# Choroid plexus protects cerebrospinal fluid against toxic metals

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ABSTRACT **Although heavy metal ions are known to be toxic to the central nervous system (CNS), the mechanisms by which the CNS may protect itself from initial challenges of such toxic ions is unknown. The choroid plexus is the principal site of formation of the cerebrospinal fluid (CSF) which bathes the brain. We have determined in rats and rabbits that after intraperitoneal administration of lead, cadmium, mercury, and arsenic compounds, these toxic metal ions accumulated in the lateral choroid plexus at concentrations of Pb, Hg, and As that were 70-, 95-, and 40-fold higher, respectively, than those found in the CSF. Cd was not detected in the CSF. In addition, concentrations of these heavy metal ions were found to be many fold greater in the choroid plexus than in the brain or blood. The accumulation of Pb in the choroid plexus was dose-dependent and time-related. When the choroid plexus was preincubated, in vitro, with ouabain (1.5 mM), the uptake of Cd from the CSF side of the choroid plexus was inhibited 57%. Cadmium metallothionein was not found in the choroid plexus. Whereas the concentration of reduced glutathione in the choroid plexus was less than that in the brain cortex, the concentration of cystine was fourfold greater. The lateral choroid plexus sequesters Pb, Cd, As, and Hg. It appears to be one of the important mechanisms that protects the CSF and the brain from the fluxes of toxic heavy metals in the blood. —Zheng, W.; Perry, D. F.; Nelson, D. L.; Aposhian, H. V. Choroid plexus protects cerebrospinal fluid against toxic metals. *FASEB J.* 5: 2188-2193; 1991.**

*Key Words: choroid plexus • heavy metals • central nervous system • neurotoxicity • blood-CSF barrier*

AFTER EXPOSURE TO HEAVY METALS OR metalloids, central nervous system (CNS)[3] symptoms are not uncommon (1). Even though much effort has been made to identify the neurological disorders resulting from toxic exposure to heavy metals, it is surprising how little has been learned about how heavy metals and metalloids interact with specific areas of the CNS and what protective mechanisms may be localized in the CNS to protect against such environmental insults.

The choroid plexus, using active transport mechansims, extracts micronutrients from the blood and manufactures and regulates the CSF. Choroidal epithelial cells have tight junctions that hinder the diffusion of small water-soluble molecules from it to the CSF (2, 3). In addition, the choroid plexus can be likened to a "kidney" for the brain in that it maintains the chemical stability of the CSF as the kidney maintains the stability of the blood. Thus, many metabolites and compounds such as iodide (4), prostaglandins (5), salicylate (6), and cephalosporin antibiotics (7) are transported by the choroid plexus in the other direction, from the CSF to the blood.

There have been suggestions, based largely on autopsy data in humans (8–10) as well as radioautographic and incomplete data in experimental animals (11–13), that the choroid plexus may concentrate heavy metals and act as a heavy metal sink. Many of these and other studies have been contradictory (8–11), incomplete (13), and/or have lacked a comprehensive, rigorous analytical or experimental approach (8, 11, 12). For example, in some studies that attempted to demonstrate that the choroid plexus concentrates Pb ions, the conclusions were based on 3–21 cpm of radioactivity per isolated choroid plexus (13). Conclusions based on such small amounts of radioactivity are of dubious value.

The aims of the present research were to *1*) determine whether the choroid plexus sequesters heavy metals or metalloids; *2*) characterize the kinetics of the accumulation of Pb in the choroid plexus; and *3*) determine the concentrations of metallothionein, glutathione, cysteine, and sulfhydryl groups in the choroid plexus. Our experimental results indicate that the lateral choroid plexus sequesters Pb, Cd, As, and Hg. One function of the choroid plexus may be to protect the CSF and brain from toxic levels of heavy metals in the blood. An energy-dependent system appears to be involved in the sequestering of cadmium by the choroid plexus.

