

Alteration of Tissue Disposition of Cadmium by Chelating Agents

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The effect of several chelating agents (diethyldithiocarbamic acid, DDC; nitrilotriacetic acid, NTA; 2,3-dimercaptopropanol, BAL; *d,l*-penicillamine, PEN; 2,3-dimercaptosuccinic acid, DMSA; ethylenediaminetetraacetic acid, EDTA; and diethylenetriaminepentaacetic acid, DTPA) on the toxicity, distribution and excretion of cadmium (Cd) was determined in mice. When chelators were administered immediately after Cd, significant increases in survival were noted after treatment with DMSA, EDTA, and DTPA. DTPA, followed by EDTA and then DMSA, were consistently the most effective in decreasing the tissue concentrations of Cd and increasing the excretion of Cd. NTA, BAL, DDC and PEN had no beneficial effects.

The effects of increasing the time interval between Cd administration and initiation of chelation therapy was determined by using a single administration of DTPA, EDTA, and DMSA. Mice treated immediately after Cd administration excreted approximately 50% of the administered dose of Cd compared to 0.2% in controls. Treatment with chelator at later times significantly increased Cd excretion but the magnitude of the effect was much less than that seen in mice treated immediately after Cd.

To determine the role of MT in the acute decrease in chelator efficacy following Cd poisoning, rats were injected IV with Cd followed by DTPA at various times after Cd. Although DTPA reduced Cd content in the various organs when given immediately after Cd, the chelator was ineffective at all later times. Increases in hepatic and renal metallothionein (MT) did not occur until 2 hr after Cd, and did not coincide with the earlier drop in chelator efficacy. Blockade of MT synthesis by actinomycin D failed to eliminate this decreased DTPA effectiveness. Therefore, it appears that MT does not play an important role in the acute decrease in efficacy of chelation therapy for Cd poisoning.

The effect of repeated daily administration of chelators on the distribution and excretion of Cd was studied by administering chelators daily for 5 days starting 48 hr after Cd. DTPA, EDTA, DMSA and BAL significantly increased the urinary elimination of Cd. Thus, mobilization of Cd into urine occurs with repeated chelation therapy, which may decrease tissue concentrations of Cd and reduce the toxicity of the metal.

The LD₅₀ values for the various chelators studied are shown in Table 1. Marked differences in the toxicity of the chelators was observed: NTA, BAL and PEN were very toxic, while DDC, DMSA and DTPA were moderately toxic, and EDTA was only slightly toxic. The dose of chelator chosen for the subsequent studies was approximately one-fourth of the respective LD₅₀ value.

The effect of the various chelating agents on the lethality of Cd is shown in Figure 1. Mice which received saline after Cd are shown as control.

Previous work in our laboratory has shown that the IV LD₅₀ for Cd is approximately 3.5 mg Cd/kg. In control mice receiving 4 mg Cd/kg, 40% survival was seen which is consistent with the reported LD₅₀. At doses of Cd greater than 4 mg Cd/kg no survival was seen in control mice. No protective effect was observed with administration of PEN, BAL or NTA. Slight protection was seen with DDC at 4 and 6 mg Cd/kg (70 and 60% survival, respectively), but no protection was seen with DDC at the two higher doses of Cd. An increase in survival was also noted with DMSA, ranging from 70 to 50% at all doses of Cd. Treatment with EDTA provided even greater protection, approximately 80% survived at all doses of

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Table 1. LD₅₀ values of chelators.^a

Chelator	LD ₅₀		Dose used, g/kg ^b
	nmole/kg	g/kg	
Sodium diethyldithiocarbamic acid (DDC)	8.35	1.40	0.35
Disodium nitrilotriacetic acid (NTA)	1.70	0.40	0.10
2,3-Dimercaptopropanol (BAL)	0.85	0.11	0.027
Pencillamine (PEN)	2.53	0.38	0.095
2,3-Dimercaptosuccinic acid (DMSA)	16.2	2.95	0.74
Calcium disodium ethylenediaminetetraacetic acid (EDTA)	16.4	6.11	1.50
Calcium trisodium diethylenetriaminepentaacetic acid (DTPA)	6.94	3.60	0.90

^aChelators were administered IP to groups of 10 mice per dose. Survival was recorded at the end of 14 days.

^bRefers to the dosage of chelators used in the subsequent comparisons of the chelators.

Cd tested. The most dramatic protection was observed with DTPA which completely protected mice up to 8 mg Cd/kg (i.e., greater than two times the IV LD₅₀ for Cd).

The effect of the chelators on the excretion of Cd into urine and feces is shown in Figure 2. The amount of Cd excreted ($\mu\text{g Cd/kg}$ body weight) in 24 hr is shown for all treatments. Significant increases in urinary excretion of Cd (top panel, Fig. 2) were caused by PEN, DMSA, EDTA and DTPA. The effects of both EDTA and DTPA are especially noteworthy as they caused excretion of 50 and 60% of the administered Cd, respectively. No increase in urinary excretion of Cd was seen after DDC, NTA or BAL. The excretion of Cd into feces (bottom panel, Fig. 2) shows that this is the major route of elimination for Cd in control ani-

mals. The agents which caused an increase in urinary excretion of Cd caused a corresponding decrease in fecal elimination of the metal. No significant effect on fecal elimination of Cd was observed after DDC, NTA, BAL and PEN.

