1974 (including covering contaminated soil with clean soil and reducing smelter emissions) and brought about a decrease in blood lead levels that were measured a year later. The details of the blood lead levels and their significance are presented in Chapter 12. The conclusion to be drawn from this study (and from the similar studies referred to above) is that people who live in the vicinity of a major industrial source of lead (e.g., a smelter) are exposed to abnormally high lead concentrations.

7.4 DIETARY EXPOSURES

7.4.1 Food

The route by which most people receive the largest portion of their daily lead intake is through foods, with estimates of the daily dietary lead intake for adult males ranging from 100 to 500 μg/day.[26] Only a fraction of this ingested lead is absorbed, as discussed in Chapter 10.

The sources of the lead content of unprocessed vegetable foods have been noted earlier (Section 6.4.3). Studies of the lead associated with crops (near highways) have shown that both lead taken up from soil and aerosol lead delivered by deposition are found with the edible portions of common vegetable crops. However, there is enormous variability in the amount of lead associated with such crops and in the relative amounts of lead in and on the plants. Several factors are involved, the most prominent of which are: the plant species, the traffic density, the meteorological conditions, and the local soil conditions.[27-33] The variability induced by differences in the above factors, coupled with the fact that many studies have neglected differentiation between lead on plants versus lead in the plants, makes it difficult to generalize. Data of Schuck and Locke[29] suggest that in some cases (e.g., tomatoes and oranges), much of the surface lead is readily removed by washing. But as noted in Section 6.4.3, this is not universally true; in some cases much more vigorous washing procedures are required.

In view of the wide variability of soil conditions (pH, organic matter, cation exchange capacity, phosphorus content, etc.), of meteorology (especially wind conditions and rainfall), and of the effects of species diversity on the routes of lead accumulation, only crude general correlations between air lead levels and food crop lead levels are possible. This is influenced by the fact that the lead associated with plants may be derived from natural sources, from automotive sources, and from other sources such as manufacturing or combustion. One study in Southern California reported that 60 to 70 percent of the lead associated with oat tops was directly attributable to automobile (aerosol) emissions, but it did not distinguish between lead in the edible portion (grain) and lead on the hulls or chaff.[27] This same study reported that lettuce grown in the Salinas Valley had 3 to 25 ppm lead (dry weight) associated with it, whereas the soil lead content was only 10 ppm. The lead content in the lettuce was reported to be 0.15 to 1.5 ppm on a fresh weight basis. The limited data accumulated were used to deduce that the excess lead was delivered to the lettuce by aerial emission from autos, and that removal of lead from automobile exhaust would reduce the lead content of the lettuce by as much as 80 percent.[27] In other areas, the contribution would be smaller. Though these figures may be accurate, they are based on some rather tenuous assumptions that are not well supported by observation and very limited data. The estimates must thus be considered with caution. Moreover, one cannot extrapolate from lettuce or oats to all crops.

The possible connection between air lead and food lead may be underscored by comparisons between leafy vegetables and food grains. Studies have shown that the edible portions of grains absorb very little air lead,[32] whereas the leafy vegetables retain appreciable quantities.[27-29] An FDA survey[34] shows that grains contain approximately 20 percent as much lead as the leafy vegetables. It cannot be concluded, however, that 80 percent of the lead in all leafy vegetables derives directly from air because the difference must also reflect species-dependent differences in uptake from soil.

An overall analysis of the data available supports the contention that plants grown near busy highways consistently have more lead in and on them than those in other areas. This difference is typically very hard to detect at distances greater than about 100 to 200 m from the highway, thereby reflecting the fact that large percentages of the aerosol lead fall out near roadways. It appears reasonable to point out that the vast majority of edible crops marketed in this country are grown at distances of more than 100 to 200 m from the highway and that much of the aerosol lead can be removed from association with the plants by processing. Clearly there are exceptions that will influence both sides of the question. For the present, however, the available data are not sufficient to permit the quantitative estimate of the contribution of automotive lead to foodstuffs on a national or even regional scale.

The concentrations of lead in various food items are highly variable, and as much variation is found

within specific food items as between different food categories. Schroeder and Balassa,[35] in a study of American foods, have found that the ranges are 0 to 1.5 mg/kg (ppm) for condiments, 0.2 to 2.5 mg/kg for fish and other seafood, 0 to 3.7 mg/kg for meats and eggs, 0 to 1.39 mg/kg for grains, and 0 to 1.3 mg/kg for vegetables. All of these values refer to unprocessed foods. A British report[36] on lead in foods describes similar ranges for meat and eggs, grain products (flour and bread), and vegetables; but concentrations up to 14 mg/kg were found in condiments, and up to 18 mg/kg in certain shellfish.

The amount of lead taken in with food varies from person to person. It depends on (a) the total amount of food eaten, (b) the history of the food during growth, (c) its opportunity to acquire intrinsic lead (absorbed from soil or water) and extrinsic lead (deposited insecticides or contaminated dusts), and (d) dietary habits (such as using fresh rather than canned foods). On a per-weight basis, the dietary intake of lead by children has been shown to be two or three times that of adults. This additional dietary intake is especially significant when the lead added to food by processing and to water by plumbing *(vide infra)* is considered. A 1974 FDA survey of heavy metals in foods[37] found relatively high lead concentrations in metal-canned foods. In the adult food category, canned foods averaged 0.376 ppm lead, and non-canned foods averaged 0.156 ppm lead. In the baby food category, canned foods (juices) averaged 0.329 ppm lead, and foods in jars averaged 0.090 ppm. The report of the survey concludes that from the age of about 1 year on, canned foods comprise 11 to 12 percent of a person's diet, but they contribute about 30 percent of the average dietary lead intake. In a comparison made in the United Kingdom,[38] lead concentrations in canned foods were found to vary widely with the precise nature of the food, but they averaged about ten times greater than those in fresh foods.

The soldered seam of tin cans is evidently the major source of this additional lead in canned foods, and increasing lead concentrations in samples of a can's contents taken progressively nearer the seam have been found.[39] Similarly, there is a correlation between increasing lead concentrations in canned products and the increasing ratio of the can's seam length to volume. Of 256 metal-canned foods examined, 37 percent contained 200 μg Pb/liter or more; 12 percent contained 400 μg Pb/liter or more. These levels are markedly above the potable water standard of 50 μg Pb/liter (0.05 mg/kg) established by the U.S. Public Health Service.

Canned pet foods have been found to contain 0.9 to 7.0 ppm lead (approximately 900 to 7,000 μg/liter),[40] and 18 products averaged 2.7 ppm (approximately 2700 μg/liter). Apart from the possible toxic effects on pets, the products pose a hazard to persons who may include them in their own diet.

The lead content in milk is of special interest because it is a major component of the diets of infants and young children. The FDA survey[37] found lead concentrations in whole milk ranging from 10 to 70 μg/liter and averaging about 20 μg/liter. In a recent study by Ziegler et al.,[41] seven samples of baby formula and three samples of whole cow's milk were analyzed in duplicate. Mean concentrations of lead were 18 μg/kg (range 15 to 20) in formula and 10 μg/kg (range 7 to 15) in milk (1 μg/kg is approximately 1 μg/liter). Lead concentration in infant fruit juices ranged from 23 to 327 μg/kg; in five varieties of strained fruits, it ranged from 13 to 131 μg/kg; and in seven varieties of strained vegetables, lead concentration was 14 to 73 μg/kg. Tolan and Elton[38] reported 30 μg Pb/liter in fresh milk in Great Britain and 50 μg Pb/liter in canned (evaporated) milk. Michell and Aldous[39] reported a comparable average for fresh whole milk purchased in New York State — 40 μg Pb/liter. But their results for evaporated milk averaged 202 μg Pb/liter and ranged as high as 820 μg Pb/liter.

Hankin et al.[42] suggest an additional food-related source of potential lead exposure, again predominantly affecting children. The colored portions of wrappers from bakery confections, candies, gums, and frozen confections have lead concentrations ranging from 8 to 10,100 ppm. The higher concentrations are attributed to lead-containing inks. No related illnesses were identified, nor was contamination of the food implied; but the eating of foods from such wrappers and the licking or chewing of the wrappers were postulated as one more avenue for an additional increment to total lead exposure.

The presence of high lead concentrations in illicit whiskey (moonshine), which is still popular in some parts of the United States despite the repeal of prohibition, causes lead poisoning in adults. The apparent source of the lead is the soldered joints in the distilling apparatus.

Another potential source of dietary lead poisoning is the use of inadequately glazed earthenware vessels for food storage and cooking. An impressive example of this danger involved the severe poisoning of a physician's family in Idaho and stemmed from drinking orange juice that had been stored in an earthenware pitcher.[43] Similar cases, sometimes in-

cluding fatalities, have involved other relatively acidic beverages such as fruit juices and soft drinks and have been documented by other workers.[44,45]

Recent reports on lead in European wines[46,47] show concentrations typically averaging from 130 to 190 μg/liter (0.13 to 0.19 ppm) and ranging as high as 299 μg/liter (0.299 ppm). Measurements of lead in domestic wines have not been undertaken; but if the European data are indicative, wines could contain lead concentrations comparable to processed foods previously discussed.

7.4.2 **Water**

The U.S. Public Health Service's standards for drinking water specify that lead should not exceed 50 μg/liter (0.05 ppm). The average adult drinks about 1 liter of water per day. The presence of detectable amounts of lead in untreated public water supplies was shown by Durum[48] to be widespread, but only a few samples contained amounts above the 50 μg/liter standard. Durfor and Becker[49] analyzed untreated and treated water for the largest U.S. cities, and almost all pairs of samples showed a substantial decrease in lead that was ascribable to treatment provided. A maximum lead concentration of 62 μg/liter was detected in finished water from one of several wells used in Salt Lake City to supplement their surface water supply. Some 95 percent of the water supplies sampled, however, had less lead than 10 μg/liter in the treated water before entering the distribution system. Eight of the water supplies distributed water with a pH of less than 7, which could be corrosive to the distribution piping; most of these were in the Northwest. A chemical analysis of 592 interstate carrier water supplies in 1975 showed only 0.3 percent to exceed the 50 μg/liter standard.[50] These samples were collected after treatment but before distribution, and they represent both suspended and dissolved lead. Interstate carrier water supplies serve planes, trains, buses, and vessels in interstate commerce, and they include almost all of the largest U.S. water supplies.

The presence of lead in drinking water may result from contamination of the water source or from the use of lead materials in the water distribution system. Although lead is a relatively minor constituent of the earth's crust, it is widely distributed in low concentrations in sedimentary rock and soils (as discussed in Chapter 3), and naturally occurring deposits may be an important source of contamination in isolated instances. Industrial waste may also contribute to the lead content of water sources, but this appears to be a local and not a widespread problem. The extensive use of lead compounds as gasoline additives has greatly increased the availability of lead for solution in ground and surface waters. For example, in a study in east-central Illinois,[51] the urban portion of an 86-square-mile watershed, which constituted 14 percent of the area, contributed about 75 percent of the lead in the drainage waters. The principal source of this lead is identified as automotive emissions. Detailed data reported for 1 month (June 1972) show that drainage waters from this urban portion contained an average total lead concentration of 69.5 μg/liter, including 6.3 μg/liter of soluble lead. The rural portion yielded an average of lead concentration of 7.4 μg/liter of drainage water, including 2.1 μg/liter of soluble lead.

The major source of lead contamination of drinking water is the water supply system itself. Water that is corrosive can leach considerable amounts of lead from lead plumbing and lead compounds used to join pipe. Several widely adopted codes, such as the ASA-A40 Code, Uniform Plumbing Code, and BOCA Code, allow the use of lead pipe and list lead as an acceptable soldering material for joining pipes that convey water. Lead pipe is currently in use in many parts of the United States for water service lines and interior plumbing, particularly in older urban areas. In a community water supply survey of 969 water systems conducted in nine geographically distributed areas of the United States in 1969 and 1970, it was found that 1.4 percent of all tap water samples exceeded the 50 μg/liter standard.[52] The maximum concentration found was 640 μg/liter total lead. The occurrence of samples exceeding the standard was more prevalent in waters with a relatively low pH and low specific conductance. It was estimated that 2 percent of the survey population of 18.2 million was exposed to high lead levels at the tap.