## MATERIAL AND METHODS

### Chemicals

Chemicals were obtained from the following sources: lead acetate, 5',5'-dithiolbis-(2-nitrobenzoic acid) (DTNB) and ouabain from Sigma, St. Louis, Mo.; cadmium chloride and monochloroacetic acid from J. T. Baker, Phillipsburg, N.J.; cadmium-109 (specific activity: 3.06 mCi/mg) from Du Pont, Claremont, Calif.; mercuric chloride from Aldrich, Milwaukee, Wis.; methyl mercuric chloride from K & K Laboratories, Cleveland, Ohio; sodium arsenate from MCB Chemists, Cincinnati, Ohio; glutathione and monobromobimane from Calbiochem-Behring, La Jolla, Calif.; 1-heptanesulfonic acid sodium salt from Eastman Kodak, Rochester, N.Y. All reagents used in this experiment were of analytical grade, HPLC grade, or the best available pharmaceutical grade.

### Animals

All rats (male, Sprague-Dawley) at the time they were used were 8–9 wk old (250–300 g). They were purchased from Harlan Sprague Dawley Inc., Indianapolis, Ind. Rabbits

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[3]Abbreviations: AAS, atomic absorption spectrophotometry; CNS, central nervous system; CSF, cerebrospinal fluid; TCA, trichloroacetic acid; HPLC, high performance liquid chromatography; DTNB, 5',5'-dithiolbis-(2-nitrobenzoic acid).



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(male, New Zealand white, 2.2–2.7 kg) were purchased from Ziela Sons Inc., Tucson, Ariz. Animals were quarantined for 1 wk after arrival and kept in a temperature-controlled, 12-h light/dark cycle facility. The rats or rabbits were fed ad libitum with a Teklad rat or rabbit diet, respectively. The diet was purchased from Teklad, Madison, Wis.

### Metal and metalloid administration and analysis

Metal salts that were injected were dissolved in saline or water. The doses that were injected i.p. in rats were as follows: 4 mg Cd (as $CdCl_2$)/kg, 27 mg Pb (as Pb acetate)/kg, 1 mg Hg (as $HgCl_2$)/kg. Because the National Research Council has recommended against using rats to study the metabolism of arsenic compounds (14), rabbits were used for the arsenic experiments and the dose was 2 mg As (as $Na_2HAsO_4$)/kg, i.v. All doses of the metal or metalloid salt injected were approximately 25–33% of its $LD_{50}$. For the Pb dose-response study, rats were administered 5.4, 10.8, 16.2, or 27 mg Pb (as Pb acetate)/kg, i.p., 4 h before necropsy. For the time course study, rats received 27 mg Pb/kg, i.p.

Twenty-four hours after administration of the metal or metalloid solution, rats or rabbits were anesthetized with 50 mg i.p. or 25 mg i.v. sodium pentobarbital/kg, respectively. Blood was obtained from the abdominal aorta. The collected blood was immediately transferred to a tube containing heparin. The animals were then decapitated. The brain was extricated from the skull and the lateral choroid plexus was removed and rinsed with 0.2 ml of saline for 15 min to remove blood.

For Pb and Cd analyses, tissues were digested with 70% $HNO_3$ (50:1 vol/wt for lateral choroid plexus and CSF; 20:1 vol/wt for brain and blood) at 110°C for 1 h in loosely capped glass tubes. A volume of 30% $H_2O_2$ (equal to half the volume of 70% $HNO_3$ used) was added dropwise to the digesting solutions which were heated for another hour at 110°C. For Hg, the digestion procedure was the same except that 12 N HCl (volume was one-fourth that of 70% $HNO_3$) was also added; the digestion tubes were tightly capped to avoid evaporation. For As, tissues were digested according to the method of Krynitsky (15).

An Instrumentation Laboratory Video 12 atomic absorption spectrophotometer (AAS) equipped with an IL-655 graphite furnace was used for Cd and Pb determinations. Absorbance was corrected for nonspecific background using the Smith-Hieftje technique. An IL model 440 atomic vapor accessory was used to measure Hg (cold vapor AA) and As (hydride generation).

