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Neurotoxic Effects and Biomarkers of Lead Exposure: A Review

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Abstract

Biological monitoring techniques are useful for risk assessment of toxic agents in the field of environmental health. Lead, a systemic toxicant affecting virtually every organ system, primarily affects the central nervous system, particularly the developing brain. Consequently, children are at a greater risk than adults of suffering from the neurotoxic effects of lead. The ability of lead to pass through the blood-brain barrier is due in large part to its ability to substitute for calcium ions. Within the brain, lead-induced damage in the prefrontal cerebral cortex, hippocampus, and cerebellum can lead to a variety of neurological disorders, such as brain damage, mental retardation, behavioral problems, nerve damage, and possibly Alzheimer's disease, Parkinson's disease, and schizophrenia. At the molecular level, lead interferes with the regulatory action of calcium on cell functions and disrupts many intracellular biological activities. Experimental studies have also shown that lead exposure may have genotoxic effects, especially in the brain, bone marrow, liver, and lung cells. This paper presents an overview of biomarkers of lead exposure and discusses the neurotoxic effects of lead with regard to children, adults, and experimental animals, updated to January 2009.

Keywords

lead poisoning; biological monitoring; neurotoxicity; neurodevelopment; genotoxicity

INTRODUCTION

Lead (Pb) is a highly toxic heavy metal occurring naturally in the Earth's crust. Lead is found in all parts of the environment, primarily deriving from such human activities as mining, manufacturing, and burning fossil fuels. Lead exists in three forms: metallic lead, inorganic lead and lead compounds (or lead salts), and organic lead (containing carbon). Lead in the environment rarely occurs in its elemental state, but rather in its +2 oxidation state (Pb^{2+}) in various ores throughout the earth. Lead has been found in at least 1,272 of the 1,684 National Priority List (NPL) sites identified by the United States (US) Environmental Protection Agency (EPA) /1/. Lead is used in a variety of products, primarily in lead car batteries. Other uses of lead include leaded gasoline, paints, ceramics, ammunition, water pipes, solders, cosmetics, hair dye, farm equipment, airplanes, shielding for x-ray machines, and in the manufacture of corrosion and acid resistant materials used in the building industry /2–3/. Despite the ban on using lead in paints or as a gasoline additive in the United States (US), human exposure to lead continues as lead does not degrade in the environment, remaining strongly absorbed to soil.

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The most common sources of current lead exposure in the US are lead-based paint in older homes, contaminated soil, household dust, drinking water, lead crystal, and lead-glazed pottery. In humans, the routes of exposure include the ingestion of lead-contaminated food or drinking water containing lead leaching from older corroding pipes and fixtures, inhalation in industrial settings, and dermal contact /2/. Children can be exposed to peeling or flaking lead-based paint or weathered powdered paint when engaging in hobbies or activities that increase exposure. Children afflicted with pica (the compulsive, habitual consumption of nonfood items) are particularly vulnerable /[4]/. The magnitude of the toxic response depends on several factors, including the dose, the age of the person exposed, the life stage of a woman (children, lactation, menopause), occupational exposure, duration of exposure, health and lifestyle, and nutritional status of the person exposed.

A remarkable explosion in the literature about the health effects of lead has occurred since the dissemination of U.S. Occupational Safety and Health Administration (OSHA) lead standards in 1993 /[5–6]/ stating that workers can attain blood lead levels up to 40 μg dL^{-1} for their working lifetime. Since then, many longitudinal studies have provided evidence that cumulative lead dose causes cognitive dysfunction or decline (reviewed in /[7]/). The neurotoxic effects of lead in workers can be induced at BPb levels below 18 μg dL^{-1}, somewhat higher than the critical level of lead neurotoxicity in children (5 μg dL^{-1}) /[8]/.

Adverse health effects caused by lead exposure include intellectual and behavioral deficits in children, including hyperactivity; deficits in fine motor function, hand-eye coordination, and reaction time; and lowered performance on intelligence tests. Recent evidence has revealed other important health effects of lead exposure, such as hypertension and other cardiovascular outcomes /[9]/ and renal disease /[10]/. Chronic lead exposure in adults can also lead to decreased fertility, cataracts, nerve disorders, muscle and joint pain, and memory or concentration problems. Extreme lead exposure can cause a variety of neurologic disorders, such as lack of muscular co-ordination, convulsions, and coma. As lead affects several enzymatic processes responsible for heme synthesis, the hematologic system is also a highly sensitive target for lead toxicity. Lead has long been recognized as a developmental neurotoxicant that can interfere with the developing brain, resulting in functional impairment. Thus, lead exposure continues to be a major public health problem, particularly in urban centers in the US and in developing nations.

BIOMARKERS/BIOLOGICAL MONITORING OF LEAD EXPOSURE

Biological monitoring has been defined as the measurement and assessment of agents or their metabolites either in tissues, secreta, excreta, expired air or any combination of these to evaluate exposure and health risks compared with an appropriate reference /[11]/. The term *biological marker* (*biomarker*) is a general term used for a system that specifically measures an interaction between a biological system and a chemical, physical, or biological environmental agent. Biological monitoring techniques are useful for risk assessment of toxic agents in the field of environmental health. Biomarkers are generally classified into three groups: biomarkers of exposure, effect, and susceptibility.

A variety of biomarkers are available to monitor human exposure to lead. Appropriate selection and measurement of lead biomarkers of exposure are critically important for health care management purposes, public health decision making, and primary prevention synthesis. Although different biologic tissues and fluids (blood, urine, bone, tooth, hair, and nail) have been used to test for lead exposure, no biomarker of bioavailable lead has been generally accepted. The present review reviews focuses on the neurotoxic effects and the biomarkers of exposure of lead in humans and experimental animals.

The difficulty in assessing the exact nature of lead exposure depends on the complex toxicokinetics of lead within various body compartments (namely, cycling of lead between bone, blood, and soft tissues). Blood lead (BPb), mainly erythrocyte lead, is a representative of soft tissue lead and the primary biomarker used for the assessment of lead exposure, both for screening and diagnostic purposes and for biomonitoring body burden and absorbed (internal) doses of the metal. In adult humans, up to 50% of inhaled lead is transferred to bloodstream and of the ~10% absorbed dietary lead, more than 98% is found in blood cells /[12–13]/. Blood lead measurements reflect both recent and past exposures, the latter resulting from mobilization from bone back into blood /14/, and even in persons without excessive exposure to lead, bone can contribute from 45% to 55% of BPb /[15–16]/. In exposed children, for example, 90% or more of BPb consists of mobilized bone-lead /[17–19]/. The time required for BPb to decline to < 10 μg dL^{-1} in non-chelated children having BPb levels between 25–29 μg dL^{-1} was about 2 years and was linearly related to the BPb peak /[16]/. In a study of the environmental, dietary, demographic, and activity variables associated with biomarkers of exposure for lead, BPb was found to be associated with: (a) housedust concentrations of lead; (b) the duration of time spent working in a closed workshop; and (c) the year in which the subject moved into the residence /[20]/. An important weakness of BPb is its poor response to changes in exposure at high levels /21/.

Other currently available biomarkers of internal lead dose have not yet been accepted by the scientific community as a reliable substitute for BPb measurement /[17]/. Nevertheless, in certain cases bone or teeth (for past exposures), feces (for current gastrointestinal exposure), or urine (for organic lead) are sometimes more useful than blood.

As the plasma fraction is rapidly exchangeable in the blood, the toxic effects of lead are assumed to be primarily associated with plasma lead (PPb) /[17,22]/. Although PPb should be more germane than BPb to lead exposure and distribution, little is known about the association between PPb and clinical outcome. The determination of PPb is problematic because erythrocyte hemolysis can shift the metal into the plasma and artificially increase PPb levels.

Many researchers accept that a cumulative lead exposure integrated over many years, in bone for example, rather than a single BPb measurement of lead dose may be the most important determinant of some forms of toxicity. Bone Pb (BnPb) accounts for > 94% of the adult body burden of lead (70% in children) /[23–25]/. Hernandez-Avila and colleagues /26/ reported a strong association between BnPb levels and serum lead levels of adults exposed to lead. The findings of this study indicated the potential role of the skeleton as an important source of endogenous labile lead that may not be adequately discerned through the measurement of BPb levels.

The most informative recent epidemiologic studies of the impact of lead on health are those that could derive estimates of both recent (BPb) and cumulative (BnPb) exposure for each participant. In a recent review of studies measuring both BPb and BnPb at exposure levels encountered after environ-mental exposure, the associations between the biomarkers of cumulative dose (mainly in tibia) and cognitive function in adults were stronger and more consistent than were the associations with BPb levels /7/. Patella (kneecap) lead, representing a pool that may capture aspects of both current bioavailable and cumulative lead dose thus offering advantages over tibia or BPb, was used by Wright et al /27/ to determine whether lead-exposure biomarkers are associated with declines in cognitive test scores in older persons. The researchers found that among subjects in the lowest quartile of patella lead levels, Mini-Mental Status Exam (MMSE) scores decreased by 0.03 points per year (CI = −0.07 to 0.005), whereas in the highest quartile, the MMSE score decreased by 0.13 points per year (CI = −0.19 to −0.07). Similar interactions were found between BPb

levels and age. Increased levels of BnPb and BPb were found inversely associated with cognitive performance among older men, suggesting that lead exposure might accelerate age-associated cognitive decline.

