Mechanisms of lead neurotoxicity, or looking beyond the lamppost

ELLEN K. SILBERGELD
Program in Toxicology, University of Maryland, Baltimore, Maryland 21201, USA

ABSTRACT **Despite several decades of research on the neurotoxicology of lead and its continued prominence as a major environmental and occupational health hazard, the mechanisms of its toxic action in the nervous system are still unknown. The differential effects of lead exposure in young children and adults, as well as inconsistencies between in vivo and in vitro studies, suggest that lead toxicity may have multiple mechanisms in the central nervous system (CNS). Two are: neurodevelopmental toxicity, possibly involving interference with cell adhesion molecules, resulting in miswiring of the CNS during early development and possibly permanent dysfunction; and neuropharma-cological toxicity, which might involve interactions between lead and calcium and lead and zinc, resulting in interference with neurotransmission at the synapse. This may be reversible.—Silbergeld, E. K. Mechanisms of lead neurotoxicity, or looking beyond the lamppost. *FASEB J.* 6: 3201–3206; 1992.**

Key Words: *brain development* • *lead neurotoxicity* • *zinc* • *calcium*

LEAD POISONING IS ONE OF THE most significant preventable diseases of environmental origin; in the United States, recent surveys by the Public Health Service have concluded that environmental lead poisoning is among the top four diseases of young children (1, 2). Exposure to lead is also common in industry, and thousands of workers are exposed to levels of lead in excess of current occupational guidelines (2).

The primary target for lead is the nervous system. Despite nearly 200 years of knowledge of the existence of lead neurotoxicity, we still do not know the specific targets of lead in the nervous system or the mechanisms by which lead exerts its neurotoxic effects. A sampling of the published literature through MEDLINE indicates that in the past 3 years more than 1000 articles on lead neurotoxicity have been published, including reviews and reports of major epidemiological studies. Arguably, we know more about lead in terms of quantity of research than any other identified human neurotoxin. Yet we are for the most part still collecting observations rather than developing and testing hypotheses on fundamental mechanisms of action of this elemental neurotoxin. Like the drunk under the lamppost, we continue to look in the same places, where the terrain is familiar but where no key—at least to date—has been found. This review summarize the past 2 decades of observations, both clinical and experimental and proposes some testable hypotheses as to the mechanisms of lead neurotoxicity.

THE CLINICAL MANIFESTATIONS OF LEAD NEUROTOXICITY

Clinical observations have driven experimental research in lead neurotoxicity since the first reports by Tanquerel des Planches nearly 300 years ago. The first phase of research was the development of appropriate animal models of lead neurotoxicity, first for the most striking sequelae of high exposures—overt encephalopathy in young children and severe peripheral neuropathy in adults. Pentschew and Garro (3) produced a rodent model of acute lead encephalopathy in which they demonstrated that breakdown of the blood:brain barrier and massive hemorrhage underlay the clinical pathology in young children dying of acute lead exposure, which had been described some 30 years earlier (4). Models for peripheral neurotoxicity were developed by Kostial and Vouk (5) and Silbergeld et al. (6), who showed that lead when added acutely to in situ or in vivo preparations, blocked stimulated release of acetylcholine from preganglionic and prejunctional nerve terminals.

Over this same period, however, clinical concerns focused on the sequelae of chronic exposures to relatively lower and lower levels of lead. In the most recent guidelines for management of childhood lead poisoning, the Center for Disease Control (CDC) concluded that blood lead levels in excess of 10 μg/dl (0.483 μmol/l, in SI units) are associated with increased risks of neurotoxicity (1). This new level, less than one-half of CDC's 1985 guideline for blood lead, is still only one order of magnitude below that associated with encephalopathy and death in young children.

Most experimental research in response to these new concerns has been based on the assumption that lower levels of lead probably affected the same targets in the nervous system as did higher levels, but with less severe consequences. Goldstein et al. (7) demonstrated that subencephalopathic lead exposure affected the integrity of capillary endothelial cells in vivo and in vitro, thus compromising the function of cellular constituents of the blood:brain barrier. Other research has continued to examine the effects of lower concentrations of lead on cholinergic neurochemistry in the central (CNS) and peripheral (PNS) nervous systems (8–10).

By the end of the 1970s, clinical studies indicated another important aspect of lead neurotoxicity: that the neurotoxic effects of lead appeared to differ in sensitivity and expression between young children and adults (11, 12). There may be even more precisely defined "critical windows" in terms of the consequences of exposures to lead from in utero through late neonatal periods for apparently specific developmental effects of lead on the CNS. The most recent results from long-term prospective studies indicate that such periods do exist: evaluations and psychometric tests related to neurobehavioral development through the first 4 years of life were highly correlated with indicators of lead exposure of the fetus (maternal and umbilical cord blood lead levels); after that

[1]Abbreviations: CDC, Center for Disease Control; CNS, central nervous system; PNS, peripheral nervous system; NCAMs, neural cell adhesion molecules; NMDA, *N*-methyl-D-aspartic acid.