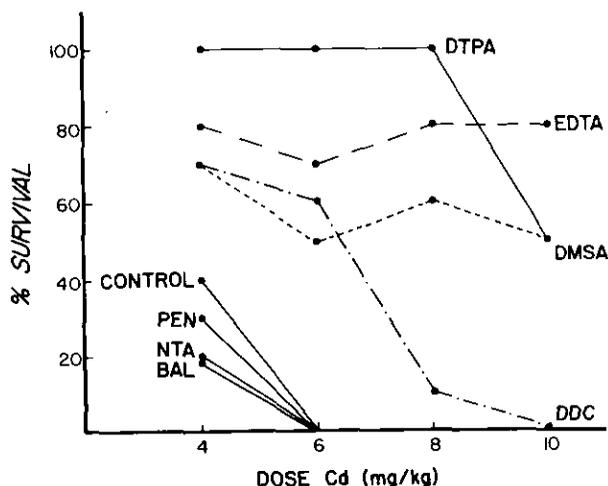


FIGURE 1. Effect of the indicated chelators on the lethality of Cd. Cd was administered intravenously, immediately followed by one of the chelators or saline (control). Chelators were given at a dose approximately equal to one-fourth of their respective LD₅₀ (shown in Table 1). Each point represents the percentage survival of 10 animals. From Cantilena and Klaassen (1).

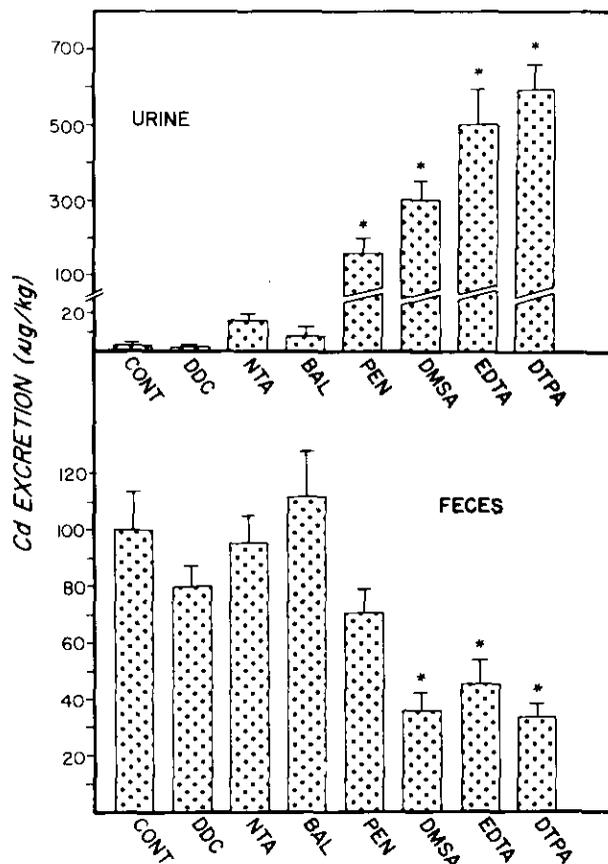


FIGURE 2. Effect of the indicated chelators on the amount of Cd excreted in 24 hr ($\mu\text{g Cd/kg}$ body weight). The chelators were given IP immediately after Cd (1 mg Cd/kg) at a dose approximately equal to one-fourth of their respective LD₅₀ (shown in Table 1). Bars indicate SE, $n = 6$. An asterisk indicates significantly different from control. From Cantilena and Klaassen (1).

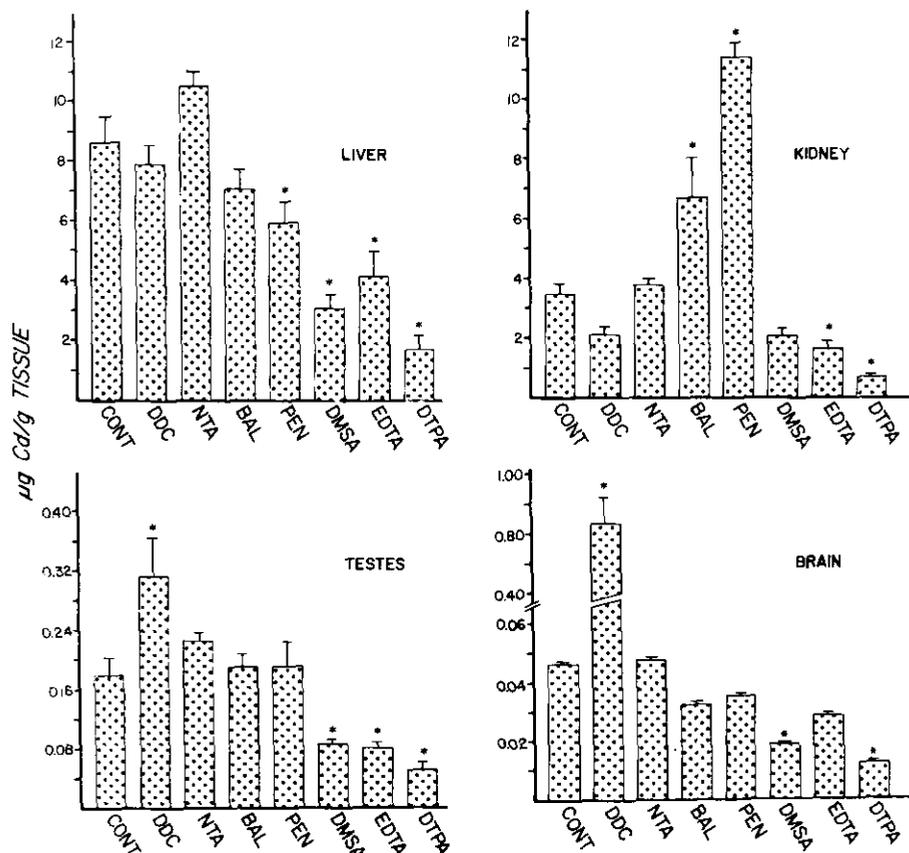


FIGURE 3. Cd concentrations in the indicated tissues of mice 24 hr after treatment with various chelators. Chelators were given IP immediately after Cd at a dose approximately equal to one-fourth of their respective LD_{50} (shown in Table 1). Bars indicate SE, $n = 6$. An asterisk indicates significantly different from control. From Cantilena and Klaassen (1).