Saliva is a convenient source and therefore a potential substitute for blood as a biomarker for lead exposure /[28]/. Nevertheless, saliva has not been generally accepted as a reliable biomarker of lead exposure because of conflicting and unreliable saliva lead (SPb) measurements. Early research suggested an association between SPb levels and BPb and PPb levels /[29–30]/. Subsequently, data from a study by Thaweboon et al /31/ compared BPb and SPb in an area highly contaminated from lead mining, Thailand. The geometric mean for the BPb content was 24.03 μg dL^{-1} (range 11.80–46.60 μg dL^{-1}) whereas the SPb content was 5.69 μg dL^{-1} (range 1.82–25.28 μg dL^{-1}), suggesting that saliva is not suitable material for biological monitoring with respect to lead exposure. Similarly, Barbosa and coworkers /[32]/ evaluated the use of parotid SPb levels as a surrogate of BPb or PPb levels to diagnose lead exposure. Age or gender did not affect SPb levels or the SPb:PPb ratio. Only a weak correlation was found between Log SPb and Log BPb ($r = 0.277$, $p < .008$), and between Log SPb and Log PPb ($r = 0.280$, $p = .006$), suggesting that SPb cannot be used as a biomarker to diagnose lead exposure or as a surrogate of PPb levels, at least for low to moderately lead-exposed populations. A later study by this group /[33]/ did show a clear relation between SPb and environmental contamination by lead. The authors suggested that further studies on SPb should be undertaken to investigate the usefulness of saliva as a biomarker of lead exposure, particularly in children.

The collection of urine lead (UPb) is favored for long-term biomonitoring, especially for occupational exposures. Urine Pb originates from PPb that is filtered at the glomerular level and excreted through the kidneys. According to certain authors /[17,34]/, UPb levels adjusted for glomerular filtration rate can serve as a proxy for PPb. Fukui et al /35/ concluded that the correlation of UPb with BPb among workers occupationally exposed to lead was close enough to suggest that UPb can be a good alternative to BPb on a group basis, but not close enough to allow UPb to predict BPb on an individual basis.

Although lead excreted in hair has been suggested for the assessment of lead exposure /36/, an extensive debate ensues about hair lead (HPb) as a biomarker (discussed in /[17]/). Hair is a biological specimen that is easily and non-invasively collected with minimal cost and is easily stored and transported to the laboratory for analysis. Such advantages should make hair an attractive biomonitoring substrate, at least superficially.

Similar to hair, nails have many superficial advantages as a lead exposure biomarker, especially as specimen collection is noninvasive and simple and specimens are very stable after collection, not requiring special storage conditions. Nail lead (NPb) is considered to reflect long-term exposure because this compartment remains isolated from other metabolic activities in the body /[37]/. Because toenails are less affected than fingernails by exogenous environmental contamination, toenails have been preferred for lead-exposure studies. The lead concentration in nails depends on the age of the subject /38/, but apparently not on the subject's gender /39/.

In comparison to bone, teeth accumulate lead over the long term. Some evidence has shown that teeth are superior to bone as an indicator of cumulative lead exposure because the losses from teeth are much slower /[40]/. Moreover, deciduous teeth are relatively easy to collect and analyze and are very stable for preservation purposes. In an early study, concentrations of BPb determined at regular 6- month intervals were related to the lead concentrations in surface tooth enamel (EPb) but correlated with SPb only in the short term /[41]/. A recent study from Brazil /28/ compared the SPb of children from a city with no reported lead

contamination and children residing in a region notoriously contaminated with lead. Inductively coupled plasma mass spectrometry revealed that SPb correlated with EPb in these two populations.

Micronuclei (MN) are chromosome fragments that are not incorporated into the nucleus at cell division. The MN assay in peripheral blood is considered a reliable biomarker of genotoxic exposure to both physical and chemical agents /42–43/; increases in MN frequency indicate exposure to clastogenic and/or aneugenic agents. Sister chromatid exchanges (SCEs), high-SCE frequency cells (HFCs), and DNA-protein cross-links (DPCs) have also been shown to be reliable biomarkers for monitoring workers exposed to lead and clearly indicate health effects from occupational exposure to lead /44/.

Several enzymatic processes responsible for heme synthesis can be used as biomarkers for the toxic effects of lead, primarily δ-aminolaevulinic acid dehydratase (δ-ALAD), which catalyzes the condensation of two molecules of 5-aminolevulinic acid to form the heme precursor, porphobilinogen. As the activity of δ-ALAD is inhibited by lead binding, this enzyme is accepted as the most sensitive measurable biological index of lead toxicity /17/.

THE BLOOD BRAIN BARRIER

The brain consists of two cell types: neurons that send/receive messages from the cell body and glia that protect neurons. Glial cells are subdivided into microglia, oligodendroglia (myelin-producing cells), and astrocytes. Synaptic transmission refers to the propagation of nerve impulses from one neuron to another at a junction between neurons (synapse) through the release of chemical neurotransmitters, such as dopamine, adrenaline, noradrenaline, or γ-amino butyric acid (GABA). Following release, neurotransmitters bind to specific receptors on the surface of presynaptic and postsynaptic cells. Structurally, the brain contains three main parts: the cerebrum, which controls voluntary movement, thought, learning, reasoning, emotions, judgment, memory, the senses, and spoken language; the cerebellum, which functions to control coordinate body movement; and the brain stem.

Astrocytes along with the cerebral micro-vascular endothelium, pericytes, neurons, and the extracellular matrix constitute a physical blood-brain-barrier (BBB) that excludes many substances from entering the brain. Transport across the BBB is strictly limited through both physical (tight junctions) and metabolic barriers (enzymes, diverse transport systems) that control the passage of water-soluble substances from the bloodstream into the CNS. The tight junctions between the epithelial cells comprise a complex of transmem-brane (junctional adhesion molecule-1, occludin, claudins) and cytoplasmic (zonula occludens-1 and occludens-2 (ZO-1, ZO-2), cingulin, AF-6, and 7H6) proteins linked to the actin cytoskeleton. Several intrinsic signaling pathways, including those involving calcium, phosphorylation, and guanine nucleotide binding (G) proteins, modulate the expression and subcellular localization of tight junction proteins. Disruption of BBB tight junctions can lead to impaired function of the BBB, thereby compromising the CNS /45–46/. Primarily due to its ability to substitute for calcium ions (Ca^{2+}), Pb^{2+} crosses the BBB rapidly and concentrates in the brain (BrPb). Picomolar concentrations of Pb^{2+} can replace micromolar concentrations of Ca^{2+} in a protein kinase C (PKC) enzyme assay, a calcium-dependent process /47–49/. Thus, at the functional level of the BBB, the ability of lead to mimic or mobilize calcium and PKC could alter the behavior of endothelial cells in the immature brain and disrupt the BBB /50–52/.

LEAD-INDUCED NEUROTOXIC EFFECTS

Knowledge of the neurotoxicology of lead has advanced in recent decades due to revelations regarding the mechanisms and cellular specificity of lead. Potential mechanisms of lead-

induced cognitive deficits have been investigated using cellular models of learning and memory. New research provides convincing evidence that exposures to lead have adverse effects on the central nervous system (CNS), that environmental factors augment lead susceptibility, and that exposures in early life can cause neurode-generation in later life.

As the main target for lead toxicity is the CNS, the brain is the organ most studied in lead toxicity. Lead neurotoxicity occurs when the exposure to lead alters the normal activity of the CNS and causes damage to the CNS. The direct neurotoxic actions of lead include apoptosis (programmed cell death), excitotoxicity affecting neurotransmitter storage and release and altering neurotransmitter receptors, mitochondria, second messengers, cerebrovascular endothelial cells, and both astroglia and oligodendroglia. Symptoms can appear immediately after exposure or may be delayed and include loss of memory, vision, cognitive and behavioral problems, and brain damage/mental retardation. Most early studies concentrated on the neurocognitive effects of lead, but recently higher exposures have been associated with such morbidities as antisocial behavior, delinquency, and violence /[53]/. Several hypotheses have been proposed to explain the mechanism of lead toxicity on the CNS.

Effect of Lead on Neurodevelopment

A child's BPb measurement is estimated to account for 2% to 4% of variance in neurodevelopment measures (approximately 4% to 8% of the explained variance) /54–55/. The Agency for Toxic Substances and Disease Registry (ATSDR) /2/ cautions, however, that when studying the effects of lead on child development, the influence of multiple factors like treatment by parents or other adult caregivers should be taken into account. A child's family and personal psychosocial experiences are strongly associated with performance on neurodevelopment measures and account for a greater proportion of the explained variance in these measures than BPb levels.

Many studies have examined the effects of lead on children's development outcomes covering varying ages at which BPb was measured and varying ages over which BPb levels were averaged. Statistically significant associations have been identified between average BPb levels over a specific period (for example, 0–5 years) and various adverse health outcomes; other studies have reported statistically significant associations with a single lead measurement at a specific age (for example, prenatal, 24 months, 6.5 years) or with a peak measurement. In contrast to adults, central nervous system effects are more prominent than peripheral effects in the developing nervous system /[56]/. The developmental effects of lead occur during a critical time window (age < 2 years of age).