Hem and Durum[53] discuss the solubility of those species of lead that may be present in drinking water and suggest that the solution of lead from environmental sources may be an important contribution in certain areas, depending on the chemical composition of the runoff water. Above pH 8.0, the solubility of lead is below 10 μg/liter, regardless of the alkalinity of the water. In waters near pH 6.5 with a low alkalinity, however, the solubility of lead could approach or exceed 100 μg/liter. Lazrus et al.[54] determined the lead content of precipitation at 32 points in the United States for a period of 6 months in 1966 and 1967. They reported an average lead concentration of 34 μg/liter after filtering the sam-

ples. Samples of rainfall at Menlo Park, Calif., during 1971 showed a wide range of lead concentrations, from a few μg/liter to more than 100 μg/liter.[53] Hem and Durum[53] hypothesize that higher lead concentrations should be anticipated in runoff water and impounded raw water supplies in the Northeast, certain urban areas of the South, and along the Pacific Coast because of low pH and alkalinity in waters. However, in much of the rest of the United States, lead fallout rates and the chemical composition of the runoff (pH >8; alkalinity >100 mg/liter) would minimize the problem. Information to test their hypothesis is limited at present. Of the few surveys of surface waters that have been conducted, most were not done after periods of heavy rainfall, and the surveys that have been done have measured dissolved rather than total lead. Durum[48] measured lead at 700 lake and river sites in the United States. These measurements were primarily single samples taken at times of relatively low stream flows in October and November 1970. Detectable concentrations of dissolved lead (>1 μg/liter) were found in 63 percent of the samples, but only three samples contained more than 50 μg/liter. A large proportion of the samples for the northeastern and southeastern states contained lead above the detection limit, and quite a few of the samples showed levels above 10 μg/liter. This regional distribution of lead in stream water is in accord with the idea that water composition in the eastern states is more commonly favorable for solution of lead. A substantial number of samples from southern California were high in lead, and these influenced the data from the southwestern states. Kopp and Kroner[55] presented data on dissolved lead in rivers and lakes of the United States. The data were gathered over a 5-year period (1962 to 1967) and represent more than 1500 samples. A detectable concentration of dissolved lead was found in 305, or 19.3 percent of the samples; the observed values ranged from 2 to 140 μg/liter. The highest concentration was detected on the Ohio River at Evansville, Indiana. Twenty-seven of their samples exceeded 50 μg/liter. Observed mean observations of >30 μg/liter dissolved lead were found in the following river basins: Ohio, Lake Erie, Upper Mississippi, Missouri, Lower Mississippi, and Colorado.

7.5 OCCUPATIONAL EXPOSURES

The highest and most prolonged exposures to lead are found among workers in the lead smelting, refining, and manufacturing industries.[56] In the work areas, the major route of lead exposure is by inhalation and ingestion of both lead-bearing dusts and fumes. Airborne dusts settle out from the air onto food, water, the workers' clothing, and other objects and are then transferred to the mouth in one fashion or another. Therefore, good housekeeping and, above all, good ventilation have a strong impact on exposure. Exposure levels have been found to be quite high in one factory and quite low in another solely because of differences in ventilation engineering or housekeeping practices and worker education.

7.5.1 Exposures in Lead Mining, Smelting, and Refining

The greatest potential for high-level exposure exists in the process of lead smelting and refining.[56] The most hazardous operations are those in which molten lead and lead alloys are brought to high temperatures, resulting in the vaporization of lead. This is because condensed lead vapor or fume has, to a substantial degree, a small (respirable) particle size range. Thus although the total air lead concentration may be greater in the vicinity of ore-proportioning bins than it is in the vicinity of a blast furnace in a primary smelter, the amount of particle mass in the respirable size range may be much greater near the furnace.

A measure of the potential lead exposure in primary smelters was obtained in a study of three typical installations in Utah.[56] Air lead concentrations near all major operations, as determined using personal monitors worn by the workers, were found to vary from about 100 to more than 4000 $\mu g/m^3$. Obviously, the hazard to these workers would be extremely serious were it not for the fact that the use of respirators is mandatory in these particular smelters.

Although there are no comparable data for exposures in secondary smelters, which are found in or near most large cities, the nature of their operation is similar to that of primary smelters except that no ore-processing is involved, since secondary smelters depend on the local supply of lead scrap in the form of discarded electric storage batteries, cable casings, pipes, and other materials for their supply of lead. Consequently, the exposure hazard to workers in secondary smelters is probably similar to that found in the primary smelter study. Hundreds, perhaps thousands, of the small scrap dealers that supply these secondary smelters have their own neighborhood or even backyard melting operations for extracting and reclaiming lead. These operations can contribute substantially to local airborne lead levels.

High levels of atmospheric lead are also found in foundries in which molten lead is alloyed with other metals. Berg and Zenz[57] found in one such operation that average concentrations of lead in various work areas were 280 to 600 $\mu g/m^3$. These levels were subsequently reduced to 30 to 40 $\mu g/m^3$ with the installation of forced ventilation systems to exhaust the work area atmospheres to the outside.

Exposures for workers involved in lead mining depend to some extent on the solubility of the lead from the ores. The lead sulfide (PbS) in galena is insoluble, and absorption through the lung may be slight. It is not really known how readily absorption takes place. In the stomach, however, some lead sulfide may be converted to slightly soluble lead chloride, which may then be absorbed in moderate amounts.

7.5.2 Exposures in Welding and Shipbreaking

When metals that contain lead or are protected with a lead-containing coating (paint or plating) are heated in the process of welding or cutting, copious quantities of lead particles in the respirable size range are emitted into the air. Under conditions of poor ventilation, electric arc welding of zinc silicate-coated steel (determined to contain some 29 mg Pb/in^2 of coating) produced breathing-zone concentrations of lead reaching 15,000 $\mu g/m^3$, far in excess of 450 $\mu g/m^3$, the current occupational short-term exposure limit (STEL) in the United States.[58] Under good ventilation conditions, a concentration of 140 $\mu g/m^3$ was measured.[59]

In a study of salvage workers using oxy-acetylene cutting torches on lead-painted structural steel under conditions of good ventilation, breathing-zone concentrations of lead averaged 1200 $\mu g/m^3$ and ranged as high as 2400 $\mu g/m^3$.[60]

7.5.3 Exposures in the Electric Storage Battery Industry

At all stages in battery manufacture except for final assembly and finishing, workers are exposed to high air lead concentrations, particularly lead oxide dust. Air lead concentrations as high as 5400 $\mu g/m^3$ have been recorded in some studies.[56] The hazard in plate casting, which is a molten-metal operation, is from the spillage of dross, resulting in dusty floors. During oxide mixing, which is probably the most hazardous occupation, ventilation is needed when the mix is loaded with lead oxide powder, and frequent cleanup is necessary to prevent the accumulation of dust. In the pasting of the plates, whether by hand or machine, the danger again is from dust which accumulates as the paste dries. Also the forming and stacking processes are dusty, and ventilation is needed there also. The data cited are sufficiently alarming to suggest that respirators must be worn in most of these operations.

7.5.4 Exposures in the Printing Industry

In a printing establishment, the exposure to lead is probably in direct proportion to the dispersion of lead oxide dust, secondary to the remelt operation. Brandt and Reichenbach[61] have reported on a 1943 study in which melting pots were located in a variety of places where used type was discarded. The pots were maintained at temperatures ranging from 268° to 446°C. The highest air lead concentration recorded was 570 $\mu g/m^3$. Since this report was published, working methods and industrial hygiene conditions have changed considerably; but a marginal degree of hazard still prevails. In 1960, Tsuchiya and Harashima[62] found in several printing shops in Japan lead levels of 30 to 360 $\mu g/m^3$ at breathing level.

7.5.5 Exposures in Alkyl Lead Manufacture

Workers involved in the manufacture of both tetraethyl lead and tetramethyl lead, two alkyl lead compounds, are exposed to both inorganic and alkyl lead. Some exposure also occurs at the petroleum refineries where the two compounds are blended into gasoline, but no exposure data are available on these blenders.

The major potential hazard in the manufacturing of tetraethyl lead and tetramethyl lead is from skin absorption, but this is guarded against by the use of protective clothing. Linch et al.[63] found a correlation between an index of organic plus inorganic air lead concentrations in a plant and the rate of lead excretion in the urine of the workers. The average concentration of organic lead in the urine was 0.179 mg/m^3 for workers in the tetramethyl lead operation and 0.120 mg/m^3 for workers in the tetraethyl lead operation. The tetramethyl lead reading was probably higher because the reaction between the organic reagent and lead alloy takes place at a somewhat higher temperature and pressure than that employed in tetraethyl lead production.

7.5.6 Exposures in Other Occupations

In both the rubber products industry and the plastics industry there are potentially high exposure levels to lead. The potential hazard of the use of lead stearate as a stabilizer in the manufacture of polyvinyl chloride was noted in the 1971 Annual Re-

port of the British Chief Inspector of Factories.[64] The Inspector stated that the number of reported cases of lead poisoning in the plastics industry was second only to that in the lead smelting industry. Scarlato et al.[65] and Maljkovic[66] have reported on other individual cases of exposure. The source of the problem is the dust that is generated when the lead stearate is milled and mixed with the polyvinyl chloride and the plasticizer.

Sakurai et al.[67] in a study of bioindicators of lead exposure, found ambient air concentrations averaging 58 $\mu g/m^3$ in the lead covering department of a rubber hose manufacturing plant. Unfortunately, no ambient air measurements were taken for the other departments or the control group.

7.5.7 Exposures Resulting from Manmade Materials

At least two manmade materials in widespread use are known to contain lead: paint and plastics.

In 1974, the Consumer Product Safety Commission collected selected household paint samples and analyzed them for lead content.[68]Analysis of 489 samples showed that 8 percent of oil-based paints and 1 percent of water-based paints contained greater than 0.5 percent lead (5000 μg Pb/g paint, based on dried solids), which was the statutory limit at the time of the study. The current statutory limit for Federal construction is 0.06 percent. This limit is equivalent to 600 μg Pb/g paint.[68] Old paint that is still on buildings will continue to pose a potential hazard for some time. It can become accessible through flaking, even though painted over with nontoxic paints.

Lead in paint constitutes a potential health problem primarily for children with pica who may habitually ingest 1 to 3 g (or more) of paint per week.[68]

Plastics contain a number of heavy metals that are constituents of organometallic stabilizers added during manufacture. The most commonly used lead-containing stabilizer is dibasic lead stearate, in amounts ranging from 0.5 to 2.0 parts per 100 parts of resin.[69] This stabilizer is normally used in rigid PVC products. Diffusion, or leaching by solvents, is estimated to be quite slow — on the order of 10^{-10} to 10^{-12} cm^2/sec at room temperature — but no definitive information is available.

Incineration of lead-containing plastics may become an increasingly significant source of localized lead pollution. It has been estimated that in the year 2000, for example, there could be approximately 2.54 times 10^9 kg of PVC plastic waste to be disposed of annually, of which about 0.59 times 10^9 kg would probably be incinerated.[70] Assuming that lead will be emitted from the uncontrolled incineration of PVC's at the rate of 0.2 g Pb/kg of waste[71] (a figure applying to all solid waste), about 1.2 times 10^5 kg of lead could be released per year. This would be an increase of more than fourteenfold over the estimate for 1975. Since the greater part of the lead in these incinerated plastic wastes will remain in the ash, electrostatic precipitators can substantially decrease the emitted fraction (to an estimated 0.03 g/kg). But this process only aggravates the difficulties of residual solid waste disposal with its attendant problems of fugitive dust and the potential contamination of soil, surface waters, and groundwaters through leaching from landfill operations.

Lead is present in other products that may constitute sources of lead exposure when used or disposed of. Lead may be found in color newsprint, craft and hobby materials, toothpaste tubes, cosmetic products, candle wicks, pewter and silver hollowware, painted utensils, and decals on glassware. For example, lead in the paint on handles of kitchen utensils has been found by Hankin et al.[72] to range from 0 to 9.7 percent (0 to 97,000 ppm). More than half the paint samples (13 of 21) exceeded the allowable limit for painted toys, which is 0.06 percent.

7.5.8 Historical Changes

Perhaps the most impressive data on the magnitude of environmental contamination by lead and its increase over time are to be found in a small group of recent historical studies that examined levels of the metal in polar snow and ice (Chapter 6). Of particular interest was the 200-fold increase in lead levels over several centuries found by Murozumi et al.[73] in the interior of northern Greenland, and the 10-fold increase through the last century of ice layers reported by Jaworowski[74] in a study of two Polish glaciers. These findings parallel the results of a study by Ruhling and Tyler,[75] which indicated an approximate fourfold increase in lead content in Swedish moss samples taken from the period 1890 to the present. These historical records reflect the increasing distribution of lead caused by man.

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8. EFFECTS OF LEAD ON ECOSYSTEMS

It has been substantiated that lead is a natural constituent of the environment, but natural, background levels of lead in the environment are not known with any degree of certainty. As a natural constituent, lead does not usually pose a threat to the organisms of natural and agroecosystems. However, the widespread use of lead in a variety of chemical forms by man has redistributed the natural lead in the environment and has consequently increased the exposure of the biotic components of ecosystems to unprecedented levels of lead. Concern now exists about the possible threat to these biotic components because of their inherent value to ecosystem stability and because of the ultimate impact that effects of the ecosystem would have on man. Figure 8-1 depicts the environmental flow of lead and the possible exposure routes for plants and animals in the ecosystem.[1]

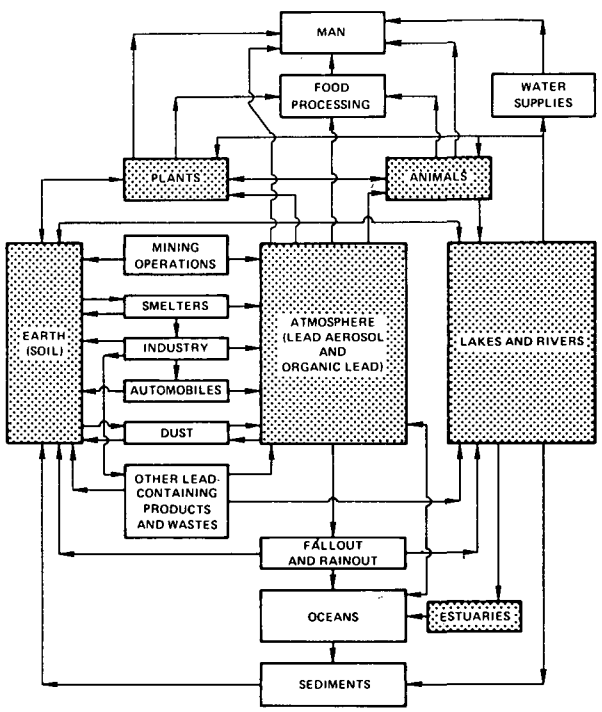

Figure 8-1. Simplified ecologic flow chart for lead showing principal cycling pathways and compartments.[1]

8.1 EFFECTS ON DOMESTIC ANIMALS, WILDLIFE, AND AQUATIC ORGANISMS

8.1.1 Domestic Animals

Lead poisoning, a frequent cause of accidental death in domestic animals for many years,[2] usually results from the ingestion of lead or lead-containing material. Substances that cause lead poisoning include lead-based paints, used motor oil, discarded oil filters, storage batteries, greases, putty, linoleum, and old paint pails. Animals such as cattle, dogs, and cats that have natural licking and chewing habits are particularly susceptible. Horses are not usually poisoned in this manner because they normally do not lick discarded materials. Animals grazing in the vicinity of smelters, mines, and industrial plants from which lead fumes and/or dusts are being emitted or that are fed vegetation harvested from such areas may also be poisoned. The possibility of animals being poisoned from ingestion of roadside pasture contaminated by mobile sources is of concern, but no case of this type has been reported in the literature.[3]

Breathing of lead dusts can be another way whereby animals are exposed to lead, but poisoning of domestic and wild animals as a result of lead inhalation has not been substantiated.