The effect of the chelators on tissue distribution of Cd is shown in Figure 3. Note that the scale of the ordinate changes in the bottom two panels of the figure. The concentration of Cd ($\mu\text{g Cd/g tissue}$) is shown for each treatment group. The upper left quadrant shows the concentration of Cd found in liver, the main storage site of administered Cd in the body. Treatment with PEN, DMSA, EDTA and DTPA significantly reduced the amount of Cd found in liver with DTPA being the most effective.

The upper right quadrant of Figure 3 shows the results from kidney, the main target organ of chronic Cd exposure. The agents which decreased tissue Cd levels in kidney were EDTA and DTPA. Penicillamine (PEN) caused a threefold increase in kidney Cd concentration. A similar increase, though not of the same magnitude, was seen after treatment with BAL. No significant change in Cd concentration in kidney was seen in mice treated with DDC, NTA, or DMSA.

The results from testes, another target organ of Cd toxicity, are shown in the lower left quadrant of Figure 3. Similar to their effects on kidney, EDTA and DTPA significantly lowered the amount of Cd in testes. A significant decrease in testicular Cd concentration was also observed after DMSA. No significant changes in testicular Cd concentrations were seen with NTA, BAL or PEN. Similar to what was observed with PEN and BAL in the kidney, DDC significantly increased the concentration of Cd in testes.

The effect of treatment with the chelators on brain Cd concentrations is shown in the lower right quadrant of Figure 3. The brain normally receives very little Cd relative to other body tissues. Treatment with DMSA and DTPA caused a significant decrease in tissue Cd concentrations. Similar to its effect in testes, DDC caused a significant increase in brain Cd, almost a 20-fold increase compared to controls.

Histopathological examination of renal tissues

from animals that received Cd only (1 mg Cd/kg), 24 hr before sacrifice, did not reveal any significant pathological lesions. Similarly, renal tissue from mice given Cd and DDC, or Cd and NTA did not show any evidence of damage. Minor focal necrosis with swelling was noted in the convoluted proximal tubules of 2/6 and 3/6 of the EDTA- and DTPA-treated animals, respectively. The focal necrosis in the EDTA group involved less than 30% of the nephrons with less than 50% of the length of the involved segments being necrotic. In the DTPA group, less than 50% of the nephrons were involved. In two of six mice receiving BAL after Cd, rare segmental necrosis of the proximal tubule was found. Less than 10% of the nephrons were involved. Treatment with DMSA after Cd resulted in varying degrees of necrosis in the convoluted proximal tubule of all six mice examined. The degree of damage varied from minor focal necrosis to extensive uniform necrosis. The most severe lesions were observed in Cd-poisoned mice treated with PEN. Treatment with PEN resulted in extensive necrosis of the distal portion of the convoluted proximal tubule (pars recta) with swelling and fragmentation of cells and some loss of nuclei. Most, but not all, of the nephrons were involved. The distal nephron and glomeruli were uninvolved, as were most of the initial segments of the convoluted tubules. Along the basement membrane, occasional residual or flattened nuclei were found. There was no evidence of mitosis or any other component of regeneration noted.

The effect of increasing the time interval between Cd administration and initiation of chelation therapy was determined using a single administration of DTPA, EDTA or DMSA. The effect of the chelating agents on daily urinary elimination of Cd for each day after administration of Cd is shown in the five panels (top to bottom) of Figure 4. The scale of the ordinate (μg Cd excreted/kg body weight) is not the same in all panels. The excretion of Cd from mice receiving either saline (control) or one of the three chelators at various times after Cd administration (0, 2, 12, 36 or 72 hr) is shown from left to right in each panel. Urinary excretion data for groups which had not yet received chelator treatment are omitted for clarity.

During the first 24 hr after Cd administration (day 1, Fig. 4), the first three groups (0, 2, and 12 hr) received chelation treatment. Saline-treated controls in these three groups excreted approximately 1 μg Cd/kg during this period. Treatment with DTPA significantly increased urinary excretion of Cd in all three groups during day 1. The

DTPA-treated mice excreted 500, 50 and 2.5 μg Cd/kg when treated at 0, 2 and 12 hr, respectively. Treatment with either EDTA or DMSA at 0 or 2 hr after Cd also markedly increased urinary elimination of Cd.

Excretion of Cd into urine during day 2 is shown in the second panel from the top of Figure 4. Mice which received either DTPA or DMSA immediately after Cd (0 hr) had an increased urinary elimination of Cd on the second day after administration. Mice which received chelation therapy 2 hr after Cd did not excrete more Cd than their respective control. The animals treated 36 hr after Cd received their respective chelator 12 hr into the day 2 collection period. Treatment with DTPA, but not with EDTA or DMSA, significantly increased the urinary elimination of Cd at this time.

The third panel from the top of Figure 4 shows the urinary elimination of Cd during the third day after Cd administration. No group received treatment on the third day. Mice which received

