cadmium content measurements were made using Student’s t-test. The assumption of unequal variances was used for comparisons of data points. Comparisons between ratios of cadmium in liver and kidney were made using both the unpaired t-test with Welch’s correction and the Mann-Whitney non-parametric test. Differences in data points with a p value of < 0.05 were considered statistically significant.

RESULTS

Consumption of Cadmium in Drinking Water

Cumulative water and 109Cd consumption during pregnancy and lactation showed little difference between the MTN and MT1,2KO mice (Table 1). Cadmium concentrations directly determined in drinking water bottles averaged 0.074 μCi 109Cd/ml (0.15 ng Cd/ml). Pregnant and NP mice all consumed 3–5 ml water/day, while lactating mice consumed 6–7 ml/day, regardless of strain (Table 2).

109Cd in Small Intestines of Dams

For all mouse groups (GD 7–LD 11, NP), the small intestines contained much more 109Cd than any of the other organs examined (Fig. 1A versus 1B–1D; compare y-axes). Furthermore, no significant difference existed between the MTN versus MT1,2KO strains in cadmium retained by the small intestines, independent of whether the data were expressed as amount of cadmium (Fig. 1A) or as percentage of ingested cadmium (Table 3). For example, on LD 11, the entire small intestine of the MTN dams contained 6.5 × 10^5 dpm 109Cd (0.60 ng 109Cd), while that of the MT1,2KO dams contained 3.7 × 10^5 dpm (0.34 ng 109Cd)—values not significantly different from one another (Fig. 1A). Finally, the distribution of cadmium among the small intestine segments was independent of MT status, with the duodena of all MTN and MT1,2KO groups containing the highest percentage (65–80% of 109Cd in entire small intestine), followed by the jejunum, then the ileum (Table 3).

109Cd in Nonintestinal Internal Organs of Dams

Blood and total nonintestinal internal organs. Very little 109Cd remained circulating in blood in all groups examined (Fig. 1B), amounting to < 0.0021% ingested dose (Table 4) and ≤ 1% of total 109Cd in the nonintestinal internal organs analyzed (Tables 4 and 5). Although small, the amount of 109Cd in blood was significantly higher in the MT1,2KO dams than the MTN dams at LD 11 (Fig. 1B, Table 4). For all groups, cadmium in nonintestinal internal organs ranged from 0.13 to 0.49% of the ingested 109Cd (Table 5, column 3) and 8 to 28% of that in the total small intestine (Table 5, column 5). Again, ingested 109Cd in internal organs was significantly higher for MT1,2KO mice than for MTN mice on GD 17 and LD 11, reaching 0.46–0.49% of the ingested dose, two- to three-fold higher than in the MTN dams on these days (Table 5).

Liver and kidneys. After the small intestine, the liver and kidney contained the most 109Cd in all groups, reaching the highest levels on LD 11 (Figs. 1C and 1D). Starting on GD 14, the amount of 109Cd was significantly higher in the livers of the MT1,2KO versus MTN dams (Fig. 1C). Conversely, 109Cd levels in kidneys of MTN dams were significantly higher than those of MT1,2KO dams by LD 11 (Fig. 1D).

The relative amounts of 109Cd in liver versus kidney (Table 6) differed between the two strains of mice during gestation and lactation. For the MTN mice on GD 7, the ratio of liver 109Cd/kidney 109Cd was 5.2 (Table 6). As gestation continued, this ratio gradually decreased, reaching 3.1 on GD 17 (Table 6). By LD 11, the amount of 109Cd in the kidney was nearly equivalent to that in the liver, and the liver/kidney 109Cd

Table 2: Daily Water Consumption by MTN and MT1,2KO Mouse Dams during Pregnancy and Lactation

<table>
<thead>
<tr>
<th>Exposure group</th>
<th>GD 7</th>
<th>GD 14</th>
<th>GD 17</th>
<th>LD 11</th>
<th>NP20</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTN</td>
<td>3.3</td>
<td>3.9</td>
<td>4.4</td>
<td>6.4</td>
<td>4.8</td>
</tr>
<tr>
<td>MT1,2KO</td>
<td>2.9</td>
<td>5.0</td>
<td>4.8</td>
<td>6.7</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Note. Water consumption was calculated from Table 1.
content ratio (1.4) was similar to that in the MTN NP mice (1.6). In contrast, in the MT1,2KO dams, liver/kidney $^{109}\text{Cd}$ ratios were close to 10 throughout gestation and lactation and, after GD 7, were significantly higher in MT1,2KO dams than in MTN dams (Table 6). This significantly greater deposition of cadmium in liver versus kidney in MT1,2KO dams was also observed in the NP controls (Table 6).