Incidents of lead poisoning in cattle and horses caused by emissions from stationary sources have been reported from Benecia, Calif.;[3] Trail, B.C.;[2] Belleville, Penn.;[4] St. Paul, Minn.;[5] and southeastern Missouri.[6] Deaths from lead poisoning of lambs and sheep in Britain[7] and of horses, sheep, and goats in continental Europe have been reported.[8-11]

Hammond and Aronson[5] have estimated that the minimal cumulative lethal dose of lead for a cow is 6 to 7 mg/kg of body weight per day. They state that this intake represents a concentration of about 300 ppm in the total diet. In cattle that consumed lead-contaminated hay and corn silage grown in a field adjacent to a smelter, fatal lead poisoning occurred after approximately 2 months. In another study,[12]

cattle were fed lead at the rate of 5 to 6 mg/kg per day for a period of 2 years without the appearance of visible clinical symptoms. A steer fed the same diet for 33 months, however, showed clinical symptoms of lead poisoning culminating in death.[13] The length of time required for the appearance of overt clinical symptoms of lead poisoning is in most instances directly related to the amount consumed per unit of time.

Horses are more susceptible than cattle to poisoning from the chronic intake of lead. In the Trail, B.C., study,[2] horses grazing near a lead smelter developed overt symptoms of lead poisoning, but cows in the same pasture were not clinically affected. The minimal toxic dosage for a horse has been estimated to be between 1.7 and 2.4 mg/kg body weight daily,[1] which is approximately 80 ppm dry weight in forage.

The reasons for the greater susceptibility of horses are complex. An early symptom of lead poisoning in the horse is paralysis of the nerves of the pharynx and larynx. This interferes with breathing, especially on exercise, and causes the animal to breathe stertorously, or to roar. When severe, this paralysis can produce suffocation and death. In addition, the faulty action of the epiglottis (which closes off the lung during inhalation) permits inhalation of food, which can result in suffocation and death or in severe pneumonia, which can also be fatal.

Colts pastured near the Trail, B.C., smelter[2] showed loss of weight, generalized muscular weakness, stiffness of joints, and harsh, dry coats. Distortion of the limbs occurred, the joints became greatly enlarged and the hocks touched. The laryngeal paralysis usually associated with lead poisoning did not occur. Hupka[9] noted the same symptoms in colts that had grazed on pasture contaminated with flue dust from a metal works. Autopsy showed that the auricular cartilages were detached and loose in the joint. Also noted was an acute catarrhal pneumonia of both lobes of the lungs, with food particles in the bronchi. Roaring typical of chronic lead poisoning did not occur until quite late.

The clinical picture described above has come under close scrutiny in various laboratory studies.[14,15] because of the lack of consensus as to whether it was the result of lead poisoning. Lead and zinc coexist in many ores, and both were present in the two situations referred to above. Under these conditions animals being reared in the area would be exposed to both elements simultaneously. Studies by Willoughby et al.[14] indicated that lameness, blindness, swelling at the epiphyseal ends of long bones, or an increase in the amount of joint fluid in foals resulted from the intake of zinc by itself and zinc and lead together, but not from lead alone. Gunther,[15] in studies based on experimental exposure of colts, reached similar conclusions.

Horses sometimes, though infrequently, pull up plants and eat roots and soil along with leaves. This practice may be a factor in increasing their lead intake[16] relative to that of cattle grazing the same pasture.

Lead poisoning in all domestic animals produces various degrees of derangement of the central nervous system, gastrointestinal tract, muscular system, and hematopoietic system. Younger animals appear to be more sensitive than older ones.[13] Calves may suddenly begin to bellow and stagger about rolling their eyes, frothing at the mouth, and crashing blindly into objects. This phase may last up to 2 hr, after which a sudden collapse occurs. In less severe cases, depression, anorexia, and colic may be observed. The animals may become blind and may grind their teeth, move in a circle, push against objects, and lose their muscle coordination. Mature cattle display fewer overt symptoms, although the syndrome of maniacal excitement is not uncommon.[1]

Clinical symptoms in sheep consist mainly of depression, anorexia, abdominal pain, and diarrhea. Anemia is also commonly associated with lead poisoning in sheep.[1] Pavlicevic[11] notes that clinical symptoms in lambs consist of paralysis of the extremities, pharynx, tongue, and larynx; a rigidly held neck; and an anemic mucous membrane. Lambs may be poisoned through the mother's milk when the ewe is on contaminated pasture. Sterility and abortion in ewes have been observed as a result of lead ingestion.[1]

Toxic dosages of lead for domestic animals other than horses and cattle have not been calculated from statistically reliable studies.

The effects of lead on biological processes in animals generally include effects on the nervous and hematopoietic systems and the kidney tissue. These effects have been studied with various types of test animals (primarily the rat) at the enzymatic, subcellular, cellular, and tissue morphology levels; and systemically at the physiological and biochemical levels.

The most sensitive indicator in rats is the decrease of the enzyme δ-aminolevulinic acid dehydrase (ALAD) that regulates heme synthesis.[17] Lead clearly affects test animals at the subcellular and enzymatic levels of biological function.

8.1.2 **Wildlife**

Lead has so permeated the environment that it is now known to be a regularly occurring constituent of all animal life. Birds and other wild animals are exposed to a wide range of lead levels. Measurable amounts of lead may be found in the tissues of these animals, and lead poisonings have occurred.

Toxic effects from the ingestion of spent lead shot were first observed in ducks in 1919 and have since been recognized as a major health problem in both aquatic and upland species of waterfowl. It has been estimated that thousands of ducks, geese, and swans die of lead poisoning each year.[18] Lead poisoning from spent shot has also been reported in game birds such as wild pheasants, mourning doves, and quail.[19] The scope of the problem becomes readily apparent when it is noted that the majority of the birds die after the hunting season is over; thus it is the breeding stock that is lost. Spent shotgun pellets have been removed from the gizzards of birds with lead poisoning.[20]

Pieces of lead metal when swallowed are normally not harmful to humans or other mammals because they pass through the digestive tract too rapidly to lose more than a minor portion of their surfaces to digestive enzymes and other substances. But it should be noted that persons who consistently eat game often have somewhat elevated blood lead levels and high fecal lead levels from swallowing lead pellets. The gizzard of a bird, however, is a comminuting organ for food that operates by grinding up the food in a muscular sack containing small stones that the bird has swallowed. Spent shotgun pellets lying in the sediment on the bottoms of lakes are picked up by the bottom-feeding ducks in the same manner as pebbles. Because the shot are soft, they are ground fine and made quite susceptible to digestive action rather than just coming into superficial contact with the digestive tract, as in most other animals.[21] Lead released from the gizzard is absorbed by the lower digestive tract. One number-6 lead shot can furnish enough lead to induce fatal lead poisoning in a duck; but, the length of time a pellet is retained in the gizzard depends partly on its size and partly on the fiber content of the diet. A high fiber diet is especially conducive to lead poisoning. The number of shot required to poison a bird also depends on the size of the bird itself. Six number-6 shot are always fatal for mallards, and four or five number-4 shot are generally fatal for Canadian geese.[22]

The symptoms generally associated with lead poisoning in waterfowl are lethargy, anorexia, weakness, flaccid paralysis, emaciation, anemia, greenish diarrhea, impaction of the proventriculus, and distention of the gall bladder.[22] Waterfowl appear to be at least twice as sensitive to the biochemical effects of lead as are man and other mammals.[23] Death appears to be associated with the inhibition of δ-aminolevulinic acid dehydrase (ALAD) by lead.[23,24] In instances where insufficient lead is ingested to cause death, sterility may result.[1]

In an attempt to prevent the deaths of waterfowl through the ingestion of lead shot, the U.S. Fish and Wildlife Service ordered the use of steel shot during the 1976 hunting season in certain areas of the states in the Atlantic Flyway. Despite strong opposition from the National Rifle Association and hunters, the plan is to extend this limitation to the Mississippi Flyway during 1977.[25]

Waterfowl mortality caused by the toxic effects of lead mine wastes coupled with environmental stress was reported by Chupp and Dalke[26] for the Coeur d'Alene River Valley of Idaho. Because of their feeding habits, feeding waterfowl consumed lead from the sediments in the shallow areas of the river along with metallic materials adhering to roots and tubers of aquatic plants. Ingestion of plants containing lead can also contribute to lead poisoning in waterfowl.

The puffin *(Fratercula arctica)*, a sea bird, is in serious decline. In studies to determine the cause, Parslow et al.[27] note the fact that puffins tend to concentrate lead through the food chain (Table 8-1). The authors were not able, however, to associate observed lead concentrations with the decline of the species.

TABLE 8-1. ACCUMULATION OF CERTAIN HEAVY METALS FROM SEAWATER BY FISH *(Ammodytes* AND *Clupea)* AND A PUFFIN[27]

	Metal levels, ppm, wet weight			Approximate accumulation factors		
Metal	Seawater	Fish	Puffin	Fish/ seawater	Puffin/ fish	Puffin/ seawater
Mercury	0.00003	0.037	0.79	1,230	21	26,300
Lead	0.00003	<0.002	0.36	<67	>180	12,000
Cadmium	0.00011	0.309	1.67	2,800	5.4	15,000
Copper	0.003	1.74	4.49	580	2.6	1,500
Zinc	0.01	53	95	5,300	1.8	9,500

Measurements of lead levels in pigeons[28,29] and in song birds[30] indicate that urban birds have higher lead levels than rural birds (Table 8-2). Bagley and Locke[19] analyzed 28 species of birds and noted the concentration of lead in the livers and bone tissue. Lead in the livers was an indication of acute exposure, and in the bone, of chronic exposure. Liver levels ranged from 0.3 to 5.0 ppm, and bone levels ranged from 0.2 to 26.0 ppm. As might be expected, the highest levels were found in aquatic waterfowl; however, the osprey *(Pandion haliaetus)*, a predator, also showed high bone levels. The bone lead levels are an indication of continued exposure to lead and serve as an indication of normal levels rather than adverse exposure.[19]

TABLE 8-2. SUMMARY OF LEAD CONCENTRATIONS IN BIRD ORGANS AND TISSUES FROM AREAS OF HIGH-LEAD AND LOW-LEAD ENVIRONMENTS (ppm, dry weight)

Species and lead level[a]	Feathers	Gut	Liver	Lung	Kidney	Bone[b]	Muscle[c]
Red-winged blackbird:							
Low(10)	26.5	2.1[d]	5.8	0.4	2.1[d]	6.9	0.8[d]
High(4)	66.8	2.6[d]	1.2	4.1	4.1[d]	9.1	0.6[d]
House sparrow:							
Low(16)	27.0	2.3	0.6	0.9	3.5	16.9	0.9
High(11)	158.3	26.2	12.0	6.9	33.9	130.4	2.1
Starling:							
Low(11)	6.4	1.3	4.0	2.8[d]	3.6	12.8	0.8[d]
High(13)	225.1	6.0	16.1	5.2[d]	98.5	213.0	2.4[d]
Grackle:							
Low(10)	36.0	1.4	2.5	2.3[d]	3.5	21.5	0.8
High(11)	81.4	10.2	12.1	2.7[d]	13.5	62.8	1.4
Robin:							
Low(10)	25.3	3.2	2.4	2.2	7.3	41.3	1.0[d]
High(10)	79.7	24.5	10.5	10.3	25.0	133.7	1.2[d]

[a] High-lead and low-lead environments are urban and rural areas respectively; high-lead area for red-winged blackbirds is 10 m from an interstate highway. Sample size in parentheses.
[b] Femur.
[c] Pectoral.
[d] Differences between low- and high-lead environments not significant at the 0.05 level; all others significant at least at the 0.05 level.

