

***THE DEPENDENCE OF Pb DISTRIBUTION IN BRAIN CELLS ON THE  
INTERACTIONS OF ASTROGLIA AND NEURONS***

A Senior Thesis

By

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Group: Biochemistry/Chemistry

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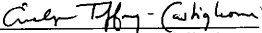
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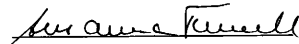
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## ABSTRACT

### **The Dependence of Pb Distribution in Brain Cells on the Interactions of Astroglia and Neurons**

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Lead (Pb) is a ubiquitous environmental contaminant which has been linked to neurobehavioral deficits, primarily in young children. A current hypothesis for Pb distribution among brain cells is that astroglia, which are interposed between the blood capillaries and neurons, protect neurons by Pb uptake and storage. This property of astroglia is thought to develop with age, and is not present in very young animals. The purpose of this study was to test three hypotheses: that maturation of neuronal cells affects their Pb accumulation, that soluble factors secreted into astroglial environment alters their Pb accumulation, and that astroglia preferentially sequester Pb over neurons. In an initial experiment, SY5Y human neuroblastoma cells were exposed to medium containing low levels of Pb and Neuronal Growth Factor- $\beta$  (NGF- $\beta$ ), a soluble factor secreted by astroglia which induces cell maturation. In a second experiment, astroglia were exposed to endothelial-, astroglia-, and neuronal-conditioned media containing low levels of Pb. In a final experiment, astroglia and neuronal cells were exposed to Pb in a bicameral co-culture system, in which cell types were physically separated but shared a common pool of medium. Graphite Furnace Atomic Absorption Spectroscopy (AAS) was used to determine Pb amounts, which were expressed per number of cells. Results showed neuronal cell maturation, induced by NGF- $\beta$ , lead to a decrease in Pb uptake. Furthermore, soluble factors secreted by neuronal cells caused a significant increase in the amount of Pb uptake by cultured astroglia, as determined in both conditioned medium and co-culture experiments. Our findings support the conclusion that astroglial accumulation of Pb increases with exposure to influencing factors from neuronal cells and that neuronal cell accumulation decreases with maturation. These findings are in agreement with the Lead sink hypothesis.

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## INTRODUCTION

Lead (Pb) has been recognized for centuries as a neurotoxin and exists in communities as a ubiquitous contaminant (Patterson and Settle, 1993). Effects of Pb on the nervous system vary widely, depending primarily on the level and duration of exposure. High levels of Pb exposure are associated with encephalopathy, severe brain damage, and death in both children and animal models (Winder, 1984). Extreme levels of Pb have been removed from most American household items, thereby decreasing the probability that single, high level exposure will cause severe detrimental effects in children and adults. Concern, however, still lies particularly with children exposed to low levels of Pb over an extended period of time. In 1991, the Centers for Disease Control (CDC) set 10 $\mu$ g/dl as a screening target Pb blood level which individuals should not exceed (Brody, *et al.*, 1994). An estimated 8.9% of American children under the age of 6 years have Pb levels that are above this level and thus of toxicologic concern, making an understanding of the effects of lead on the brain cells critical (Brody *et al.*, 1994). Young children are much more vulnerable to brain damage from Pb exposure than adults, even at levels previously thought to be harmless. Pb contamination of the environment still exists at low levels in most communities and has been linked to, decreased prenatal cognitive development, reduced IQ, decreased attention span, and increased aggression (Needleman *et al.*, 1979; Bellinger *et al.*, 1989). Because low-level Pb exposure is a realistic concern in the United States, our studies have been performed with chronic, low-level Pb

exposures of  $1\mu\text{M}$ . This level is comparable to blood Pb levels which are of toxicologic concern.