$^{109}\text{Cd in Fetuses and Whole Pups}$

The amounts of $^{109}\text{Cd}$ in fetuses of both mouse strains were near the limit of detection. On GD 17, the MT1,2KO fetuses retained 0.001% ingested dose (equivalent to 0.1 pg $^{109}\text{Cd}$), 100 times the amount retained by the MTN fetuses (Table 4). In addition, the $^{109}\text{Cd}$ content of the MT1,2KO but not the MTN fetuses increased about six-fold from GD 14 to GD 17 (Table 4). The corresponding placental $^{109}\text{Cd}$ content at GD 14 and GD 17 also showed a significant difference between mouse strains, giving measurements of 0.008 and 0.028% ingested dose in MT1,2KO dams, amounts 2.7 and 7 times higher, respectively, than in MTN dams (Table 4).

The amount of $^{109}\text{Cd}$ transferred from both MTN and MT1,2KO dams to pups during 11 days of lactation (0.047–0.062% ingested dose) was much greater than that transferred to fetuses during 17 days of gestation (Table 4). Furthermore, on LD 11, the $^{109}\text{Cd}$ content of a litter of MT1,2KO pups was not significantly different from that of a litter of MTN pups (Table 4). There was, however, a three-fold higher level of $^{109}\text{Cd}$ in the mammary tissue of MT1,2KO dams than MTN dams on L11 (Table 4). The $^{109}\text{Cd}$ in mammary tissue on GD 7 and GD 14 was in range of that in the NP20 controls and was similar in both mouse strains (0.001–0.004% ingested dose).

$^{109}\text{Cd in Pup Tissues}$

As was observed in dams, the pup intestines contained a major fraction of the $^{109}\text{Cd}$ present in the whole animal (Table 7, Fig. 2). In addition, this distribution was essentially the same for MTN and MT1,2KO animals ($^{109}\text{Cd}$ in intestine $= 89\%$ of that in whole pup for MTN; 84% for MT1,2KO; Table 7). The milk-filled stomachs also had a very similar level of Cd in both types of mouse. Less than 1% of pup total body $^{109}\text{Cd}$ was in the pup feces for both MTN and MT1,2KO mice (Table 7).

Although there was a tendency for somewhat higher $^{109}\text{Cd}$ levels in all tissues of the MT1,2KO pups, the strain difference was statistically significant only for the liver (Fig. 2) and whole pup minus intestinal tract (Table 7). The amount of Cd in the kidney of pups of both types of mice was close to the limit of detection (Fig. 2). However, long count times indicated that, as for the dams, the ratio of liver/kidney $^{109}\text{Cd}$ content was significantly higher for the MT1,2KO than MTN pups (Fig. 2, Table 6).

$^{115}\text{METALLOTHIONEIN DURING GESTATION AND LACTATION}$

FIG. 1. Cadmium content of organs of mouse dams at various stages of gestation or lactation. Values are mean $\pm$ SE. Closed squares give results from MTN dams; open squares from MTN NP20 controls (20 days of $^{109}\text{Cd}$ exposure, nonpregnant mice, equivalent to LD 1). Closed circles give results from MT1,2KO dams; open circles from MT1,2KO NP20 controls. GD 7, GD 14, GD 17, NP20, and LD 11 MTN values are means from six, four, four, six, and eight mice, respectively, with exception of LD 11 blood ($n=7$) and NP20 liver ($n=5$). Corresponding MT1,2KO group sizes are three, six, six, six, and five mice, respectively, with the exception of LD 11 blood ($n=4$). Cadmium exposures and abbreviations as in Table 1. *Indicates MT1,2KO value significantly different from corresponding MTN value ($p < 0.05$, Student’s $t$-test, using the assumption of unequal variances).