### Tissue sulfhydryl analysis

Rats were anesthetized with 50 mg sodium pentobarbital/kg, i.p. The right side of the internal carotid artery was cannulated cranially with a PE-50 polyethylene tubing (Clay Adams) and the brain was perfused with 10 ml of isotonic mannitol through the cannula at a rate of 1.0 ml/min. The rats were exsanguinated immediately after perfusion was completed. The choroid plexus and brain cortex were homogenated in 0.02 M EDTA, 1:50 and 1:40 wt/vol, respectively. The concentrations of total thiol, low molecular weight thiol (LMW-, MW < 3,000), and high molecular weight thiol (HMW-, MW > 3,000) in the tissue were measured by using Ellman's reagent (16). Total thiol was estimated by mixing tissue homogenates directly with the assay solution containing 0.1 mM DTNB in absolute methanol. The LMW-thiol was assayed by first adding 50% trichloroacetic acid (TCA) (1/10 of homogenate volume) to precipitate protein. After centrifugation, the supernatant was ultrafiltered through a microconcentrator (Centricon-3, MW cutoff 3000) at 5000 × *g* for 2 h at 25°C. The filtrate was then reacted with DTNB. The absorbance was read in a Zeiss model PMQ II spectrophotometer at 412 nm against a reagent blank. GSH was used as the standard. The HMW-thiol was obtained by subtracting LMW-thiol from total thiol.

### Determination of glutathione, cysteine, and Cd-metallothionein

To prevent autoxidation during sample preparation, the choroid plexus and brain cortex were homogenized in 10% sulfosalicylic acid/1.0 mM EDTA. After centrifugation at 15,000 × *g* for 30 s, the supernatant was adjusted to pH 7–8. GSH and cysteine content of the supernatant were determined by reaction with monobromobimane followed by HPLC and fluorescence detection (17). Total cysteine was measured by incubating the supernatant with dithiothreitol followed by bimane derivatization and HPLC. Cystine was calculated by subtracting cysteine from the total. The HPLC conditions for determination of GSH and related thiols were as follows: Samples were chromatographed on an Ultrasphere-ODS IP column (25 cm × 4.6 mm, 5 μ). Buffer A was 20 mM monochloroacetic acid, 10 mM heptanesulfonic acid sodium salt in methanol; buffer B 20 mM monochloroacetic acid, 10 mM heptanesulfonic acid sodium salt in water, pH 3.3. Chromatographic conditions were: 70% B for 2 min followed by a linear gradient to 65% B over a duration of 15 min; 65% B for 2 min followed by a linear gradient to 10% B over a duration of 5 min; 10% B for 5 min; then returned to 70% B over a duration of 2 min followed by equilibrium at 70% B for 10 min. The flow rate was 1.0 ml/min. Retention times of the fluorescent bimane derivatives were: GSH, 18.9 min; CySH, 10.9 min; γ-glutamylcysteine, 19.9 min; cysteinylglycine, 22.2 min.

For determination of Cd-metallothionein in the choroid plexus, rats were injected with 4 mg Cd (as $CdCl_2$)/kg, i.p. Twenty-four hours later, the choroid plexus from three rats were removed, pooled, and homogenized in 10 mM Tris · HCl (pH 7.4) (18). The homogenate was then saturated with $^{109}Cd$. Separation and identification of Cd-MT was performed using an HPLC DEAE anion-exchange column (Waters) coupled with a Beckman model 164 uv detector and a Beckman model 171 radioisotope detector. The standard rat liver Cd-MT was prepared according to Lehman and Klaassen (18).

### $Na^+,K^+$-ATPase assay

The choroid plexus from both lateral ventricles of normal rats was removed and immediately incubated for 1 min at 37°C in artificial CSF (19). Various concentrations of ouabain were added and the incubation continued at 37°C for 15 min. At the end of the incubation, the choroid plexus was transferred onto a filter (1.27 μ) in a tiny Buchner funnel and washed, using vacuum, with 0.1 ml cold artificial CSF three times. The tissue was then homogenized in 0.2 ml of 5% glucose/0.2 M EDTA and assayed for the $Na^+,K^+$-ATPase according to Esmann (20). All samples were run in duplicate. Protein was measured using a Bio-Rad Protein Assay Kit (Bio-Rad Lab., Richmond, Calif.) using bovine serum albumin as the standard.

