

Effects of Chelating Agents on Oral Uptake and Renal Deposition and Excretion of Cadmium

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The gastrointestinal absorption, transport, tissue deposition and excretion of cadmium was studied in adult male mice given a single oral LD₅₀ dose of ¹⁰⁹Cd-labeled CdCl₂ alone or in combination with nitrilotriacetic acid (NTA), sodium tripolyphosphate (STPP) or ethylenediaminetetraacetic acid (EDTA). Blood, intestinal mucosa, liver and kidneys were analyzed for ¹⁰⁹Cd at different times after exposure and the influence of the chelating agents on Cd binding to metallothionein and other tissue ligands was also studied. Acute toxicity was noted.

Complex formation between Cd and EDTA was studied in solutions containing Cd:EDTA at 1:04 and 1:4 molar ratios. Adult male mice were exposed orally or by direct infusion into the stomach to either of the two solutions (containing an LD₅₀ dose of Cd). Body retention and tissue deposition of Cd was recorded after 4 (direct infusion) or 21 days (oral exposure), and the mortality in different exposure groups observed.

Adult male were also exposed to a low oral dose of ¹⁰⁹Cd-labeled cadmium (0.5 mg/kg), followed by 18 months continuous administration of NTA, (500 ppm) STPP (500 ppm) or EDTA (50 ppm) in the drinking water or the chelating agent in combination with Cd (50 ppm), Cd alone (50 ppm) or deionized water. Whole-body retention of ¹⁰⁹Cd, tissue deposition of ¹⁰⁹Cd and total Cd and development of proteinuria were observed.

When cadmium was given with an excess of EDTA, all Cd ions were bound in a 1:1 Cd-EDTA complex. Decreased acute toxicity was observed which was related to increased body elimination of cadmium. The Cd passes though the body still bound to EDTA and is excreted via the kidneys in this form. Similar results were found in mice exposed to Cd + NTA, while gavage of Cd + STPP led to an initially decreased systemic uptake of Cd and thereafter to a prolongation of the biological half-time and thus a comparatively higher body retention of the metal.

Cd may form a 2:1 complex with EDTA in the presence of excess cadmium. An increased retention and toxicity of cadmium was seen after direct infusion of this solution, while gavage resulted in a decreased toxicity. The effect of different chelating agents on acute cadmium toxicity and metabolism seemed to be due to changes in the stability of the administered chelate complexes, due to variation in pH and to the availability of metal binding ligands such as metallothionein *in vivo*. NTA, STPP and EDTA had no effect on the metabolism or toxicity of cadmium after long-term low dose oral exposure.

Introduction

The role of chelating agents in heavy metal metabolism and toxicity has been much discussed and subjected to several investigations. Due to its metal-binding properties, EDTA has found wide application in several fields and is also used as an antidote for metal poisoning in human beings (1). Several studies have shown that EDTA is superior to BAL (British Anti-Lewisite) in the protection against acute cadmium poisoning in animals

(2-6). However, EDTA is also known to be potentially nephrotoxic to animals (4,7-10), and renal lesions have been observed in human beings given chelation therapy with EDTA against lead poisoning (11,12). Other toxic effects including hepatic lesions and degenerative changes in the intestinal cells have also been reported (10,13,14). EDTA can further exert adverse effects by forming complexes with many essential metals in the body (such as Ca, Mg, Cu and Zn), resulting in alterations of the homeostasis of calcium in humans (15) and teratogenic effects in rats due to zinc deficiency (16). It has been re-

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Table 1. Designs of the various experiments reviewed in this chapter.

| Experiment | Groups and number of animals (sex) | | Routes of single exposure to radioactive cadmium | Single dose of cadmium, dose/kg body weight | Single dose of chelating agents, dose/kg body weight | Cd or chelating agents in drinking water (ppm) | Time of observation |
|------------|------------------------------------|--|--|---|--|--|---------------------|
| I | 4+4+4+4 (M) | | Oral | 60 mg | — | — | 5 min |
| | 4+4+4+4 (M) | | " | 60 mg | 600 mg NTA | — | 30 min |
| | 4+4+4+4 (M) | | " | 60 mg | 600 mg STPP | — | 1 hr |
| | 4+4+4+4 (M) | | " | 60 mg | 600 mg EDTA | — | 5 hr |
| II | 4 + 9 (M) | | Oral | 60 mg | — | — | 1 + 5 + 96 hr |
| | 4 + 9 (M) | | " | 60 mg | 60 mg EDTA | — | |
| | 4 + 9 (M) | | " | 60 mg | 600 mg EDTA | — | |
| | (3+3+4) (M) | | Direct infusion | 60 mg | — | — | |
| | (3+3+4) (M) | | into the stomach | 60 mg | 60 mg EDTA | — | |
| | (2+2+2) (M) | | | 60 mg | 600 mg EDTA | — | |
| | 5 (M) | | | 0.9% NaCl (controls) | — | — | 14 days |
| III | 4 + 9 (M) | | Oral | 60 mg | — | — | 5 hr + 21 days |
| | 4 + 9 (M) | | " | 60 mg | 600 mg NTA | — | |
| | 4 + 9 (M) | | " | 60 mg | 600 mg STPP | — | |
| IV | a 8+8 (F) | | Oral | 15 µg ^a | — | — | 18 months |
| | b 8+8 (F) | | " | 15 µg ^a | — | Cd 50 | |
| | c 8+8 (F) | | " | 15 µg ^a | — | NTA 500 | |
| | d 8+8 (F) | | " | 15 µg ^a | — | Cd + NTA 50 + 500 | |
| | e 8+8 (F) | | " | 15 µg ^a | — | STPP 500 | |
| | f 8+8 (F) | | " | 15 µg ^a | — | Cd + STPP 50 + 500 | |
| | g 8+8 (F) | | " | 15 µg ^a | — | EDTA 50 | |
| | h 8+8 (F) | | " | 15 µg ^a | — | Cd + EDTA 50 + 50 | |

^aDose/animal.

ported in animal experiments that simultaneous exposure to Cd and EDTA by subcutaneous injection markedly reduced cadmium retention in kidney (4,5) and resulted in increased urinary excretion of cadmium about 500-fold (4). However, when EDTA was administered some time after the Cd dose, the effect of the chelating agent was less dramatic (4,17). These results indicate that EDTA cannot interfere when the metal is already bound to metallothionein in the tissue. EDTA has also been shown to enhance the gastrointestinal absorption of lead (18–21) which has also been seen for cadmium (22) in *in vitro* perfusion studies on rat intestine. In contrast to these observations, decreased absorption of cadmium from the gastrointestinal tract of rats has been reported after direct infusion of Cd-EDTA into the duodenum (23).

The common use of either NTA (nitrilotriacetic acid) or STPP (sodium tripolyphosphate) in modern detergents resulted in studies to clarify the role of these chelating agents in heavy metal toxicity. NTA was shown to increase the acute toxicity of cadmium in rats when administered together with the metal as a single subcutaneous dose (24). This was later confirmed in studies on mice (25), where also STPP given subcutaneously

with Cd increased the acute toxicity of the metal due to extensive necrotic changes in the livers of the exposed animals (25). In contrast to these results, NTA given orally together with cadmium has been shown to reduce deposition of cadmium in liver, kidney and muscle in rats and to increase urinary and fecal excretion of cadmium (26,27), thereby reducing the toxic effects of the metal. The reason for the different effect of NTA, which is influenced by the route of administration, is probably a change in stability of the chelate complex. It has been shown that the toxic effects of many metals *in vivo* depend on the metal:chelant ratio (28) due to a competition between the chelating agent and various tissue ligands for the metal ion.