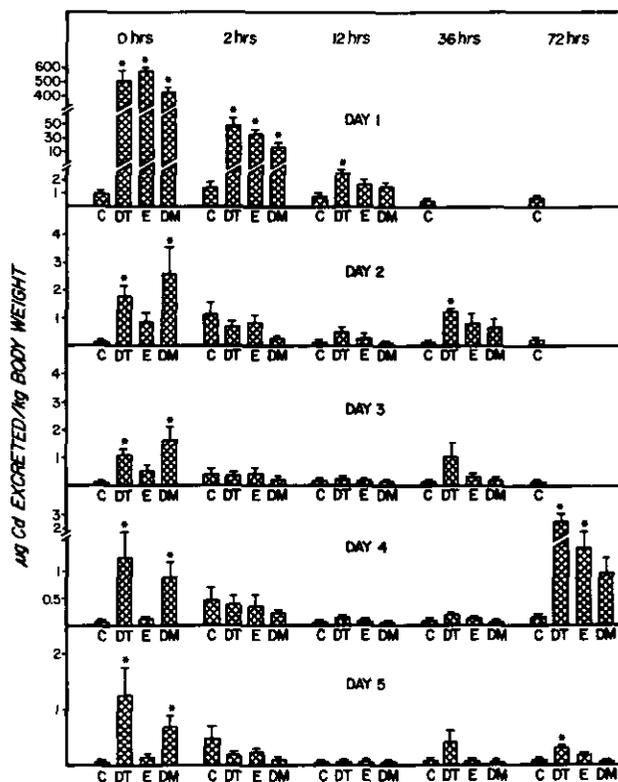


FIGURE 4. Effect of DTPA (DT), EDTA (E), and DMSA (DM) administered at the indicated times after Cd (0 to 72 hr) on the urinary elimination of the metal. The daily excretion of Cd of treated groups is shown for 5 consecutive days after Cd (days 1-5). Bars indicate SE, $n = 5$ to 8. An asterisk indicates significantly different from control ($p < 0.05$). From Cantilena and Klaassen (2).

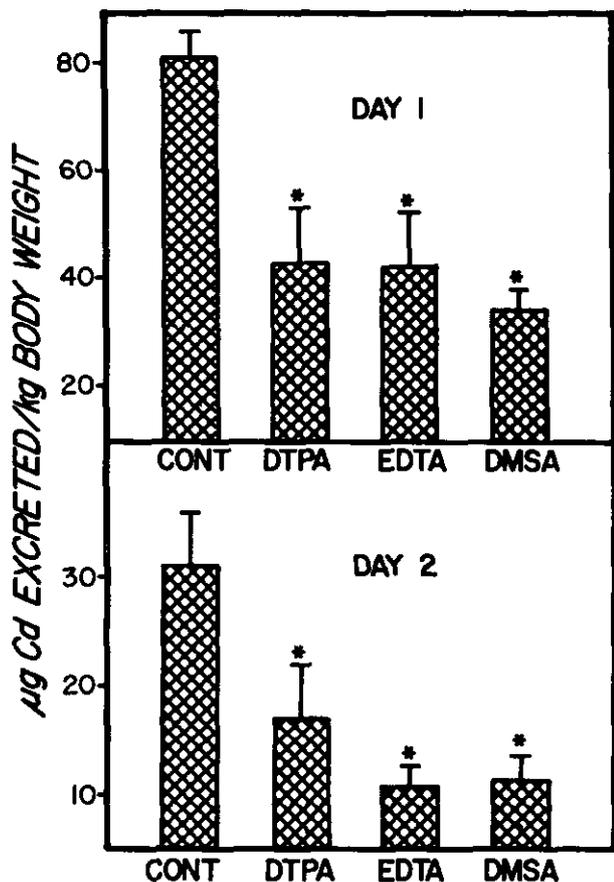


FIGURE 5. Effect of the indicated chelators on the fecal elimination of Cd. Chelating agents were administered immediately after Cd. Fecal elimination during the first 2 days post-Cd is shown. Bars indicate SE, $n = 5$ to 8. An asterisk indicates significantly different from control ($p < 0.05$). From Cantilena and Klaassen (2).

either DTPA or DMSA 0 hr after Cd still excreted more Cd than the control group. However, during this period the mice were excreting only 1 to 2.5 $\mu\text{g Cd/kg}$ compared to the 500 $\mu\text{g Cd/kg}$ they eliminated during day 1.

The urinary elimination of Cd during day 4 is shown in the fourth panel from the top of Figure 4. Significant increases in the amount of Cd eliminated by mice given DTPA or DMSA immediately after Cd (0 hr) were observed. During day 4, the 72-hr group received chelation therapy. The mice treated at this time with DTPA or EDTA excreted more Cd into the urine than their respective controls.

The bottom panel of Figure 4 shows the urinary elimination of Cd during day 5. After 5 days, mice which received DTPA or DMSA immediately after Cd (0 hr) continued to excrete more Cd than controls. Similarly, mice which received DTPA 72

hr after Cd excreted more Cd than controls.

The effect of administration of DTPA, EDTA, or DMSA immediately after Cd on its elimination into feces is shown in Figure 5. Treatment with DTPA, EDTA, or DMSA decreased the fecal elimination of Cd by approximately 50% during day 1 (top panel) and approximately 50 to 70% during day 2 (bottom panel). The excretion of Cd into feces by control mice diminished with time from 80 $\mu\text{g Cd/kg}$ during day 1 (Fig. 5) to 2 $\mu\text{g Cd/kg}$ during day 5 (data not shown). The excretion data of the 0-hr group for day 1 (top panel) and day 2 (bottom panel) are the only fecal excretion data shown because all other groups (2, 12, 36 or 72 hr) and days (3 to 5) showed no statistically significant effects.

The effect of chelation treatment at various times after Cd administration on the concentration of Cd in kidney, the main target organ of Cd toxicity, is shown in Figure 6. Administration of DTPA, EDTA, or DMSA immediately after Cd (0 hr) resulted in a significant decrease in the concentration of Cd in kidneys 5 days later. DTPA, EDTA and DMSA produced 70, 60 and 30% decreases in renal Cd content, respectively. Chelator treatment at later times did not significantly affect the concentration of Cd in kidneys.