Studies of lead exposure and effects in wild animals other than birds are infrequent. Braham[31] studied the distribution and concentration in the California sea lion *(Zalophus californianus)*. He noted that accumulation was occurring in the species but could detect no adverse effects at the time of the study. The exposure of small mammals and selected invertebrates near roadways has also been studied.[32-38] In general, gradients in body lead concentrations declined with increasing distance from the road. The body lead gradients usually were similar in pattern to, though lower than, the soil level gradients; but interesting exceptions were observed in some cases. No evidence of toxicity was observed in any of these animals individually or in relation to population distributions.

An analysis of lead concentrations in 3 species of small mammals from 11 sites in Huntingdonshire, Great Britian,[32] showed that the lead concentrations in the animals were more closely associated with the type of food consumed than with nearness to the highway where the air lead concentrations were highest.

One hundred and one mammals — 51 long-tailed field mice *(Apodemus sylvalicus)*, 27 bank voles *(Clethrionomys glariolus)*, and 23 field moles *(Microtus agrestis)* — were trapped along roadsides.[32] The concentration of lead was significantly higher in *Microtus* than in *Clethrionomys* or *Apodemus* (Figure 8-2). The marked differences among the lead concentrations of the three species can be accounted for by species behavior and food consumed. *Microtus* eats grass as its staple food, whereas *Apodemus* feeds on grain, seedlings, buds, fruit, hazel nuts, and animals such as snails and insects. The range of food for *Clethrionomys* encompasses that of both the other species, but the habitat is restricted to hedges rather than the open field *(Apodemus)* or the roadside *(Microtus)*. Differences in food and food contamination may therefore ac-

count for the differences in lead concentrations in the three species.

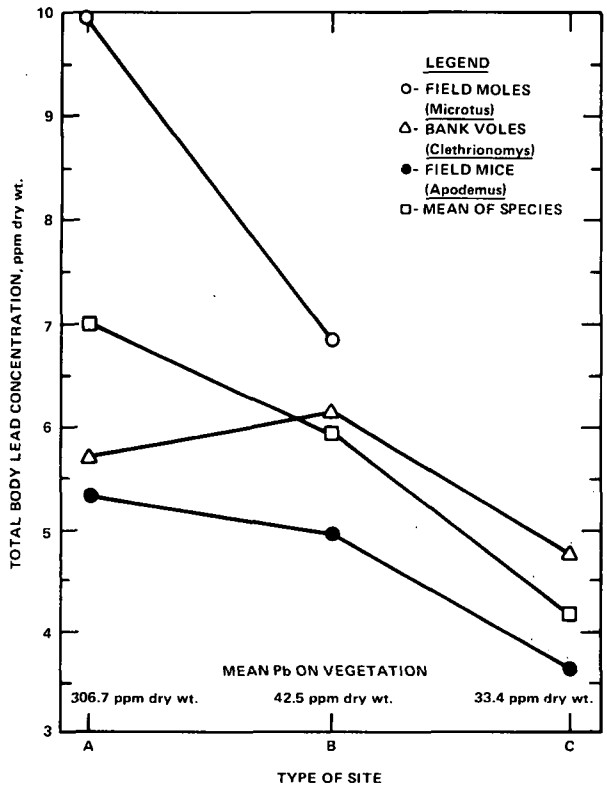

Figure 8-2. Concentration of lead in three species of small mammals trapped beside major and minor roads and at arable and woodland sites.[33]

In a study in central Illinois, samples of small mammals were obtained from a range of environments including those within 10 m of a high-traffic-volume road (>12,000 vehicles/24hr), those within 5 m of medium-use roads (2000 to 6000 vehicles/24 hr), those within 5 m of low-use roads (<2000 vehicles/24 hr), and those in urban areas (approximately 100,000 inhabitants).[38]

All species except the white-footed mouse *(Peromyscus leucopus)*, showed higher concentrations of lead in habitats adjacent to high-traffic-volume situations, especially in urban areas. Since the home range of this species averages more than 50 m in diameter, even those individuals caught nearest the highway were undoubtedly spending considerable time much further removed from the traffic source, possibly accounting for the low lead levels in these animals.

There was also a correlation between habitat requirements and lead concentrations in small mammals captured near high-traffic roadways. Species requiring dense vegetation — the prairie vole *(Microtus ochrogaster)*, the short-tailed shrew *(Blarina brevicauda)*, the least shrew *(Cryptotis parva)*, and the white harvest mouse *(Reithrodontomys megalotis)* — had higher total body lead burdens and higher levels in selected tissues than did those species such as the white-footed mouse *(Peromyscus maniculatus)* and the house mouse *(Mus musculus)* that extend their home ranges into cultivated fields. There was also a correlation between feeding habits and lead concentrations in body tissues. Insectivores (the shrews) had the highest lead concentrations; herbivores (voles) had intermediate concentrations; and granivores (deer mice, white-footed mice, and house mice) had the lowest concentrations, reflecting the fact that lead concentrations in seed tissue are usually extremely low (<1 ppm).

It is highly doubtful that the very low concentrations of lead in these mammals (Table 8-3) could be having a significant impact on their population dynamics.

Studies of lead concentrations in insects in central Illinois ecosystems have shown positive correlations with lead emission levels, decreasing from areas adjacent to heavily traveled roads to areas remote from roads (Table 8-4).[39] There was also a strong trend of increasing lead content from sucking to chewing to predatory insects collected near high-traffic roadways (Figure 8-3). Chewing insects probably ingested more lead from deposits on leaves than did insects that suck liquids from the internal vascular tissues of plants. Data on predatory insects that feed on lead-containing herbivores suggest that lead is selectively retained in the body, leading to biological concentration in this two-trophic (feeding-level) system.[39]

Definitive studies correlating toxicity with environmental lead concentration have not been done.

8.1.3 Aquatic Organisms

Acute lead toxicity in aquatic organisms has been observed and studied experimentally. Lead toxicity in fish is partially related to drainage from metallic wastes into streams. Early experiments were carried out in England where contamination of natural waters by lead mining caused the disappearance of fish from streams.[1] Although the effect of lead on lower forms of life is not well documented, it appears to be less toxic than in higher forms.[1,40]

Apparently, lead and other metals are irritating to the skin of many freshwater fish and cause an unusual reaction. The presence of metal in the water around them causes a copious secretion of mucus over the whole body surface, particularly in the gill

TABLE 8-3. MEAN LEAD CONCENTRATIONS IN ORGANS AND TISSUES OF SMALL MAMMALS FROM INDICATED AREAS OF ENVIRONMENTAL LEAD EXPOSURE[38]
(ppm, dry weight)

Species and exposure area	Total body	Gut	Spleen	Liver	Lung	Kidney	Bone[a]	Muscle[b]
Blarina brevicauda:								
High	18.4	24.0	4.5	4.6	16.9	12.4	67.1	9.7
Medium	6.7	7.0	3.6	2.0	5.6	5.8	19.9	5.7
Low	5.7	3.1	2.3	1.0	7.8	3.9	12.2	5.4
Microtus ochrogaster:								
High	5.1	11.0	5.3	1.6	2.8	8.1	16.6	8.2
Medium	5.9	18.4	2.2	1.2	1.8	7.6	23.2	3.0
Low	1.9	2.8	2.4	1.0	1.3	2.8	4.6	2.0
Peromyscus maniculatus:								
High	6.3	19.2	19.4	3.5	6.4	7.9	24.6	6.8
Medium	4.3	6.0	3.0	1.7	2.4	9.0	8.0	7.4
Low	3.3	4.5	6.5	1.8	6.1	3.0	6.4	1.8
Control[c]	3.1	4.3	3.7	1.1	1.5	1.8	5.7	2.1
Mus musculus:								
High	6.8	18.6	12.1	2.9	2.8	8.1	19.2	5.9
Medium	6.0	8.8	3.1	1.6	3.4	6.6	21.0	3.9
Low	6.7	4.8	5.1	1.6	1.7	3.1	23.5	3.4
Control[c]	2.0	2.7	2.1	1.9	3.4	3.4	9.3	3.8
Reithrodontomys megalotis:								
High	12.3	17.8	145	4.7	20.9	—	109.5	27.5
Medium	3.0	6.2	9.2	1.1	4.2	2.1	—	4.4
Low	2.7±	3.5	5.6	2.3	4.7	4.8	18.4	—

[a] Femur.
[b] Thigh.
[c] Control areas are fields more than 50 m from a road.

TABLE 8-4. LEAD CONCENTRATIONS OF INSECTS AT TWO DISTANCES FROM A HIGH-TRAFFIC-VOLUME ROAD (INTERSTATE HIGHWAY)[39]
(ppm)

Feeding type	Distance: 0 to 7 m from pavement	Distance: 13 to 20 m from pavement
Sucking	15.7	9.8
Chewing	27.3	10.4
Predatory	31.0	20.0
Mean	24.7	13.4

area. Analysis of this mucus has revealed the presence of "considerable quantitities" of lead.[41] The mucus does not, however, prevent absorption of lead into the fish. If the metal level is low, the process is harmless because the excreted film is readily shed; but if higher levels are present, the mucus blanket generated may suffocate the animal before it can be shed. The phenomenon has been observed only in freshwater fish and is subject to modification by water hardness, temperature, and other factors. It should be emphasized that the process described above is entirely external and unrelated to lead levels in the body of the fish. In some cases of lead poisoning in freshwater fish, mucus formation has not been observed.

Symptoms of chronic lead poisoning in fish include anemia, functional damage to the inner organs, possible damage to the respiratory system, growth inhibition, and retardation of sexual maturity.[1,42]

A study in central Illinois[43] of both urban and rural tributaries of the Saline Branch of the Vermilion River showed that lead appears to be taken up by aquatic organisms by means of external contact rather than by ingestion. Lead concentrations in aquatic organisms were found to be related to the amount of contact with substrates, such as sediments (Figure 8-4), that contain the highest lead concentrations in the streams. Thus species differences in concentrations are determined in part by habitat preference and feeding habits.

Filtered water from the two streams had concentrations of lead varying from 0 to 15 mg/liter of water (ppm), and suspended solids in the water contained 15 to 200 ppm lead. The highest levels occurred in the urban stream. The upper 10 cm of sediments in the urban stream contained an average lead concentration of 387.5 ppm, more than 10 times greater than that in the rural stream. Fish in the rural stream contained an average of 1.4 to 4.1 ppm

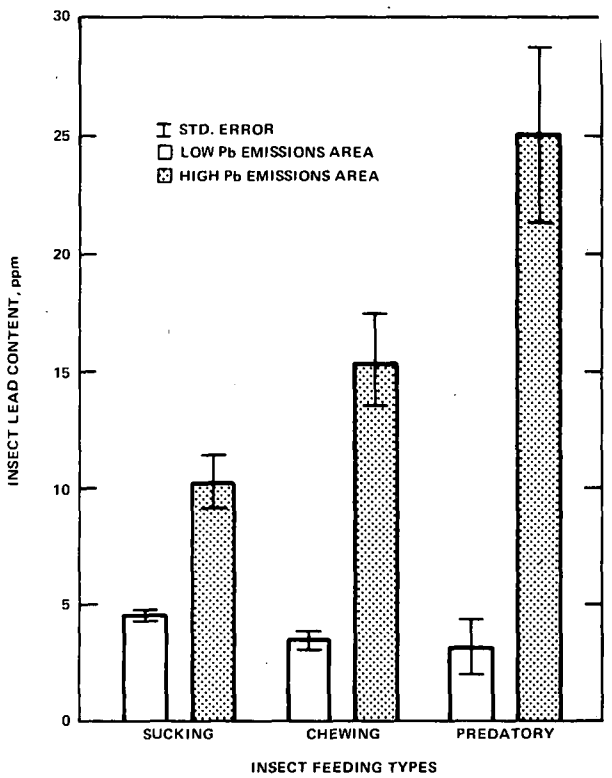

Figure 8-3. Mean lead content in grouped insect samples taken from low- and high-lead-emission areas for sucking, chewing, and predatory feeding types.[39]

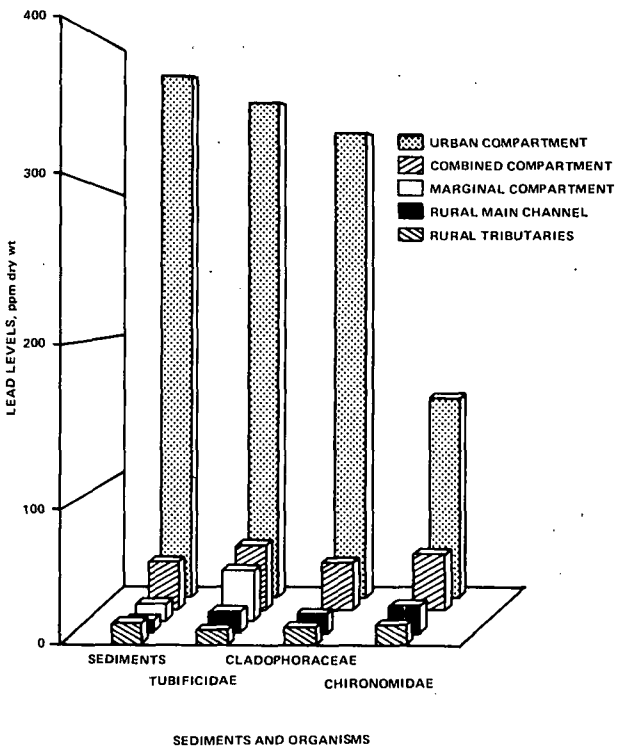

Figure 8-4. Mean lead levels in representative organisms and sediments of the compartments of the drainage basin of the Saline Branch of the Vermilion River, Ill.[43]

of lead in dry tissue (Table 8-5).[43] No fish were found in the urban stream. Lead levels in the invertebrates ranged from about 5 to 20 ppm in the rural stream to more than 350 ppm in the urban stream. These data appear to be in agreement with relative lead levels found in tubificid worms, clams, and fish in the Illinois River.[40]

Hardisty et al.[44] studied lead levels in estuarine fish, but were unable to determine any biological effects of lead. Merlini and Pozzi[45] studied lead accumulation by freshwater fish. Ionic lead (as Pb^{++}) was concentrated threefold when the pH of the lake water was lowered. Lead toxicity in fish was not reported.