The present paper summarizes some further results from experiments on mice concerning gastrointestinal absorption, transport, tissue deposition and excretion of cadmium after single and repeated exposure alone or in combination with either EDTA, NTA or STPP.

Methods

Experimental Design

CBA mice, inbred for more than 200 genera-

tions (Institute of Genetics, Stockholm University), were used in all experiments. In the long-term experiment, animals of both sexes were studied (Table 1). The other experiments were performed on male mice, since, in pilot studies of the LD₅₀ of cadmium, male mice were found more susceptible to adverse effects of cadmium compared to females. Animals were housed in plastic cages at a constant light and dark cycle, with a temperature of 20°C. Air humidity varied between 25 and 40%. The animals were fed an ordinary pelleted diet and had free access to deionized water. All animals were 3 months of age at the beginning of each experiment.

Chemicals

A carrier-free solution of ¹⁰⁹CdCl₂ ($T_{1/2} = 1.3$ yr, Radio-Chemical Center, Amersham, U.K.) was mixed with nonradioactive cadmium chloride in such proportions that concentration and activity would fit the purpose of each study (listed in Table 1).

The chelating agents EDTA, NTA and STPP were administered alone or simultaneously with cadmium as a single dose either by gavage (experiments I and III) or by direct infusion into the stomach (experiment II), or given in the drinking water for 18 months (experiment IV). Routes of exposure and concentration of chelating agents and cadmium used in each experiment are given in Table 1.

Complex Formation between Cd(II) and EDTA

The complex formation between Cd(II) and EDTA was determined by a potentiometric method in solutions containing varying amounts of cadmium (6–50 mmole/L) and EDTA (1–100 mmole/L) in a pH range from 1.8 to 6.5. Stability constants for the formation of several complexes (including Cd–EDTA and Cd₂–EDTA) were evaluated and equilibrium distribution of the various complexes formed between Cd(II) and EDTA (as a function of pH and the concentration of complex-forming components) was calculated in the solutions used for animal exposure in experiments I and II. Further details concerning these mechanisms are given in a separate publication (29,30).

Metal Analysis

Radiolabeled Cadmium (¹⁰⁹Cd). Whole-body and organs was measured by use of gamma scintillation spectrometry (29).

In experiment I the duodenum and small intes-

tine were removed immediately after sacrifice, cut open and carefully washed with isotone saline. The concentration of radiolabeled cadmium in these organs was also determined by gamma scintillation spectrometry with a Nuclear Chicago 8742 Autogamma counting system. All countings were compared to appropriate standards containing a defined amount of ¹⁰⁹Cd-labeled cadmium.

Whole blood (about 0.5 mL/animal) was taken from mice in experiment I by heart puncture under light ether anesthesia immediately before sacrifice, and centrifuged for 2 min at 20,000 rpm in a microcentrifuge. The concentration of radiolabeled cadmium was determined in whole blood, plasma and red cells according the technique described above for organ samples.

Total Concentration of Metals (Cd and Zn). Metal concentrations in all solutions used for exposure were analyzed by flame atomic absorption spectrometry (AAS) as described by Elinder et al. (31).

Total concentrations of cadmium and zinc in liver, kidney, kidney cortex and pancreas (experiment IV) were determined by flame-AAS or, in

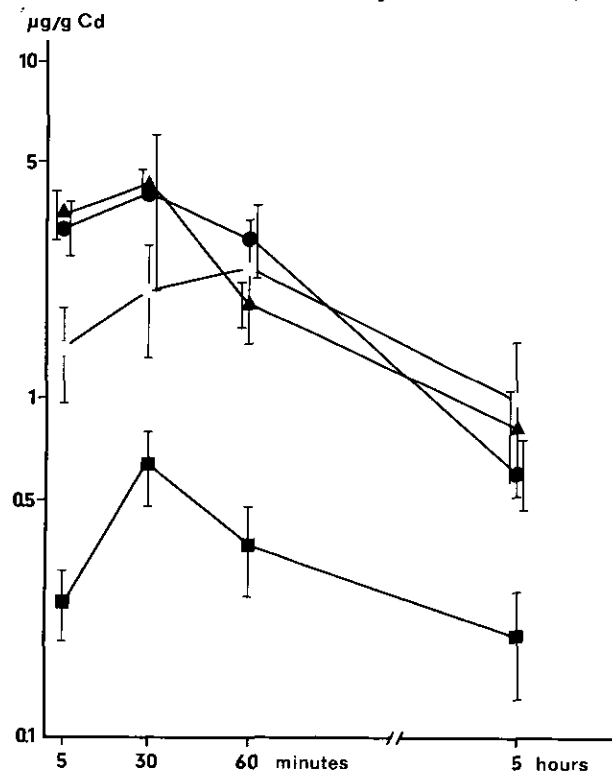


FIGURE 1. Concentration of cadmium in whole blood at different times after a single oral dose of cadmium alone or in combination with chelating agents: (▲) Cd + EDTA; (○) Cd; (●) Cd + NTA; (■) Cd + STPP. Bars denote standard error.