Under constant or repeated experimental exposure conditions, brain Pb concentrations are roughly 1-3 times the concentration in whole blood (Bradbury and Deane, 1993). The mechanism of Pb entry into the brain is still unclear. However, before Pb may disturb the functions of neurons, it must pass several protective and supportive layers of cells. Blood carries nutrients,  $\text{O}_2$ , and various contaminants into the capillaries of the brain. Most toxins and foreign bodies are inhibited from passing into the tissues of the brain by endothelial cells, which line the capillaries and form an impermeable barrier via tight junctions (Fig 1). Pb must therefore pass through this layer of cells before entering the brain, where it then encounters astroglia. Astroglia function as a mechanical support system for the neurons. Studies have more recently revealed that astroglia have a host of functions, including assisting in metabolism and modifying neurotransmitter functions (Kimelberg *et al.*, 1992). Astroglia communicate indirectly with neurons via astrocyte-to neuron signaling and neurotrophic factor secretion (Nedergaard, 1994; Rudge *et al.*, 1992). Pb may travel either through or between the astroglia to reach the neurons. Following exposure to astroglia, Pb may finally encounter neurons, inflicting functional damage.

Interest in astroglial roles in Pb neurotoxicity was chiefly stimulated by the work of Holtzman *et al.* (1984), who proposed that astroglia serve as Pb sinks in the mature mammalian brain. These investigators showed that as rat brains

matured *in vivo*, astroglia gained the ability to preferentially sequester Pb over neurons and to store this Pb in non-mitochondrial areas of the cell, preventing functional damage. The authors therefore proposed that astroglia serve as Pb sinks as animals mature and in this manner prevent neurons from Pb exposure and damage. Pb was detected by *x-ray diffraction microanalysis* (EDAX), which identify discrete Pb inclusions. Formation of inclusions, however, requires high doses of Pb. Therefore, EDAX cannot be utilized to analyze low-level Pb exposure and thus cannot test the Pb sink hypothesis under low-level conditions.

Cell culture techniques have allowed for the isolation of both neurons and astroglia for individual analysis, and therefore serve as a suitable technique for determination of cell-type specific levels of Pb uptake. Cell may be placed in tissue culture treated flasks, exposed to Pb, and subsequently removed for analysis of selected cellular properties or toxic endpoints. Additionally, better analytical methods, such as *graphite furnace Atomic Absorption Spectroscopy* (AAS), have allowed low-level Pb detection and measurement in cell cultures. Astroglia studies *in vitro* have confirmed Pb uptake at low-level concentrations (Tiffany-Castiglioni, 1989). Neuronal cell uptake of Pb at low-level exposure had not been previously confirmed prior to the present study.

In a prior study, cultured astroglia, possessing many of the same features of astroglia *in vivo*, were prepared from 1 day old Sprague-Dawley rat pups (Levison and McCarthy, 1991). The human neuroblastoma clonal cell line SH-SY5Y, hereafter referred to as SY5Y neuronal cells, was used as a model for neurons.



This cell line has been used in previous Pb studies and exhibits neuronal characteristics, including response to nerve growth factor (Perez-Polo et al., 1979; Sonnenfeld and Ishii, 1982). The purpose of cell culture is to provide a method of isolating a particular cell type, altering a specific factor in the environment of the cell, and subsequently determining the quantitative effect of changing the environment. This allows investigators to determine many of the probable features of cells *in vivo*. Realistically, however, cells do not act independently from one another and are continuously adjusting their biochemical and physiologic processes according to input from surrounding cells.

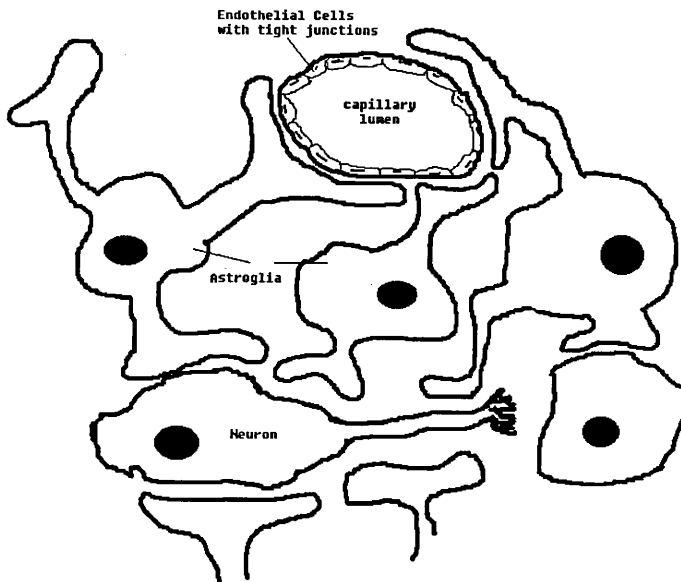
Three hypotheses were tested with chronic low-level (1  $\mu$ M) Pb exposure. First, that mature neuronal cells in culture have the capacity to take up Pb and that this capacity is altered by differentiation into a more mature state. Second, that the ability of astroglia to take up lead in culture is influenced by factors secreted by other cell types, specifically neurons and endothelial cells, which are known to influence astroglia maturation. Lastly, that astroglia preferentially take up Pb over neurons.