### In vitro uptake of cadmium by the choroid plexus

The choroid plexus from one side of the lateral ventricle was placed in medium containing 1.5 mM ouabain and in-

cubated for 15 min at 37°C. As a control, the choroid plexus from the other side of the lateral ventricle was placed in medium without ouabain. After the 15-min incubation, 5 μl of $^{109}CdCl_2$ solution was added to a final concentration of 50 μg Cd/ml (0.1 μCi $^{109}Cd$) and the incubation was continued for 10 min at 37°C. The choroid plexus was removed and washed with 0.1 ml cold artificial CSF three times. The radioactivity of the choroid plexus and that of medium was then counted using an LKB type-1282 CompuGamma counter. The tissue was then homogenized in 0.2 ml of 5% glucose/0.2 M EDTA and the protein concentration was determined.

### Statistics

The student *t* test was used to compare two means. Differences between two groups were regarded as significant if *P* values were less than or equal to 0.05.

## RESULTS

### Sequestering of heavy metals and metalloids by the choroid plexus

The results clearly demonstrated that 24 h after injection of the heavy metal ions, the lateral choroid plexus contained much higher concentrations of the metal or metalloid ions than did the CSF or brain cortex (**Table 1** and **Fig. 1**). For example, the Pb concentration in the lateral choroid plexus was 70-fold greater than in the CSF, inorganic mercury 95-fold and As 40-fold greater (Table 1). Cd was not detected in the CSF. The Pb concentration in the lateral choroid plexus was 57-fold greater than in the brain cortex (Fig. 1). Cd was 33-fold greater; inorganic mercury 12-fold, and As 13-fold greater (Table 1). On the other hand, the concentrations of Hg, Pb, and As in the CSF were 1/76th, 1/13th and 1/12th, respectively, of those found in the blood. The concentrations of Pb and Cd in the lateral choroid plexus were 6- and 13-fold greater than that found in the blood. The action of the choroid plexus in concentrating these metals from the blood appears to be against a concentration gradient.

When tissues were removed from rabbits 4 h after i.v. administration of 2 mg As/kg, the amount of As in the lateral choroid plexus was 0.279 ± 0.05; CSF, 0.005 ± 0.001; brain cortex, 0.044 ± 0.007, and blood, 0.106 ± 0.027 μg/g or ml. In addition, when rats were given 175 μCi $^{210}Po$ s.c. daily for 3 days, the radioactivity in the lateral choroid plexus was found to be fivefold greater than in the brain cortex (data not shown). Polonium-210 is a daughter of radon-222.

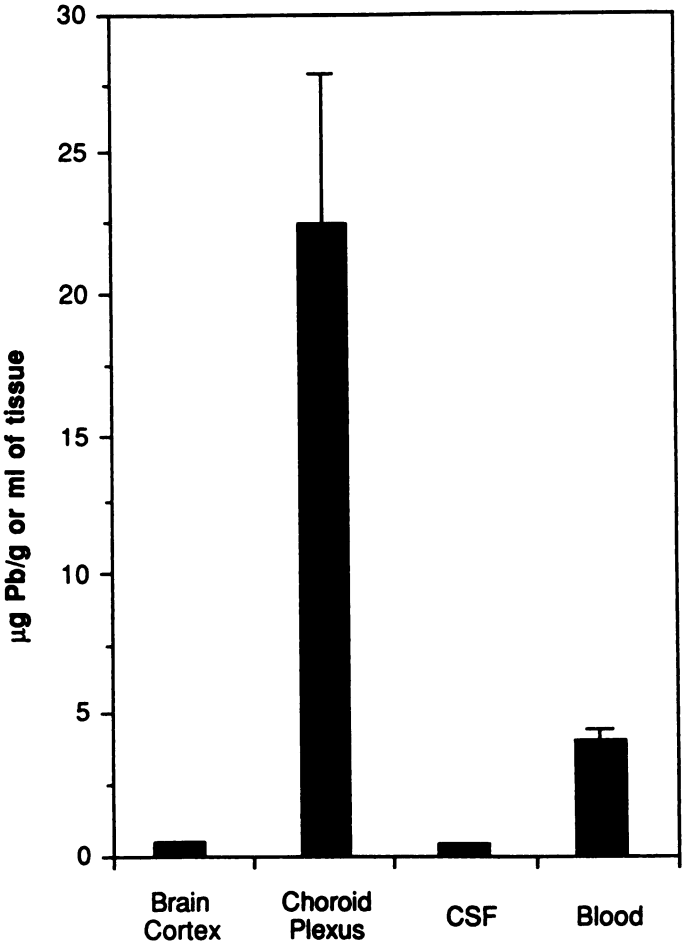


**Figure 1.** Accumulation of Pb by the lateral choroid plexus. Rats were injected i.p. with 27 mg Pb (as Pb acetate)/kg. Twenty-four hours later, tissues were removed and analyzed for Pb by AAS. For choroid plexus and CSF, each experimental measurement was performed on a pool of tissues from 3 rats. Data represent mean ± SE of three such pools ($n$ = 3); $n$ = 4 for all other tissues.