0892-6638/92/0006-3201/$01.50. © FASEB

time neurobehavioral performance was no longer predicted by prenatal exposure, but rather was correlated with blood lead levels of the child at 24 months of age (13). Interpretation of these studies, both clinical and experimental, is somewhat limited by the long-term toxicokinetics of lead in that exposures cannot be precisely terminated, and by the effects of maturation itself on behavior and its measurement in children from 12 months to 12 years of age. Some experimental studies, using rodents and primates, have confirmed this significant interaction between timing of exposure to lead and the nature and persistence of neurotoxic effects (14–16). The great importance of neurodevelopmental status for lead neurotoxicity was demonstrated in experiments on the effects of lead on *N*-methyl-D-aspartic acid (NMDA) receptor-mediated function. The potency of lead to inhibit NMDA-mediated conductance changes in organotypic cell cultures depends on the age of the fetus at the time of collecting neural tissue for in vitro exposure (17, 18).

In summary, the manifestations of lead neurotoxicity appear to be highly age- and dose-dependent. Young organisms, including children, may be more sensitive than adults; moreover, the expression of toxicity after exposure early in development (possibly specifically to prenatal development in humans) is on memory and attention. Adult organisms are not immune to lead toxicity, but the effects of lead may be more clearly expressed as peripheral neurotoxicity in adults, and the CNS effects (at low doses) are manifest as changes in mood and affect (12, 19).

EFFECTS OF LEAD ON THE NERVOUS SYSTEM: CURRENT STATE OF KNOWLEDGE AND LIMITS

Lead exposures in vivo are associated with many changes in neurochemical parameters, alterations in brain structure, and deficits in learning and behavior. There are few clues as to how these effects may be related to each other or to a single or limited set of molecular actions of lead in the CNS. Experimental studies have been conducted with differences in exposure dose and timing such that integration across studies is not possible. There is no general hypothesis for a mechanism sufficient to explain how cellular events are specifically localized or how events at cellular and regional levels underlie the behavioral and cognitive dysfunction observed in lead-exposed human or experimental subjects. Of course, one reason for this lack of integration is the more general level of uncertainty in explaining learning, memory, and other behavior in structural or molecular terms—a challenge that continues for neuroscience as a basic discipline.

Nevertheless, I shall attempt to draw some conclusions and eliminate some possible hypotheses through a critical examination of the available data. As part of this analysis, a warning should be given toward using in vitro systems to understand all aspects of lead neurotoxicity. In several instances lead exposure in vivo produces effects on brain neurochemistry that have not been duplicated by exposing tissue preparations from the same regions to lead in vitro (for instance, effects on cholinergic and GABAergic neurochemistry; see ref 8). There are three possible reasons for these apparent inconsistencies between in vivo and in vitro lead exposure. *1)* Some of these effects may require *chronic* exposure of target sites. *2)* Some of lead's neurotoxic actions may be the result of indirect effects on the CNS, such as alterations in porphyrin biochemistry in other organs, resulting in the overproduction of δ-aminolevulinic acid or other neuroactive precursors of heme (20) or effects on hypothalamic-pituitary-gonadal systems with extensive feedback loops (21). *3)* The effects of lead on certain neural events, such as transmitter release, may be indirect and occur only when the inter- and intraregional wiring of the CNS is intact.

It should not be assumed that the event associated with the lowest concentration of lead in vitro is necessarily the primary mechanism of lead neurotoxicity. Equating the most sensitive in vitro response to lead with the most significant or primary effect runs the dangers inherent in using in vitro systems already noted with an additional potential problem. At its critical target (or targets), lead may be concentrated in small subcellular compartments where its local concentration may be quite high, analogous to the compartmentation of calcium within neurons. Thus, the critical site or mechanism may respond only when lead concentrations are relatively high, in the micromolar range, even though overall lead concentrations in the brain may be several orders of magnitude lower. We do not know with precision the subcellular distribution of lead, although there are endogenous and induced proteins that transport and sequester lead in nuclei and vesicles of glia and other cells (22–24). As shown in **Fig. 1,**