Current cell culture methods make it difficult to simulate an *in vivo* situation by allowing cells to communicate through secretions in the medium, while maintaining physical isolation to allow analysis of Pb intake by different cell types. Our study, therefore, used three types of experimental models in which to mimic selected cell-cell interactions and their effect on Pb accumulation:

1) SY5Y neuronal cells were first tested for a capacity to take up Pb at low levels of exposure. A comparison was made of Pb accumulation by cells differentiated with Neuronal Growth Factor (NGF- $\beta$ ) and undifferentiated cells. NGF- $\beta$  is a soluble factor secreted from astroglia which induces neuronal cell maturation, as seen by neurite extension (Fig 2) (Hong, unpublished).

2) Astroglia were exposed to the conditioned medium to determine the secreted factor effect on their Pb uptake. The cells were exposed to factors secreted by both endothelial and neuronal cells, allowing investigation of Pb uptake as influenced by cells naturally found in an astroglia *in vivo* environment. Exposure to soluble factors was achieved by exposing astroglia to medium previously conditioned by neuronal and endothelial cells. Neuronal and endothelial cells are known to alter astroglia maturation (Legare, *et al.*, submitted; Gebhardt and Goldstein, 1988).

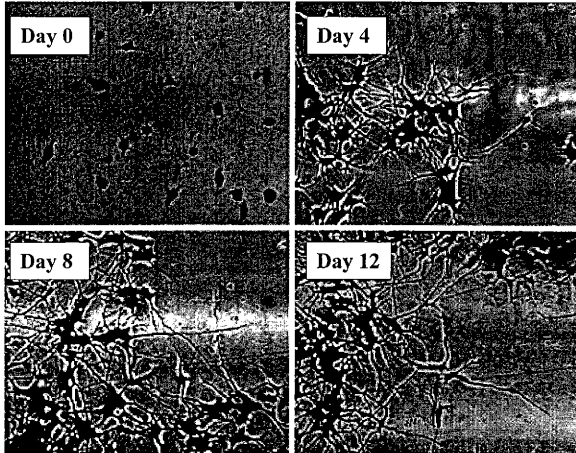
3) Astroglia and SY5Y neuronal cells were exposed to Pb in a bicameral co-culture system in which cell types were physically separated, but shared a common pool of medium (Fig 3). Pb and secreted soluble factors could travel freely in the medium between the physically separated cells, allowing quantitative determination of Pb distribution between astroglia and neuronal cells.



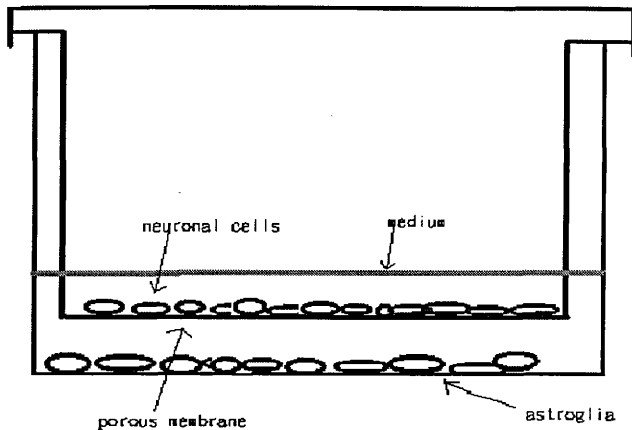
**Fig. 1 Astroglial relationship to neurons and endothelial cells in the brain. Astroglia are interposed between the capillaries and neurons. Capillaries entering the brain are lined by endothelial cells sealed with tight junctions to promote selective permeability. Pb encounters the astroglia before encountering the neurons.**