Toxicity varies with pH, temperature, hardness, and other water properties.[40] The concentrations of lead injurious to fish and other aquatic and marine life may be found in *Water Quality Criteria, 1972.*[46] The 96-hr LC_{50} value in soft water for rainbow trout *(Salmo gairdneri)* has been reported to be 1 mg/liter. Pickering and Henderson[47] list the soft water LC_{50} values for fathead minnows *(Pimephales promelas)* and bluegills *(Lepomis macrochirus)* as being 5 to 7, and 23.8 mg/liter, respectively. In hard water, the LC_{50} values for the last two species are reported as being 482 and 442 mg/liter, respectively;[47] whereas for rainbow trout, Davies and Everhard[42] report 471 mg/liter. Detrimental effects on fish species occur at concentrations as low as 0.1 mg/liter. In studies of rainbow trout, mortalities attributed to lead occurred at the high test concentrations, which in soft water were 95.2 μg Pb/liter and in hard water, 3.24 mg/liter total lead or 0.064 mg/liter free lead.[42] Physical abnormalities occurred between 11.9 and 6.0 μg Pb/liter in soft water and between 0.12 and 0.36 mg/liter total lead (0.018 and 0.032 mg/liter free lead) in hard water. In *Daphnia magna,* an effect on reproduction has been observed at 0.03 mg Pb/liter.[48]

A study of long-term effects of lead exposure on three generations of brook trout *(Salvelinus fontinalis)* indicated that all second generation trout exposed to 235 and 474 μg Pb/liter developed severe spinal deformities (scoliosis), and 34 percent of those exposed to 119 μg Pb/liter developed scoliosis. Of the newly hatched third generation alevins exposed to 119 μg Pb/liter, 21 percent developed scoliosis. Analysis of residues in eggs, alevins, and juveniles indicated that accumulation of lead occurred during these life stages.[49]

Weir and Hine[50] utilized a conditioned avoidance

TABLE 8-5. LEAD CONCENTRATIONS OF PREDOMINANT ORGANISMS FROM THE RURAL, URBAN, AND COMBINED COMPARTMENTS OF THE DRAINAGE BASIN OF THE SALINE BRANCH OF THE VERMILION RIVER, ILL.[43] (ppm, dry weight)

Compartment and organism	Arithmetic mean	Number of samples	Standard deviation
Rural:			
Plants:			
Cladophora	20.1	11	5.1
Potamogeton	30.0	15	5.4
Invertebrates:			
Hirudinea	12.6	12	14.8
Oligochaeta	13.2	7	7.6
Tubificidae	16.0	13	11.8
Sphaeriidae	5.5	11	3.1
Lirceus fontinalis	7.6	5	3.4
Hexagenia limbata	10.4	15	9.7
Anisoptera	6.6	6	2.4
Chironomidae	20.1	12	18.2
Decapoda	4.7	23	5.2
Fish:			
Catostomus commersoni (white sucker)	2.4	19	1.6
Etheostoma nigrum (Johnny darter)	4.1	9	2.4
Ericymba buccata (Silverjaw minnow)	1.8	29	0.9
Notropis umbratilus (Redfin shiner)	1.9	29	0.6
Pimephales notatus (Bluntnose minnow)	2.7	83	2.7
Semotilus atromaculatus (Creek chub)	1.4	35	0.6
Urban:			
Plants:			
Cladophora	347	6	139
Invertebrates:			
Tubificidae	367	29	373
Chironomidae	153	5	106
Decapoda	11	4	
Combined:			
Plants:			
Elodea	89.9	22	93.3
Cladophora	34.9	7	7.5
Invertebrates:			
Tubificidae	48.6	71	33.9
Chironomidae	42.7	12	16.4
Physa	41.7	10	25.5
Psychodidae	32.3	4	25.5

technique to assess the deleterious effects of four metal ions, including lead, on goldfish. Behavioral impairment was noted following sublethal concentrations of lead nitrate (Table 8-6). The lowest concentration that gave significant impairment was 0.07 ppm.

Shellfish have been reported to concentrate lead.[1]

TABLE 8-6. SUMMARY OF GOLDFISH TOXICITY DATA[50]

Ion	LC_{50}, ppm[a,b]	LC_1, ppm[b]	Slope
Arsenic	32.0 (41.6 to 24.6)	1.5	1.4
Lead	110.0 (121.0 to 100.0)	60.0	1.2
Lead (without calcium carbonate)	6.6 (9.2 to 4.7)	1.5	1.6
Mercury	0.82 (0.90 to 0.75)	0.36	1.2
Selenium	12.0 (17.0 to 7.9)	1.0	2.5

[a] Based on a 7-day survival time.

[b] Lowest concentrations of ions that significantly impaired conditioned avoidance responses and their fractions of the 50- and 1-percent lethal concentrations.

Two species of marine gastropods were shown to concentrate lead in their shells.[51] However, Valiela et al.[52] reported that three species of estuarine bivalves showed no increase in lead when sludge fertilizer containing lead was added to experimental plots in a salt marsh. Furthermore, the lead apparently was trapped by the marsh surface sediments, since no increases were recorded downstream in the detritus on creek bottoms or in filter-feeding bivalves. Lead uptake by the cordgrass *Spartina alterniflora* reduces by a small amount the quantity of lead in the sediments.[53]

Aquatic lead concentrations of 0.5 mg/liter have been shown to be toxic to flagellates and infusoria, the microscopic animals that frequently develop in decaying organic matter. Bacterial decomposition of organic matter is inhibited by lead concentrations of 0.1 to 0.5 mg/liter.[54]

8.2 EFFECTS ON PLANTS

8.2.1 Routes of Plant Exposures

Plants may be exposed to lead through the leaves, stems, bark, or roots. The extent of exposure depends on the amount of lead in the immediate environment and the form and availability of that lead.

8.2.1.1 LEAVES, STEMS, AND BARK

Particulate substances in the air, including lead, are deposited on plant surfaces by fallout, impaction, and precipitation.[55] Precipitation may, in addition to depositing material, wash off or leach out material from the plant surface or tissue. Wind and sloughing of the cuticle wax may also remove deposited material. Thus, meteorological factors are important in determining the fate of compounds that come into contact with plant surfaces. The morphology of the plant surface, however, plays the major role in determining the type and quantity of material that will be retained by that plant surface.[56,57] Epidermal cells of the aerial parts of plants are usually coated with a waxy substance, cutin, and often form unicellular or multicellular hairs, spines, or glands. In addition, secretions from glandular

hairs may make leaf surfaces sticky; thus, particulate material can accumulate on the plant surface, particularly the leaves. However, particulate matter must enter the internal plant tissues to affect the plant.[56] Franke[58] suggests that the cutin is penetrable via intermolecular spaces and that cuticle has been shown to be permeable to both organic and inorganic ions and to undissociated molecules. The capability of an ion to penetrate is determined by its charge, adsorbability, and radius.

Vascular plants have small surface openings, called stomata and lenticels, that function in gas exchange. Stomata are also sites of exchange of aqueous substances under certain conditions.[58] Stomata occur in the epidermis of leaves and young stems. Lenticels are found on older stems of woody species. Surrounding the stomatal pores are guard cells that open and close the pores through changes in their turgidity. Gas exchange, which takes place through the stomata as the result of a concentration gradient, is a passive phenomenon unlike the active breathing of animals.

Large deposits of inert, insoluble metal compounds on the leaves are probably of little consequence to a plant. The most important factor in determining foliar penetration is the solubility of the individual metal.[56,58] The insolubility of lead is undoubtedly a major reason that little incorporation and accumulation occur through the leaf surface.[56]

Carlson et al.[57] experimentally fumigated soybean *(Glycine max* L.) with $PbCl_2$ aerosol particulate; no intraplant movement was noted. Simulated rainfall removed up to 95 percent of the topically applied lead. Studies by Arvik and Zimdahl[59,60] indicated that only extremely small amounts of lead could penetrate plant cuticles, even after extended exposure. Increased penetration of some cuticles occurred after the removal of waxes, but penetration seemed more related to plant species than to cuticle thickness. Oats *(Avena sativa* L.) and lettuce (*Lactuca sativa* var. Black-Seeded Simpson) are two species, according to Rabinowitz, whose leaves atmospheric lead is capable of penetrating.[61] The only conclusion possible from current data is that airborne lead can be taken up by the foliage of some plants, but only in extremely small amounts.

A recent report[62] suggests that grasses and small herbaceous plants in the field, as well as pea plants (*Pisum sativum* L.) and pine tree seedlings (*Pinus sylvestris* L.) in the laboratory, when grown in nutrient solution, release lead and zinc into the air. It is questionable whether the lead exuded from plant leaves adds appreciably to the atmospheric burden. The complexities of lead movement from soil into plants make it unlikely that plants are capable of exuding large amounts into the air.

Particles containing lead, in addition to chlorine and bromine, have been found embedded in the bark of both pine and elm trees growing near highways. The lead content of the particles on the trees decreased over a 3-year period, and the particles on the bark of elm trees showed less lead than those on pine trees.[63] No evidence exists to show that lead entered the trees through the bark.

Trees have been used as indicators of increasing environmental lead concentrations with time.[64] Studies in central Illinois have shown a fivefold increase in lead in tree rings during the last 50 years (Figure 8-5). This graphically illustrates the increase in environmental lead uptake that has occurred in that time. However, data reported by Holtzman on four hardwood trees in a rural suburban area and on one tree in a suburban area near Chicago (tree ages were 100 to 120 years) showed no consistent increase or decrease in lead in the tree rings.[65]

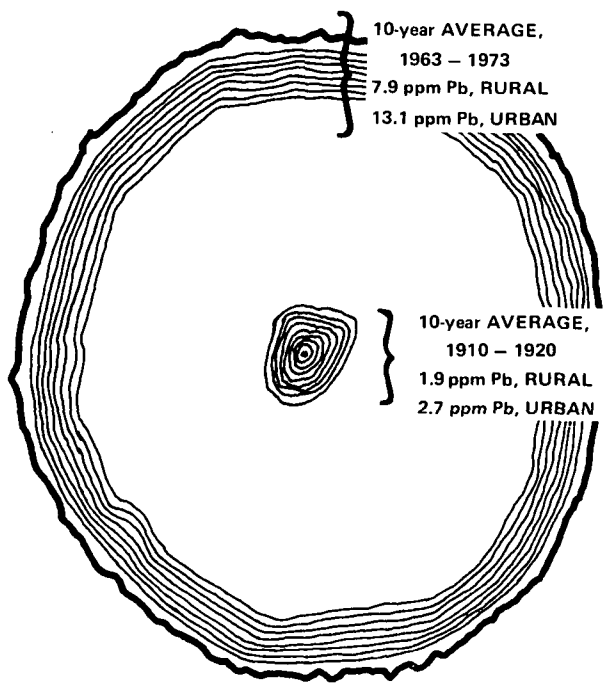

Figure 8-5. Tree ring analysis of lead concentrations in urban and rural trees.[63]

8.2.1.2 ROOTS

The root system is the major pathway of plant exposure to lead, as lead in the soil may be absorbed by the roots and moved into the plant.[60,66-77] But the total lead content of the soil is only one of several in-

teracting factors determining plant uptake. These factors are not well understood, but uptake is known to be influenced by plant species and by the available lead pool in soils.[66,71-74] The pool of available lead is determined by soil pH, soil organic and clay fractions, and the cation exchange capacity as well as other soil sorption characteristics.[66,70-81] Absorption of lead by plant roots is inversely related to cation exchange capacity.[74,79,80] The total lead in the soil in relation to cation exchange capacity determines lead availability.[80] Roots take up minerals that are in the soil solution;[60,82] thus lead movement into roots is in the form of ions from the soil solution or from weak sorption sites.[60,66,74]

Baumhardt and Welch[70] have shown that the percentage of soluble lead in soil decreases with time as sorption occurs. Soil phosphate[60,75,77,78] and liming,[60,75,83,84] as well as cadmium[85] in soil, reduce the uptake of lead by plant roots. Olson and Skogerboe[86] have identified the primary lead species in their soil tests as lead sulfate (Chapter 6). At present, the capability of plants to take up this relatively insoluble form is not understood.[74] Jones et al.[87] have reported that lead uptake is enhanced by a deficiency of sulfate in the soil. In this study, the lead in rye grass tops was higher than that in the roots when the soil was deficient in sulfur.

Once lead enters the plant from the soil solution, most of it remains in the roots.[60,66,72,77] Distribution to other portions of the plant does occur, but it is uneven and variable among species.[71,72,77] Plant age and season of the year also affect internal distribution.[66,72] In all cases, the levels are quite low because of the small amounts of available lead in the soil solution.