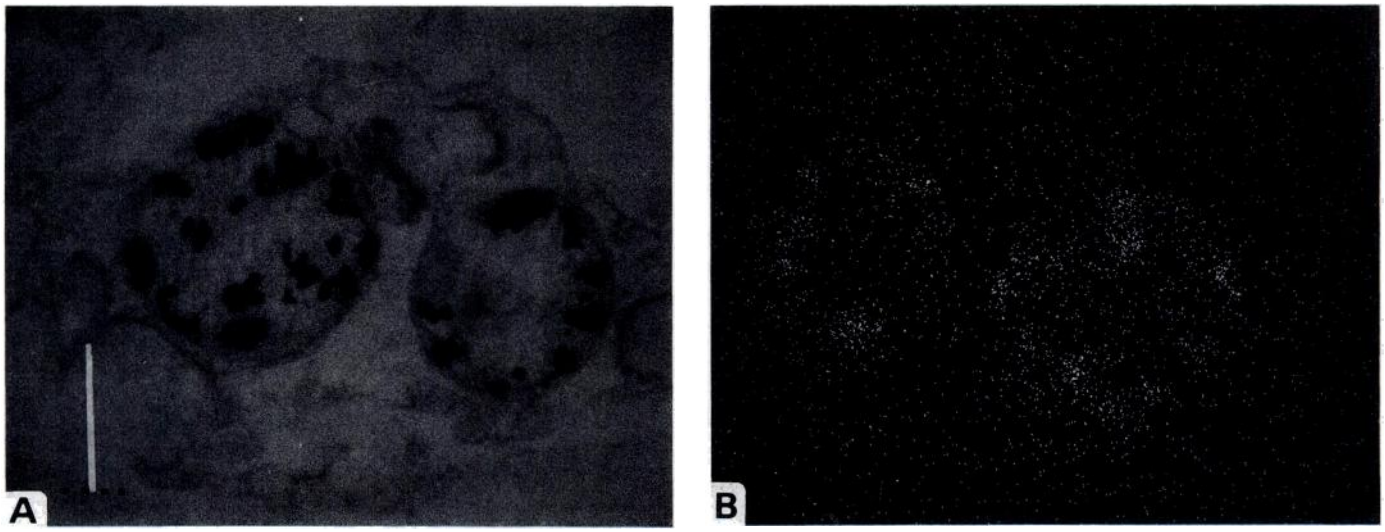

Figure 1. Localization of lead in neural tissue exposed to lead (1 μM) in vitro; STEM microscopy with ion probe microanalysis was used to visualize tissue without osmication (A). Thirty minute mapping of the spatial distribution of lead was done by collecting X-rays characteristic of the K X-rays of lead (B). ×40,000. For details, see ref 25.

mapping of lead in neural tissue exposed to lead in vitro demonstrates a high degree of intracellular compartmentation that can be visualized by electron probe X-ray fluorescence (25).

Lead does not appear to affect the CNS in a simple dose-dependent manner. Some effects observed at relatively high doses are not expressed at lower doses. For instance, the effects of lead on cerebellar blood:brain barrier integrity, first noted in the earliest animal models of lead encephalopathy (3), are not observed in animals with blood lead levels below 40 μg/dl (26). Some effects observed at low doses are not seen at higher doses. For instance, lead appears to affect hippocampal development and dopaminergic neurotransmission at low doses, but at higher doses these changes are not consistently observed (27, 28).

Although lead has many actions on the CNS, it does not affect all brain regions or all neurotransmitter pathways in a similar manner. Some neurochemical effects of lead vary with brain region, as in the case of dopaminergic neurotransmission (27). The regional localization of lead neurotoxicity is not clear, but the basal ganglia and motor cortex appear to be relatively unaffected by lead exposure in vivo. This is consistent with the absence of lead-induced effects on most motor functions of central origin. The wrist drop and other signs of lead intoxication in lead workers involve effects on the peripheral nervous system (6, 19). Recent reports of the effects of lead on physical balance in children (28) appear to involve proprioceptive mechanisms of postural sway rather than those motor pathways controlling gait and locomotion.

Over the past decade, attention has been focused on the hippocampus as a target for lead. However, there is insufficient research to exclude other regions, particularly the mesolimbic system, where low level effects of lead have been reported (27, 29). The rationale for selecting the hippocampus seems to be as follows: the hippocampus contains relatively high concentrations of zinc, and zinc-dependent functions may be sensitive to lead (see below); the hippocampus contains a dense plexus of cholinergic fibers that are affected by lead exposure; and the hippocampus is functionally related to behaviors involving memory and learning (see ref 30 for a review). Earlier reports had suggested that the hippocampus selectively accumulates lead, but this is not the case when changes in regional brain lead concentrations are expressed relative to concentrations in controls (9). At high doses, lead affects the growth of the hippocampus in vivo and in vitro (28, 30, 31). At lower doses, lead alters long-term potentiation responses in hippocampal slices (32). Yet even though the hypothesis of a hippocampal localization of lead neurotoxicity is attractive, it should not be adopted in the absence of a comparably sensitive examination of other brain regions.