### Dose-response and time course of Pb accumulation in the choroid plexus

The concentration of Pb in the lateral choroid plexus increased proportionally with the increase in dose when rats

TABLE 1. *The lateral choroid plexus sequesters Pb, Cd, Hg, and As*

| Tissues | Group | μg/g or μg/ml[a] | | | | |
|---|---|---|---|---|---|---|
| | | Pb | Cd | As | Hg[c] | Hg[d] |
| Choroid plexus | Treatment | 22.3 ± 5.4 | 4.33 ± 1.54 | 0.079 ± 0.029 | 0.572 ± 0.160 | 1.57 ± 0.32 |
| | Control | 1.2 ± 0.3 | 0.04 ± 0.01 | <dl[b] | <dl | <dl |
| CSF | Treatment | 0.32 ± 0.08 | <dl | 0.002 ± 0.001 | 0.006 ± 0.002 | <dl |
| | Control | <dl | <dl | <dl | <dl | <dl |
| Brain cortex | Treatment | 0.39 ± 0.06 | 0.13 ± 0.02 | 0.006 ± 0.003 | 0.046 ± 0.015 | 0.31 ± 0.09 |
| | Control | 0.07 ± 0.00 | <dl | <dl | <dl | <dl |
| Blood | Treatment | 4.0 ± 0.28 | 0.33 ± 0.04 | 0.023 ± 0.009 | 0.456 ± 0.107 | 20.7 ± 0.89 |
| | Control | 0.09 ± 0.04 | <dl | <dl | 0.029 ± 0.003 | 0.03 ± 0.00 |

[a] All values are μg/g tissue except for CSF and blood which are μg/ml. Data represent mean ± SE; each experimental measurement was performed on a pool of 3 choroid plexus; for Pb and MeHg, three such pools ($n$ = 3) were used to obtain the mean of choroid plexus data and $n$ = 4 for all other tissues. For Cd, $n$ = 3; for Hg, $n$ = 4; for As, $n$ = 2 for the choroid plexus and $n$ = 3 for all other tissues. [b] dl, detection limit. [c] $HgCl_2$ was injected. [d] MeHgCl was injected.

were administered various doses of Pb acetate (**Fig. 2**). Pb concentrations in the brain cortex and CSF, however, were not significantly changed (Fig. 2). A time study of Pb being sequestered in the choroid plexus showed that during the 24-h period after injection of Pb, the lateral choroid plexus continued to concentrate this neurotoxic heavy metal ion whereas the CSF and brain had much lower concentrations of Pb (**Fig. 3**).

### Sulfhydryl, GSH, and Cd-MT concentration in the choroid plexus

The conventional method for determining non-protein-bound thiols in tissues requires the precipitation of protein with TCA. If the metals in the choroid plexus are bound to a ligand such as metallothionein or other soluble, small molecular weight polypeptides, the acid treatment would be expected to release metals from their binding sites, thus artificially elevating the level of non-protein-bound thiols. To avoid this possible artifact, instead of measuring non-protein-bound thiols, we measured low molecular weight thiol (LMW-SH, MW < 3000). The concentrations of the total free thiol and the LMW-thiol in the lateral choroid plexus were found to be 20 and 61% lower, respectively, than those in the brain cortex (**Table 2**). Although concentrations of GSH in the choroid plexus were 2.3-fold lower than in the brain cortex (Table 2), the concentration of total cysteine (cysteine + cystine) in the choroid plexus was 2.6-fold greater than that in the brain cortex (Table 2). Moreover, the concentration of cystine in the choroid plexus was fourfold higher than in the brain cortex (Table 2). Our results confirm the GSH value in the brain cortex reported by Anderson and Meister (21). Neither cysteinylglycine nor γ-glutamylcysteine could be detected in the choroid plexus (data not shown).