## Neurite Extension in SY5Y Cells Differentiated with 7 ng/ml NGF

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**Fig 2.** Neurite extension in SY5Y neuronal cells induced by NGF- $\beta$ . SY5Y cells were exposed to 7ng/ml NGF- $\beta$  over a 12 day period. Neurite extension began day 4, with rapid neurite growth occurring between days 8 and 12.



**Fig. 3** Astroglia and Neurons maintained on a bicameral co-culture system. Media levels covered both the neurons and astroglia. A  $.4\ \mu\text{m}$  porous membrane prevented cell movement, while allowing soluble factors and Pb to pass freely.

## MATERIALS AND METHODS

### EXPERIMENT 1

#### Experimental Approach and Rationale

**Experiment 1:** SY5Y neuronal cells were exposed to Pb in order to confirm Pb uptake and to measure the effect of differentiation on Pb accumulation. Pb measurements have been determined through the use of graphite furnace Atomic Absorption Spectroscopy (AAS). Neuronal cells were exposed to 1 $\mu$ M Pb for periods of 1, 4, or 7 days. Cell differentiation was induced through NGF- $\beta$  for a period of 4 days prior to and continuing throughout Pb exposure. SY5Y cells were counted and submitted for AAS analysis. Results showed that SY5Y cells do take up sufficient amounts of Pb to warrant further low-level Pb studies *in vitro*. More importantly, results showed that NGF- $\beta$  induced maturation caused a decrease in Pb uptake.

**Experiment 2:** Conditioned media exposure allowed astroglia to receive soluble factors secreted from neuronal and endothelial cells. Astroglia were exposed to neuronal conditioned medium (SY5YCM), endothelial conditioned medium (ECM), astroglia conditioned medium (ACM), and non-conditioned medium (NonCM) for periods of 1, 4 and 7 days. ACM and NonCM were both used as controls. Conditioned media was supplemented with fresh medium and all media contained 1 $\mu$ M Pb. Pb levels present in the astroglia were determined via graphite furnace atomic absorption spectroscopy (AAS). Results were then

expressed per million cells. No significant difference in Pb uptake was shown between the controls and endothelial conditioned mediums. Neuronal conditioned medium, however, lead to a significant increase in astroglia Pb uptake.

**Experiment 3:** SY5Y neuronal cells and astroglia were maintained in a co-culture system allowing communication via soluble factor secretion, while maintaining physical isolation for subsequent individual analysis. Pb levels present in the astroglia and neuronal cells were again determined via graphite furnace atomic absorption spectroscopy (AAS) and results then expressed per million cells. Astroglia appeared to preferentially sequestered Pb over neurons at both 4 and 7 days of exposure.

### **SY5Y Neuroblastoma Culture and Pb Exposure**

SY5Y neuroblastoma cells (gift from Dr. J. Regino Perez-Polo, the University of Texas Medical Branch at Galveston), passage 69, were maintained in a 1:1 Dulbecco's Modified Medium (DMEM; Sigma Chemical Co., St. Louis, MO) and Nutrient Mixture F-12 (F12; Sigma), which was supplemented with 10% Fetal Bovine Serum (FBS; Gibco BRL, Gaithersburg, MD). NGF- $\beta$  was also obtained from Sigma. Neuronal cells were seeded in 75 cm<sup>2</sup> tissue culture treated flasks (Sigma) at an initial density of 1-2 million cells per flask. Cells were maintained in 10 ml of DMEM/F12 supplemented with 10% FBS and incubated with 5% CO<sub>2</sub> at 36.5°C. Medium was changed at two day intervals throughout Pb and NGF- $\beta$  exposure. Four test groups were examined; control, NGF- $\beta$ , Pb, and

NGF- $\beta$  + Pb. Each test group included 3 samples for analysis. Cells which were to receive NGF- $\beta$  exposure began treatments four days prior to Pb exposure. This allowed NGF- $\beta$  induced maturation to occur sufficiently before Pb entered the environment. Neurite extension of SY5Y neuronal cell dendrites begins 4 days after initial exposure, as shown in Fig. 2 (Hong, unpublished). A concentration of 7nM for NGF- $\beta$  treatments was used throughout the experiment.