Because lead is an ion and its toxicity is related to its ionic nature rather than its chemical or physical formulation (9), hypotheses for molecular mechanism have been based on consideration of interactions between lead and physiologically important ions. At present, the molecular hypothesis most often advanced as the basic mechanism for lead neurotoxicity is an ionic interaction between lead and calcium. According to this hypothesis, lead acts on mechanisms where calcium is the physiological ion regulator or activator (33). Another ionic interaction of biological importance may be between lead and zinc, based on the known actions of lead to substitute for zinc in cysteine-rich, metal-binding sites of such proteins as δ-aminolevulinic acid dehydrase and α_2-microglobulin (24). Another molecule whose activity is affected by lead is protein kinase C (34), recently reported to have four cysteine-histidine zinc-binding sites per molecule (35). Although lead, zinc, and calcium are all divalent cations, a fundamental biophysical objection to either ionic hypothesis is the greatly different sizes and differing mass:charge ratios of these ions.

Even if one or both of these ionic interactions were involved mechanistically in lead neurotoxicity, by themselves they do not provide an explanation for any specificity to the effects of lead. Calcium- and zinc-dependent events abound in cell physiology, from regulation of genetic transcription through *jun/fos* gene products and DNA zinc-binding finger loop proteins to regulation of receptor-gated ion conductance channels. Because calcium- and zinc-dependent events are found in many cells and in many organ systems, other events at the molecular level must be important local determinants of lead neurotoxicity within organs and cells.

TWO DISTINCT FORMS OF LEAD NEUROTOXICITY

The complexity of lead neurotoxicity in exposed humans and the interactions of age with dose suggest there may be several mechanisms of toxicity, and at least two distinct forms of lead neurotoxicity, involving specific mechanisms and resulting in specific expressions of altered neuronal function. Both expressions of neurotoxicity may occur at the same time within an organism at a given exposure, but the long-term consequences are likely to differ. Basically, I propose that lead exerts neurotoxic effects in the following distinct ways: first, as a neurodevelopmental toxicant, interfering with the hard wiring and differentiation of the CNS; second, as a neuropharmacological toxicant, interfering with ionic mechanisms of neurotransmission.

Mechanisms of neurodevelopmental lead toxicity

During neurodevelopment, the CNS undergoes highly programmed changes involving overall growth in cell number and size in the organ and proliferation and outgrowth of cells to establish connections between cells. Many factors regulate these processes, including growth factors, neurotransmitters functioning as trophic agents, and glycoprotein cell adhesion molecules (36). Exposure of fetal animals to lead affects both overall regional growth and neuron-specific differentiation/synaptogenesis in the CNS. Of these processes, synaptogenesis appears to be relatively more sensitive to low levels of lead exposure (9, 37). The effects of lead on differentiation of capillary endothelial cells from the fetal brain may be analogous to its effects on neurons undergoing development (38).

Thus the first hypothesis is that as a neurodevelopmental toxin, lead interferes with the programmed establishment of cell:cell connections. This action is precisely timed because of the precise timing of events in this process. It does not require the continued presence of lead in the CNS. Even if this effect is only to delay rather than to block a normal process, it can have persistent consequences unless another opportunity for recapitulating these time-dependent events is offered.

What molecular mechanisms may be involved in this action of lead? As shown by Regan (37), lead delays the ontogenic switch from the neonatal to the adult form of the glycosylated neural cell adhesion molecules (NCAMs). Lead could act by inhibiting the molecular events of cell:cell attachment, involving among other things the presence of cal-

cium; or it could inhibit glycosylation through actions on specific sialyltransferases; or it could exert higher level effects, possibly on transcription of genes for NCAMs.

Some insight may be gathered from examining data from another organ system where cell:cell communication and adhesion are also affected bv lead. In mineralized tissue, where interactions between cells are important to the growth and maintenance of bone, lead can interfere with the secretion of osteonectin/SPARC, a protein expressed by osteoblasts (39). Osteonectin/SPARC is also found in the developing CNS where it may perform a similar function (40). Thus this may be a general mechanism of lead toxicity, the consequences of which are determined by the tissue in which these proteins are expressed and the developmental stage in which they play a role.

Lead as a neuropharmacologic toxin

Lead also functions pharmacologically, interfering with synaptic mechanisms of transmitter release and signal transduction. These effects are directly related to the presence of lead within the synapse. They are potentially reversible if lead is removed from this milieu, although continuous lead exposure may result in long-term modulation of cellular responsiveness at pre- and postsynaptic levels, such as alterations in long-term potentiation in the hippocampus (see previous section). These pharmacologic effects include effects of lead to inhibit and facilitate transmitter release, modulate ion conductance, and thereby to affect electrophysiological output of the neuron (see ref 10).