Low-level BPb and development—Although the toxic effects of high levels of lead have been well documented for centuries, of great concern is the relative recent discovery that low levels of blood lead (BPb < 10 μg dL^{-1}) are associated with adverse effects in the developing organism. In 1991, Centers for Disease Control and Prevention (CDC) in the US declared that a BPb level of 10 μg dL^{-1} should prompt public health actions /[57]/, while concurrently recognizing that although useful as a risk management tool, 10 μg dL^{-1} BPb should not be interpreted as a threshold for toxicity. Indeed, no threshold has yet been identified. Subsequently, low-level exposure to lead during early childhood was shown to be inversely associated with neuropsychological development through the first 7 years of life /[58]/.

In 2007, the CDC /[59]/ summarized the findings of a review of clinical interpretation and management of BLLs < 10 μg dL^{-1} conducted by CDC's Advisory Committee on Childhood Lead Poisoning Prevention and concluded that research conducted since 1991 has

strengthened the evidence that children's physical and mental development can be affected at BLLs <10 μg dL^{-1}.

In utero lead exposure—The possibility of intra-uterine exposure to lead began to be addressed only in the late 1970s. Correlations between maternal and umbilical cord blood lead (UCPb) levels confirmed the transfer of lead from the mother to the fetus /[60–61]/, and a newborn infant's BPb was shown to reflect that of the mother /62/. Moreover, the increase in lead level in breast milk with increasing maternal BPb levels represents an additional risk to the newborn infant /63/.

Prenatal lead exposure, assessed using UCPb as a biomarker, has long been known to impair the cognitive development of the infant. Strong evidence on the early developmental effects of exposure to lead was first provided by Bellinger and colleagues /[64]/. Scores from the Bayley Scales of Infant Development (BSID) revealed that high cord blood levels were associated with lower covariance-adjusted scores on the Mental Development Index (MDI) but not on the Psychomotor Development Index (PDI). The level of BPb at 6 months of age was not associated with scores on either MDI or PDI, consistent with the hypothesis that low levels of lead are delivered transplacentally and are toxic to infants. In a later study covering 6 and 12 months of age /[65]/, the lead concentration of capillary blood measured at both ages showed that MDI scores, adjusted for confounding, were inversely related to infants' UCPb levels. As the scores were not significantly related to postnatal BPb levels at either age, the prenatal exposure to lead level was deemed to be associated with less favorable development through the first year of life.

In a prospective longitudinal cohort study of 249 children from birth to two years of age, Bellinger et al /[66]/ assessed the relation between prenatal and postnatal exposure to low levels of lead and early cognitive development. The development of children with UCPb lead levels less than 3 μg dL^{-1} (low), 6 to 7 μg dL^{-1} (medium), or ≥ 10 μg dL^{-1} (high) was assessed semiannually, beginning at the age of 6 months, with the MDI (mean ±SD, 100 ±16). Infants in the high-prenatal-exposure group scored lower than those in the other two groups at all ages, and as before, the scores were not related to the infants' postnatal BPb levels.

A nonlinear relation between the first trimester of pregnancy BPb and the MDI at age 24 months was reported /67/. The results of the analyses showed that both maternal PPb and whole BPb levels during the first trimester (but not in the second or third trimester) were significant predictors ($p < .05$) of poorer postnatal MDI scores. Additionally, the effect of first-trimester maternal PPb was substantially greater than the effects of second- and third-trimester PPb. On the other hand, another study excluding the first trimester showed that the IQ of 6–10-year-old children decreased significantly ($p < .0029$; 95% CI, −6.45 to −1.36) with increasing natural-log third trimester PbB, but not with PbB at other times during pregnancy or postnatal PbB measurements /[68]/. Although a causal association between lead exposure and impaired cognitive functioning was most likely in early studies, the potential for residual confounding, particularly by social factors, made the strength and shape (linear or nonlinear) of this association across BPb levels uncertain /52/.

A direct link exists between low-level lead exposure during early development and deficits in neurobehavioral-cognitive performance evident late in childhood through adolescence /[69]/. Strong evidence for an association between low BPb levels and intellectual impairment in children, especially for those having maximal measured BPb levels of < 10 μg dL^{-1}, emerged from a pooled analysis of 1,333 children followed from birth or infancy until 5–10 years of age /[70]/. Of these, 18% had a maximal BPb concentration of < 10 μg dL^{-1} and 8% had a maximal blood lead concentration of < 7.5 μg dL^{-1}. After adjustment for covariates,

an inverse relation was found between BPb concentration and the full-scale IQ score. A log-linear model revealed a 6.9 IQ point decrement [95% confidence interval (CI), 4.2–9.4] associated with an increase in concurrent BPb levels from 2.4 to 30 μg dL^{-1}. The respective estimated IQ point decrements associated with an increase in BPb from 2.4 to 10 μg dL^{-1}, 10 to 20 μg dL^{-1}, and 20 to 30 μg dL^{-1} were 3.9 (95% CI, 2.4–5.3), 1.9 (95% CI, 1.2–2.6), and 1.1 (95% CI, 0.7–1.5). For a given increase in BPb, the lead-associated intellectual decrement for children with a maximal BPb level < 7.5 μg dL^{-1} was significantly greater than that observed for those with a maximal blood lead level ≥ 7.5 μg dL^{-1} ($p = .015$).

Téllez-Rojo et al /[71]/ also studied the longitudinal associations between low concentrations of BPb and neurobehavioral development in environmentally exposed children in Mexico City in 294 children having a BPb < 10 μg dL^{-1} at both 12 and 24 months of age, with a gestation ≥ 37 weeks and a birth weight > 2,000 g. The MDI and PDI of the BSID II translated into Spanish were used for the evaluation. Also included in the multivariate models were maternal age and IQ and children's gender and birth weight. The finding of inverse associations between 24-month BPb level and concurrent MDI and PDI scores on the BSID II indicated that children's neurodevelopment is inversely related to their BPb levels in the range of < 10 g dL^{-1}, providing further evidence that 10 μg dL^{-1} should not be viewed as a biological threshold for lead neurotoxicity.

An association was found between prenatal and childhood BPb concentrations and criminal arrests in early adulthood. Between 1979 and 1984, Wright et al /[72]/ recruited pregnant women living in poor areas of Cincinnati, which had a high concentration of older, lead-contaminated housing, into the Cincinnati Lead Study. The researchers measured the women's BPb concentrations during pregnancy, as an indication of the prenatal lead exposure of the offspring, and the child's BPb levels regularly until the children were six and half years old. The authors then obtained information from the local criminal justice records on how many times each of the 250 offspring had been arrested between becoming 18 years old and the end of October 2005. Increased blood lead levels before birth and during early childhood were associated with higher rates of arrest for any reason and for violent crimes. For example, for every 5 μg dL^{-1} increase in BPb levels at six years of age, the risk of being arrested for a violent crime as a young adult increased by almost 50% (RR = 1.48) (see Hwang /[53]/).

NEUROTOXICITY STUDIES IN CHILDREN

The extent and rate of absorption of lead through the gastrointestinal tract depend on the characteristics of the individual and on the physicochemical characteristics of the medium ingested. Children are at higher risk because they are more likely to play in the dirt, put their hands and other objects into their mouths, and absorb about half of an oral dose of water-soluble lead /73/. Absorption of lead in soil is less than that of dissolved lead /2,[74]/. Experimental animal studies in juvenile Rhesus monkeys (38% absorption) versus adult female monkeys (26% absorption) /[75]/ and in rat pups absorbing ~40–50 times more lead than adult rats /76–78/ provided evidence for an age-dependency of gastrointestinal absorption of lead.

The effects of lead exposure are a health concern for all humans, but especially during early childhood because children are most at risk. According to the CDC /79/, during 1999–2002 approximately 310,000 children aged 1 to 5 years remained at risk for exposure to harmful lead levels. The preponderance of experimental and human evidence indicates that lead has persistent and deleterious effects on brain function form the basis for subsequent cognitive impairments in lead-exposed children. The specific effects on glutamatergic transmission, which is critically involved in development, neuronal plasticity, learning and memory, and

mood consolidation, are of particular concern. Impairment of dopaminergic functioning (involved in motor control, attention, memory, and executive functioning /[80]/) could induce a myriad of behavioral problems and cognitive impairments.

Exposure in utero in infancy or exposure in early childhood can slow mental development and cause lower intelligence later in childhood that can persist beyond childhood. As the nervous system of a child is still developing, the effects of lead are more toxic than on a mature brain. In children, lead poisoning can cause brain damage/mental retardation, behavioral problems, low IQ, hearing loss, hyperactivity, developmental delays, behavioral problems, diminished school performance, as well as deficits suggestive of Attention Deficit Disorder (ADD) /[70,81–87]/.

Recent meta-analyses conducted on cross-sectional studies or a combination of cross-sectional and prospective studies suggest that an IQ decline of 1 to 5 points is associated with each increase in PbB of 10 μg dL^{-1}, and identified no threshold for the effects of lead on IQ /[2]/. Collectively, the results of a pooled analysis of additional studies provided suggestive evidence of lead effects on cognitive functions in children at PbBs < 10 μg dL^{-1} and, possibly as low as 5 μg dL^{-1} /[1]/. A threshold below which lead has negligible influence could not be determined /[88]/.