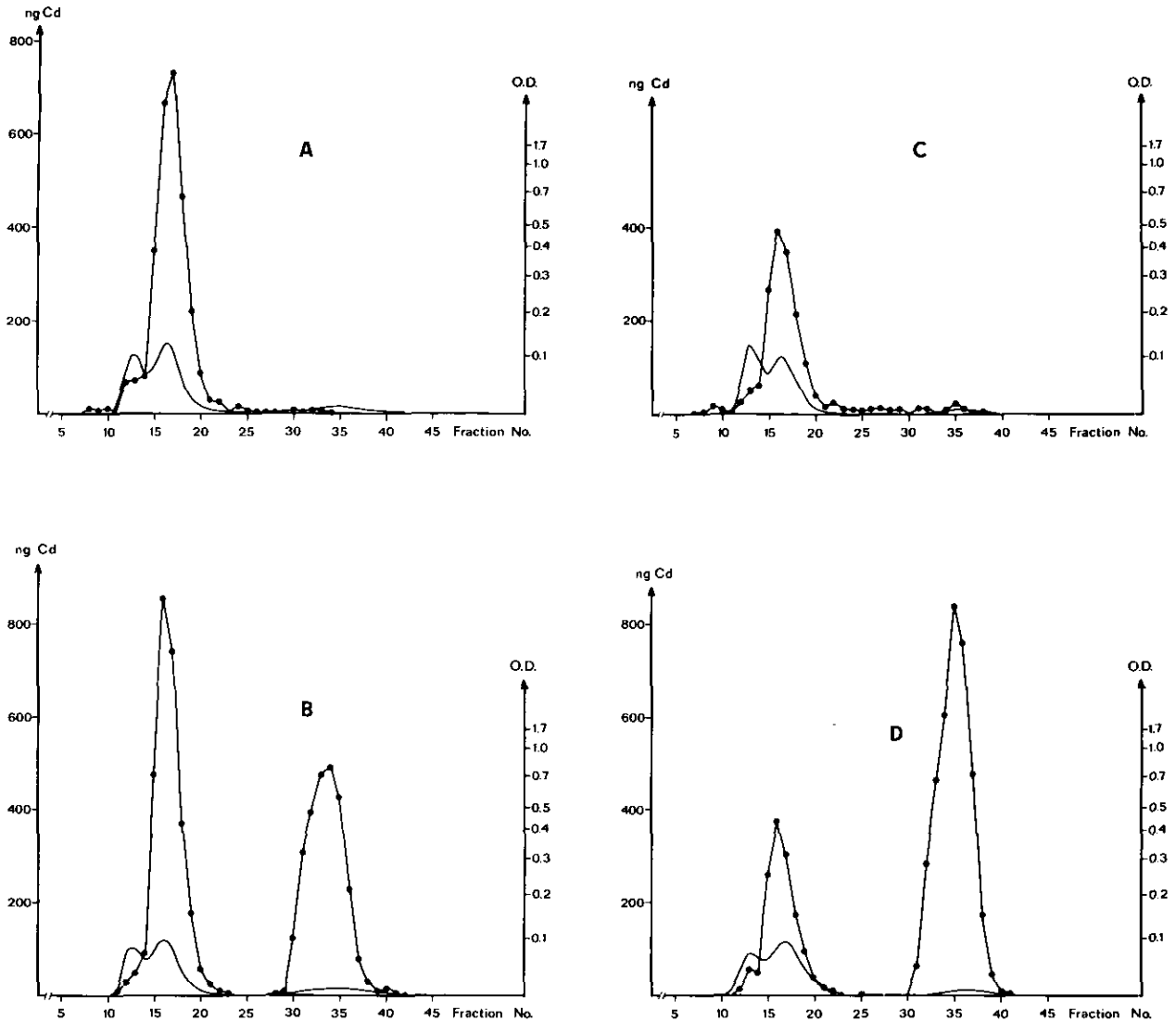


FIGURE 2. Distribution of cadmium among mouse plasma proteins 30 min after exposure to a single oral dose of: (A) Cd alone, (B) Cd + NTA, (C) Cd + STPP or (D) Cd + EDTA: (●—) ^{109}Cd -labeled cadmium in 5 mL fraction; (—) O. D. at 254 nm.

cases of low metal content, heated-graphite atomizer (HGA)-AAS was used after dry ashing (32).

The accuracy of the cadmium analysis in tissues has been controlled by measurements of cadmium in reference materials (NBS liver and Orchard leaves). Furthermore, comparison with other laboratories using AAS or neutron activation analysis has shown consistent results (33).

Analytical Technique for Studying Metal Protein Binding

Preparation of tissue samples (duodenum and small intestine, experiment I; liver and kidneys, experiments II-III) for analysis of content and distribution of cadmium among different pro-

teins, included homogenization, ultracentrifugation and separation of the organ supernatants on a G-75 Sephadex column. Fractions of 5 mL were eluted with 0.01 M Tris-HCl buffer. In experiment I, samples of duodenum from two mice in the same exposure group were pooled and analyzed accordingly.

Plasma samples from two mice in the same exposure group (experiment I) were pooled and separated on a G-75 Sephadex column in fractions of 5 mL by elution with 0.01 M Tris-HCl buffer. The content of radiolabeled cadmium in each fraction was determined by gamma scintillation counting as described above for organs.

The G-75 Sephadex column used for separation of proteins was calibrated using a protein calibra-

tion kit with proteins of specified molecular weights (29,34).

Evaluation of Proteinuria

Proteinuria (experiment IV) was determined qualitatively and quantitatively according to the method described by Nordberg and Piscator (35). A densitometer was used for evaluation of electrophoretic patterns. Patterns having a β -globulin scanned surface exceeding 8% of the prealbumin scanned surface were classified as suspected pathological, and patterns with a β -globulin area exceeding 24% of the peak with the largest area were designated as clearly pathological proteinuria. These figures are in approximate agreement with the β -peak heights of 10% or 30% of the highest peak in patterns designated as suspected or clearly pathological by Nordberg and Piscator (35).

Statistical Analysis

The Student's *t*-test was used to calculate the significance of differences in metal content in organs of mice in different exposure groups. Analysis of variance was used for calculation of statistically significant differences of the same parameters among several groups. When comparing

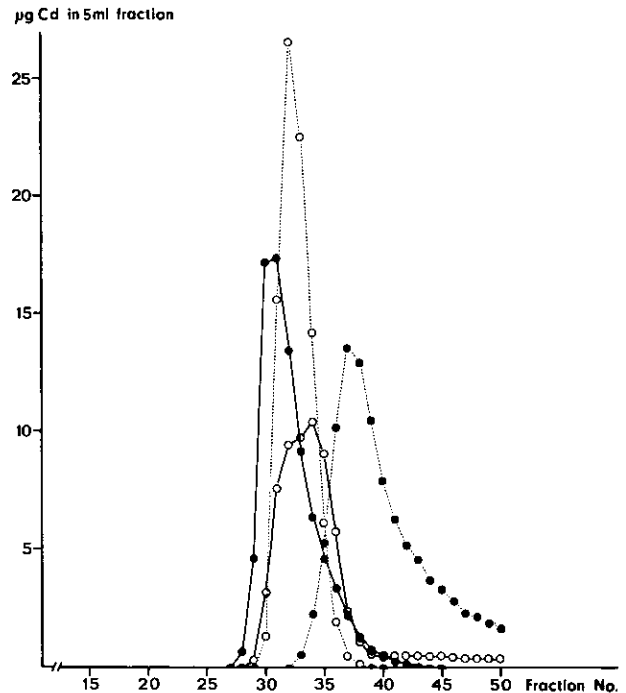


FIGURE 3. Distribution of radiolabeled cadmium in 5 mL fraction after separation of the different solutions used for animal exposure on a G-75 Sephadex column: (●- -) Cd; (●-) Cd + EDTA; (○- -) Cd + NTA; (○-) Cd + STPP.