Using cadmium-109, anion exchange high performance liquid chromatography (HPLC), and optical absorbance at 254 nm as means of detection, we did not find any Cd-metallothionein in either the lateral choroid plexus or brain cortex (Table 2). Our results do not rule out the presence of other types of metallothioneins. Because it has been reported that the metallothionein level in liver homogenates is 10-fold higher than that in the brain homogenates (22), our failure to detect metallothionein in the choroid plexus may be due to its presence at levels below the detection limit of the assay.

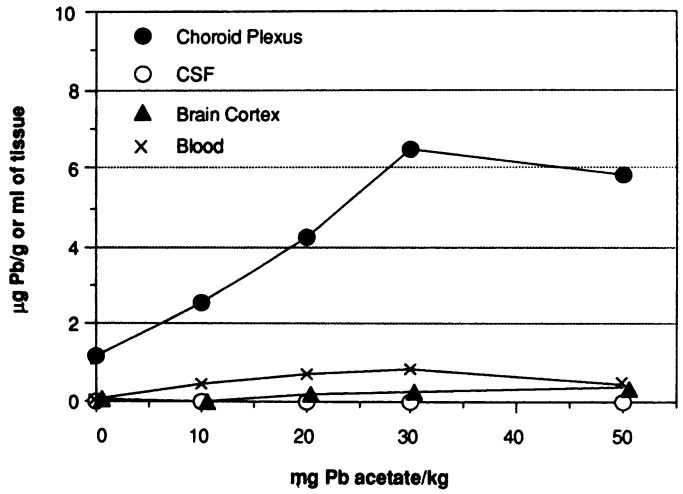


**Figure 2.** Dose response of Pb accumulation in the lateral choroid plexus. Rats were injected i.p. with appropriate doses of Pb acetate. Four hours later, tissues were removed and analyzed for Pb by AAS. For choroid plexus and CSF, each measurement was a pool of tissues from 4 rats. Data represent means of two such pools; $n = 2$ for all other tissues.

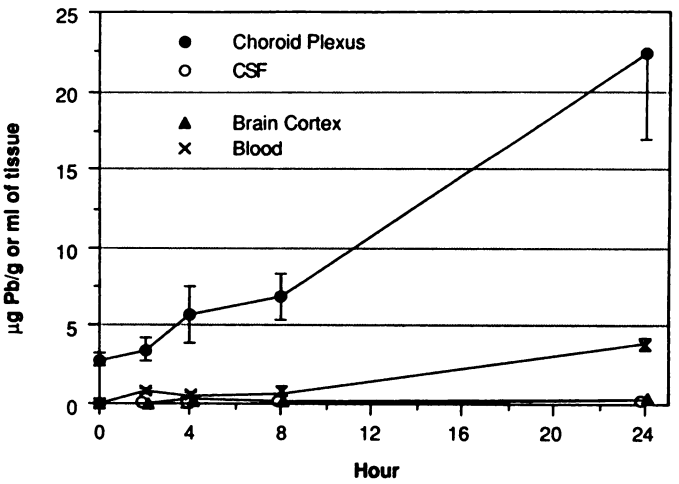


**Figure 3.** Time course of Pb accumulation in the lateral choroid plexus. Rats were given 27 mg Pb (as Pb acetate)/kg, i.p. Tissue samples were removed at appropriate time and analyzed for Pb by AAS. For choroid plexus and CSF, each measurement was performed on a pool of tissues from 4 rats. Data represent mean ± SE of three such pools ($n = 3$); $n = 4$ for all other tissues.

### Inhibition by ouabain of Cd uptake by the choroid plexus

When ouabain was incubated in vitro with rat lateral choroid plexus in artifical CSF, ouabain did not completely inhibit the $Na^+,K^+$-ATPase activity of the choroid plexus (data not shown). Maximum inhibition of the enzyme by ouabain was approximately 40%. When the choroid plexus was pretreated in vitro with 1.5 mM ouabain and the incubation continued with $^{109}Cd$, the radioactivity of the ouabain-treated group was 43% of control (**Fig. 4**). This indicates that ouabain significantly inhibited Cd uptake or binding by the choroid plexus.