The time course for effectiveness of the chelators in removing Cd from liver, the main storage site for Cd in the body, is shown in Figure 7. Treatment with DTPA, EDTA or DMSA at 0 hr

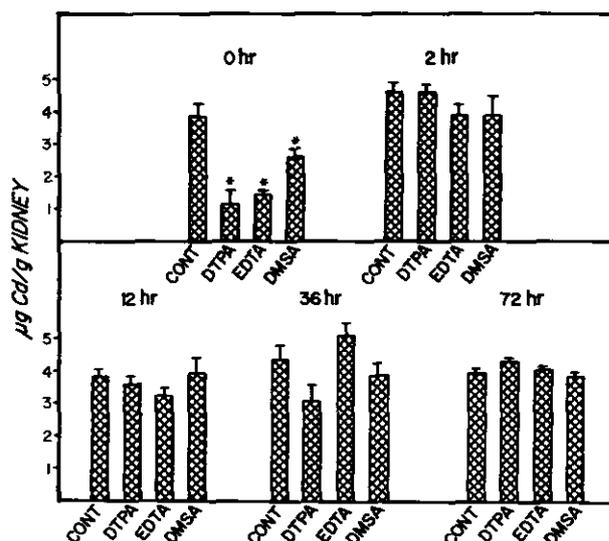


FIGURE 6. Effect of administration of the indicated chelators at various times (0 to 72 hr) after Cd exposure on the concentration of Cd in kidney. Bars indicate SE, $n = 5$ to 8. An asterisk indicates significantly different from control. From Cantilena and Klaassen (2).

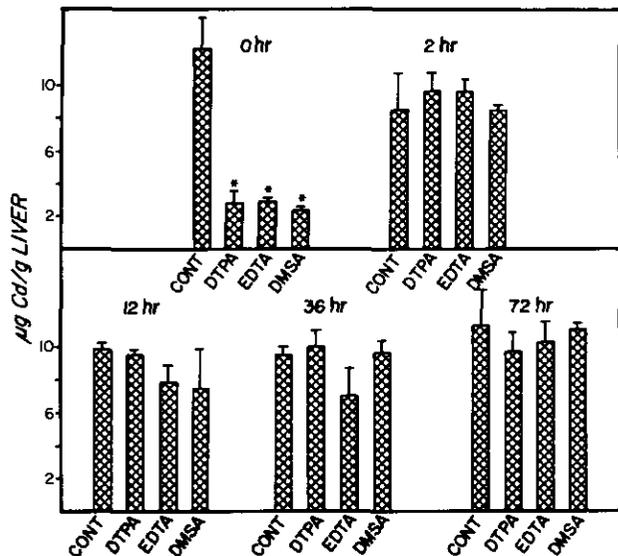


FIGURE 7. Effect of administration of the indicated chelators at various times (0 to 72 hr) after Cd exposure on the concentration of Cd in liver. Bars indicate SE, $n = 5$ to 8. An asterisk indicates significantly different from control. From Cantilena and Klaassen (2).

resulted in a significant decrease (75 to 80%) in concentration of Cd in liver; administration of chelators at later times had no significant effect. Similar results were obtained in testes (Fig. 8) and in brain (Fig. 9), in that administration of the chelators only immediately after Cd (0 hr) resulted in a significant decrease in concentration

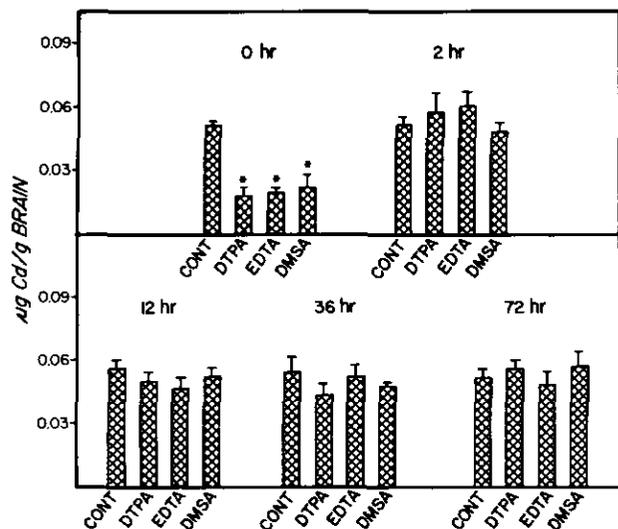


FIGURE 9. Effect of administration of the indicated chelators at various times (0 to 72 hr) after Cd exposure on the concentration of Cd in brain. Bars indicate SE, $n = 5$ to 8. An asterisk indicates significantly different from control. From Cantilena and Klaassen (2).

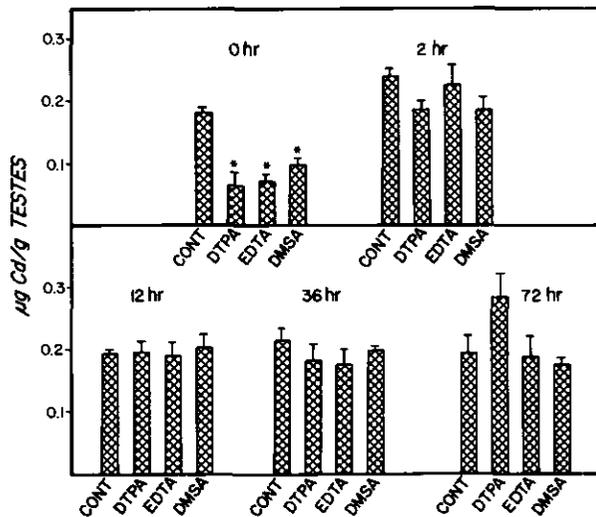


FIGURE 8. Effect of administration of the indicated chelators at various times (0 to 72 hr) after Cd exposure on the concentration of Cd in testes. Bars indicate SE, $n = 5$ to 8. An asterisk indicates significantly different from control. From Cantilena and Klaassen (2).

of Cd in these tissues. This trend was observed for the other tissues examined (data now shown) in that statistically significant decreases in tissue