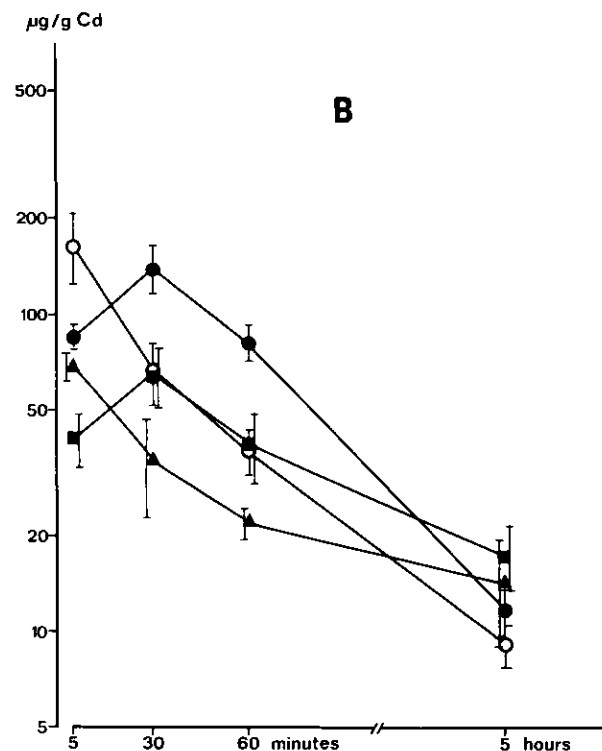
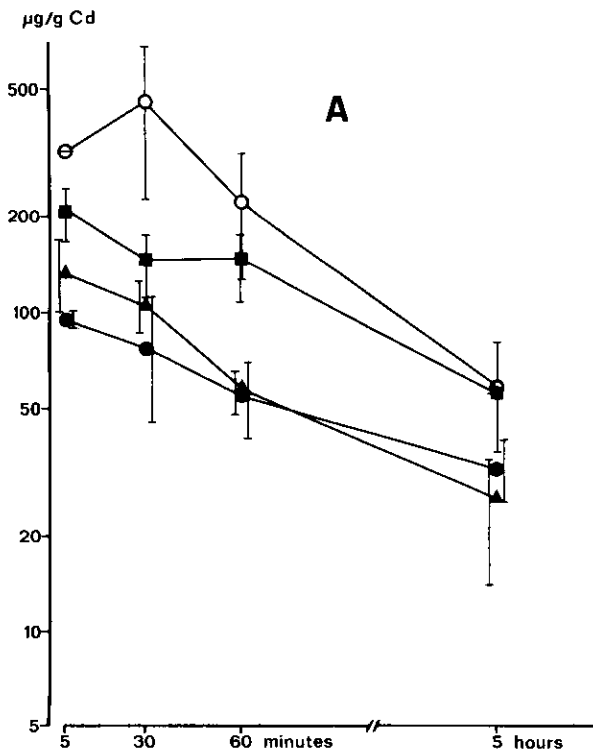


FIGURE 4. Concentration of cadmium in (A) duodenum and (B) small intestine of mice at different times after exposure to a single oral dose of cadmium alone or in combination with chelating agents. Symbols as in Fig. 1.

groups of less than five animals in each the Mann-Whitney *U*-test and Kruskal-Wallis one-way analysis of variance by ranks were used (36).

Results

Acute Exposure

Absorption and Transport in Blood. Cadmium concentrations in whole blood during the first hours after a single oral dose of cadmium alone or in combination with either EDTA, NTA or STPP (experiment I) are shown in Figure 1. At 5 and 30 min after exposure, twice as high blood cadmium concentrations were observed in mice given Cd + EDTA and Cd + NTA compared to those given Cd alone. However, at 1 and 5 hr after exposure no such differences were seen among these groups. At all times of observation, lower concentrations of cadmium in blood were observed in mice exposed to Cd + STPP compared to all other exposure groups.

Cadmium was recovered mainly in plasma during the first hours following exposure. The distribution of cadmium among plasma proteins 30 min after exposure is seen in Figure 2. After exposure to Cd alone or Cd + STPP (Fig. 2A and 2C), cadmium was eluted together with high molecular weight proteins. Mice exposed to cadmium in combination with either NTA or EDTA (Figs. 2B and 2D) had a substantial part of the cadmium bound in the respective chelate complexes as well (see Fig. 3). Distribution of cadmium in plasma 5 and 60 min after exposure was similar to that observed after 30 min.

To gain further information about the mechanisms by which these chelating agents act in the transport of cadmium from the intestine to blood, the concentration and distribution of cadmium in the intestinal mucosa was studied. Generally, higher concentrations of cadmium were seen in the duodenal mucosa (Fig. 4A) compared to the that of small intestine (Fig. 4B). Maximum cadmium concentrations in the intestinal mucosa was noted between 5 and 30 min after exposure in all mice. Cadmium distribution among proteins in the mucosa of the small intestine was determined 30 min and 5 hr after exposure by gel chromatography, and the results are given in Figure 5. Mice exposed to cadmium alone or Cd + STPP had the main part of the cadmium in the intestinal mucosa bound to high molecular weight proteins 30 min after exposure (Fig. 5A and 5C). Cadmium in the Cd + STPP-exposed mice was also partly eluted with a low molecular weight protein. Mice given Cd + NTA or Cd + EDTA had most cadmium bound in the chelate

form (Fig. 3) in the intestinal mucosa 30 min after exposure (Figs. 5B and 5D).

At 5 hr after exposure, a general transfer of mucosal cadmium from the high to a low molecular weight protein was seen in all of the exposed mice (Figs. 5E-H). The molecular weight of this protein was estimated to about 10,000 daltons, which corresponds to that of metallothionein observed after gel chromatography (37). Thus, in spite of no further characterization, this protein will be called metallothionein in the following text.

Retention and Distribution. In experiment II, mice were exposed to single doses of CdCl₂ alone or in combination with varying amounts of EDTA [in Cd(II)EDTA molar ratios of 1:0.4 or 1:4, respectively], either via stomach tube (gavage) or by direct infusion into the stomach. At 1, 5, 24 and 96 hr after exposure the retention of cadmium in liver and kidney was determined. Distribution of cadmium among proteins in these organs was also studied 5 hr after exposure.

At 1 hr after gavage the liver/kidney cadmium concentration ratio was about 1:0.25 in mice given Cd alone, about 1:0.5 in mice given Cd + EDTA (1:0.4) and about 1:1 in mice given Cd + EDTA (1:4).

About four times higher cadmium concentrations were found in liver and kidney 1 hr after direct infusion into the stomach compared to gavage ($p < 0.05$) (Table 2). Mice exposed to Cd + EDTA (1:0.4) by direct infusion had more than twice as high liver cadmium concentrations and almost five times higher kidney cadmium concentrations 1 hr after exposure compared to mice given Cd alone ($p < 0.05$), while mice given Cd + EDTA (1:4) had very low concentrations of cadmium in these organs.

In order to obtain an explanation of these results, the complexation between Cd(II) and EDTA was determined in the different solutions used for exposure. In the solution with an excess of EDTA in relation to cadmium, almost all Cd(II) ions were bound to EDTA in a stable 1:1 complex, Cd-EDTA (with a logarithmic stability constant of 15.30 ± 0.04) (Fig. 6).