Socioeconomic status (SES) has received attention due to its possible effect on a child's lead exposure. The Third National Health and Nutrition Examination Survey (NHANES III) studied BPb levels in the U.S. between 1991 and 1998. The results showed that 21% of children in the inner city versus 5.8% of children in other areas had BPb levels equal to or greater than the maximum allowable level of 10 μg/dL determined by the CDC. When observed according to income level, 16.3% of children from low-income level families had BPb levels of ≥10 μg/dL compared with 5.4% and 4.0% of children from middle-and high-income families /[89]/.

As discussed before, BPb levels < 10 μg dL^{-1} have been associated with changes in neurochemistry and behavior. Tang and colleagues /[90]/ investigated the prenatal effects of lead exposure on the behavior of 9-month-old infants. The authors used plasma from the samples to evaluate the concentrations of the dopamine metabolite homo-vanillic acid and the serotonergic metabolite 5-HIAA (5-hydroxy indoleacetic acid), along with measuring UCPb levels at delivery. The mean UCPb level was 3.9 μg dL^{-1}, with the 5^{th} and 95^{th} percentiles of the range 2.5 and 7.0 μg dL^{-1}, respectively. Both 5-HIAA levels and measures of sociability were negatively associated with UCPb levels, as shown by correlation analysis, suggesting that low-level prenatal lead exposure could produce neurotoxic effects on the developing serotonergic system and may affect an infant's sociability.

A study conducted by Trope and colleagues /[91]/ examined two male cousins who were living in the same household. One subject, a 10-year-old boy had elevated BPb levels. His cousin, a 9-year-old boy, had not been exposed to lead. A comprehensive neuropsychological evaluation revealed that difficulties in reading, writing, and arithmetic were found only in the lead-exposed child. Difficulties in linguistics and attention mechanisms were found as well. Although high-resolution MRI and MRS (magnetic resonance spectroscopy) showed normal brain MRIs in both subjects, the lead-exposed child had vast alterations in brain metabolites.

Sciarillo and colleagues /[92]/ evaluated the influences of early lead poisoning on socio-emotional development. The authors observed an increase in a variety of behavioral problems in lead-exposed in 4-to-5-year-old children and at a BPb level of 15 μg dL^{-1}, an increase in aggression. Mendelsohn and colleagues /[93]/ carried out a study on children aged

12–36 months who were too young to have experienced academic failure. Children who had lead levels of 25 μg dL^{-1} were evaluated using the BSID to measure factors related to social/emotional function. The scores of the lead exposed children were significantly worse than those of non-exposed children in measures of emotional regulation and orientation engagement.

The effects of lead on the development of the nervous system establish the basis for cognitive impairments in lead-exposed children. On the other hand, specific effects on glutamatergic transmission, which is critically involved in both development and neuronal plasticity, portend impairments in learning and memory. Behavioral problems, including attention deficit hyperactivity disorder, as well as cognitive impairments, are produced by a disruption of dopaminergic functioning. Needleman and colleagues /55/ have also associated lead exposure with juvenile delinquency and criminal behavior (also see /53،72/).

NEUROTOXICITY STUDIES IN ADULTS

Recent studies

The documentation of lead as a toxin for adults preceded the first description of childhood lead poisoning by several millennia, having been recorded as early as 2000 BC /94/. In adults, lead poisoning can cause nerve damage to the sense organs and nerves controlling the body, leading to neurodegenerative diseases like Alzheimer's and Parkinson's disease /95/, hearing and vision impairment, schizophrenia, and impaired cognitive function. Which cognitive domains are affected has only begun to be explored in detail. Weisskopf et al /96/ found that low-level cumulative exposure to lead in nonoccupational settings can adversely affect cognitive function, particularly in the visuospatial/visuomotor domain. Bleecker et al /97/ administered the Rey Auditory Verbal Learning Test (RAVLT), a test of verbal learning and memory, to 256 English speaking lead smelter workers (mean age of 41±9.4 years and employment duration of 17±8.1 years). Lead exposure variables, based on up to 25 years of prior BPb data, included a mean current BPb of 28±8.8 μg dL^{-1}, working lifetime time weighted average blood lead (TWA) of 39±12.3 μg dL^{-1}, and a working lifetime integrated blood lead index (IBL) of 728 (434.4) μg-y dL^{-1}. The results indicated that BPb was not associated with any of the RAVLT variables, but TWA and IBL contributed significantly to the explanation of variance of measures of encoding/storage and retrieval but not immediate memory span, attention, and learning. Thus, lead exposure over years but not current BPb interfered with the organization and recall of previously learned verbal material. Associations between PbB and/or BnPb and poorer performance in neurobehavioral tests have been reported in older populations having a current mean BPb < 10 μg dL^{-1} /2/.

Early Studies

Pioneering long-term follow-up studies of children who had been exposed to lead showed that deficits in neuropsychology (IQ changes) can continue into adulthood. A study by Stokes and colleagues /98/ evaluated young adults (mean age 24.3 years) 20 years after lead exposure as children. The exposed group grew up around a lead smelter which was operated without emission-reducing devices. The average BLL for children in this area was 50 μg dL^{-1} in 1974 and 39.6 μg dL^{-1} in 1975. The BPb level (49.3 μg dL^{-1}) was known for only 25% of the exposed group. At the time of evaluation both groups had low BLLs. The exposed group performed significantly worse on each test of cognitive functioning as well as on tests of fine motor functioning and postural stability. The neuropsychological functioning of a group of adults 50 years after hospitalization for lead poisoning at the age of 4 years or younger was evaluated in a study by White and colleagues /99/. Each individual in the exposed group had a history of lead exposure. When tested, the lead-exposed group had

poorer performance on tasks of abstract reasoning, cognitive flexibility, verbal memory, verbal fluency, and fine motor speed.

In the late 1970s, Tonge et al /100/ found microscopically a significant correlation between cerebellar calcification and raised BnPb lead levels in 10 to 15% of autopsies. A decade later Reyes and colleagues /[101]/ described computed tomographic (CT) findings of cerebral and cerebellar calcification in three adults with known lead exposure for ≥ 30 years and elevated SPb levels at admission (54–72 μg dL^{-1}; normal range, 0–30 μg dL^{-1}). Punctiform, curvilinear, speck-like, and diffuse calcification patterns were found in the subcortical area, basal ganglia, vermis, and cerebellum. All three patients showed nonspecific neurologic symptoms of dementia, loss of visual acuity, and peripheral neuropathy. Schroter and colleagues /[102]/ reported a case of a 59-year old potter in Germany who presented lead neuropathy after 37 years of occupational exposure. The patient had a 25-year history of normochromic normocytic anemia with moderate basophilic stippling. The patient also reported history of 3 short psychotic episodes. Cranial CT showed extensive, bilateral, symmetrical calcification in the cerebellar hemispheres and minor calcification in the subcortical area of the cerebral hemispheres and basal ganglia. T2-weighted MRI showed high signal intensity in the periventricular white matter, basal ganglia, insula, posterior thalamus, and pons.

Most research on lead exposure has focused on deficits in memory and learning. A large body of evidence shows, however, that lead also influences other behaviors such as mood (depression), anxiety, and violence/aggression. Observations of the relations between early lead-exposure and neuropsychological abnormalities have been carried out throughout the course of life. Chronic lead exposure has been linked to the development of neurodegenerative diseases such as Alzheimer's and Parkinson's disease (reviewed by Monnet /[95]/). Alzheimer's disease is characterized by the formation between neurons of waxy plaques consisting predominantly of β-amyloid protein, and lead increases the expression of the amyloid precursor protein.

Schizophrenia is also a candidate due to its features that closely resemble the behavioral deficits linked to lead exposure /103–104/. Opler et al /[104]/ conducted a study of prenatal lead exposure and schizophrenia using the biomarker of exposure δ-aminolevulinic acid in archived maternal serum samples collected from subjects enrolled in the Childhood Health and Development Study (1959–1966) based in Oakland, California. The authors found a possible association between prenatal Pb exposure and the development of schizophrenia in later life. Although several limitations constrained generalizability in a second study in 2008 by the same group, the results provided further evidence for the role of early environmental exposures in the development of adult-onset psychiatric disorders.

NEUROTOXICITY STUDIES IN EXPERIMENTAL ANIMALS

Animal data on lead toxicity are generally considered less suitable as the basis for health effects assessments than are the human data /[2]/. No absolutely equivalent animal model exists for the effects of lead on humans. Nevertheless, studies of lead toxicity in experimental animals are important as an adjunct to non-experimental human studies, particularly if a question remains of whether the associations observed in human studies could be attributable to residual confounding. Similar to human studies, research in animals has clearly demonstrated that learning and memory deficits can be a consequence of developmental lead exposure.