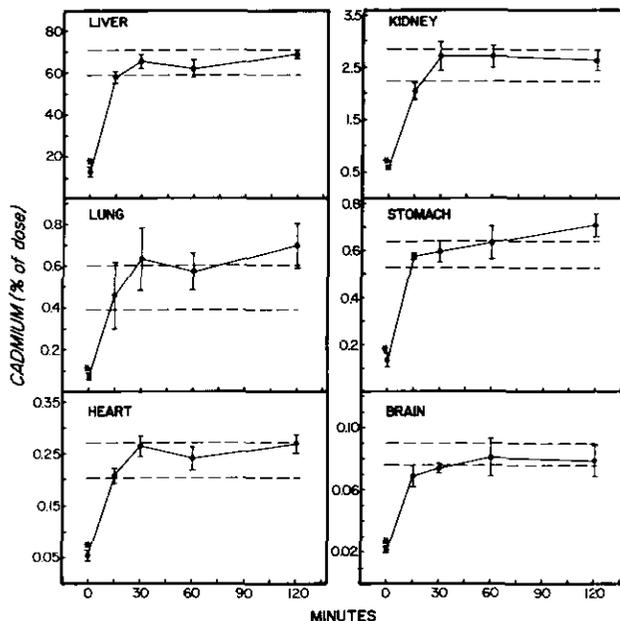


FIGURE 10. Effect of DTPA treatment (90 mg/kg, IP) at various short intervals (0, 15, 30, 60 and 120 min) on the organ retention of Cd 24 hr following IV administration (1 mg Cd/kg; time 0) in young adult rats. Values represent $\bar{x} \pm$ SE for six rats. The broken line represents $\bar{x} +$ SE for controls (no DTPA). An asterisk indicates significantly different from control ($p < 0.05$). From Waalkes et al. (3).

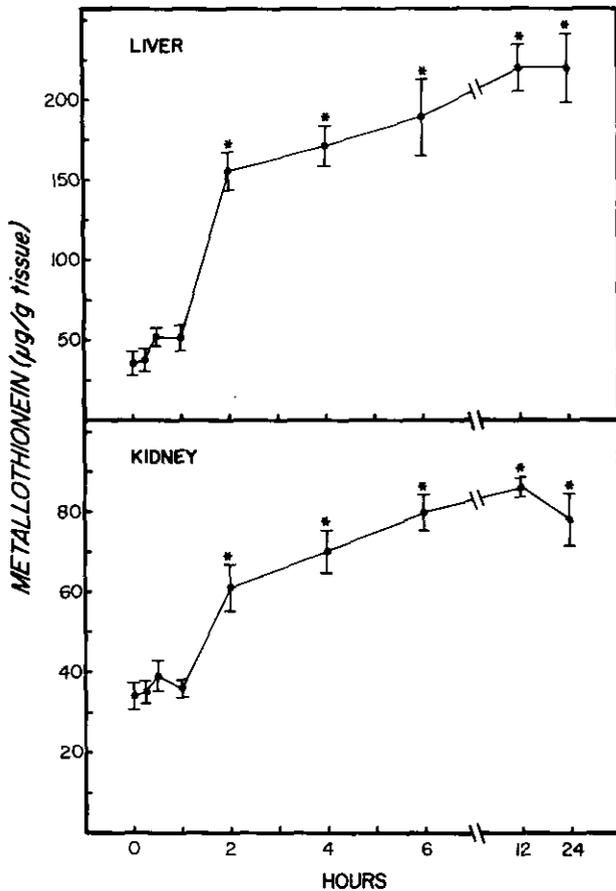


FIGURE 11. Time course of MT concentration in liver and kidney following IV administration of Cd (1 mg Cd/kg). Values represent $\bar{x} \pm SE$ for six rats. An asterisk indicates significantly different ($p < 0.05$) from basal concentrations (time 0). From Waalkes et al. (3).

Cd concentrations were only noted in mice that received chelation therapy immediately after Cd.

To determine the time of onset for decreased effectiveness of chelators after Cd exposure, rats were injected with DTPA (90 mg/kg, IP) at various times (0, 15, 30, 60 and 120 min) after Cd exposure (1 mg/kg, IV; time 0), and 24 hr later the amount of Cd in various tissues was quantified (Fig. 10). DTPA markedly lowered the amount of Cd remaining in liver, kidney, lung, stomach, heart, and brain at 24 hr when given immediately after Cd. However, if DTPA was delayed as little as 15 min, the chelator was ineffective. The amount of Cd in the various tissues of rats receiving DTPA at 15, 30, 60 or 120 min following Cd was not different from control (no chelator). The retention of Cd in the spleen (data not shown) was not affected by DTPA therapy.

The concentration of MT in liver and kidney at

various times following Cd (1 mg Cd/kg, IV) treatment is shown in Figure 11. There was no significant increase in MT concentration in either organ until 2 hr after Cd injection. Between 2 and 24 hr there was a significant increase in MT in both liver and kidney. MT levels reached a plateau in both organs between 4 and 12 hr after Cd injection which was 6.4 and 2.5 times higher than basal levels for liver and kidney, respectively.

The effect of actinomycin D pretreatment (1.25 mg/kg, IP) on the induction of hepatic and renal MT 4 hr following Cd (when MT levels are approximately maximal) is shown in Figure 12. In animals pretreated with vehicle 1 hr prior to Cd, there was a marked increase in both hepatic and renal MT concentrations. However, rats receiving actinomycin D had no significant increase in MT over controls in either organ.