It was further shown that EDTA may form a 2:1 complex Cd₂-EDTA (with a logarithmic stability constant of 16.58 ± 0.07) in the presence of excess cadmium. This binuclear complex accounted for about 35% of all complexes in the Cd + EDTA (1:0.4) solution within the pH range 4-7 (Fig. 6). The rest of the Cd(II) ions in this solution were either free (about 45%) or bound in a 1:1 complex with EDTA (about 20%). Further details about complexes formed between Cd(II) and EDTA have

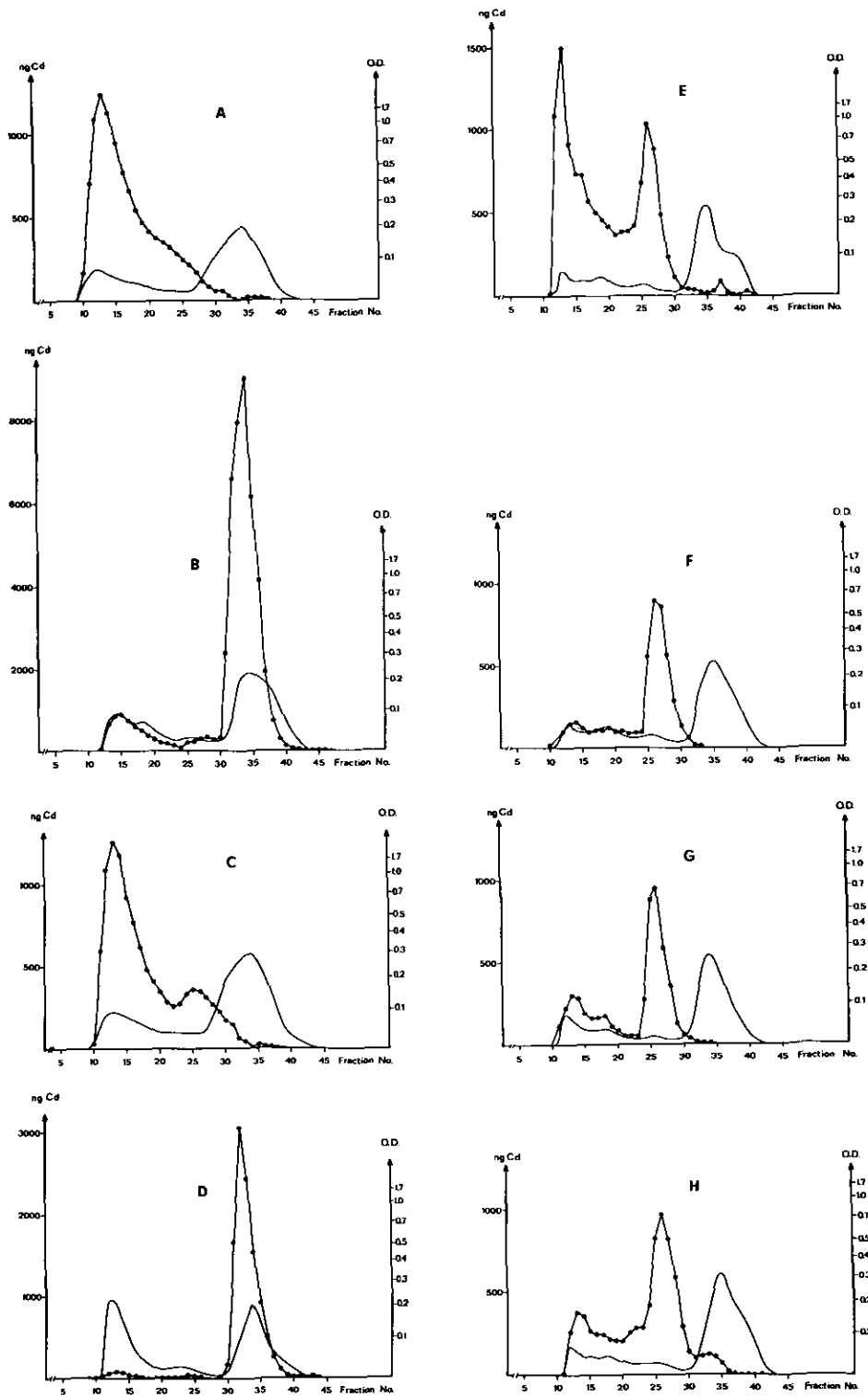


FIGURE 5. Distribution of cadmium among proteins in mouse small intestinal mucosa (A-D) 30 min or (E-H) 5 hr after a single dose of Cd alone, Cd + NTA, Cd + STPP or Cd + EDTA, respectively. Symbols as in Fig. 2.

Table 2. Concentrations of cadmium in organs of mice 1 hr after a single dose of Cd alone or Cd in combination with various amounts of EDTA by either oral exposure or direct infusion into the stomach (under narcosis).

| Groups (molar ratio) | Cd concn, mg/kg | | | | | | | |
|-------------------------|-----------------|-------|---------|-----------------|-------|---------|----------|---|
| | Oral exposure | | | Direct infusion | | | | |
| | | Liver | Kidneys | <i>n</i> | Liver | Kidneys | <i>n</i> | |
| Cd | MV | 16.34 | 5.47 | 4 | MV | 68.47 | 29.48 | 3 |
| | SD | 6.80 | 2.32 | | SD | 3.02 | 6.60 | |
| Cd + EDTA (1:0.4) | MV | 6.42 | 3.30 | 4 | MV | 158.54 | 151.07 | 3 |
| | SD | 3.42 | 1.70 | | SD | 60.97 | 55.77 | |
| Cd + EDTA (1:4) | MV | 10.10 | 10.52 | 4 | MV | 5.43 | 8.12 | 2 |
| | SD | 4.11 | 6.17 | | SD | 0.60 | 1.38 | |

been reported by Engström et al. (29) and Jawaïd (30).

The distribution of cadmium different proteins in liver and kidney was determined at all times of sacrifice. At 1 hr after exposure, most cadmium in the liver of all mice was bound to high molecular weight proteins. Mice exposed to Cd alone had most cadmium in kidneys bound to high molecular weight proteins (Fig. 7). Almost all cadmium in kidneys of mice exposed to Cd + EDTA (1:4) was bound in the Cd-EDTA complex, while only part of the cadmium given in the Cd + EDTA (1:0.4) solution was bound to the chelant, and the rest bound to high molecular weight proteins. An increased 24-hr elimination of cadmium was seen when given together with EDTA, which accounted for about 45% of the dose in mice given Cd + EDTA (1:4), about 35% in mice given Cd + EDTA (1:0.4) compared to only about 20% in mice given Cd alone.

At 5 hr after exposure, mice given Cd + EDTA had more Cd bound to metallothionein in the liver than mice given Cd alone (Figs. 8A-C). The distribution of cadmium among proteins in the kidneys after gavage of Cd + EDTA did not show any marked differences to that of Cd alone, i.e., the largest part was recovered in the high molecular weight protein fractions, except for a minor peak corresponding to the elution of the complexes (Figs. 8D-F).

In another study (experiment III) the influence of NTA and STPP on cadmium retention in liver and kidney of mice was studied 5 hr and 21 days following single oral doses of Cd alone or in combination with the respective chelating agents.

At 5 hr after exposure no difference in retention of cadmium was seen among mice given Cd alone or Cd + NTA while animals given Cd + STPP had accumulated less cadmium (about one-third in liver and one-fifth in kidney) compared to mice given Cd alone ($p < 0.001$).