A series of studies in primates conducted by Rice /[105–106]/ showed that similar behavioral problems are seen in lead-exposed primates and in lead-exposed children: increased distractibility, inability to inhibit inappropriate behavioral response, and perseveration in

inappropriate behaviors. For example, lead-treated monkeys were impaired in their ability to perform discrimination reversal task, but not on the initial visual discrimination task. Deficits were more severe in the presence of distracting irrelevant stimuli. Lead-treated monkeys displayed severe perseveration on one button on a task requiring them to alternate responding between two buttons. Lead-treated monkeys displayed memory impairment on a task requiring them to remember a previously observed stimulus or position, which was at least in part the result of interference from responses from previous trials. Lead-treated monkeys exhibited a higher rate of response on an intermittent schedule of reinforcement, and had difficulty inhibiting responding when required. Thus, for many of the tasks on which monkeys have been found to display learning and/or memory impairment, the deficit can be attributed at least in part to an attentional deficit and/or perseverative behavior. This unusual behavioral pattern of response was demonstrated in monkeys with a steady-state BPb level of 11–13 μg dL^{-1} following long-term exposure, and at higher blood lead levels, the behavior was shown to be dose-dependent /[107]/. Monkeys exposed to lead only during infancy were impaired on both spatial and non-spatial tasks of learning and memory /[105,108]/.

In other animal studies, lead affected the hippocampus, cerebellum, and messenger systems in rats. As children of lower SES are known to have a disproportionately higher risk of being exposed to lead, Schneider and colleagues /[109]/ examined the extent to which different environmental surroundings can modify the effects of lead on the developing rat brain. Young rats were raised in either enriched or impoverished environments. Half the animals in each environment were exposed to lead via drinking water half drank distilled water. Lead-exposed rats raised in the impoverished environment had spatial learning deficits and significantly decreased neurotrophic factor gene expression in the hippocampus. In contrast, the animals raised in the enriched environment performed similarly to their unexposed counterparts and were significantly protected against the behavioral and neurochemical toxicity of lead. Lead-exposed rats in the impoverished environment had significantly decreased neurotrophic factor gene expression in the hippocampus. Taken together, the results demonstrated that an impoverished environment can accentuate and an enriched environment can protect against neurobehavioral and neurochemical toxicity from developmental lead exposure.

In mammals, one germinal region in which neurons are born is the subgranular zone (SGZ) of the hippocampal formation. In the SGZ, the stem cells involved in adult neurogenesis are believed to be a subset of astrocytes, which gives rise to intermediate progenitors, which then locally mature into granule neurons integrate into the existing circuitry of the hippocampus /110/. Gilbert and colleagues (cited in /111/) reported that although developmental lead exposure reduced the viability of newly generated neurons in the dentate gyrus, developmental lead exposure did not alter spatial learning and memory in adult rats tested in the Morris Water Maze (MZM). Successful performance in this assay has been linked to the hippocampus, cerebellum, striatum, basal fore-brain, and neocortex. Among the possible explanations for this failure could be that neurogenesis in the adult dentate gyrus granule cell layer of the hippocampal formation might have little to do with MWM performance. Also possible is that other learning and memory assays like fear conditioning or inhibitory avoidance might have revealed deficits.

A significant amount of CNS myelination takes place during the first 2 months of life, and during chronic lead intoxication, the myelin fraction Pb level increases significantly. Oligodendroglia can impair brain function by direct or indirect responses to lead. In lead-poisoned rats, three months of lead exposure (mean BPb, 38.2 μg dL^{-1}; mean brain level, 0.03 μg g^{-1}) caused morphologic abnormalities in the brain and the oligodendrocytes also appeared grossly abnormal /[112]/. The destruction of the myelin sheaths in lead-exposed rats

could be secondary to lead-induced damage to oligodendrocytes during early life. Lead also has a direct effect on myelin proteins; for example, acute lead exposure decreases the activity of an enzyme preferentially located in myelin, CNPase, which has been shown to be an integral protein for myelin synthesis during development.

Ruan and Gu /113¯114/showed that in the BBB, lead accumulating on the plasmalemma of the endotheliocyte endosurface could damage the close junctions and seep between endotheliocytes. The results of a later study suggest that lead exposure disrupts the structure of the BBB in young animals /[115]/. Lead exposure significantly increased BPb concentrations in male weanling Sprague-Dawley rats by 6.6-fold ($p < .05$) and brain tissue Pb by 1.5–2.0-fold ($p < .05$) over those of controls. Electron microscopy suggested a leakage of cerebral vasculature, manifested as an extensive extra-vascular staining of lanthanum nitrate in brain parenchyma. Western blot analysis showed a 29% to 68% reduction ($p < .05$) in occludin expression below the controls. The data suggest that lead exposure disrupts the structure of the BBB in young animals, and that the increased BBB permeability could facilitate the accumulation of lead in the brain. When systematically administered at low doses, lead also induces BBB dysfunction /[116]/.

Experimental animal studies have shown that at nanomolar concentrations, lead induces the mitochondrial release of calcium, initiating apoptosis /117/, which has been particularly well studied in the retina. Exposure to low to moderate concentrations (10 nM to μM) of lead ions induced apoptosis in rod and bipolar cells both in cell culture /130/ and in developing and adult rats /118/. Exposure to low to moderate levels of lead during development (0–21 days), resulting in BPb levels of 19–60 μg dL^{-1} at 21 days of age, produced selective loss of rod and bipolar cells, the dying cells exhibiting signs of apoptosis. Adult rats exposed to low to moderate lead levels for 6 weeks showed similar results. The degree of cell death was age-and dose-dependent, the developing retina being particularly sensitive to lead exposure. Lead-induced retinal degeneration also appeared to be related to rod-specific effects of lead and calcium on rod mitochondria, suggesting that lead and calcium bind to the internal metal-binding site of the mitochondrial transition pore, subsequently open the transition pore, and initiate the cytochrome C-caspase activation cascade leading to apoptosis. These effects of lead on retinal cell apoptosis may have functional significance as long-term visual system deficits occur in humans, monkeys and rats following low to moderate developmental exposure to lead (20–60 μg dL^{-1}) /[125,117]/.

MECHANISMS OF LEAD-INDUCED NEUROTOXICITY

The mechanisms of lead-induced neurotoxicity are complex. Oxidative stress, membrane bio-physics alterations, deregulation of cell signaling, and the impairment of neurotransmission as key aspects involved in Pb neurotoxicity.

Like other commonly found persistent toxic metals (mercury, arsenic, cadmium) lead damages cellular material and alters cellular genetics. The mechanism that all toxic metals have in common, however, involves oxidative damage. Lead can cause toxicity by oxidative stress and by directly or indirectly-produced lipid peroxidation /119¯122/. Lead toxicity leads to free radical damage via two separate, albeit related, pathways: (1) the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxide, and (2) the direct depletion of antioxidant reserves. One effect of lead exposure is on glutathione, a cysteine-based molecule produced in the interior compartment of the lymphocyte. The sulfhydryl complex of glutathione directly binds to toxic metals that have a high affinity for sulfhydryl groups. Thus, lead can effectively inactivate the glutathione molecule, making is unavailable as an antioxidant.

Lead binds to enzymes that have functional sulfhydryl groups, rendering them nonfunctional, further contributing to an impairment in oxidative balance. The levels of two specific sulfhydryl-containing enzymes that are inhibited by lead—ALAD and glutathione reductase—were depressed in both animal and human lead-exposure studies. Depressed levels of glutathione reductase, glutathione peroxidase, and glutathione-S-transferase correlated with depressed glutathione levels in occupationally lead-exposed workers (cited in /[123]/).

Role of Calcium

Under physiological conditions, calcium ions govern a multitude of cellular processes like cell growth, differentiation, and synaptic activity. Although physiological elevations in intracellular Ca^{2+} are salient to normal cell functioning, the excessive influx of Ca^{2+}, together with any Ca^{2+} release from intracellular compartments, can overwhelm Ca^{2+}-regulatory mechanisms and lead to cell death.

Lead accumulates in and damages mitochondria /[124]/. Mitochondria have an important role in the regulation of the intracellular calcium concentration. An increased entry of Ca^{2+} into mitochondria is believed to enhance mitochondrial electron transport, increasing the production of reactive oxygen species (ROS) like $\cdot O_2^-$. Consequently, homeostatic mechanisms exist to maintain a low intracellular Ca^{2+} concentration so that Ca^{2+} signals remain spatially and temporally localized. This permits multiple independent Ca-mediated signaling pathways to occur in the same cell. A decrease in the functioning of the mitochondria can alter the ordinarily benign synaptic transmission mediated by glutamate into neuron-killing excitotoxicity /[125–126]/.

Excitotoxocity—Arundine and Tymianski /[127]/ have reviewed the molecular mechanism of excitotoxicity, the pathological process by which nerve cells are damaged and killed by glutamate and similar substances. Excitotoxocity contributes to neuronal degeneration in many acute CNS diseases, including ischemia, trauma, and epilepsy. The key mediators of excitotoxic damage are calcium ions. In excitotoxicity, excessive synaptic release of glutamate can lead to the disregulation of Ca^{2+} homeostasis. Glutamate and other amino acids can activate both ionotropic (ion channel-forming receptors) and metabotropic (G-protein-coupled) receptors. Metabotropic receptors may contribute very little to the actual acquisition of new information. The ionotropic receptors are subdivided into three receptor classes named by their selective agonists: AMPA (a-amino-3-hydroxy- 5-methyl-4-isoxazolepropionic acid) receptors, kainate receptors, and NMDA-r, which upon activation open their associated ion channel to allow the influx of Ca^{2+} and Na^+ ions. AMPA and kainate receptors trigger rapid excitatory neurotransmission in the CNS by promoting entry of Na^+ into neurons, but a subset of neurons in the hippocampus, cortex, and retina express AMPA receptors that are also permeable to Ca^{2+}. Blockage of AMPA-r will shut down neuronal communication and affect various components essential for learning /[128]/.