Despite the block of MT synthesis with actinomycin D, the pronounced decrease in DTPA efficacy was still evident at 15 min (Fig. 13). The amount of Cd in various tissues in the actinomycin D-pretreated rats given DTPA 15 min after Cd

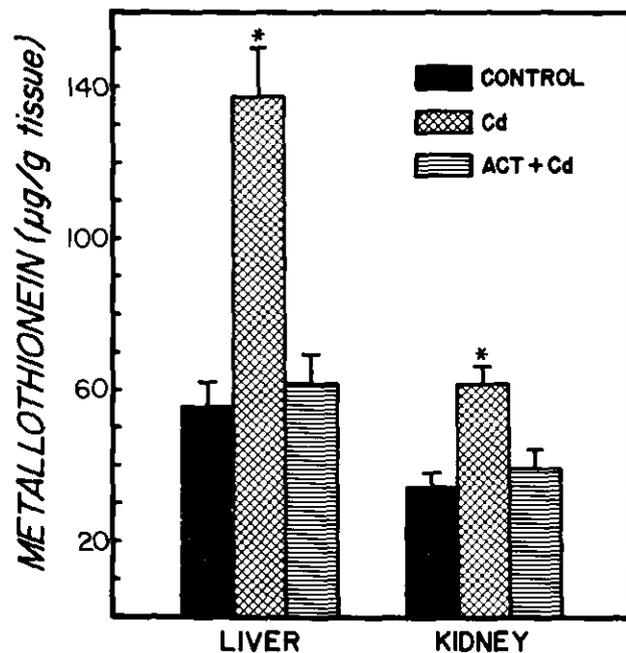


FIGURE 12. Effect of actinomycin D (ACT) pretreatment on the concentration of MT in liver and kidney 4 hr following Cd. Values represent $\bar{x} \pm SE$ of four rats. The ACT + Cd group received 1.25 mg actinomycin D/kg 1 hr prior to Cd (1 mg Cd/kg, IV); the Cd group received actinomycin D vehicle (10 mL/kg) 1 hr prior to Cd; and controls received actinomycin D vehicle (10 mL/kg) 1 hr prior to saline (10 mL/kg). An asterisk indicates significantly different from control ($p < 0.05$). From Waalkes et al. (3).

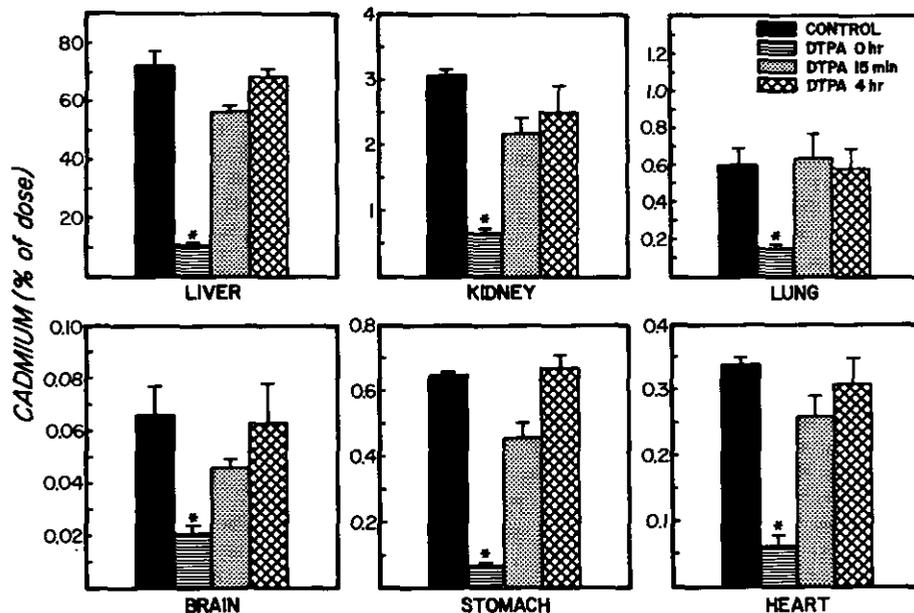


FIGURE 13. Effect of actinomycin D (ACT) pretreatment (1.25 mg/kg, IP) 1 hr prior to Cd (1 mg/kg, IV; time 0) on the efficacy of DTPA (90 mg/kg) given at various times following Cd as assessed by organ retention of the metal 24 hr later. All groups received actinomycin D and Cd treatments followed by DTPA at various times (0, 15 min or 4 hr) or saline. Values represent $\bar{x} \pm SE$ of four rats. An asterisk indicates significantly different ($p < 0.05$) from the group receiving no DTPA. From Waalkes et al. (3).

was not significantly different from that in rats that did not receive the chelator.

The efficacy of DTPA was also determined in newborn rats (5-day-old) following Cd injection (1 mg Cd/kg, IV). Hepatic MT concentration in 5-day-old rats without any pretreatment was $1260 \pm 45 \mu\text{g MT/g wet weight}$ ($\bar{x} \pm SE$), which is approximately 36 times the basal levels in young adult rat liver (35.4 ± 7.5). Despite these high levels of hepatic MT in newborn rats, DTPA was still effective if given immediately following Cd (Fig. 14). However, when DTPA administration was delayed to 2 hr after Cd injection, it became ineffective in both the newborn and adult rats.

The effect of repeated daily administration of chelators on the distribution and excretion of Cd was studied by administering chelators daily for 5 days starting 48 hr after Cd. Daily Cd excretion ($\mu\text{g/kg body weight}$) by mice that received one of the various chelators or saline from day 3 to day 7 (post-Cd administration) is shown in Figure 15. During the first day of treatment (day 3) significant increases in urinary elimination of Cd were found in mice treated with DTPA, EDTA, DMSA, and BAL. DTPA produced the greatest increase in urinary elimination (approximately 100-fold). Although this amount represents only 0.2% of the total Cd dose, results obtained during successive

days (days 4 to 7) indicate DTPA, EDTA, DMSA and BAL significantly increased the urinary excretion of Cd, with the exception of DMSA on day 7 which was not significantly different from controls. No significant effects on urinary elimination of Cd were observed in mice treated with DDC, NTA, or PEN.

The amount of Cd eliminated into urine as a result of treatment with DTPA was remarkably constant on successive days. Approximately $2 \mu\text{g Cd/kg}$ was eliminated daily during DTPA treatment. In contrast, the urinary elimination of Cd in mice treated with BAL progressively increased during the course of treatment.