## EXPERIMENT 2

### Astroglia Culture and Conditioned Medium (CM) Collection

Astroglia cultures were taken from 1 day old Spraque-Dawley rats from Charles River (Houston, TX). Procedures for cerebral hemisphere extraction and seeding in 75cm<sup>2</sup> flasks were followed according to Tiffany-Castiglioni, *et al*(1987). Briefly, cerebral hemispheres were removed from 1 day old rat pup brains, triturated, and trypsined. Waymouth's medium with 10% FBS was then added and the following suspension filtered through 130 and 35  $\mu$ m mesh filters to separate the fibroblasts and blood cells from the astroglia. Cell suspensions were then centrifuged at 1250 x g for 5 minutes. Medium containing contaminants was then removed and replaced with Waymouth's medium containing 10% FBS and 1% antibiotic solution containing 1% penicillin, streptomycin, and neomycin (PSN;Gibco BRL).. Cells were then seeded 1 hemisphere per 75cm<sup>2</sup> flask. All cell cultures were incubated in 10mL Waymouth's medium with 5% CO<sub>2</sub> at



36.5°C. Cells were allowed to settle and attach for four days before first media change. Subsequently, medium changes continued at two-day intervals throughout the remaining portion of the study, without antibiotics. Differences in medium types between cells required a gradual change from Waymouth's medium with 10%FBS to IMDM solution. This was done by gradually changing to Waymouth:IMDM ratios to 1:0, 1:1, 2:8, and finally 0:1 beginning 4 days after culture and ending 10 days after culture. At this point, cells were maintained completely in IMDM medium containing 1% L-glutamine, 1% Antibiotic-Antimycotic, and 10% heat-inactivated FBS. FBS was heat-inactivated by submersing the bottle in a water bath set to 56°C for 30 minutes. After allowing medium to become conditioned to astroglia secretions, medium was sterilely removed into 50 ml centrifuge tubes and placed in a 4°C freezer for storage until use. Astroglia were provided fresh medium. The collected CM was then thawed, combined, and centrifuged at 1250 x g for 5 minutes. This collected any floating astroglia debris and possible contamination in a pellet at the bottom of the centrifuge tube. Medium from each 50 ml tube was then combined in a sterile media bottle, carefully leaving the pellet and 2-3 ml of medium in the tube. NonCM IMDM solution was then added to the conditioned media to yield a 1:1 solution of fresh and conditioned media. A prepared 1mM Pb solution was then added to the medium, yielding a final concentration of 1µM.

#### **SY5Y Neuronal Cell Conditioned Medium (CM) Collection**

Prior to Pb testing, SY5Y neuroblastoma cells, passage 71, were maintained in DMEM/F12 with 10% FBS. In order to collect conditioned medium (CM), medium was gradually changed to Iscove's Modified Dulbecco's Medium (IMDM; Gibco BRL), supplemented with 10% heat-inactivated FBS, 1% Antibiotic-Antimycotic (Gibco BRL), and L-glutamine (Gibco BRL) over an 8 day period following the astroglia media change method. Similar to the astroglia procedure, conditioned media was sterilely collected, frozen until used, thawed, centrifuged, and prepared with 1 $\mu$ M Pb.

#### **Endothelial Culture and Conditioned Medium (CM) Collection**

Cloned cerebrovascular endothelial cells (CVE) were derived from the brain microvessels of SJL mice (Sapatino et al., 1993) and cultured in IMDM supplemented with 10% heat-inactivated FBS, 1% Antibiotic-Antimycotic, and 1% L-glutamine. Similar to the astroglia procedure, conditioned medium was sterilely collected, frozen until use, thawed, centrifuged, and prepared with 1 $\mu$ M Pb.