What are the mechanisms of these events? Here, the ionic hypothesis may be relevant inasmuch as lead can to some extent substitute for calcium, and possibly zinc, in ion-dependent events at the synapse. These would include sodium and calcium channels, calcium-binding modulators such as calmodulin, and secondary and tertiary messengers such as adenyl cyclase and protein kinase C—all of which are reported to be affected by lead (38). Lead may affect NMDA-mediated channel currents by occupying zinc-binding sites in the channel and occluding ion movements (17).

The pharmacologic effects of lead seem to vary in sensitivity on an "inside-out" basis with respect to neuronal function, as shown in **Fig. 2.** That is, the cellular event most sensitive to lead identified so far is protein kinase C, which is activated by in vitro concentrations of lead as low as 1 pM (34). Mitochondrial release of calcium is also quite sensitive to lead in the nanomolar range (41). Dopamine-sensitive adenyl cyclase is inhibited by lead in the submicromolar range (42). The membrane-bound enzyme Na,K-ATPase is also a relatively sensitive target for lead, with slightly greater sensitivity on the ATP-binding site on the intracellular side of the protein (43). In contrast, lead interferes with neurotransmitter release or transmitter-gated ion channels only at concentrations in the high micromolar range (5, 6, 10, 17, 44).

As long as lead is excluded from entering the neuron, relatively high concentrations may be required to affect its function. However, based on ion microprobe analysis, lead can enter certain neurons (22, 25). Under these conditions, intracellular sites such as second-messenger and ion storage systems, which are highly sensitive to lead, would become accessible. The differential ability of neurons to prevent lead entry may be important determinants of neurotoxicity. This would be a mechanism that could confer specificity to the neurotoxic effects of lead.

Neither the neurodevelopmental hypothesis nor the neuropharmacologic hypothesis in itself confers any particular specificity. If lead exposures were precisely timed during neurodevelopment, then this might determine outcome, but in reality lead toxicokinetics are rather fuzzy given the long half-life of lead in certain compartments and its mobilization and redistribution, particularly during pregnancy (45). One possible mechanism is a lead-binding protein, which has recently been identified in the rat brain (46). This protein, which normally binds zinc, may serve to transport and concentrate lead within specific brain regions where the protein is synthesized. The fact that it is normally a zinc-binding protein suggests that factors governing zinc distribution may regulate lead as well. Research is needed to determine the role of lead-binding proteins in brain and the distribution of these proteins on a regional basis.

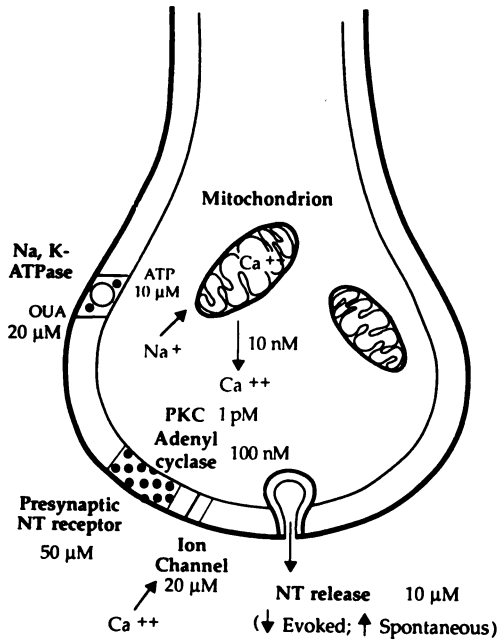

Figure 2. Schematic of the effects of lead on various parameters of neuronal function. Numbers shown are approximate ED50 values for inhibition or stimulation of specified processes; for references, see text. OUA, ouabain-binding site of Na,K-ATPase; PKC, protein kinase C. Locations of various functions are schematic only; no functional relationship between neurotransmitter receptors and calcium channels is implied.

SOME UNANSWERED QUESTIONS ABOUT LEAD NEUROTOXICITY

There are several new issues related to lead neurotoxicity for which we still need appropriate animal models and clearly focused experiments. First, as already discussed, new data on the effects of lead on events at the genetic level suggest that these effects may be important in the nervous system, as they appear to be in kidney (24) and bone (39). Second, the precise interaction between lead and neurodevelopment needs further research. How precisely timed are the events that are sensitive to lead? Does their sensitivity change or are different sites affected by lead later in life? Is neurodevelopmental toxicity determined by the status of important proteins in cell development and adhesion, such as the NCAMs, as suggested by Regan (37)? Are the postdevelopmental effects of lead primarily pharmacologic, as proposed here? Third, and potentially of greatest importance, what is the natural history of lead poisoning?