8.2.2 **Effects on Vascular Plants**

Lead is a normal soil constituent, but it has not been shown to be essential for plant growth.[66,69] The response of plants to lead is therefore dependent on the extent to which normal metabolic processes are disturbed. Metabolic disturbances manifest themselves as growth abnormalities (the visible symptoms of which may be growth stimulation, stunting, yellowing or purpling of leaves) or, in the event of acute toxicity, senescence and death.[82] Metabolic disturbances are most likely to occur in response to high available lead levels and to highly soluble forms of lead entering the plant.[66,69]

Antonovics, Bradshaw, and Turner[88] state that lead uptake is constant with increasing levels of soil lead until a certain point is reached at which lead uptake becomes unrestricted and rises abruptly. Little is known, however, about the mechanism of lead uptake. Undoubtedly, lead in solution moves into the plant through the root hairs along with mineral nutrients and is translocated to other areas of the plant. The vascular tissue is the pathway of water in the root, through the stem, into the petioles, and into the leaf veins.[89] After entering the root hairs, water containing lead and nutrients must pass through the root cortex to reach the central core of vascular tissue. Because the movement of water through the cortex is from cell to cell with no specific pathway such as vascular tissue, lead possibly may not pass easily through cell membranes. This may explain why plant roots show higher lead contents than other plant organs. Malone et al.[90] have shown that some of the lead that enters the root is concentrated in dictyosome vesicles and subsequently moves via the vesicles to the cell wall, where fusion with the cell wall occurs.

Lead has been reported to have both a beneficial and an inhibitory effect on plant growth.[66,69] Brewer[69] cites studies in which lead nitrate resulted in increased nitrification and increased plant growth when added to the soil. However, when lead nitrate was added to solution cultures, retardation of root growth occurred. In neither case was the metabolic action of lead nitrate observed in the plants. The chemical identity of lead in plants is as yet not known.[60]

Most studies[60,66,69] that describe growth inhibition and plant toxicity caused by lead compounds are based on visible growth responses resulting from lead added to soils or to solution cultures and do not deal with specific metabolic processes.

The effects of lead compounds on such plant processes as photosynthesis,[60,91-93] mitosis,[1,60,66] and water uptake[92] have been reported. Miles et al.,[91] using isolated chloroplasts from spinach leaves, found that lead salts inhibit photosynthetic electron transport. Wong and Govindjee[94] have shown in isolated maize chloroplasts that lead salts affect photosystem I, inhibiting P700 photooxidation and altering the kinetics of re-reduction of P700. In laboratory studies, lead has been found to have a damaging effect on cell walls, nuclei, and mitochondria. Lead retarded cell proliferation but permitted an increase in size.[1] Spindle disturbances and chromatid formation in root tips of *Allium cepa* induced by lead nitrate have been found to be indistinguishable from those induced by colchicine.[1]

Miller and Koeppe[95] and Bittell et al.[96] studied the effects of lead on mitochondrial respiration. Lead effects are related to the phosphate status of

the respiratory system as well as to the oxidation of nicotinamide adenine diphosphonucleotide monohydrogen (NADH). The lead levels used in this study approximated the levels found near heavily traveled highways. The form of lead used, $PbCl_2$, and the isolated mitochondria were not typical of field conditions, but lead in plants does associate with mitochondria and chloroplast membranes.[60,66,75]

The presence of lead in plants has also been shown to have indirect effects on plant growth. For example, absorption of phosphorus and manganese, two essential elements for growth, is inhibited when lead is present in the plant.[97] Lead may also contribute to copper deficiency in plants.[98]

The majority of the studies reporting lead toxicity have been conducted with plants grown in artificial nutrient culture. As a result of the studies, the concept has emerged that the effects of lead, whether stimulatory or inhibitory, depend on a variety of environmental factors, including associated anions and cations within the plant and in the growth media, and the physical and chemical characteristics of the soil itself. Because lead interacts with so many environmental factors, specific correlations between lead effects and lead concentrations are extremely difficult to predict.[60]

Lead toxicity has not been observed in plants growing under field conditions. This observation may be explained by the fact that ambient lead concentrations in the environment have not been high enough, except under unusual conditions (near mines and smelters) to cause a toxic effect or a decrease in crop yield.[60]

In summary, the effects of lead on vascular plants appear at this time to be minimal. The most important effects may be those resulting from ingestion of topical and internal plant lead by grazing animals (the next trophic level). From the standpoint of economic consequences, evidence developed on the effects of lead in agroecosystems indicates that topical lead contamination of plants is more likely to have economic consequences than internal lead (see Section 6.4.3).

8.2.3 **Effects on Nonvascular Plants**

8.2.3.1 MOSSES AND LICHENS

Mosses have been shown to have unique capacity for sorbing heavy metal ions to their surfaces. Traces of copper and lead are sorbed readily even in the presence of other metal ions (calcium, magnesium, potassium, sodium).[99] These ions are sorbed through the leaves of the moss because mosses have no root system for uptake of nutrients from soil or other substrates. Mosses also have neither epidermal cells nor a cuticle (waxy layer), so the internal parenchymal cells are readily exposed to substances from the air.[99,100] Accumulation of heavy metal ions in mosses is generally from precipitation, which is a very dilute solution of metals and water. The accumulation occurs because of the chemical complexes formed between these heavy metal ions in precipitation and negatively charged organic growth.[101]

Lichens, like mosses, have no roots; therefore all minerals are absorbed through the cell membranes. Mechanisms similar to those found in mosses may also be responsible for the uptake of metal ions by lichens. But lichen accumulation of lead is not as extensive as that in mosses.[101]

8.2.3.2 ALGAE

Trollope and Evans,[102] in a study of algal blooms in the Lower Swansea Valley, Wales, noted the sensitivity of algae to the heavy metal content of water. A marked difference was observed in the nature of algal blooms found in three different groups of waters at 12 different stations (Table 8-7). The algae most tolerant to high lead concentrations were: *Coccomyxa, Mougeotia, Tribonema,* and *Zygnema.* Less tolerant were *Microspora, Oscillatoria* and *Ulothrix,* and the least tolerant were *Cladophora, Oedogonium,* and *Spirogyra.* All of these algae were subject to contamination from run-off water and dust from the zinc smelter. The concentrations of lead in the plants varied among genera and within a genus. The uptake of individual metals by algal blooms appears to be regulated. Mean metal concentrations in the three groups of algae are ordered Fe >Zn >Pb >Cu >Ni, whereas the mean metal concentrations in the aquatic bodies were ordered differently: Adjacent to the source, Zn >Pb >Fe >Ni >Cu; near, Zn >Ni >Pb >Fe >Cu; distant (6 to 10 km), Fe >Zn >Ni, Pb >Cu. Concentrations of metals in the algae were directly related to the concentrations in the water, with the algae in the most polluted waters having the highest concentrations. No experiments were conducted to determine whether the algae found in the least polluted waters would grow in more polluted ponds.

Observations in the New Lead Belt of southeastern Missouri[103] have shown that relatively high concentrations of lead in stream bottom sediments do not have much effect on algal growth in these relatively hard natural waters. Under these conditions, the dissolved lead salts are in very low concentrations and well below the limits of tolerance of most

TABLE 8-7. CONCENTRATIONS OF SELECTED HEAVY METALS IN FRESH WATER AND IN FRESHWATER ALGAL BLOOMS[102]

	Concentrations in fresh waters, μg/ml						Concentrations in freshwater algal blooms, μg/mg				
Area of water	Cu	Fe	Ni	Pb	Zn	Algal bloom	Cu	Fe	Ni	Pb	Zn
Water adjacent to zinc smelting waste:											
1	0-03	0-24	0-15	0-31	34-1	Mougeotia	0-38	17-61	0-24	6-19	44-94
						Tribonema a	0-4	9-97	0-16	5-45	19-93
						Tribonema b	0-7	33-92	0-26	4-94	17-61
2	0-02	0-11	0-1	1-24	19-61	Tribonema c	0-67	23-37	0-29	3-68	21-11
						Tribonema d	1-33	30-6	0-24	14-19	17-44
3	0-01	0-25	0-12	0-1	11-44	Coccomyxa	0-65	49-51	0-15	3-23	19-05
						Zygnema	0-46	39-85	0-7	2-6	45-89
4	0-02	0-28	0-15	0-1	1-96						
Mean	0-02	0-22	0-13	0-44	16-78	Mean	0-66	29-26	0-29	5-75	26-57
Water near zinc smelting waste:											
5	0-02	0-27	0-12	0-1	1-96	Oscillatoria	0-34	2-8	1-07	0-58	1-88
6	0-05	0-56	2-2	0-31	4-9	Ulothrix	0-48	7-78	0-3	2-38	3-56
7	0-06	0-56	2-94	2-91	4-9	Microspora	1-02	42-31	0-11	2-16	9-26
Mean	0-04	0-46	1-75	1-11	3-92	Mean	0-61	17-63	0-49	1-70	4-89
Water distant from zinc smelting waste:											
8	0-03	0-39	0-07	0-1	0-21	Cladophora a	0-06	3-94	0-03	0-23	0-89
						Spirogyra a	0-22	3-03	0-13	0-4	1-59
						Spirogyra b	0-29	7-66	0-12	0-11	1-92
9	0-02	0-1	0-06	0-1	0-08	Oedogonium	0-11	0-7	0-07	0-06	0-12
10	0-01	0-39	0-12	0-1	0-08	Cladophora b	0-05	2-91	0-1	0-09	0-97
11	0-02	0-39	0-12	0-1	0-16	Spirogyra c	0-23	0-46	0-09	0-13	1-09
12	0-02	0-11	0-12	0-1	0-05	Spirogyra d	0-05	8-93	0-03	0-04	0-32
Mean	0-02	0-28	0-1	0-1	0-12	Mean	0-14	3-95	0-08	0-15	0-98

algae, including the sensitive *Cladophora*. Extensive blooms of *Cladophora* were observed in one stream where bound lead associated with the filaments exceeded 5000 ppm. These results indicate that lead chelated or bound to the cell envelopes apparently had no major physiological effect on algae under the existing natural conditions.

8.2.3.3 BACTERIA

The response of certain bacteria to lead has been studied by Tornabene and Edwards.[104,105] *Micrococcus luteus* and *Azotobacter* sp., when grown in lead-containing media under experimental conditions, were able to take up substantial quantities of lead with no apparent effects on cell viability. Most of the lead became associated with the cell membrane. Several studies[106,107] indicate that bacteria in lake sediments under anaerobic conditions react differently. Methylation of mercury and arsenic by microorganisms is a well-known phenomenon,[108,109] but the methylation of lead is not. The first evidence for the methylation of lead was demonstrated experimentally by Wong et al.[110] Microorganisms in lake sediments were able to transform certain inorganic and organic lead compounds into a volatile tetramethyl lead (Me_4Pb) when the sediment was enriched with nutrient broth and glucose to stimulate growth and growth occurred under anaerobic conditions. The Me_4Pb lead production was greatly increased when inorganic lead nitrate or organic trimethyl lead acetate (Me_3PbOAc) was added at 5 mg per liter of sample. The biological methylation from trimethyl lead (Me_3Pb) to Me_4Pb appeared to proceed quite readily. This conversion was demonstrated using pure species of bacterial isolates from lake sediments without the sediments being present. They were able to show that *Pseudomonas, Alcaligenes, Acinetobacter, Flavobacterium,* and *Aeromonas* sp. growing in a chemically defined medium could transform lead nitrate, lead chloride, and trimethyl lead acetate (Me_3PbAc) into volatile tetramethyl lead (Me_4Pb). None of the bacteria were able to convert insoluble lead to Me_4Pb. Schmidt and Huber,[107] however, have observed that Pb^{2+} can be biologically alkylated and transformed to Me_4Pb (see Section 6.4.2.2).

Generally, most naturally occurring bacteria can tolerate lead without toxicity.[104,110] However, there is wide variation in effects. For example, lead has been shown to stimulate growth of a bacterium iden-

tified as *Micrococcus flava* Strevisan,[111] producing an insoluble lead metabolite; but lead has also been shown to be widely inhibitory to aerobic activated sludge bacteria,[112] aerobic river water bacteria,[113] and marine sulfate-reducing bacteria.[114] These reports seem to indicate that lead has a relatively casual relationship with bacterial cells, with no specific inhibitory role; but this should be viewed with caution.

Previously, the presence of lead in estuarine sediments was mentioned. The methylation of lead in this environment and its effects on the biota existing there have not been studied.

Effects of the addition of 1000 ppm of copper, nickel, lead, and zinc on carbon dioxide release during aerobic incubation of soil alone and after treatment with straw were studied.[115] Carbon dioxide release during incubation from soils without added straw was decreased by all metallic elements. Carbon dioxide release from soil plus straw was decreased by lead. The toxic effects of the high concentration of elements on the activity of the microorganisms attacking organic matter were believed to be caused by the ability of the elements to compete with essential elements (manganese, iron, and zinc) for the active sites (SH, NH_2, = NH) of enzymes. Nickel and lead were slightly more inhibitory than copper and zinc.[115]

Cole[116] found that addition of lead to soil resulted in 75- and 50-percent decreases in net synthesis of amylase and α-glucosidase, respectively. The decrease in amylase synthesis was accompanied by a decrease in the number of lead-sensitive, amylase-producing bacteria, whereas recovery of synthesis (usually in 24 to 48 hr) was associated with an increase in the number of amylase-producing bacteria, presumably lead-resistant forms. The results indicated that lead is a potent but somewhat selective inhibitor of enzyme synthesis in soil and that highly insoluble lead compounds such as PbS may be potent modifiers of soil biological activity.