Although Ca^{2+} dysregulation is paramount to neurodegeneration, the exact mechanism by which Ca^{2+} ions actually mediate excitotoxicity is less clear. One hypothesis outlined in /[127]/ suggests that Ca^{2+}-dependent neurotoxicity occurs following the activation of distinct signaling cascades downstream from key points of Ca^{2+} entry at synapses, and that triggers of these cascades are physically co-localized with specific glutamate receptors.

Reactive oxygen species—Oxidative stress is recognized as accountable for redox regulation involving ROS and reactive nitrogen species (RNS). The of role oxidative stress is key for the modulation of critical cellular functions, notably for neurons astrocytes and microglia, such as apoptosis program activation and excitotoxocity, the two main causes of

neuronal death. Because they have a reduced capacity to detoxify ROS, neurons are particularly vulnerable to increases in ROS levels /[129]/. Oxidative stress kills neurons by stimulating the Forkhead box, class O transcription factor FOXO3, a pivotal player in cell death/life pathways /130–131/. Neurons in the hippocampal CA1 region are particularly sensitive to oxidative stress. The respiratory chain of mitochondria that by oxidative phosphorylation is the source ATP synthesis is responsible for most ROS, and notably $\cdot O_2^-$. Mitochondrial dysfunction is a final common pathogenic mechanism in aging and in neurode-generative diseases. Nitric oxide synthase (NOS) activity is involved in learning and memory. Low-level lead exposure inhibits NOS activity in the rat hippocampus, the cerebral cortex, and the cerebellum /132–133/. The cascade of events that leads to neuronal death is complex. In addition to mitochondrial dysfunction (apoptosis), excitotoxicity, and oxidative stress (inflammation), the mechanisms from gene to disease involve protein misfolding leading to aggregates and proteasome dysfunction on ubiquinited protein material.

Although mitochondria are the main source of ROS in the excitotoxic process, many enzymatic systems primarily or secondarily increase the presence of these compounds in the CNS /[134]/. Calcium-dependent enzymes convert xanthine dehydrogenase to xanthine oxidase, leading to the production of the superoxide anion $\cdot O_2^-$ and peroxide (H_2O_2). Moreover, Ca^{2+} activates the enzyme phospholipase A2, which leads to the production of arachidonic acid that in turn, is transformed by cyclooxygenases, increasing the formation of $\cdot O_2^-$. Calcium activates NOS, increasing the presence of NO in the neuron and in surrounding areas as well. Nitric oxide has a double effect because it activates guanylylcyclases and reacts with $\cdot O_2^-$ to form the highly toxic compound peroxynitrite, a strong oxidizing agent that causes nitration in proteins and the oxidation of lipids, proteins, and DNA, leading to a form of cell death that has the characteristics of apoptosis. Lipid peroxidation alters the structure of lipidic membranes, and leakage occurs in the cytoplasmic membrane. Apart from the loss of ionic gradients, glutamate release from presynaptic terminals is enhanced, thus exacerbating the adverse effects.

Glutamate and the NMDA Receptor

Glutamate, an excitatory amino acid, activates ionotropic receptors and metabotropic receptors to develop their essential role in the brain. The physiologic role of NMDA-r seems to be related to synaptic plasticity. In addition, working together with metabotropic glutamate receptors, NMDA-r ensures the establishment of the long-term potentiation phenomenon (LTP), a process believed to be responsible for the acquisition of information. These functions are mediated by calcium entry through the NMDA-r-associated channel. Calcium activates a number of Ca^{2+}-dependent enzymes that influence a wide variety of cellular components, like cytoskeletal proteins or second-messenger synthases. High concentrations of glutamate or neurotoxins acting at the same receptors cause cell death through excessive receptor activation. The over-activation of NMDA receptors triggers an excessive entry of Ca^{2+}, initiating a series of cytoplasmic and nuclear processes that promote neuronal cell death. For instance, Ca^{2+}-activated proteolytic enzymes can degrade essential proteins. Ca^{2+}/calmodulin kinase II is activated, and a number of different enzymes are phosphorylated, increasing their activity. Transcription factors like c-Fos, c-Jun, or c-Myc are also expressed. Furthermore, Ca^{2+}-dependent endonucleases can degrade DNA. All these mechanisms, together with enhanced oxidative stress, can induce cell death through necrosis or apoptosis.

Cellular Studies

The direct effects of lead on isolated biological substrates have been investigated using cellular models. Lead exposure has a toxic effect on oligodendroglia and astroglia /[90]/;

astrocytes have been suggested to serve as a lead sink in the mature and developing brain /[135]/. Tiffany-Castiglioni /[135]/ and colleagues have shown that cultured astrocytes accumulate lead at much higher levels than measured in the culture medium, and that more lead is accumulated and retained in younger astrocytes. The astrocytes' ability to accumulate lead is attributed to its maturation of interactions with neuronal cells /[136–137]/. Brain astrocytes accumulate and sequester lead in non-mitochondrial sites far from the site of action, potentially protecting not only their own respiratory processes but those of the more vulnerable neurons, as well /[133,138]/. Although the astrocytic accumulation of lead may serve initially to protect neurons from the toxicity of lead, this glial store of lead may constitute a reservoir for the continuous release of lead into the brain and may contribute neuronal damage.

Studying the fine structural localization of glutamine synthetase in cultured rat brain astrocytes, Norenberg and Martinez-Hernandez /[139]/ found that astrocytes modulate synaptic activity and potential excitotoxicity by taking up glutamate after its release and converting it to glutamine in the presence of the glial-specific enzyme glutamine synthetase. Research using an in vitro system to study the effects of lead on astrocyte-endothelial cell interactions showed that astrocytes in cell cultures are sensitive to the toxic effect of lead at low concentrations while endothelial cell cultures are not /[140–141]/. The authors suggested that lead disrupts the main structural components of the BBB by primary injury to astrocytes followed by secondary damage to the endothelial micro-vasculature.

In primary rat astrocytes, lead does not appear to interfere with intracellular calcium transients /[142]/. The back-transport of Pb^{2+} via the Ca-ATPase pump plays an important role in the ability of the ion to pass through the BBB /[143–145]/. In vitro experiments measured lead uptake into epithelial cells by monitoring the fluorescence of cells loaded with indo-1 at a wavelength at which indo-1 fluorescence is independent of calcium but stopped by the binding of lead. The extracellular medium containing lead caused fluorescence quench over time and was reversed upon addition of a membrane permanent heavy metal chelator. Both time and concentration independence were exhibited in the lead uptake by cells in suspension. The depletion of intracellular calcium stores activated the entry of lead into these cells, possibly occurring via store-operated cation channels.

The 78-kDa molecular chaperone glucose-regulated protein (GRP78) is an interesting target. The GRP78 protein chaperones the secretion of the cytokine interleukin-6 (IL-6) by astrocytes. The ability of lead to bind strongly to GRP78, to induce GRP78 aggregation, and to block IL-6 secretion in astroglial cells provides evidence for a significant chaperone deficiency in lead-exposed astrocytes in culture /[146]/.

Although the BBB and the blood cerebrospinal fluid barrier (BCB) share a common character (tight junctions between adjacent cells), the barriers are entirely different (endothelia in BBB and epithelia in BCB). In an investigation by Shi and Zheng /[147]/, lead exposure on the tightness of BCB was examined. The authors found that early exposure to Pb (before the formation of the tight barrier) at 5 and 10 μM, significantly reduced the tightness of BCB, as evidenced by a 20% reduction in transepithelial electrical resistance values ($p < .05$), and > 20% increase in the paracellular permeability of [$^{(14)}$C]sucrose ($p < .05$). No detectable barrier dysfunction in lead exposure was found after tight-barrier formation. Both RT-PCR and Western blot analyses on typical TJ proteins revealed that lead exposure decreases both the mRNA and protein levels of claudin-1, with the membrane-bound claudin-1 being more profoundly affected than cytosolic claudin-1. Lead exposure had no significant effect on ZO-1 or occluding, however. The data suggest that lead exposure selectively alters the cellular level of claudin-1, which, in turn, reduces the tightness and augments the permeability of tight blood-CSF barrier.

Molecular Studies

Among the most relevant molecular targets of lead neurotoxicity are membrane ionic channels and signaling molecules. Lead transport through the erythrocyte membrane is mediated by the anion exchanger in one direction and by the Ca-ATPase pump in the other. In other tissues, lead permeates the cell membrane through voltage-dependent or other types of calcium channels. Due the similarity between Pb^{2+} and Ca^{2+}, low concentrations of inorganic Pb^{2+} can disrupt transmitter release by causing an aberrant augmentation of basal release and suppression of evoked release /[148]/, which could affect the synaptic connections in the brain during the first few years of development and disrupt brain plasticity. The Pb^{2+} mediated inhibition of evoked transmitter release is largely attributable to an extracellular block of the voltage-gated calcium channels.