## DISCUSSION

Our experiments clearly indicate that the choroid plexus sequestered and concentrated heavy metals and metalloids (Table 1, Fig. 1). In the case of Cd, even though the blood Cd concentration remained high 24 h after exposure, there was virtually no detectable amount of Cd in the CSF (Table 1). The action of the choroid plexus in concentrating these metals from the blood appears to be against a concentration gradient (Table 1, Fig. 1). In addition, our findings (Table 1) clearly show that the previously published (9) statement: "unlike other toxic chemicals, cadmium does not accumulate in the lateral choroid plexus" is incorrect.

Our results present a number of questions. First, what is the mechanism by which the choroid plexus sequesters these heavy metals and metalloids and protects the homeostasis of the CSF? What are the ligands for these heavy metals in the choroid plexus? Unlike a simple barrier, the choroid plexus retains the metals rather than just excluding them from the brain. Thus, a specific ligand (or ligands) must be present that retains heavy metal or metalloid ions. Although tissue sulfhydryl groups are most commonly the ligands to which a variety of heavy metals are bound (23), the concentrations of total thiol, LMW-thiol, as well as HMW-thiol in the lateral choroid plexus were found to be lower than those in the brain

cortex (Table 2). On the other hand, GSH, proposed as a cell's primary defense against cadmium toxicity (24), has a concentration in the choroid plexus lower than that of the brain cortex. There have been no previous reports in the literature as to the GSH content of the choroid plexus. Concentrations of total glutathione and cysteine in the CSF of rats, as reported by Anderson et al. (25), are 0.005 and 0.004 μmol/ml, respectively, about 176- or 25-fold less than those in the choroid plexus. Metallothionein, which has a high cysteine content and binds essential metals such as Zn and Cu as well as toxic metals such as Cs and Hg, has been suggested to have a role in metal homeostasis (26). In the present work, however, Cd-MT was not detected in either the choroid plexus or the brain cortex. Of the various naturally occurring thiol- or sulfur-containing compounds implicated in protecting cells from toxic metals, only cystine has been found in the lateral choroid plexus at concentrations exceeding that found in the brain cortex (Table 2). The significance of this higher cystine level in the lateral choroid plexus is not understood at present. As we did not examine the consequences of depleting or altering GSH, metallothionein, or thiol content in the choroid plexus after the metal has accumulated, we cannot completely exclude the possible role of these thiol-containing molecules in the sequestering of metals in the choroid plexus. The metal binding ligand (or ligands) in the choroid plexus, however, may be other than these well-known molecules.

Second, what is the role of the channels for essential metals and any other transport systems in the accumulation of heavy metals in the choroid plexus? Is there a unique pathway for the transport of heavy metals from the blood to the choroid plexus or, vice versa, from the CSF to the choroid plexus? The rapid passive transport of Pb ions into brain endothelium and thus into the brain has been demonstrated (27, 28). However, the accumulation of Pb in the choroid plexus seems unlikely to be due to the passive diffusion mechanism, because sequestration of Pb by the choroid plexus appears to be against a blood Pb concentration gradient (Fig. 1). On the other hand, active transport systems that remove metabolites and wastes from the CSF and carry them to the choroid plexus and then to the blood have been demonstrated in the mammalian choroid plexus (4–7). The low level of metal ions in the CSF (Table 1, Figs. 1–3) may also be due to the uptake of metal ions by the choroidal epithelium from the CSF. Ouabain inhibited the uptake of Cd into the choroid plexus (Fig. 4). This indicates that an energy-required mechanism might be involved in the uptake of metal from the CSF side of the choroid plexus.