8.3 EFFECTS ON RELATIONSHIPS BETWEEN ARTHROPODS AND LITTER DECOMPOSITION

A study[117] of the impact of a lead smelting complex in southeastern Missouri focused on forest-floor litter arthropod fauna. Litter-arthropod food chains and the possible transfer of lead through plant-herbivore-carnivore food chains were studied as a means of detecting perturbations in this ecosystem. Both point and fugitive sources contributed to heavy metal levels in the study area. Lead, cadmium, zinc, and copper were the primary elements studied. Litter mass, heavy metal and macronutrient content (Ca, P, K, and Mg), cation exchange capacity, and pH were studied to characterize the arthropod food base. Arthropods were removed from litter by Von Tullgren funnel extraction. The arthropods were taxonomically classified according to their feeding habits or levels: detritivore, fungivore, littergrazer, omnivore, and predator. Level refers to the sequential location of a particular organism in the food chain or web. Their population density at each trophic level, biomass, and heavy metal and macronutrient content was determined.

Changes in litter decomposition and nutrient cycling were reflected in the population dynamics of litter arthropods and macronutrient pools. Reduced arthropod density, biomass, and richness (an estimate of maximum diversity) were observed. The macronutrients Ca, K, and Mg in the 01 and 02 litter layer at a site 0.4 km from the smelter were significantly reduced. Two litter layers or horizons are recognized by the Soil Science Society of America. The 01, or surface layer, is that in which dead plant material still retains its original conformation. The 02 layer is that layer in which the material is fragmented and no longer recognizable as to species or origin.

Mean heavy-metal concentrations were greater in the undecomposed 02 litter layer collected at 0.45 and 0.8 km from the smelter. The Pb concentration was 103,000 ppm; Zn was 4910 ppm; Cu was 6080 ppm; and Cd was 179 ppm. At these sites, heavy-metal concentrations correlated with 02 litter layer accumulations. A change from the normal was also noted in the cation exchange capacity and pH of the soil.

In summary, the results of this study[117] indicate that the dynamics of forest-nutrient cycling processes are seriously disturbed near these lead smeltering complexes.

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9. QUANTITATIVE EVALUATION OF LEAD AND BIOCHEMICAL INDICES OF LEAD EXPOSURE IN PHYSIOLOGICAL MEDIA

In this chapter, the state of the art regarding measurement of lead and biochemical markers of lead exposure is summarized, and the accuracy and precision of various methods of intra- and inter-laboratory comparison are examined. The relative diagnostic merits of one type of measurement with respect to another — blood lead versus urinary lead, for example — are discussed elsewhere in the document.

9.1 GENERAL SAMPLING PROCEDURES FOR LEAD IN BIOLOGICAL MEDIA

The occurrence of lead in physiological media of interest in this discussion is at the trace level, even under conditions of marked exposure. Therefore, a number of sample collection and handling precautions are called for, both to minimize loss of lead from samples and to avoid the contamination of samples by this ubiquitously distributed element.

Sample-gathering problems are of special concern in blood lead screening programs in which great numbers of samples must be gathered, transported, and processed in the shortest possible time.[1] Sample collection details for blood lead and other biological indicators of exposure have been reviewed in the clinical literature.[2]

In blood studies it is often desirable to use capillary blood by finger prick instead of venous puncture, especially when young children are involved. A number of studies have shown that capillary and venous blood are essentially identical in lead content: Mitchell et al.[3] obtained a correlation factor of 0.92. However, it should be emphasized that such results can be achieved only when extreme care is used in cleaning the skin, and when contamination of capillary tubes and syringe is avoided.

A number of workers have used filter paper discs for blood sample collection as an alternative to handling discrete blood volumes. The paper for this purpose must be selected for uniformity of manufacture, low lead content, and uniform blood dispersal.

In the methods of Cernik and Sayers involving lead workers,[4,5] for example, whole blood obtained by finger prick is spotted onto Whatman No. 4 qualitative paper. Either 9.0-mm or 4.0-mm discs are then punched out and analyzed by the Delves cup microtechnique or the carbon-cup flameless atomic absorption procedure. These methods correlate well with blood obtained by venous puncture.

Joselow and Bogden[6] have employed a micromethod for routine mass screening of children using a paper disc-in-Delves-cup technique. Capillary blood is allowed to drop onto an 8.2-cm disc of Schleicher and Schwell No. 903 filter paper so as to form a spot somewhat larger than 1/4 in. in diameter, and discs of 1/4-in. diameter are punched out directly into previously conditioned Delves cups. A correlation coefficient of 0.9 was obtained when results of this method were compared with a macroprocedure using venous blood and the procedure of Hessel.[7]

Cook et al.,[8] in their investigation of capillary blood collected on paper and of blood volume gathered by venous puncture, obtained a correlation approaching 0.8.

Puncture-site preparation is also important in sampling. Marcus et al.[1] report that a preliminary cleaning with ethanolic citric acid solution followed by a 70-percent ethanol rinse is satisfactory, whereas Cooke et al.[8] chose to employ a vigorous scrubbing with low-lead soap solution and deionized water rinsing.

A second precaution with finger-prick sampling is the way in which the blood flow is expedited for sampling. Gravity flow or direct uptake (filter paper) is preferable to squeezing the finger tip, as hard squeezing might dilute the blood drop with tissue fluid.

Further precautions include the use of polypropylene syringes[9] with needles of stainless steel and polypropylene hubs for puncture sampling.

Urine-sample collection requires acid-washed plastic containers (and caps) and should include a low-lead bacteriocide if samples are stored.

Hard- and soft-tissue gathering from laboratory animals or human sources entail surface debridement of ambient lead encountered in the process of sampling. Hair cleaning before analysis may be carried out via the method of Hammer et al.;[10] bone and teeth may be given a quick rinse in EDTA solution. With soft tissue, the outer layer may be removed or a segment of underlying matter excised. For organs of heterogeneous morphology such as kidney, it may be more desirable to subject the sample to a quick rinse in an ionic chelant, such as EDTA, that does not penetrate beyond the outer membrane.

Regardless of specific methodology employed, all reagents used in lead determinations in biological media should be certified for low-lead content; and samples should be stored in a manner that minimizes lead contamination from air or surfaces. Standard lead solutions should be prepared frequently, either from stock solutions from analytically certified sources or from the pure metal. Solution preparation in glass should be minimized, particularly when analysis is at the sub-part-per-million level.

9.2 BLOOD LEAD

The first generally accepted practice for measuring lead in blood and other biological media involved a spectrophotometric technique based on the binding of lead with a chromogenic agent to yield a chromophoric product. In this connection, the classic ligating agent has been dithizone-1, 5-diphenylthiocarbazone. The lead dithizonate is measured spectrophotometrically at 510 nm.

Two reliable variations of the spectrophotometric technique when dealing with lead content of 1 to 10 ppm are the USPHS and APHA procedures.

The USPHS assay[9] is a double-extraction, mixed-color procedure having bismuth as the chief interferent. Blood (and urine) samples are wet-ashed using concentrated nitric acid certified as to low-lead content. After digest treatment with hydroxylamine and sodium citrate, the pH is adjusted to 9 to 10, and cyanide ion is added. Formation and extraction of lead dithizonate is carried out using a chloroform solution of dithizone. Lead is then re-extracted into dilute nitric acid (1:99 water), and the aqueous layer is treated with ammonia-cyanide solution and re-extracted with dithizone-containing chloroform. The organic extracts are read in a spectrophotometer at 510 nm. Although bismuth interferes, this element is encountered infrequently in biological media.

The APHA procedure[11] varies from that described above mainly in permitting removal of bismuth at pH 3.4 as the dithizonate.

At present, the colorimetric method has been largely supplanted by two other techniques: atomic absorption (AA) spectrometry in all its variations and anodic stripping voltammetry (ASV).

Of these two analytical approaches, the more technically popular, by far, is AA spectrometry, which is used for both macro- and micro-scale analyses. The theoretical basis for AA and its instrumental design are beyond the scope of this presentation; basic reviews are provided by Christian and Feldman[12] and by L'Vov,[13] however.

Macro-scale AA analysis involves direct aspiration of suitably treated lead-containing samples into a flame for lead-atom generation and excitation. Micro-AA, which is being used more widely as accessories and instrument refinements become commercially available, is of two types: flame and flameless, with the latter employing thermoelectric systems in lieu of a flame.

Of the flame microtechniques for AA analysis of blood samples, the most widely used is Delves cup procedure[14] in which small volumes of blood, 10 to 100 μl, are placed in lead-free nickel crucibles. After the organic matrix is destroyed, the cups are inserted into a flame. The overall configuration of the system permits the optical path to be maximally occupied by the lead atom population originally present in the sample. Destruction of the organic matrix in blood may be either partial, using hydrogen peroxide, or total, with pre-ignition of the organic matter caused by placing the cups near the flame.[15]

Increasing use is being made of flameless AA, particularly the heated graphite furnace accessory, whereby volumes of blood are reduced to $\sim$ 1 μl, and also whereby *in-situ* destruction of organic matter may be achieved.

The electrochemical technique known as ASV[16] is also coming into common use in a number of laboratories, particularly for blood and urine lead analysis. As developed by Matson and Roe,[16] the technique involves concentrating an ion such as divalent lead on a negative electrode during a predetermined plating time (5 to 60 min) followed by polarity reversal and increase for short periods to yield a discrete current peak that is proportional to ion concentration.

Also in current use are X-ray fluorescence spectrometry and neutron-activation analysis, two sophisticated instrumental methods for trace analysis. The considerable expense of the equipment

and the amount of operator expertise involved rather limit their use to that of regional service facilities or central laboratories. The two methods have a distinct advantage, however, in that they permit multiple-element analysis, a feature that will be of increasing importance as more is unveiled about the complex interactions of lead with other metals in man and other organisms.

9.3 URINE LEAD

Precautions to be taken for urine sample collection were noted earlier. Because of the considerable amount of ionic matter in urine, it is usually necessary to manipulate urine samples in various ways before analysis.

All of the methods employed for blood lead analysis as described above may also be applied to assessment of urine lead levels. Care, however, should be exercised in the analysis of urine samples from patients undergoing chelation therapy, with special attention to how a specific procedure will accommodate or be interfered with by lead excreted as a complex, e.g., lead-EDTA. Prior ashing of urine samples will minimize complications in this regard, but partial degradation or no prior treatment might necessitate co-analysis of lead in the complex form for standards.

9.4 SOFT-TISSUE LEAD

Because of the nature of this medium, it is usually necessary either to ash or to solubilize tissue samples. Wet-ashing is rapid and avoids lead loss via volatilization, but it requires use of corrosive acids and procedural care to avoid contamination of reagents and other problems. Dry ashing, on the other hand, is simple and uses no contaminating reagents. The drawbacks are mainly those of lead volatilization and retention of the element in refractory residues. Newer techniques of ashing include (1) low-temperature ashing, in which dry samples are mineralized in an evacuated chamber that is bathed in an energy-rich plasma via r-f discharge in an oxygen stream, and (2) use of the combustion bomb, in which samples are heated in acid an at elevated temperature in a sealed inert vessel.

A newer method of tissue handling, solubilization, entails the treatment of samples with quaternary ammonium compounds and analysis of aliquots, chiefly by AA spectrometry.[17]

The bulk of the current literature centers on the use of atomic absorption spectrometry as the method of choice for assessing lead levels in soft tissue.

9.5 HAIR LEAD

An attractive feature of the clinical use of hair-lead levels is its noninvasive nature and the feasibility of assembling a rough time frame for lead exposure by isolating discrete segments of the total hair length.

A serious drawback in hair analysis, however, is the level of contamination by ambient air lead, lead in hair preparations, etc., as discussed by Hammer et al.[10] Hair measurements without prior treatment of the sample include both exogenous and endogenous sources. Examples of the former are dyes, shampoos, sweat, and dust. Pre-analysis hair washing with detergent and EDTA removes most of the externally found lead; but there is still no definitive way to determine whether any cleaning technique removes the contamination portion of lead and leaves the internal lead content undisturbed.

Hair is usually wet-ashed before analysis, and the digest is diluted and analyzed by AA spectrometry. Because relatively high levels of lead are encountered in hair, small sample sizes can be used when a sensitive procedure such as AA spectrometry is employed.

9.6 LEAD IN TEETH AND BONE

The biochemical significance of lead levels in teeth and bone is discussed elsewhere. From an analytical standpoint, bone samples are usually obtained from experimental animal studies. Bone samples must first be debrided of muscle and connective tissue and chemically rid of surface lead contamination by rinsing with EDTA or other chelant solution.

Bone and teeth are usually wet- or dry-ashed before analysis, and the relative merits of these mineralizing procedures are as noted above with soft tissue assays. Because of the high mineral content of bone and teeth, care must be taken to avoid spectrochemical interference by calcium, phosphate, etc. The relatively high levels of lead in these two hard tissues, however, permit dilution of the samples for testing. Because the effect of the high mineral content on analytical signals is probably sufficient to preclude the use of simple aqueous lead standards, it is advisable to employ workup solutions of bone samples that have first been analyzed for lead content and to which known amounts of lead from a stock solution are then added. Matrix standards prepared in this fashion are assumed to reflect the influence of mineral content on all of the samples.