Neurotransmitter release—Lead causes variable changes in several neurotransmitter systems. Predominantly, lead interferes with the most common neurotransmitter in the brain, glutamate, which is critical for learning in the developing brain /[149]/. The N-methyl-D-aspartate receptor (NMDA-r) is an ionotropic receptor for glutamate. The activation of the NMDA-r results opens an ion channel that is nonselective to cations, thereby allowing small amounts of Ca^{2+} into the cell. This calcium flux is thought to play a critical role in the development of synaptic plasticity, a cellular mechanism for learning and memory. Pb^{2+} acts as a non-competitive, voltage-independent antagonist of the NMDA-r channel, disrupting long-term potentiation, a process believed to be responsible for the acquisition of information, thereby compromising the permanent retention of newly learned information /[150]/. Inhibition of heme synthesis increases the level of δ-aminolevulinic acid, which has a structure similar to that of the inhibitory neurotransmitter GABA, hence interfering with GABA neurotransmission /[2]/.

PKC activation—Protein kinase C is involved in receptor desensitization, in modulating membrane structure events, in regulating transcription, in mediating immune responses, in regulating cell growth, and in learning and memory among many other functions /[151]/, and in the regulation or modulation of neurotransmitter release /[152–153]/, synaptic and neuronal plasticity /[154–155]/, neuronal ion channels /[156]/, cerebral microvascular function /[157]/, and cognition /[158–160]/. Excessive PKC activation can disrupt prefrontal cortical regulation of behavior and thought, possibly contributing to signs of prefrontal cortical dysfunction such as distractibility, impaired judgment, impulsivity, and thought disorder /[161]/.

Calcium is the natural physiologic activator of PKC, but the ability of picomolar concentrations of Pb^{2+} to substitute for micromolar concentrations of Ca^{2+} in the activation of PKC has been implicated in lead-induced neurotoxicity. In a study of lead workers, higher tibia BnPb levels and longer job durations were associated with higher in vivo PKC activity, and high BPb levels were associated with concomitant decrements in neurobehavioral test scores, but only in those lead workers with higher in vivo PKC activity /[162]/.

Impaired PKC function can compromise the second messenger systems within the cell, leading to changes in gene expression and protein synthesis. Lead binds more avidly than calcium to PKC, however, further interfering with the release of neurotransmitters. To observe the effect of chronic lead contaminant on mRNA expression of PKC and calmodulin in hippocampus of baby rats, Wang et al /[163]/ exposed Wistar pregnant rats to water containing 0.2% or 1.0% lead acetate from day 0 of pregnancy to the day when the offspring weaned. When baby rats were fed with lead-contaminated water at the same concentration as their mothers, the BrPb content of the test groups was much higher than that of the control group; the completion rate of the cliff-avoidance reflex and the score of the step-

down test of experimental groups were lower than those in the control group ($p < .05$). Compared with control group, PKC and calmodulin mRNA expression of chronic lead exposed baby rats in the hippocampus showed a downtrend ($p < .05$). The authors suggested that the linkage between the decrease of PKC and calmodulin mRNA expression level in the hippocampus and the impairment of learning and memory induced by lead in baby rats might be one of the molecular mechanisms of lead-induced impairment of learning and memory.

Inhibition of gene expression—Lead-induced changes in hippocampal NMDA-r subunit mRNA expression can lead to modifications in receptor levels or subtypes and alter the development of defined neuronal connections that require NMDA-r activation /[165]/. Another proposed mechanism for lead-induced neurotoxicity concerns the Brn-3a POU transcription factor, which is associated with the survival and differentiation of sensory neuronal cells during development. To explore the effects of lead on the Brn-3a expression level in the neurons of CNS, a group in China /[165]/ exposed pregnant rats to 0.5 g L^{-1}, 1.0 g L^{-1}, 2.0 g L^{-1} lead acetate solution in drinking water from the 15th day after pregnancy to the 21st day, when the offspring began to be weaned. Brn-3a mRNA transcription levels were monitored by RT-PCR and Brn-3a protein expression levels were observed using an immuno-histochemistry method in various brain sections. Brn-3a mRNA transcription level decreased significantly in neural cells from the cerebral hippocampus in every lead-treated group versus the control group ($p < .05$); the Brn-3a protein level also decreased significantly in lead-poisoned animals compared with controls ($p < .05$ or $p < .01$). The authors concluded that the decreased Brn-3a mRNA transcription and protein expression implies that lead exposure can impede the normal differentiation of neuronal cells. The results could explain why prenatal exposure to low-level lead impaired the space learning and memory ability of offspring of the rats reported in an earlier study /[166]/, in which the BPb and hippocampus BrPb concentrations of 1-day old and 21-day old offspring in lead-exposed rats were significantly increased compared with the control group ($p < .05$).

Similarly, exposure to low-level lead during pregnancy was shown to inhibit the growth associated protein GAP-43 mRNA and protein expression in hippocampus of rats' offspring /[167]/. In that study, the rats' offspring were 1 and 21 days old, the content of lead in hippocampus in treatment groups was significantly higher than that of the controls ($p < .05$), and the integral optical densities of GAP-43 protein and mRNA expression were significantly decreased compared with the controls ($p < .01$, $p < .05$).

In a study presented by Liu and colleagues /[168]/ using the human glioma cell line U-373MG with 10% fetal bovine serum (FBS) in the culture, lead at concentrations ranging from 0.01–10 μM affected gene expression in RT-PCR in a dose-dependent manner. Lead enhanced the expression of tumor necrosis factor (TNF) but decreased those of interleukin-1β (IL-1β), interleukin-6 (IL-6), GABA, transaminase, and glutamine synthetase. The highly sensitive changes of gene expression of these cytokines or metabolic enzymes after lead treatment confirmed their usefulness as biomarkers for the monitoring of lead-induced neurotoxicity.

Claudins are transmembranal proteins that form the backbone of the tight junctions at the BBB. Both RT-PCR and Western blot analyses on typical tight junction proteins revealed that lead exposure decreases the mRNA and protein levels of claudin-1, with the membrane-bound claudin-1 being more affected than cytostolic claudin-1. No significant effect was found upon lead exposure in ZO-1 or occludin. The data suggest that lead exposure selectively alters the cellular level of claudin-1, which in turn, reduces the tightness and augments the permeability of tight blood-CSF barrier. This possibly contributes to lead-induced neurotoxicity among young children /[147]/.

Besides Ca^{2+}, Pb^{2+} can replace other polyvalent cations like Zn^{2+} in the molecular machinery of living organisms. For example, the regulation of genetic transcription through sequence-specific-DNA-binding Zn finger protein or Zn binding sites in receptor channels is altered by Pb by displacing Zn /[169]/. Lead accumulates in cell nuclei and associates with nuclear proteins and chromatin /[170]/, which could have adverse effects on gene function if Pb^{2+} at low concentration is capable of affecting gene regulatory proteins /171/. DNA-binding proteins, Sp1 and TFIIIA, can be affected by lead, at micromolar concentrations (2.5 μM), by acting at the Zn-binding sites of these proteins /172/. Among the several genes that are controlled by Sp1 are ornithine decarboxylase, myelin basic protein, NMDAR1 subunit, and metallothionein /173/ . The nucleic acid binding potential of TFIIIA-type Zn finger proteins shows that they may have a role in regulating gene expression, signal transduction, cell growth and differentiation, and/or chromosome structure /171/. Cellular differentiation and Sp1 expression have a strong association, particularly with differentiation of oligodendrocytes in the brain. Other studies have shown that Egr-1 (the product of an early growth response gene), which is functionally involved in cell proliferation and differentiation in the brain, is also altered by lead exposure /169/.

Genetics—At least three genes have been identified in humans that can influence the accumulation and toxicokinetics of lead /[174]/.

1. Lead has long been known to alter the hematologic system by inhibiting the activities of several enzymes involved in heme biosynthesis. Particularly sensitive to lead action is the δ-ALAD protein, which has two isoforms, ALAD1 and ALAD2. ALAD2 has a higher affinity than ALAD1 for lead. Whether ALAD2 increases vulnerability by raising the BPb level or keeps lead sequestered in the blood is not known /[125]/. Bellinger and coworkers /[175]/ have suggested the latter. The evaluation of an association between lead burden and psychiatric symptoms and its potential modification by an ALAD poly-morphism augmented the evidence of a deleterious association between lead and psychiatric symptoms /[176]/.
2. When lead levels are high enough to compete with the available calcium, the second gene, the vitamin D receptor (VDR), is involved in calcium absorption through the gut and into calcium-rich tissues like bone. The binding of the blood-borne variant of vitamin D to VDR in the nuclei of intestinal cells, kidney, or bone, activates genes encoding calcium-binding proteins that promote calcium transport, increasing the absorption of calcium and lead, if present /[177]/. The VDR genotype contains at least two alleles (b and B) and three variants, bb, BB, and Bb. In occupationally exposed adults, the highest chelatable lead levels and highest lead levels in blood and bone (tibia) have been reported in those having the B allele / 178/.
3. The HFE gene is responsible for hereditary hemochromatosis, the deposit of large quantities of iron in internal organs, and might influence lead absorption. The HFE protein is a type 1 transmembrane protein that acts as a major regulator of iron absorption by binding to the transferrin receptor and decreasing its affinity for iron-loaded transferrin. Polymorphisms in HFE could influence lead absorption because lead can be mistaken for iron and be incorporated into processes requiring iron /[125, 173]/.

Genotoxic Effects

Since the first inconsistent results of investigations on the genotoxicity of lead began to emerge over three decades ago, a wealth of data has accumulated implicating lead as a genotoxic agent. Although generally non-mutagenic in bacterial assays, lead has often been shown to be genotoxic in eukaryotic cells /[179–180]/.