The subcellular distribution of cadmium in liver and kidney 5 hr after exposure showed that

more of the total liver cadmium in mice given Cd + STPP was found in the supernatant fraction (about 60%) compared to 35-40% in mice given either Cd + NTA or Cd alone. A similar distribution pattern was also observed in the kidneys. Mice exposed to Cd + NTA had relatively more cadmium bound to high molecular weight proteins in the liver compared to that seen in mice given Cd alone (Figs. 9A and B). Cadmium given in combination with STPP was mainly recovered in the metallothionein fraction (Fig. 9C). Most cadmium in kidney of all mice was bound

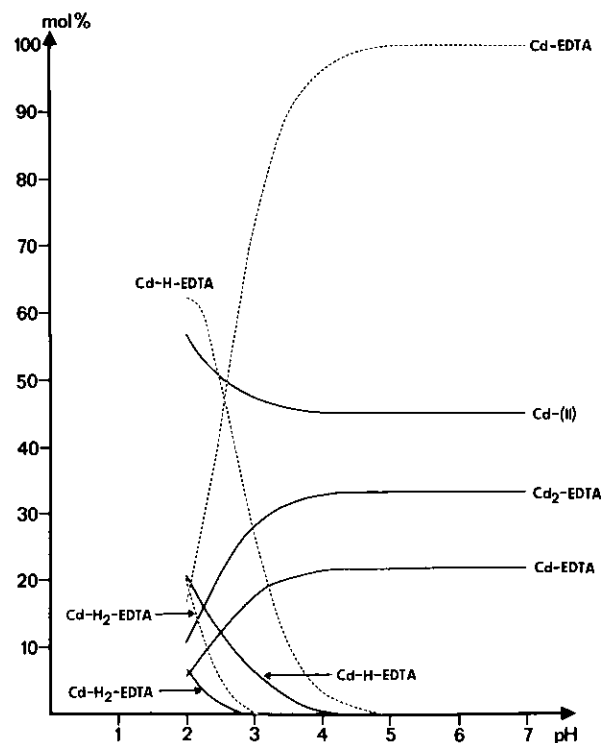


FIGURE 6. Mole per cent distribution of different Cd-EDTA complexes as a function of pH in solutions with different concentrations of Cd (II) and EDTA, respectively: (—) $C_{Cd} = 8.90 \times 10^{-2}$, $C_{EDTA} = 3.42 \times 10^{-2}$ M; (---) $C_{Cd} = 8.90 \times 10^{-2}$, $C_{EDTA} = 0.342$ M.

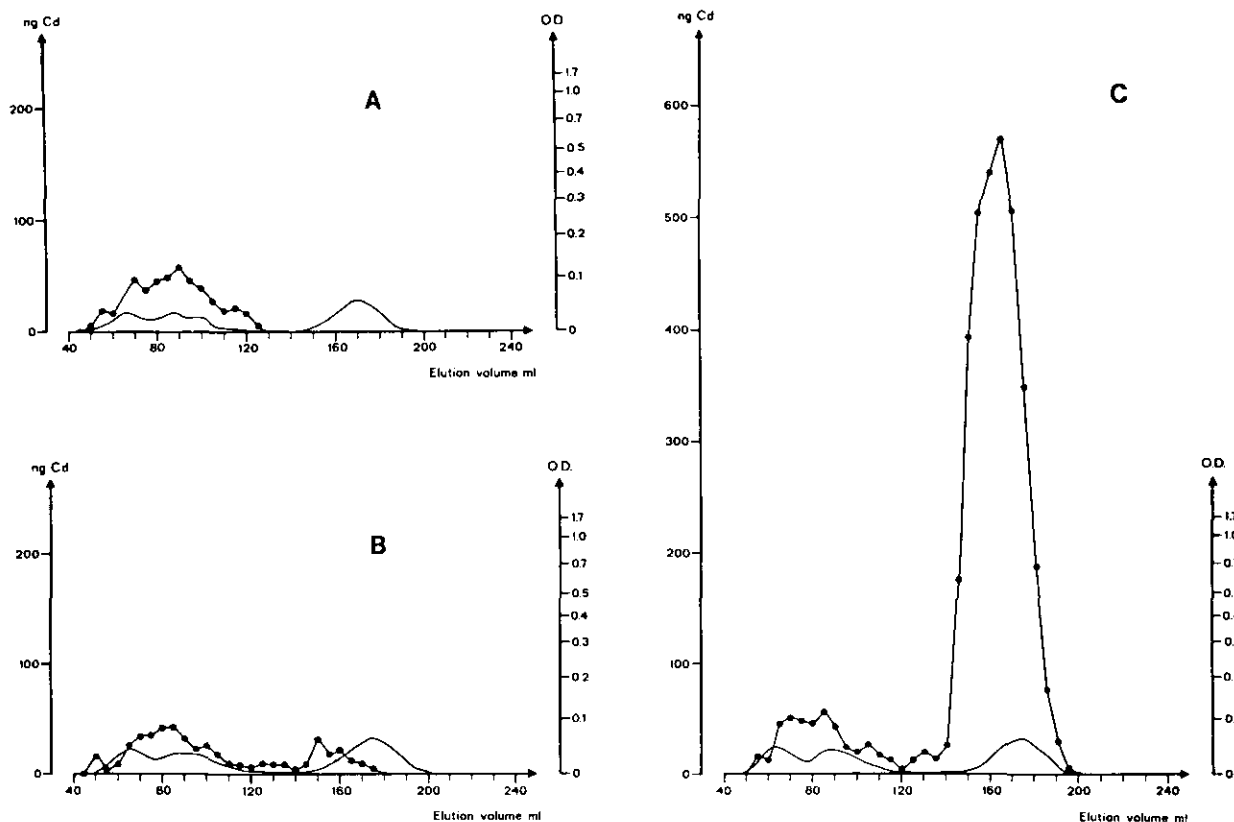


FIGURE 7. Distribution of cadmium among mouse kidney proteins, 1 hr after exposure to (A) a single oral dose of Cd alone, (B) Cd + EDTA (1:0.4) or (C) Cd + EDTA (1:4). Symbols as in Fig. 2.

mainly to high molecular weight proteins. Cd + NTA-exposed mice (Fig. 9E) did also have a prominent radioactive Cd-peak corresponding to the elution volume of the chelate complex.

Between 5 hr and 21 days after exposure, a 3- to 5-fold decrease in the cadmium concentration was observed in liver and kidney of mice given Cd + NTA, while mice given Cd + STPP on the contrary showed about a 2-fold increase of the cadmium concentration in liver and about a 7-fold increase in kidney ($p < 0.001$).

No marked change in the cadmium concentration was seen during this period in mice given Cd alone. The observed 21-day whole-body retention was 4.4% of the dose in mice given Cd alone, 2% in mice given Cd + NTA and 5.5% in mice given Cd + STPP.

Repeated Exposure

In experiment IV mice were given a single oral dose of ^{109}Cd -labeled cadmium. Some groups were thereafter administered continuous low doses of nonradioactive cadmium alone or in combination

with NTA, STPP or EDTA, in drinking water for 18 months. Other mice were given deionized water or water with addition of NTA, STPP or EDTA (see Table 1).

The total concentration of cadmium and zinc was determined in liver, kidney, kidney cortex and pancreas at sacrifice. The concentration of cadmium was highest in kidney cortex (120–160 $\mu\text{g/g}$) of all mice given cadmium in the drinking water. These animals also had higher organ concentrations of zinc. However, no substantial differences in whole-body or organ retention of either radioactive or nonradioactive cadmium were seen among groups after treatment with chelating agents.

Toxicity

The acute and chronic toxicity of cadmium was studied (by means of mortality rates) in all of these experiments for which metabolism has been described above.