#### **Astroglia Exposure to Conditioned Medium (CM)**

Astroglia began treatments 14 days after culture. Test groups included Astroglia conditioned medium (ACM), endothelial conditioned medium (ECM), neuronal conditioned medium (SY5YCM), and non-conditioned medium (NonCM). Each test group contained 1M Pb and were treated for periods of 1, 4, and 7 days.

### EXPERIMENT 3

#### **Astroglia and Neuronal Co-Culture**

Astroglia were maintained in 10 ml Waymouth's medium with 10% FBS eight days following culture from 1 day old Sprague-Dawley rat pups. SY5Y neuronal cells were maintained in 10 ml DMEM/F12 with 10% FBS prior to the experiment. Astroglia and SY5Y neuronal cells were slowly adjusted to a 1:1 Waymouth's: DMEM/F12 with 10%FBS medium over a period of four days. Three days prior to Pb addition, astroglia were seeded on the tissue culture treated dish. Simultaneously, neuronal cells were seeded at a density of 250,000 cells per tissue culture treated membrane insert and placed in a sterile dish, completely separated from the astroglia. After the cells had been allowed to attach and proliferate for three days, the insert containing SY5Y neuronal cells was placed in the dish containing astroglia. The media level was then raised to cover both cell types. This allowed Pb and soluble factors secreted between the cells to travel through the membrane. Cell counts were taken on days 4 and 7. Pellets were frozen at 4°C, until submission for graphite furnace AAS.

#### **Pb ANALYSIS PROCEDURE – ALL EXPERIMENTS**

##### **Pb Solution Preparation**

Pb acetate (Fisher Scientific, Fair Lawn, NJ) was added to sterile milli-Q<sup>R</sup> water (Millipore Corp., Bedford, MA) to make a 1mM solution. Each Pb treated

media then received the appropriate amount of Pb to reach a final concentration of  $1\mu\text{M}$ .

### **Cell Count**

Cell counts were taken on days 1, 4, and 7 after Pb addition. Briefly, cells from each sample were dislodged and suspended in 10 ml of Puck's solution, used for detachment and dissociation of cells. The pellets were washed to remove external Pb from the samples by two cycles of re-suspension in Puck's solution and centrifugation at 1250 rpm for 5 minutes. After the second wash, cells were counted with a hemacytometer, and the number of cells per sample was determined. The counted sample was then centrifuged, the remaining solution removed, and the resulting pellet allowed to dry in a sterile environment for at least 24 hours. After drying, the pellet was stored at  $-20^{\circ}\text{C}$  until AAS analysis.

### **Pb Assay by Graphite Furnace AAS**

Two hundred microliters of concentrated nitric acid (Ultrex, J.T. Baker, Phillipsburg, NJ) were added to each tube containing a dried pellet of SY5Y cells and the suspension was vortexed for 20 sec and allowed to digest overnight at room temperature. The digested cells were then diluted with matrix modifiers, vortexed for 20 sec and centrifuged for 5-6 min at 2000 rpm. Total Pb was measured by AAS with a Thermo Jarrell Ash Smith-Heifteje 12 spectrometer with furnace atomizer model 188. Pb determination was by injection of 10-20  $\mu\text{l}$  of digestion solution with drying, ashing, and atomization in accordance with

optimum parameters for Pb suggested by the Methods Manual for Furnace Operation, Volume II. All materials that came in contact with the samples, such as flasks and tubes, were Pb-free, disposable materials.

### **Statistics**

Means were compared between treated groups and controls using the Tukey-Kramer multiple comparison test. Sample sizes are provided for each experiment in the respective figure legend. If  $p \leq .05$ , the difference was considered statistically significant. Groups which varied from one another are differentiated by alphabetical markers for each day tested. Standard deviations for each sample mean are presented in each graph.