The effect of repeated daily administration of chelators on the fecal elimination of Cd was also determined. Fecal excretion, the major route of elimination of Cd, decreased with time after administration of Cd in control animals. The excretion of Cd into feces of control mice during the first and second days was 96.4 ± 11.7 and $40.5 \pm 3.6 \mu\text{g Cd/kg}$, respectively. Only DDC significantly increased the fecal elimination of Cd (Table 2), ranging from a 2-fold (day 3) to a 10-fold increase (day 7). All other chelators had no effect on fecal Cd elimination (data not shown).

Histopathological examination indicated that no significant pathological lesions were present in

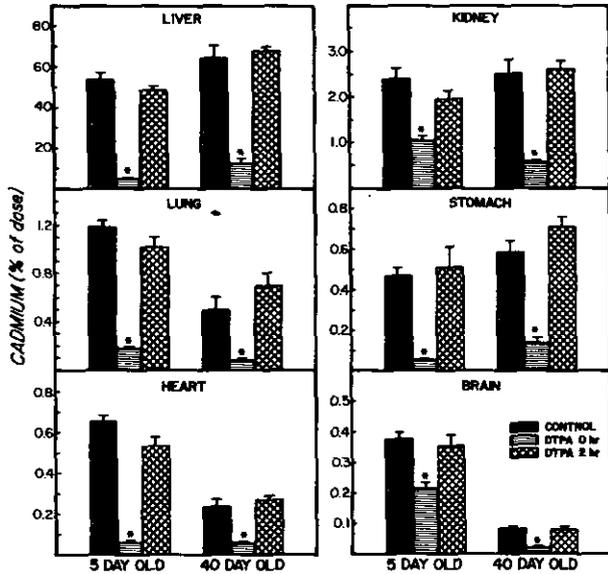


FIGURE 14. Effect of DTPA treatment (90 mg/kg, IP) at various intervals (0 and 2 hr) following IV Cd administration (1 mg/kg; time 0) on the organ retention of the metal 24 hr later in young adult (40-day-old) and newborn (5-day-old) rats. Values represent $\bar{x} \pm SE$ of six rats which, in the case of the newborns, were derived from three or more litters. An asterisk indicates significantly different ($p < 0.05$) from the appropriate control (no DTPA). From Waalkes et al. (3).

renal tissue of any of the treatment groups.

In summary, chelation therapy of Cd intoxication is extremely effective when administered immediately after Cd exposure. However, the effectiveness of chelation therapy decreases markedly with time after exposure. This rapid decrease in effectiveness occurs much quicker than the increase in metallothionein after Cd exposure. The decreased effectiveness of chelators with time after Cd exposure appears to be due to the intracellular distribution of Cd and the extracellular distribution of the chelators. While chelators are much less effective when not administered immediately after Cd exposure, the present study dem-

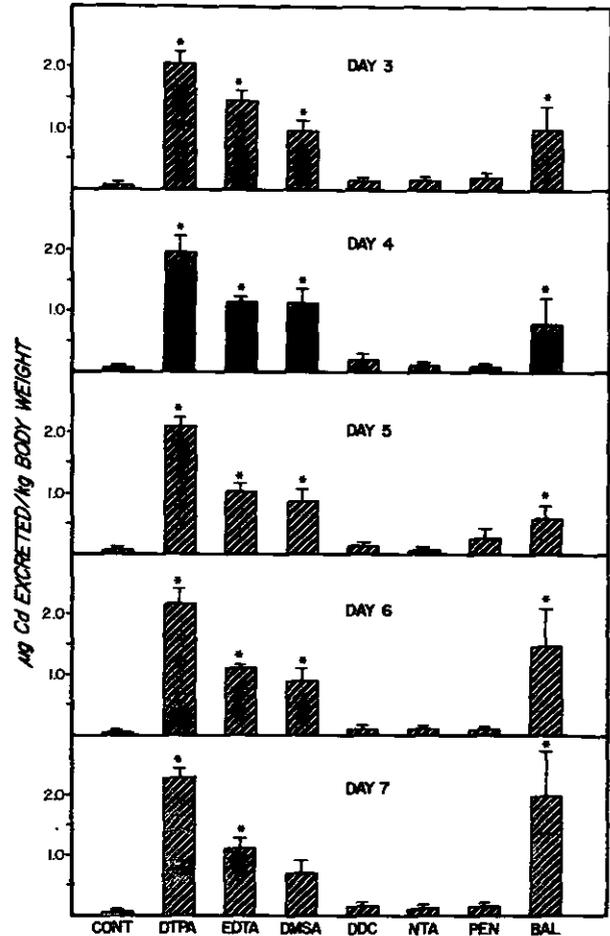


FIGURE 15. Effect of repeated daily administration of various chelating agents on the daily urinary elimination of Cd (μg Cd excreted/kg body weight). The indicated chelating agents or saline (control) was administered for 5 days beginning 48 hr after Cd administration (days 3 to 7). Bars indicate SE, $n = 5$. An asterisk indicates significantly different from control. From Cantilena and Klaassen (4).

onstrates that repeated administration of chelators does enhance the urinary excretion of Cd and provide hope that long-term therapy may decrease body burden and toxicity of Cd.

Table 2. Effect of DDC on the fecal elimination of Cd.^a

Day	Cd excretion, $\mu\text{g}/\text{kg}$ body weight	
	Control	DDC
3	8.77 \pm 0.27	20.3 \pm 1.27*
4	3.30 \pm 0.30	22.7 \pm 2.80*
5	2.01 \pm 0.26	17.3 \pm 1.12*
6	1.28 \pm 0.21	14.5 \pm 1.43*
7	1.38 \pm 0.20	15.8 \pm 0.61*

^aDaily administration of DDC (0.35 g/kg IP) was initiated 48 hr after Cd (1 mg Cd/kg, IV) and continued for 5 days. Cd excretion is shown for each 24-hr period.

*Significantly different from control ($p < 0.05$).

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