Metallothionein Assay Applied to Cadmium-Gavaged Metallothionein-Normal Mice

MT concentrations in the tissues of MTN mice four days after cadmium gavage (200 $\mu$g/mouse), were 94 $\pm$ 12 (liver), 43 $\pm$ 5 (kidney), and 142 $\pm$ 18 (duodenum) $\mu$g/g wet weight of tissue (mean $\pm$ SE, $n=5$).
Metallothionein Concentrations in MTN Mice during Pregnancy and Lactation

In MTN dams, liver MT concentrations were significantly higher than in NP20 controls on GD 7 and increased to a peak of 14.8 μg MT/g on GD 14 (Table 8); by LD 11, liver MT was at a concentration similar to that in the nonpregnant mouse (2.6 μg MT/g). In pups on LD 11, the liver MT concentration was about the same as in the GD 7 dams and higher than in the LD 11 dams. In contrast, kidney MT concentrations remained low (~1 μg MT/g) throughout gestation and lactation in dams, and were more than 10-fold higher in LD 11 pups (Table 8). Duodenal MT concentrations in the dam were higher than in the jejunum and ileum; on GD 7 they were significantly higher than in the NP20 controls and rose steadily and significantly until LD 11. MT concentrations in the duodena of LD 11 pups were significantly higher than in the LD 11 dams. Placental and duodenal MT concentrations were similar to one another on GD 14, but placental MT decreased significantly by GD 17. Although mammary MT concentrations were low, they were three-fold higher on LD 11 than in the NP mice (1.0 versus 0.3 μg MT/g, respectively, Table 8).

The MT concentrations in the liver, kidney, and duodenum of the NP MTN mice were low (0.8–3.6 μg/g) and were comparable to the MT values obtained for the MT1,2KO dams...
DISCUSSION

This study focused on the role of MT1 and MT2 in pathways for intestinal absorption and distribution of low levels of cadmium in the mouse dam and neonate during gestation and lactation. We showed for the first time that early and substantial binding of oral Cd by small intestinal tissue, particularly the duodenum, was an MT-independent pathway (Figs. 1 and 2). The nearly complete uptake and sequestration of milk-derived cadmium by the pup intestine (Table 7) demonstrated the great efficiency of cadmium absorption by the mouse neonatal intestine, shown earlier by Sasser and Jarboe in rat neonates (1977); here we showed for the first time that this striking pathway in pup intestines was not dependent on the presence of MT (Fig. 2). Finally, in both dams and pups during lactation, we demonstrated an MT-dependent restriction of oral Cd movement across the intestinal tract to blood and liver and an MT-dependent enhancement of Cd transfer to kidneys (Figs. 1 and 2, Tables 5–7).

Pathways of Cd Absorption in Dams

MT-independent cadmium absorption from intestinal lumen to small intestine cells. Oral cadmium at low concentrations was sequestered by intestinal epithelium, primarily duodenum, as observed by others in MT wild-type animals (Lind and Wicklund-Glynn, 1997; Min et al., 1991). Our results demonstrate that this cadmium pathway was independent of MT in both NP females and mouse dams (Fig. 1, Table 3).

Cadmium absorption into intestinal epithelial cells recently has been shown to utilize the divalent metal ion transport

on GD 17, which represent heat-stable Cd-binding activity due to molecules other than MT1 and MT2 (Table 8).

### TABLE 5

<table>
<thead>
<tr>
<th>Strain</th>
<th>Gestation/lactation stage</th>
<th>A</th>
<th>B</th>
<th>A/B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTN</td>
<td>GD 7 (n = 6)</td>
<td>0.18 ± 0.05</td>
<td>1.63 ± 0.44</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GD 14 (n = 4)</td>
<td>0.16 ± 0.02</td>
<td>1.48 ± 0.38</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GD 17 (n = 4)</td>
<td>0.14 ± 0.03</td>
<td>1.10 ± 0.32</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LD 11 (n = 7)</td>
<td>0.23 ± 0.03</td>
<td>2.97 ± 0.59</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MT1,2KO</td>
<td>0.13 ± 0.04 (n = 5)</td>
<td>0.62 ± 0.23</td>
<td>0.21</td>
<td></td>
</tr>
</tbody>
</table>

Note. Values are mean ± SE. Exposures and abbreviations as in Table 1. A, sum of nonintestine internal organs, dams. B, sum of small intestine segments, dams. Pup data from Table 7. C, ratio of whole pup minus intestinal tract to small intestine sum.

*MT1,2KO value significantly different from corresponding MTN value (p < 0.05, Student’s t-test, using the assumption of unequal variances).