## RESULTS

### **Effect of Differentiation by NGF- $\beta$ on Neuronal Pb Accumulation**

Total Pb content of SY5Y neuronal cells exposed to Pb at days 1, 4, and 7 following exposure was determined by graphite furnace atomic absorption spectroscopy. AAS results are expressed as nanograms per two million cells, as shown in Fig. 4, with a detection level of 5 ppb. Control cells showed background levels of Pb which reflect the limit of detection of the methodology. Significantly greater amounts of Pb were detected in the Pb treatment groups. This finding establishes that even at low-level concentration of 1 $\mu$ M, neuronal cells accumulated Pb within 24 hours of exposure. However, cells differentiated with NGF- $\beta$  accumulated significantly less Pb than did undifferentiated cells by days 4 and 7. Also noticeable was an apparent peak in Pb absorption on day 4, followed by a decrease in Pb absorption on day 7. Statistical significance was determined by the Tukey-Kramer test (n=4).

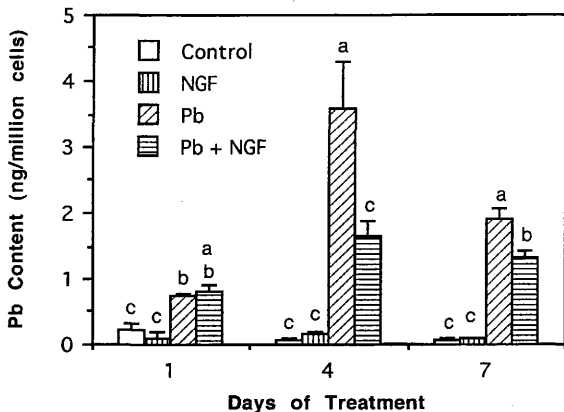
### **Effect of Conditioned Medium on Astroglial Pb Accumulation**

Total Pb content of astroglia exposed to Pb was determined by graphite furnace AAS. Results are expressed in nanograms per million cells, as shown in Fig. 5, with a detection level of 5 ppb. Astroglia exposed to endothelial conditioned media (ECM) showed no significant alteration in Pb uptake when compared to astroglia conditioned medium (ACM). However, Pb accumulation significantly increased when astroglia were exposed to conditioned medium from

neuronal cells (SY5YCM). This appears to support the Pb sink hypothesis, specifically in respect to astroglia Pb uptake in the presence of neuronal cells. . Statistical significance was determined using the Tukey-Kramer test (n=3).

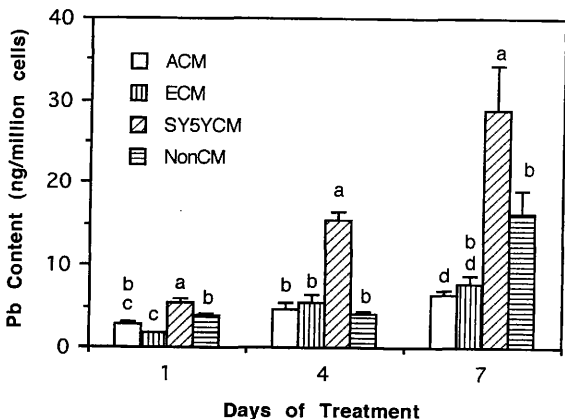
### **Effect of Astroglia and Neuronal Cell Co-Culture on Pb Distribution**

Total Pb content of astroglia and neuronal cells exposed to Pb for 4 and 7 days was determined by graphite AAS. Pb accumulation results are expressed in nanograms per million cells, as shown in Fig. 6, with a detection level of 5 ppb. Astroglia took up significantly larger quantities of Pb than neuronal cells. On both days, neuronal cell exposed to Pb in co-culture showed negligible amounts of Pb uptake. Astroglia cells, however, showed significant levels of uptake when compared to both the controls and the neuronal cells from their Pb sharing co-culture environment. This also supports the Pb sink hypothesis, confirming that astroglia preferentially sequester Pb over neurons. Statistical significance was determined using the Tukey-Kramer test (n=3).