Human studies—Sister chromatid exchanges (SCEs), usually performed on peripheral blood lymphocytes, involve the breakage of both DNA strands, followed by an exchange of whole DNA duplexes. Such exchanges occur during the S phase and are efficiently induced by mutagens that form DNA adducts or that interfere with DNA replication. The formation of SCEs has been correlated with recombinational repair and the induction of point mutations, gene amplification, and cytotoxicity.

In early studies, no detectable increase in SCE frequency relative to controls (BPb <10μg dL^{-1}) was found in a group of 18 workers with a mean BPb of 49 10μg dL^{-1} at air lead concentrations ranging from 0.05 to 0.5 mg m^3 /[181]/. Nor were significant differences in SCE rates found between 19 exposed children (BPb, 30–60 10 μg dL^{-1}) living near a lead smelting plant and 12 controls (BPb, 10–21 10μg dL^{-1}) /182/. Grandjean et al /[183]/ found that BPb and SCE rates decreased in lead workers after summer vacation. Many studies on lead workers have shown that despite increases in both BPb and Zn photoporphyrin (ZPP), newly employed workers did not show any increase in SCE rates during the first 4 months of employment, suggesting that genotoxic effects might occur only after long exposure to lead. Another interpretation of this finding could be that the current BPb level is not a good biomarker of genotoxic effects.

During the new millennium, however, positive results began to emerge. In 2002, a significant increase in SCE was detected in 23 lead workers in China whose mean BPb was approximately 32 μg dL^{-1} /[184]/. The control group (PbB levels < 15 μg dL^{-1}) was selected from an uncontaminated area. Similar results were obtained for 31 workers in a storage battery plant in Turkey who had a mean PbB of 36 μg dL^{-1}; the genotoxicity of lead was measured using SCE, erythrocyte ALAD, urinary delta-aminolevulinic acid (U-ALA), and BPb /[185]/. Decreased ALAD activity in erythrocytes and increased U-ALA excretion was observed at statistically higher BPb levels than the control group. A statistically significant correlation was observed between BPb and SCE frequencies ($p < .05$). Moreover, the correlation between U-ALA excretion and SCE frequencies ($p < 0.01$) was relatively higher than the correlation between BPb and SCE frequencies, suggesting a possible mechanism of ALA mediation in the genotoxic effects of lead.

In a study in India /[186]/, DNA damage was detected in the peripheral blood of workers exposed to lead using the alkali single cell gel electrophoresis assay comet assay /[187–188]/. The mean lead content was found to be significantly higher in the study group (248.3 μg L^{-1}) than in the controls (27.49 μg L^{-1}). Significantly more cells with DNA damage (44.58%) were observed in the study group than in the control group (21.14%). Similarly, using the same assay Steinman-Beck in Poland /[189]/ found that chronic exposure to high BPb levels can induce DNA damage in peripheral blood lymphocytes. Mean BPb concentrations in workers exposed to lead were significantly higher than in controls (422.6 ± 181.2 μg L^{-1} vs. 81.0 ± 37.84 μg L^{-1}, $p < .01$). Both the level and the grade of DNA damage were significantly higher in workers exposed to lead than in controls ($p < .05$). The highest level and the degree of DNA damage were observed in workers with BPb levels > 500 μg L^{-1} and the lowest in workers with PbB < 200 μg L^{-1}.

To clarify the in vivo mechanism(s) responsible for the effects observed in the comet assay in lymphocytes of battery plant workers, Fracasso et al /[190]/ determined ROS production and glutathione levels in living cells using the fluorescent probe (2',7'-dichlorofluorescein and monochlorobimane, respectively). The results indicated that lead-exposed workers have significantly elevated levels of DNA breaks compared with the unexposed group. A significant positive correlation with ROS production was found and a negative correlation with glutathione levels. The content of PKC alpha in cytosol and membranes decreased 40%, indicating a down-regulation of the protein.

In a large group of Bulgarian workers exposed to lead, Vaglenov et al /191–192/ used the MN assay in peripheral blood lymphocytes as endpoint in an investigation of genetic damage. The results indicated that workers occupationally exposed to lead showed clear evidence of genetic damage. A similar study evaluating the genotoxic effects of workers exposed to lead was conducted in the People's Republic of China /193/. The results of the MN test showed a respective mean MN rate and mean micronucleated-cells rate in workers of 9.04±1.51 per thousand and 7.76±1.23 per thousand, which were significantly higher than those (2.36±0.42 and 1.92±0.31 per thousand) in controls ($p < .01$).

In the comet assay, the respective mean tail lengths of 25 workers and 25 controls were 2.42± 0.09 and 1.02±0.08 μm, a significant difference ($p < .01$). Additionally, the difference of the mean tail moment between workers (0.85±0.05) and controls (0.30±0.09) was very significant ($p < .01$). A study in Upper Silesia, Poland showed that lead induces cytogenetic effects on MN in peripheral lymphocytes of 5- to 14-year-old children who had a BPb of 7.69 μg dL^{-1} /194/.

The induction of mutations, SCE, and strand breaks by Pb^{2+} alone or in combination with UV light as a standard mutagen were determined to investigate whether the genotoxicity of lead is due to indirect effects like as interference with DNA-repair processes /195/. Lead acetate alone did not induce DNA-strand breaks in HeLa cells or mutations at the HPRT locus and SCEs in V79 Chinese hamster cells. At all endpoints tested, however, Pb^{2+} interfered with the processing of UV- induced DNA damage. Pb^{2+} inhibited the closing of DNA-strand breaks after UV irradiation and enhanced the number of UV-induced mutations and sister-chromatid exchanges, indicating an inhibition of DNA repair.

Lead acetate genotoxicity on human melanoma cells has been demonstrated as well /196/. In this study, chromosomal damage induced in vitro by lead acetate in human melanoma cells (B-Mel) was evaluated using the cytokinesis-blocked MN assay and SCE analysis. Lead acetate (10^{-6}, 10^{-5} and 10^{-3} mM) induced both MN and SCE formation in a dose-dependent manner. Treated cells showed a decrease in cell viability but not concomitant cell death by apoptosis (lead acetate failed to induce internucleosomal DNA fragmentation at any dose tested). One important observation emerging from this study was that low-level lead exposure in vitro induces significant cytogenetic damage in human melanoma cells, indicating an increased sensitivity of B-Mel cells to lead acetate.

Animal studies—Several experimental studies have reported that lead has a moderate genotoxic potential. In a study conducted by Valverde and colleagues /197/, a lead inhalation model in CD-1 mice was used to detect the induction of genotoxic damage as single-strand breaks and alkali-labile sites in several mouse organs (nasal epithelial cells, lung, whole blood, liver, kidney, bone marrow, brain, and testes), assessed by the comet assay. Following single and subsequent inhalations, differences were found among the organs studied. The distribution of damage from high to low susceptibility was lung>bone marrow>liver>brain> kidney>testicle>nasal cells> leukocytes. A positive induction of DNA damage in the liver and the lung after a single inhalation was observed. The response was positive in all organs, except the testicle, in subsequent inhalations. DNA damage induction over time varied for each organ. The brain and bone marrow showed the highest damage induced. DNA damage, and metal tissue concentration was observed for lung, liver, and kidney. Differences in DNA damage occurred in organs when lead was administered acutely or sub-chronically. The results confirm that inhaling lead induces systemic DNA damage, but certain organs, such as the lung and the liver, are special targets of this metal, partly depending on the duration of exposure. As the lead concentrations used in this work were lower than those used in prior studies /198–199/, the authors concluded that even at low

levels of inhalation exposure, this metal could induce DNA damage and should be considered a risk for living organisms.

A subsequent study conducted in Croatia /[200]/ evaluated the genotoxic effect of lead acetate in the early period of life when the organism is extremely sensitive to toxic effects of lead. Six-day-old suckling Wistar rats were exposed to lead (as acetate) either orally for 9 days (daily dose 2 mg lead/kg b.wt., 18 mg/kg b.wt. total dose) or by a single intraperitoneal injection (5 mg lead/kg b.wt.). DNA damage was investigated using the comet assay and in vivo MN. The results of the comet assay showed statistically significant differences between the unexposed animals and the two groups of exposed animals ($p < .05$), which were also significantly different from each other. Orally lead-exposed animals showed a significant increase of MN frequencies in reticulocytes and erythrocytes compared with unexposed animals ($p < .05$).

CONCLUSION

Lead pervades almost every organ and system in the human body, but the main target for lead toxicity is the CNS, both in adults and in children. Blood is the most common tissue used as a biomarker of lead exposure although many other tissues and body fluids including the bone, hair, nail, saliva, tooth, urine, and umbilical cord blood have been considered. Lead is more toxic in young and unborn children than in older children and adults. In children, lead poisoning has been associated with brain damage, mental retardation, behavioral problems, developmental delays, violence, and death at high levels of exposure. The metal has also been related to the damage of sense organs and nerves controlling the body, impaired cognitive function, as well as hearing and vision impairment in adults. Studies have shown that lead exposure in children persists into adulthood. Experimental studies with animals have shown that lead exposure causes genotoxic effects, especially in brain, bone marrow, lung, and liver cells.

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