### TABLE 6

<table>
<thead>
<tr>
<th>Exposure group</th>
<th>GD 7</th>
<th>GD 14</th>
<th>GD 17</th>
<th>LD 11</th>
<th>NP20</th>
<th>Pups at LD 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTN</td>
<td>5.2 ± 1.9 (n = 6)</td>
<td>3.4 ± 0.2 (n = 4)</td>
<td>3.1 ± 0.4 (n = 4)</td>
<td>1.4 ± 0.2 (n = 8)</td>
<td>1.6 ± 0.3 (n = 5)</td>
<td>6.7 ± 3.1 (n = 7 litters)</td>
</tr>
<tr>
<td>MT1,2KO</td>
<td>10.6 ± 5.1 (n = 3)</td>
<td>10.5 ± 0.9* (n = 6)</td>
<td>10.9 ± 1.1* (n = 6)</td>
<td>8.2 ± 1.0* (n = 5)</td>
<td>4.0 ± 0.3* (n = 6)</td>
<td>54.0 ± 22.2* (n = 4 litters)</td>
</tr>
</tbody>
</table>

Note. Values are mean ± SE of ratios of 109Cd in individual organs of dams and pups. Number of dams or litters shown in parentheses for each value. Exposures and abbreviations as in Table 1. A ratio of 109Cd in liver/kidney was calculated for each individual mouse to obtain each group mean; the means are therefore not the same as those calculated from average organ content values reported in Figure 1.

*Indicates an MT1,2KO value significantly different from corresponding MTN value (Mann-Whitney non-parametric test and unpaired t-test with Welch’s correction, p < 0.05).
TABLE 7
$^{109}$Cd Content in MTN and MT1,2KO Mouse Pup Tissues on LD 11

<table>
<thead>
<tr>
<th>Sample</th>
<th>MTN (n = 7 litters)</th>
<th>MT1,2KO (n = 4 litters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole pup minus intestinal tract</td>
<td>263 ± 41</td>
<td>514 ± 95*</td>
</tr>
<tr>
<td>Stomach</td>
<td>58 ± 16</td>
<td>66 ± 26</td>
</tr>
<tr>
<td>Duodenum</td>
<td>624 ± 205</td>
<td>753 ± 186</td>
</tr>
<tr>
<td>Remaining intestine</td>
<td>1480 ± 423</td>
<td>1805 ± 409</td>
</tr>
<tr>
<td>Intestinal tract sum</td>
<td>2162 ± 635</td>
<td>2624 ± 532</td>
</tr>
<tr>
<td>Whole pup $^{109}$Cd</td>
<td>2425 ± 666</td>
<td>3138 ± 619</td>
</tr>
<tr>
<td>Total pup feces</td>
<td>23 ± &lt;1</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>Total milk-derived $^{109}$Cd</td>
<td>2448 ± 666</td>
<td>3359 ± 619</td>
</tr>
</tbody>
</table>

Note. Values adjusted for dams’ ingested doses as described in the text and expressed as mean dpm ± SE on per-pup basis for each litter. Duodenum samples rinsed; stomachs and remaining intestine segments not rinsed. Whole pup $^{109}$Cd, sum of $^{109}$Cd in all tissues analyzed. Mean value ± SE of whole-body $^{109}$Cd content calculated on a per-pup basis for the litter of each dam (7 dams, MTN; 4 dams, MT1,2KO). Total pup feces analyzed separately on LD 10 and LD 11 did not differ in $^{109}$Cd content; total feces calculated for LD 8–LD 11 as 2x the counts for feces on days 10 and 11. (Fully-formed pup feces were first observed on LD 8.) Total milk-derived $^{109}$Cd, sum of whole pup $^{109}$Cd + total $^{109}$Cd in pup feces.

*Indicates MT1,2KO value is significantly different from corresponding MTN value (Student’s t-test, p < 0.05, using the assumption of unequal variances).

Pathways of Cd Absorption and Distribution in Pups

Nearly all the $^{109}$Cd in pups (85–90%) was retained in intestinal tissues on LD 11 and, as in the dams, the capacity to synthesize MT had no significant effect on this pathway (Figs. 1 and 2).
time-related changes in MT levels during gestation and lactation.

Pathways of Cd Transfer from Dams to Pups (Fig. 2, Table 6) again illustrate the high efficiency of Cd transfer from maternal blood to fetus was extremely low during gestation in both mouse types, demonstrating that MT was not the main barrier to transplacental Cd transport. Cd clearance from blood, which was extremely rapid in dams of both strains (Fig. 1B), may have been a determining factor in restricting this transfer pathway.