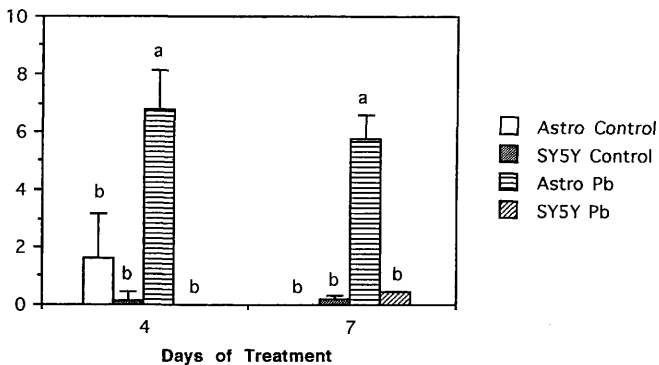


**Figure 4.** Lead accumulation of SY5Y neuroblastoma cells. SY5Y cells were differentiated with NGF (7 ng/ml/day) for four days, after which cultures were exposed to 1  $\mu$ M Pb acetate in the medium for 1, 4, and 7 days, in the continued presence of NGF- $\beta$ . Statistical significance was determined with the Tukey-Kramer test ( $n=4$ ). Bars accompanying each column represent standard deviations.





**Figure 5.** Effect of conditioned media on lead accumulation by cultured astroglia. Astroglial cultures (aged 14 d at the beginning of treatment) were exposed to 1  $\mu$ M Pb acetate in the medium for 1, 4, and 7 days. ACM = astroglia conditioned medium, ECM = endothelial cell conditioned medium, SY5YCM = SY5Y conditioned medium, and NonCM = fresh medium. CM was used in a 1:1 mixture with fresh medium. Statistical significance was determined with the Tukey-Kramer test ( $n=3$ ). Bars accompanying each column represent standard deviations.



**Fig. 6.** Lead distribution between astroglia and neuronal cells in a bicameral culture system. Co-cultures were exposed to  $1\mu\text{M}$  Pb acetate in medium for 4 and 7 days. Statistical significance was determined with the Tukey-Kramer test ( $n=3$ ). Bars accompanying each column represent standard deviations.

## DISCUSSION

These results collectively indicate that astroglial and neuronal cell Pb uptake capability is significantly influenced by secretions from cells found in their surrounding environment, specifically causing Pb to localize in astroglia rather than neurons as an animal matures. Neuronal cells showed decreased Pb accumulation after differentiation by NGF- $\beta$ , a soluble factor secreted by astroglia. The decrease in Pb uptake caused by NGF- $\beta$  may be attributed to maturation, as evidence by dendritic extension of neuronal cells in its presence. This evidence reveals a new facet to the Pb sink hypothesis developed by Holtzman (1984). Not only do astroglia appear to increase Pb uptake to protect neurons, neurons apparently release or exclude Pb as they mature. The general decrease in Pb accumulation after day 4 may either indicate a peak uptake and subsequent release of Pb into the medium or may be attributed to increasing numbers of cells reaching a level of Pb saturation. Further studies are currently underway to determine which factor is actually responsible for the apparent drop in Pb accumulation on day 7 compared to day 4.

Astroglia appear to increase Pb uptake in the presence of soluble factors secreted by neuronal cells. This increase may also be attributed to maturation of the astrocytes. *In vivo* studies at high levels of Pb have shown that levels of uptake may increase with maturation (Holtzman, 1984). Neuronal cells are known to induce astroglial maturation, which may thereby have caused an increase in Pb

uptake. However, endothelial cells, which also increase astroglial maturation in culture had no effect. This suggests that the decrease is a specific effect of neuronal cells.

The co-culture experiment corroborated both the individual neuronal and astroglia experiments, further supporting the Pb sink hypothesis. Astroglia preferentially took up Pb over neurons when each cell was given equal exposure in through shared medium. This validated the assertion that astroglia develop mechanisms which act to protect the neurons by serving as a Pb sink in the brain. Therefore, as neurons mature in the brain they are protected by extrinsic factors decreasing their Pb accumulation, while simultaneously being exposed to less Pb due to increased uptake by astroglia.

These cell models appear useful for further examination of predictions proposed by the Pb sink hypothesis. Such models may be particularly beneficial for examining the redistribution of Pb among brain cells after chelation therapy in children. A major concern of chelation therapy is that Pb freed from bone may redistribute to soft tissues, such as the brain. We hypothesize that Pb redistribution in brain cells can be manipulated pharmacologically, particularly in order to avoid deposition of Pb in neurons. The described cell culture model will be useful for testing pharmacologic agents. Work is in progress in this lab to develop such treatments.

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