Acute Toxicity. No mortality was seen in mice exposed to cadmium, and an excess of EDTA by

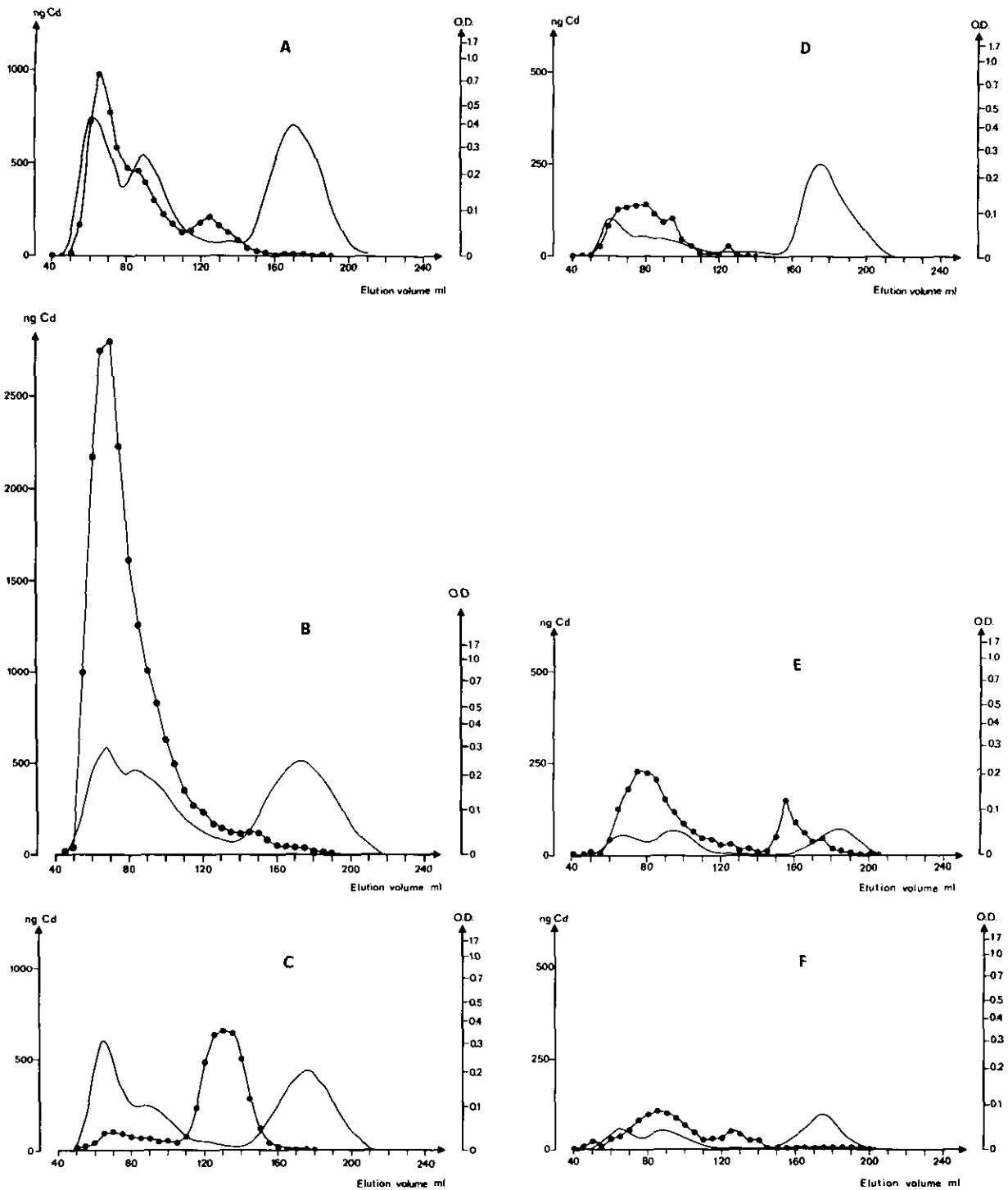


FIGURE 8. Distribution of cadmium among proteins in (A-C) mouse liver and (D-F) kidneys 5 hr after exposure to a single oral dose of Cd alone, Cd + EDTA (1 = 0.4) or Cd + EDTA (1:4), respectively. Symbols as in Fig. 2.

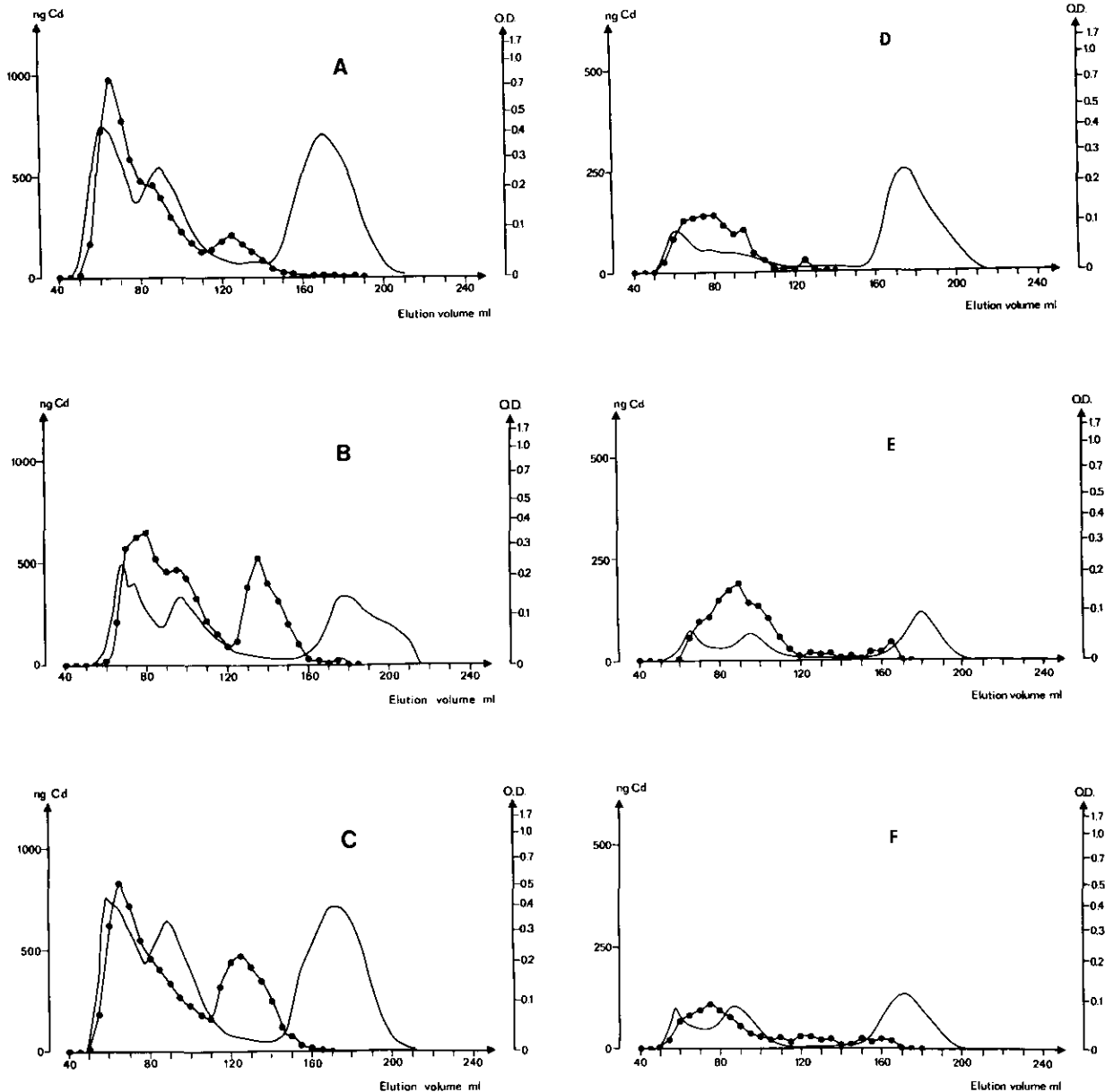


FIGURE 9. Distribution of cadmium among proteins in (A–C) mouse liver and (D–F) kidneys 5 hr after exposure to a single oral dose of Cd alone, Cd + NTA or Cd + STPP, respectively. Symbols as in Fig. 2.