Accumulation of cadmium in maternal mammary tissue during gestation and lactation has been reported by a number of workers (Bhattacharyya et al., 1982; Floris et al., 1992; Petersson and Oskarsson, 2000). The measurements of Cd in whole pups, however, indicate little or no role for MT in restricting cadmium transfer via milk. Although absolute magnitudes of Cd in mammary tissues as well as Cd content of blood were higher in MT1,2KO dams than in MTN dams, Cd levels in whole pups were similar, including the milk-filled stomachs, reaffirming that MT1,2KO dams did not provide their pups a significantly greater amount of Cd via their milk than did MTN dams (Tables 4 and 7).

Roles of Other Metallothionein Isoforms

For completeness, the other isoforms of mouse MT should be discussed here, because, although total MT concentrations were very low in the MT1,2KO mouse tissues tested (Table 8), MT3 and MT4 were not specifically evaluated. However, Palmiter and coworkers (1992) have shown that the cadmium-binding capacity of small intestine tissue did not increase in MT1,2KO mice, even after ip injection of 15 μmol Cd/kg, although MT3 detected in brain did respond to exogenous Cd. The MT4 isoform is believed to play a role in differentiation of

### TABLE 8

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MTN dams</th>
<th>MTN controls (NP20)</th>
<th>MTN pups (LD 11)</th>
<th>MT1,2KO dams (LD 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>8.8 ± 1.7</td>
<td>14.8 ± 1.9</td>
<td>11.7 ± 1.9</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.9 ± 0.4</td>
<td>1.0 ± 0.6</td>
<td>1.1 ± 0.5</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Mammary</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.04</td>
<td>0.2 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Placenta</td>
<td>6.1 ± 0.8</td>
<td>3.3 ± 0.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Duodenum</td>
<td>4.4 ± 0.7</td>
<td>6.1 ± 1.7</td>
<td>7.1 ± 0.5</td>
<td>8.5 ± 1.8</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1.5 ± 0.4</td>
<td>1.7 ± 0.4</td>
<td>2.4 ± 0.8</td>
<td>2.6 ± 0.8</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.8 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>3.1 ± 0.6</td>
<td>3.5 ± 0.8</td>
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<td>3.5 ± 0.8</td>
</tr>
</tbody>
</table>

Note. Metallothionein concentration is given as μg MT/g wet weight. MT was analyzed using Cd-hemoglobin affinity assay of Eaton and Cherian (1991) in samples taken from mice administered 0.15 ng Cd/ml as 109CdCl2 (0.074 μCi/ml) in drinking water, as described in Table 1. Abbreviations as in Table 1. Values are mean ± SE, n = 4.

* MT values for MT1,2KO mice represent heat-stable Cd-binding activity due to molecules other than MT1 and MT2.

**x,y,z,v** Values in row with same superscript letter are not significantly different (ANOVA + LSD, p < 0.05). Statistical analysis results show significance of time-related changes in MT levels during gestation and lactation.
stratified squamous epithelium (Quaife et al., 1994). The whole body of MT1,2KO pups did contain significantly more $^{109}$Cd than that of MTN pups (Table 7), but no evidence exists in our data (Table 8, MT1,2KO mouse tissues) or that of others (Liu et al., 1996a; Quaife et al., 1994) to indicate that MT3 or MT4 induction might be a compensatory mechanism in the MT1,2KO animal.

In summary, the results of this study indicate that some aspects of cadmium absorption are independent of MT, including (1) early uptake and sequestration of oral cadmium by the duodenum, (2) early transfer of $^{109}$Cd from intestine to internal organs, and (3) nearly total uptake and sequestration of milk-derived cadmium by pup intestinal tract. The presence of MT in the dam’s duodenum at pregnancy-induced levels appears to (1) restrict the movement of oral Cd across the gastrointestinal tract to blood by a factor of two to three, (2) reduce transfer of $^{109}$Cd to liver, mammary, and placental tissues, and (3) in conjunction with hepatic MT, enhance transfer of $^{109}$Cd to kidney. $^{109}$Cd distribution in nonintestinal organs of the pups was similarly influenced by MT, although net absorption of cadmium from intestinal lumen to pup organs was proportionally higher than in dams. The functions of the divergent metal transport system in cadmium uptake, the biochemical mechanism for restricted cadmium transport through the basolateral side of intestinal epithelium, and the role of MT in this latter process all require additional study.

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REFERENCES