TABLE 2. *Thiols, glutathione, cysteine, and metallothionein concentrations in the lateral choroid plexus and brain cortex*

| | Lateral choroid plexus,[a] μmol/g | Brain cortex,[b] μmol/g |
|---|---|---|
| Total free thiol | 169.8 ± 10.7 | 212.8 ± 5.2 |
| LMW-thiol (MW < 3000) | 10.1 ± 1.8 | 25.8 ± 3.5 |
| HMW-thiol (MW > 3000) | 159.7 ± 9.4 | 187.0 ± 3.1 |
| GSH | 0.69 ± 0.06 | 1.58 ± 0.12 |
| Cysteine + cystine[c] | 0.26 ± 0.04 | 0.10 ± 0.00 |
| Cysteine | 0.10 ± 0.02[d] | 0.07 ± 0.01 |
| Cystine[c] | 0.16 ± 0.04 | 0.04 ± 0.01 |
| Cd-metallothionein | nondetectable | nondetectable |

[a] Each experimental measurement was a pool of 3–4 choroid plexus. Data represent mean ± SE of three such pools. [b] For the brain cortex, the data represent the mean of four separate experiments. Unless otherwise indicated, all the choroid plexus values are significantly different from those of the brain cortex ($P < 0.05$). [c] CySH equivalents. LMW-thiol, low molecular weight thiol; HMW-thiol, high molecular weight thiol; GSH, reduced glutathione. [d] No significant difference compared with the values of the brain cortex.

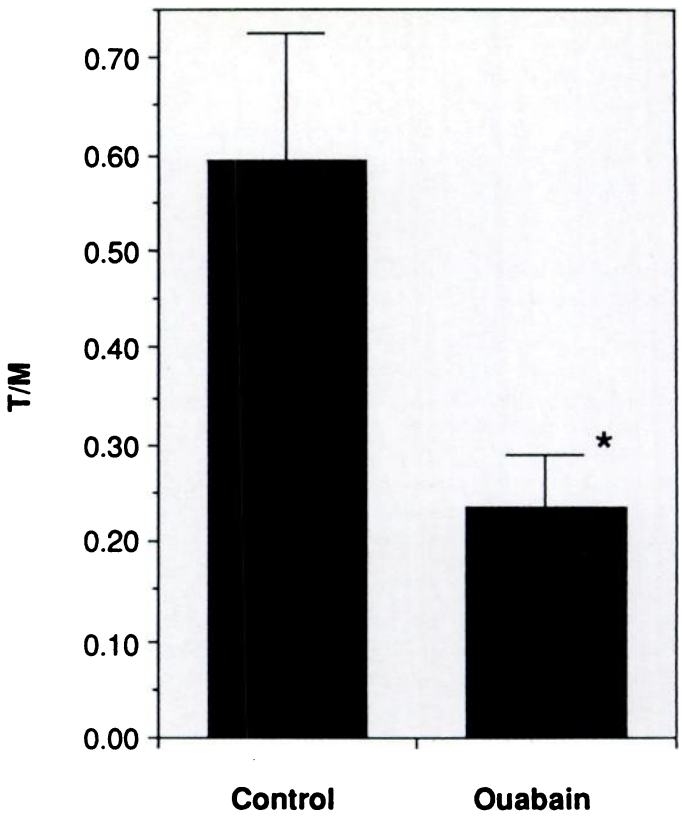


**Figure 4.** Ouabain inhibits the uptake of Cd by rat choroid plexus in vitro. The lateral choroid plexus was preincubated with 1.5 mM ouabain at 37°C for 15 min and then $CdCl_2$ was added to a final concentration of 50 μg Cd/ml (0.1 μCi of $^{109}Cd$). The incubation was continued for 10 min, after which the radioactivity of the tissue and medium was counted. Control: same incubation but without ouabain. T/M, cpm in 1.0 mg protein of tissue divided by cpm in 0.1 ml of incubation medium. Data represent mean of four separate measurement ± SE. *$P < 0.05$.

Finally, what are the consequences of heavy metals accumulating in the choroid plexus? Is there a threshold above which the capacity of the choroid plexus is exceeded or saturated? At the dose of 27 mg Pb/kg, the accumulation of Pb ions in this tissue seems saturated (Fig. 2). Therefore, it would be of interest to determine what pathological changes occur and whether CNS function is altered once a spillover takes place.

In summary, these experimental results demonstrate a definite capacity of the choroid plexus to sequester toxic heavy metal and metalloid ions. As the major site for the formation of the CSF is the choroid plexus, a major function of this highly vascular tissue may be to protect the CNS from fluxes of toxic metal ions. The implications of these findings and the eventual identity of the ligands to which these heavy metals and metalloids are bound in the choroid plexus should be of interest. FJ

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