either route of exposure (gavage or direct infusion). Administration of Cd + EDTA (1:0.4) resulted in a mortality rate of 0% (after oral exposure) and 75% (after direct infusion), while in mice exposed to Cd alone, the rate was 45% and 100%, respectively. The times of observation are given in Table 3.

Single oral exposure to Cd + NTA did not influence the toxicity of cadmium, while gavage of Cd + STPP markedly reduced toxicity of cadmium.

Chronic Toxicity. Mortality in experiment IV

was not increased in groups given cadmium in combination with chelating agents compared to those given Cd alone.

The number of mice showing pathological proteinuria increased during the last 9 months of the study, but only one of the Cd-exposed animals developed clearly pathological proteinuria. This low frequency may be due to the fact that the total cadmium concentration in the kidney cortex was below 200 $\mu\text{g/g}$ the level at which tubular dysfunction has been reported to appear in mice (35).

Table 3. Mortality in mice after single exposure to Cd alone or in combination with chelating agents by different routes of exposure.

| Groups (molar ratio) | Route of exposure | Mortality rate at various times | | | |
|------------------------------|----------------------|---------------------------------|-------|-------|---------|
| | | 5 hr | 24 hr | 96 hr | 21 days |
| Cd | Oral | 0/13 | 4/9 | 4/9 | 4/9 |
| Cd + NTA (1:6) | " | 0/13 | 4/9 | 4/9 | 4/9 |
| Cd + STPP (1:3) | " | 0/13 | 0/9 | 0/9 | 0/9 |
| Cd + EDTA (1:0.4) | " | 0/8 | 0/4 | — | — |
| Cd + EDTA (1:4) | " | 0/8 | 0/4 | — | — |
| Cd | Stomach infusion | 4/7 | 4/4 | 4/4 | — |
| Cd + EDTA (1:0.4) | " | 2/7 | 3/4 | 3/4 | — |
| Cd + EDTA (1:4) | " | 0/4 | 0/2 | 0/2 | — |
| NaCl (controls) ^a | " | 0/5 | 0/5 | 0/5 | — |

^aNo mortality was found in these mice during an observation time of 14 days.

Chelating agents given in combination with cadmium did not increase the frequency of either suspected or clearly pathological proteinuria.

Discussion

The decrease in acute cadmium toxicity observed in the mice given Cd and an excess of EDTA (by various routes of administration) was related to an increased body elimination of cadmium. A similar observation in rats has previously been reported after subcutaneous exposure (4). Since all Cd(II) ions in the administered solution were bound to EDTA in a 1:1 Cd-EDTA complex, it is possible that most cadmium was excreted via urine while still bound in this complex with EDTA. Further support for this assumption is provided by data showing that cadmium was transported in blood mainly bound to EDTA and the high accumulation of cadmium bound to EDTA in the kidneys 1 hr after oral exposure. In spite of a higher gastrointestinal absorption of cadmium, also reported for lead (18-21) the body burden of cadmium was decreased after oral exposure to Cd + EDTA (1:4). This was due to the increased elimination of absorbed cadmium, which has also been shown for lead (21).

In spite of the fact that most Cd(II) is detached from EDTA in the low pH of the stomach after oral exposure, the 1:1 Cd-EDTA complex is probably re-established in the intestine in the presence of excess EDTA. It is further probable that the weak Cd₂-EDTA complex is not re-established in the intestine in the presence of mucosal ligands with large metal-binding capacities. However, some 1:1 Cd-EDTA complexes will probably be found which may explain the low toxicity of cadmium following oral exposure in these mice.

The reason for an increased retention and toxicity of cadmium following direct infusion of Cd + EDTA (1:0.4) is not fully understood. However, it

can be assumed that the complexes in this solution will pass intact via the stomach into the intestinal lumen, since the gastric secretion of hydrogen ions is decreased under Mebumal narcosis (38,39). Fewer hydrogen ions are thus available to compete with cadmium for the binding to EDTA. Furthermore as the Cd₂-EDTA complex is uncharged, it will penetrate cell membranes more easily and thereby cause an increased body accumulation of cadmium. The increased accumulation in liver might exceed the upper limit for the metallothionein synthesis in response to cadmium, which has been shown to be dose-related after single intraperitoneal doses up to 3 µg Cd/kg body weight (40). When metallothionein is not available in quantities large enough to bind cadmium, the metal may cause tissue damage. This would be a probable explanation for the high mortality in the Cd + EDTA (1:0.4)-exposed mice following direct infusion.

The toxicity of cadmium after gavage of Cd alone and Cd + NTA was the same, while exposure to Cd + STPP caused a markedly decreased toxicity. Gastrointestinal absorption was increased by NTA and, as was the case in Cd + EDTA (1:4)-exposed mice, part of the cadmium was still bound to the chelant in intestinal mucosa and blood. According to whole-body measurements, total elimination of cadmium was enhanced by NTA. Since most kidney cadmium was bound to NTA 5 hr after exposure, the main route of elimination is probably via urine.

STPP, on the other hand, decreased the systemic uptake of cadmium during the first hours after exposure, as demonstrated by the low blood concentrations of cadmium in these mice. As the polyphosphate is being hydrolyzed into monophosphate in the low pH in the stomach, an insoluble cadmium orthophosphate salt (41) is probably formed by cadmium and monophosphate in the intestinal lumen. This would explain the lower absorption of cadmium following this type

of exposure. The longer retention time of cadmium in the intestinal mucosa of mice given Cd + STPP is probably related to the binding of cadmium to intestinal metallothionein.

Most cadmium in the liver was already bound to metallothionein 5 hr after oral exposure to Cd + STPP. This may be due to the fact that a very low amount of cadmium was initially absorbed in these mice. Relatively more cadmium probably binds to metallothionein already present in the liver. The relatively high cadmium content in the liver supernatant fraction 5 hr after oral exposure to Cd + STPP, compared to the other groups, also sustains this hypothesis. The binding of cadmium to metallothionein in the liver which is known to prolong the biological half-time of cadmium (42,43) would explain the higher 21-day body retention of cadmium in mice given Cd + STPP compared to Cd alone. Another explanation for this might be that the mice retaining the highest amounts of cadmium after gavage of Cd alone died within the 21-day period.

Body retention and toxicity following long-term oral low-level cadmium exposure was not shown to be influenced by either chelating agent during an observation time of 18 months. This may be due to an inability of chelating agents to interfere with cadmium already bound to metallothionein in tissue.

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