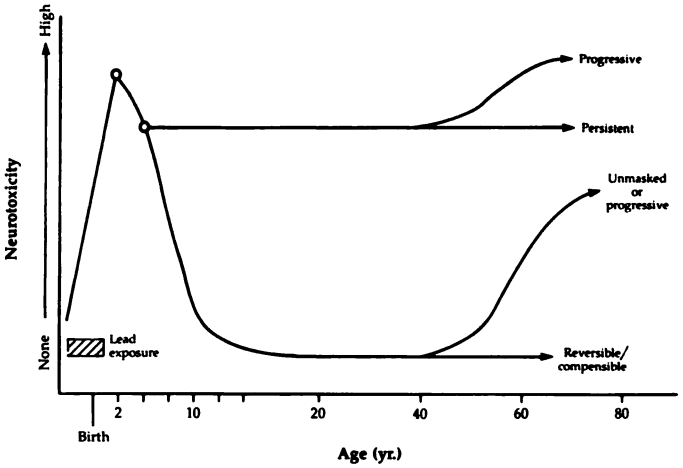

Figure 3. Possible timetable of human response to lead exposure. In this schematic, lead exposure is most intense during the first 2 years of postnatal life; after that time, the organism may continue to express neurotoxic effects or some recovery or compensation may occur. Later in life, neurotoxic damage may progress or be unmasked as biological compensation is diminished.

In 1943, Byers and Lord (47) revolutionized concepts of lead neurotoxicity by reporting that older children with prior lead exposures considered at that time to be subclinical were afflicted with a range of mental disabilities and learning problems. Needleman and colleagues (48) have reported persistent problems in school achievement in teenagers who had been found to have elevated tooth lead levels early in childhood. In the United States, there is a large cohort of persons—probably including many readers of this journal as well as the author of this article—who as children were exposed to levels of lead that are now considered toxic. The consequences of this early exposure over the human life span may be considered as the alternatives shown in **Fig. 3:** they may be reversed or completely compensated for; their neuronal targets may be desensitized; or they may be expressed in different ways, depending on the functional demands placed on the nervous system as well as age-related changes in its repertoire. This damage may be progressive in nature, so that neurotoxicity later in life may occur in the presence or absence of earlier compensation or repair. What should be of greatest concern is the possibility that as the nervous system loses plasticity and functional reserve during aging, the residual damage associated with early lead intoxication may be unmasked. Added to this, or overlaid upon this, is the possibility that lead stored in the body, mostly in the mineral compartment, may be mobilized during aging. Efflux of lead from bone stores has been reported in postmenopausal women and in retired lead workers (39, 45). The consequences of reexposure of the aging brain (14), added onto or in addition to persistent neurological damage from earlier exposure, require consideration and investigation.

SUMMARY

Lead is a ubiquitous element in the biosphere. As a consequence of past and ongoing uses of lead, millions of children and thousands of workers continue to be overexposed yearly in the U.S. (2). Well-defined animal models exist for studying neurotoxic mechanisms at low doses, and public health programs of disease prevention and clinical management are based on recent advances in our understanding of toxic effect and dose:response (1). Nevertheless, our lack of knowledge of lead neurotoxicity at the molecular level needs resolution in order to clarify four important issues related to lead poisoning as a public health problem. Are there significant differences in the effects of lead depending on the age of the exposed organism? Are all effects of lead irreversible, or is this age- and/or dose-dependent? How does lead affect events in the nervous system at the gene level? And what is the natural history of lead poisoning, or the long-term sequelae of lead exposure as the organism ages? FJ

Much of the research discussed in this paper was recently supported by a grant from the Carnegie Corporation. A remarkable group of colleagues over the past two decades have stimulated much of my own research and challenged my thinking on lead neurotoxicology: among these, I acknowledge the particular contributions of Alan Goldberg, Joel Pounds, Karen Florini, Paul Mushak, Herb Needleman, Julian Chisolm, and Bruce Fowler. With them, I look forward to the day when lead poisoning will be studied only in the laboratory as a model for understanding the nervous system.

REFERENCES

1. Centers for Disease Control. (1991) *Preventing Lead Poisoning in Young Children.* U.S. D.H.H.S., Public Health Service, Centers for Disease Control, Atlanta, Georgia
2. Agency for Toxic Substances and Disease Registry. (1988) *The Nature and Extent of Lead Poisoning in Children in the United States: A Report to Congress.* Agency for Toxic Substances and Diseases Registry, Atlanta, Georgia
3. Pentschew, A., and Garro, F. (1966) Lead encephalo-myelopathy of the suckling rat and its implications for the porphyrinopathic nervous diseases. *Acta Neuropathol.* **6,** 266-278
4. Blackfan, S. S. (1937) The lesions of lead encephalitis in children. *Bull. Johns Hopkins Hosp.* **61,** 1-61
5. Kostial, K., and Vouk, V. B. (1957) Lead ions and synaptic transmission in the superior cervical ganglion of the cat. *Br. J. Pharmacol.* **12,** 219-226
6. Silbergeld, E. K., Fales, J. T., and Goldberg, A. M. (1974) Lead: evidence for a prejunctional effect on neuromuscular function. *Nature (London)* **247,** 49-50
7. Goldstein, G. W., Asbury, A. K., and Diamond, I. (1974) Pathogenesis of lead encephalopathy: uptake of lead and reaction of brain capillaries. *Arch. Neurol.* **31,** 382-389
8. Winder, C., and Kitchen, I. (1984) Lead neurotoxicity: a review of the biochemical, neurochemical and drug-induced behavioral evidence. *Prog. Neurobiol.* **22,** 59-87
9. Silbergeld, E. K. (1991) A neurological perspective on lead toxicity. In *Human Lead Exposure* (Needleman, H. L., ed) pp. 89-103, CRC Press, Boca Raton, Florida
10. Audesirk, G. (1985) Effects of lead exposure on the physiology of neurons. *Prog. Neurobiol.* **24,** 199-231
11. Davis, J. M., and Svendsgaard, D. J. (1987) Lead and child development. *Nature (London)* **329,** 297-300
12. Cullen, M. R., Robins, J. M., and Eskenazi, B. (1983) Adult inorganic lead intoxication: presentation of 31 new cases and a review of recent advances in the literature. *Medicine* **62,** 221
13. Bellinger, D., and Needleman, H. L. (1991) Neurodevelopmental effects of low level lead exposure in children. In *Human Lead Exposure* (Needleman, H. L., ed) pp. 191-208, CRC Press, Boca Raton, Florida
14. Cory-Slechta, D. A., Weiss, B., and Cox, C. (1983) Delayed behavioral toxicity of lead with increasing exposure concentration. *Toxicol. Appl. Pharmacol.* **71,** 342-352
15. Cory-Slechta, D. (1988) Chronic low-level lead exposure: behavioral consequences, biological exposure indices, and reversibility. *Sci. Total Environ.* **71,** 433-440
16. Shigeta, S., Miyake, K., and Misawa (1989) Critical period of

brain development in learning caused by lead exposure in rats. Tokai *J. Exp. Clin. Med.* **14,** 147-152
17. Alkondon, M., Costa, A. C. S., Radhakrhishman, V., Aronstam, R. S., and Albuquerque, E. X. (1990) Selective blockade of NMDA-activated channel currents may be implicated in learning deficits caused by lead. *FEBS Lett.* **261,** 124-130
18. Ujihara, H., and Albuquerque, E. X. (1991) Age-dependent inhibition by lead of NMDA-induced current in cultured hippocampal neurons. *Neurosci. Abstr.* **17,** 1535
19. Seppalainen, A. M. (1988) Neurophysiological approaches to the detection of early neurotoxicity in humans. *CRC Crit. Rev. Toxicol.* **18,** 245-298
20. Silbergeld, E. K. (1987) Role of altered heme synthesis in chemical injury to the nervous system. In *Mechanisms of Chemical-Induced Porphyrinopathies* (Silbergeld, E. K., and Fowler, B. A. eds) pp. 297-308 Ann. N. Y. Acad. Sci. Vol. 514
21. McGivern, R. F., Sokol, R. Z., and Berman, N. G. (1991) Prenatal lead exposure in the rat during the third week of gestation: long-term behavioral, physiological, and anatomical effect associated with reproduction. *Toxicol. Appl. Pharmacol.* **11,** 206-215
22. Sulzer, D., Piscopo, I., Ungar, F., and Holtzman, E. (1987) Lead-dependent deposits in diverse synaptic vesicles: suggestive evidence for the presence of anionic binding sites. *J. Neurobiol.* **18,** 467-483
23. Klann, E., and Shelton, K. (1989) The effects of lead on the metabolism of a nuclear matrix protein which becomes prominent in lead-induced intranuclear inclusion bodies. *J. Biol. Chem.* **264,** 16969-16972
24. Fowler, B. A. (1989) Biological roles of high-affinity metal-binding proteins in mediating cell injury. *Comments Toxicol.* **3,** 27-46
25. Silbergeld, E. K., Adler, H. S., and Costa, J. L. (1977) Subcellular localization of lead in synaptosomes. *Res. Commun. Chem. Pathol. Pharmacol.* **17,** 715-725
26. Moorhouse, S. R., Carden, S., Drewitt, P. N., Eley, B. P., Hargreaves, R. J., and Pelling, D. (1988) The effect of chronic low level lead exposure on blood-brain barrier function in the developing rat. *Biochem. Pharmacol.* **37,** 4539-4547
27. Moresco, R. M., Dall'Olio, R. Gandolfi, O., Govoni, S., Di Giovine, S., and Trabucchi, M. (1988) Lead neurotoxicity: a role for dopamine receptors. *Toxicology* **53,** 315-322
28. Bhattacharya, A., Shukla, R., Bornschein, R., Dietrich, K., and Kopke, J. (1988) Postural disequilibrium quantification in children with chronic lead exposure: a pilot study. *Neurotoxicology* **9,** 327-340
29. Lasley, S. M., and Lane, J. D. (1988) Diminished regulation of meolimbic dopaminergic activity in rat after chronic inorganic lead exposure. *Toxicol. Appl. Pharmacol.* **95,** 474-483
30. Petit, T. L., Alfano, D. P., and LeBoutillier, J. C. (1983) Early lead exposure and the hippocampus: a review and recent advances. *Neurotoxicology* **4,** 79-94
31. Audesirk, T., Audesirk, G., Ferguson, C., and Shugarts, D. (1991) Effects of inorganic lead on the differentiation and growth of cultured hippocampal and neuroblastoma cells. *Neurotoxicology* **12,** 529-638
32. Altman, L., Svensson, K., and Wigand, H. (1991) Long-term potentiation in rat hippocampal clices is impaired following acute lead perfusion. *Neurosci. Lett.* **128,** 109-112
33. Pounds, J. (1984) Effect of lead intoxication on calcium homeostasis and calcium-mediated cell function: a review. *Neurotoxicology* **5,** 295-322
34. Markovac, J., and Goldstein, G. W. (1988) Picomolar concentrations of lead stimulate brain protein kinase C. *Nature (London)* **334,** 71-73
35. Hubbard, S. R., Bishop, W. R., Kirschmeier, P., George, S. J., Cramer, S. P., and Hendrickson, W. A. (1991) Identification and characterization of zinc binding sites in protein kinase C. *Science* **254,** 1776-1779
36. Jacobson, M. (1990) *Developmental Neurobiology,* 4th ed. Plenum, New York
37. Regan, C. M. (1989) Lead-impaired neurodevelopment: mechanisms and threshold values in the rodent. *Neurotoxicol. Teratol.* **11,** 533-537
38. Bressler, J. P., and Goldstein, G. W. (1991) Mechanisms of lead neurotoxicity. *Biochem. Pharmacol.* **41,** 479-484
39. Silbergeld, E. K., Sauk, J., Sommerman, M., Todd, A., McNeill, F., Fowler, B. A., Fontaine, A., and van Buren, J. (1992) Lead in bone: storage site, exposure source and target organ. *Neurotoxicology* In press
40. Nomura, S., Willis, A. J., Edwards, D. R., Heath, J. K., and Hogan, B. L. (1988) Developmental expression of 2ar (osteopontin) and SPARC (osteonectin) RNA as revealed by in situ hybridization. *J. Cell Biol.* **106,** 441-450
41. Silbergeld, E. K., and Adler, H. S. (1978) Subcellular mechanisms of lead neurotoxicity. *Brain Res.* **148,** 451-467
42. Ewers, U., and Erbe, R. (1980) Effects of lead, cadmium, and mercury on brain adenylate cyclase. *Toxicology* **16,** 227-237
43. Fox, D. A. (1991) Visual and auditory system alterations following developmental or adult lead exposure: a critical review. In *Human Lead Exposure* (Needleman, H. L., ed) pp. 105-123, CRC Press, Boca Raton, Florida
44. Minnema, D. J., Greenland, R. D., and Michaelson, I. A. (1986) Effect of in vitro inorganic lead on dopamine release from superfused rat striatal synaptosomes. *Toxicol. Appl. Pharmacol.* **84,** 400-411
45. Silbergeld, E. K. (1991b) Lead in bone: implications for pregnancy and lactation. *Environ. Health Perspect.* **91,** 63-70
46. DuVal, G. E., and Fowler, B. A. (1989) Preliminary purification and characterization studies of a low molecular weight high affinity cytosolic lead binding protein in rat brain. *Biochem. Biophys. Res. Commun.* **159,** 177-184
47. Byers, R., and Lord, E. (1943) Late effects of lead poisoning on mental development. *Am. J. Dis. Child.* **66,** 471-481
48. Needleman, H. L., Schell, A., Bellinger, D., Leviton, A., and Allred, E. N. (1990) The long-term effects of exposure to low doses of lead in childhood: an eleven year follow-up. *N. Engl. J. Med.* **322,** 83-88