9.7 COMPARATIVE STUDIES OF METHODS FOR MEASUREMENT OF LEAD IN BIOLOGICAL MEDIA

In an interlaboratory study of the USPHS colorimetric method for lead in blood and urine, Keenan et al.[18] reported the results from 10 participating laboratories. For blood, a mean lead value of 26 ± 0.82 μg/dl was obtained; spiked samples gave virtually identical correspondence among the groups. Urine samples with lead added gave values from the reporting groups with a mean of 679 ± 5.5 μg/liter.

Microscale AA techniques for whole-blood lead have been found to show good correspondence with results obtained using conventional flame procedures.[19,20]

Matson[21] has reported good correlations for lead levels in blood and urine when comparing ASV with a colorimetric and an AA procedure. Similarly, Horiuchi et al.[22] saw little difference in lead levels for blood and urine when contrasting ASV, AA, and polarography.

Interlaboratory studies of various methods for lead analysis of biological media have yielded somewhat disappointing results.[23-25] In a recent study involving 66 laboratories throughout Europe, blood and urine determination variance was observed to be unacceptably high.[25]

Presently, the Center for Disease Control (CDC) is carrying on a monthly proficiency testing program for blood lead involving approximately 200 laboratories. Results are made available through monthly reports for analysis of bovine blood samples.[26] The criterion used by CDC for assessing unsatisfactory performance is: greater than 15 percent relative deviation at levels of 40 μg/dl or above and greater than 6 μg/dl at lower levels.

In a recent CDC report (Survey 1977I)[27] covering 130 laboratories for testing and 26 reference laboratories using three bovine blood samples (cows fed lead acetate), 72 percent of the tested laboratories were within the acceptable range for a sample having a mean of 16.6 μg/dl. The acceptable percentage was lower, interestingly, at higher sample means of 48.2 and 54.6 μg/dl (64 and 67 percent, respectively). When results were tabulated as a function of method, the Delves cup AA spectrometric and ASV methods furnished the smallest coefficients of variation.

A number of other proficiency programs are presently operating in the United States, and the results of these have been included, along with the European program, in a recently published monograph by Pierce et al.[2] These same authors describe the state of the art critically and offer some recommendations for improving the quality of blood lead analyses:

1. Every laboratory should have established quality-control procedures.
2. Control procedures should include replicate analyses, recovery of known additions or spikes, participation in interlaboratory tests, and analyses of known materials.
3. Testing samples that the analysts analyze blind should be used to minimize bias.

It has been suggested[28] that the acceptable agreements in blood lead levels found when a single laboratory employs different techniques compared with the wide variance found when different laboratories employ different methods on portions of common blood samples relate primarily to preparation and state of the blood samples before and during distribution and subsequent analysis. The results of Grimes and coworkers[29] bear this out because a large number of carefully controlled blood samples analyzed by their laboratories, using the paper disc technique, provided results that compared well with those of other laboratories using other instrumentation with the disc technique.

9.8 MEASUREMENT OF URINARY δ-AMINOLEVULINIC ACID (ALA-U)

Some comments regarding sample collection for ALA-U are necessary. ALA is stable in acidified urine (pH 1 to 5), so that acetic or hydrochloric acid addition is satisfactory at the time of sample collection. If samples are stored in the dark at 4°C, the ALA content remains relatively constant for several months.[30]

ALA-U measurement usually entails the classic method of Mauzerall and Granick.[31] In this approach, ALA-U is condensed with a β-dicarbonyl compound such as acetylacetone or ethyl acetoacetate to yield a substituted pyrrole derivative; this intermediate is then caused to react with Ehrlich reagent (*p*-dimethylaminobenzaldehyde) to yield an intense ionic chromophore. This procedure is not specific for ALA-U, however, since aminoacetone interferes. Though the significance of such interference is marginal when ALA-U levels are markedly elevated, it becomes very important when only slight elevations of ALA-U are being measured.

First, urine samples are chromatographed on ion-exchange resin columns (Dowex-2), the ALA-U being co-eluted with urea using water as eluent. Eluate transfer to a Dowex-50 column is followed

by sequential elution with water to remove the urea and then with acetate solution to permit removal of the ALA-U. Treatment with acetylacetone and heating to effect complete condensation is followed by treatment of sample aliquots with modified Ehrlich reagent (*p*-dimethylaminobenzaldehyde in perchloric/acetic acid). The resulting chromophoric salt is allowed to achieve maximum intensity (*ca.* 15 min) after which the sample is read in a spectrophotometer at 553 nm. The detection limit is 3 μmoles/liter urine, and chromophore stability is limited to about 15 min.

A number of modifications to the above basic approach have been reported. Several reports attempt to take into account the interference posed by aminoacetone. The quantitative corrections to be used are described by the authors of these reports.[32,33]

The initial isolation of porphobilinogen is omitted (in cases where porphyria is not suspected) in the modification of Williams and Few,[34] in which a correlation of 0.99 was observed with the reference technique using samples from 39 lead workers. Doss and Schmidt[35] report that use of commercially available dual ion-exchange columns offers results that compare favorably with the Mauzerall and Granick method.[31]

The assay has been automated with good correspondence of results to the manual method in the laboratories of Grisler et al.[36] and Lauwerys et al.[37]

In another variation of the ALA-U procedure, the method of Schlenker et al.[38] removes aminoacetone which interferes with the assay. In the procedure of MacGee et al.,[39] urinary and blood ALA is determined by gas-liquid chromatography, a highly specific technique.

9.9 MEASUREMENT OF δ-AMINOLEVULINIC ACID DEHYDRATASE (ALA-D)

Located in erythrocytes, δ-aminolevulinic acid dehydratase (ALA-D) catalyzes the conversion of ALA to porphobilinogen in the heme biosynthetic pathway. Its inhibition by heavy metals such as lead indicates that it is a sulfhydryl enzyme and also forms the biochemical basis for assessing its activity in lead-exposed organisms.

Blood collection requires the use of low-lead tubes containing anticoagulant, whereas for micromeasurement, blood is collected in a heparinized microhematocrit tube.[40] Obviously, use of strong chelants, such as EDTA, as anticoagulants is not advisable because competition for lead may reactivate the lead-inhibited enzyme.

Minimal time lapse should occur between collection and enzyme assay—no more than 24 hr if samples of heparinized blood are held at 4°C.

The chemical basis for measuring enzyme activity involves spectral measurement of the amount of porphobilinogen generated from ALA, the porphobilinogen being condensed with *p*-dimethylaminobenzaldehyde to yield a chromophore that is measured at 553 nm. Mercury (II) is employed to minimize the interference effect of sulfhydryl entities present in the medium.

The micromethod of Granick and co-workers[40] requires only 5 μl of whole blood and appears to be of value in a screening program. Enzyme incubation is done at 37°C for about 60 min; ALA of the highest possible purity is necessary as a substrate.

Termination of the reaction (enzyme activity) is done via trichloroacetic acid.

The activity of the enzyme may be calculated in two ways:[40,41]

$$\text{Activity} = \Delta OD_{553}(\text{sample-tissue control}) \times \frac{138{,}000\ \text{nMol}}{HCl}\ \text{PBG/ml RBC/hr}$$

$$= \Delta OD_{553}(t_{60}' - t_0') \times \frac{100}{HCL} \times 131.48\ \text{nMol PBG/ml RBC/min}$$

In the European standardized method for ALA determination[42] aimed specificially at enzyme levels in blood corresponding to low levels of exposure to environmental lead, incubation of the enzyme in three aliquots of blood (0.2 ml cooled to 4°C) is carried out in the presence of excess δ-aminolevulinic acid. An aliquot blank is also carried through the procedure. Hemolysis of the cells and incubation with substrate is followed by quenching with mercuric chloride-trichloroacetic acid solution. Centrifugation and treatment with modified Ehrlich's reagent is followed at 5 min by absorbance measurement. Blood samples are preferably run within 3 hr and in no case after 24 hr when held at 4°C.

In a study by Granick et al.[43] the activity of ALA-D before and after treatment with dithiothreotol (DTT) is determined. The DTT (added *in vitro*, 20 mM) provides -SH groups and reactivates the enzyme completely at all concentrations of blood lead. Because DTT-reactivated ALA-D yields total enzyme activity, variation in levels of the unactivated enzyme may be normalized by determination of the rates of both activities. Hence a person having

a high ALA-D for genetic reasons at a given blood level of lead will have relatively high activity for both activated and nonactivated enzyme, and the activity ratio will depend less on genetic factors than on lead inhibition. Consequently, correlation is markedly improved. This study also shows that inhibition by lead is of the noncompetitive type.

9.10 MEASUREMENT OF FREE ERYTHROCYTE PROTOPORPHYRIN (FEP)

Another reaction that lead inhibits in the human heme biosynthetic pathway is heme formation. As a result of blocking this reaction, porphyrins, particularly protoporphyrin IX (actually zinc-protoporphyrin), accumulate in the erythrocytes. Measurement of protoporphyrin IX specifically, or all erythrocyte prophyrins together, is generally referred to as the free erythrocyte protoporphyrin (FEP) test.

The spectrochemical properties of FEP that form the basis for its measurement include its lability to light and strong acids, its metal coordinating ability, its possession of an absorption spectrum in the Soret band region, and its marked intensity of fluorescence. Spectral methods used, however, must take into account the fact that copro- and uroporphyrin provide considerable interference in FEP measurement. Although both absorption spectrophotometric and fluorometric methods may be employed for FEP assessment,[44-46] fluorometric techniques carried out on a microscale are more frequently used because of the relatively cumbersome nature of absorption spectrometry in terms of time and materials. These microfluorometric techniques, in particular, provide a rapid, relatively accurate means of screening pediatric populations.

In the microtechnique of Granick et al.[47], several microliters of whole blood are placed in 1-ml test tubes that also serve as fluorometric cuvettes. Addition of ethyl acetate/glacial acetic acid (2:1) is then rapidly followed by treatment with 0.5N HCl and vigorous shaking. The acidic phase (bottom layer) contains the bulk of the porphyrins. The cuvettes are scanned over the range 560 to 680 nm, using excitation at 400 nm. The ratio of the two-band maxima at 605 and 655 nm is measured for each sample with a ratio of 2:1 indicating only FEP. Any lesser value indicates copro- and/or uroporphyrins. In the latter case, an extraction with 0.05N HCl on analysis of a second sample removes copro- and uroporphyrin.

The technique of Piomelli,[48] using 20 μl of blood added to a 5-percent Celite suspension in saline, uses essentially the same initial extraction procedures as those noted above, but it is varied to employ 1.5N HCl to generate the fluorescing acid layer. Measurement is at 610 nm and excitation at 405 nm with coproporphyrin employed as the standard.

Observing that FEP is actually the zinc complex (ZPP), Lamola and coworkers[49] have devised a rather rapid and sensitive fluorometric procedure in which 20 μl of whole blood is worked up in a detergent-phosphate buffer solution (dimethyldodecylamine oxide) and fluorescence measured at 594 nm with excitation at 424 nm.

In the procedure of Chisolm and Brown,[50] which has been evaluated as a selected method by Schwartz and Piomelli, 20-μl blood volumes are treated with ethyl acetate/acetic acid solution (3:1) and agitated for 30 sec. After centrifugation, the layers are extracted with 3N HCl, and the acid layer is diluted with more 3N HCl. The acid extracts are analyzed spectrofluorometrically with coproporphyrin employed as the quantitating standard.

A portable hematofluorometer that utilizes front-face optics, internal standards, and built-in computational capabilities permits the assessment of erythrocyte zinc protoporphyrin (ZPP). As developed by Bell laboratories[51] and subsequently made available commercially,[52] the apparatus permits the analysis of a drop of blood applied to a cover slip directly from finger pricking. The ZPP level (μg ZPP/dl blood) is automatically calculated and displayed on a digital readout.

A number of micromethods for FEP analysis have been critically evaluated by Hanna et al. [53]: Double extraction with ethyl acetate/acetic acid-HCl,[48] single extraction with ethanol, single extraction with acetone,[54] and direct solubilization with detergent buffer.[49] Of these, the ethyl acetate and ethanol procedures were satisfactory; the complete extraction of FEP makes the former the technique of choice when an absolute value rather than technical simplicity is of primary concern.

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10. METABOLISM OF LEAD

10.1 INTRODUCTION

The metabolism of lead in man may be defined as the physiological processes relating to absorption, distribution, translocation, and net retention. The metabolism of lead in man is discussed in this chapter in terms of routes of exposure and of the physiological distinctions, existing within population classes, that modify metabolic processes, especially with reference to children versus adults.

Most of the material discussed in the following pages addresses the dietary habits of adults in the United States. For children, however, there are dietary habits that are distinct from those of adults and that have implications for differences in exposure between adults and children.

For example, in assessing the indirect contribution of airborne lead to diet, one must consider the hand-to-mouth activity of young children, i.e., sucking of dirty fingers in contact with environmental dust and dirt; retrieving foodstuffs, such as lollipops, that fall into dirt; and a host of other childhood activities by which airborne lead may contribute to the intake of lead by children but not by adults.

In this chapter, the physiological processes that control the uptake of lead by man (absorption) will be discussed first. Then the movement of lead through the body into its depot tissues (distribution) and its eventual elimination (excretion) will be treated.

10.2 ABSORPTION

The quantities of lead absorbed from environmental sources are determined not only by the amount ingested or inhaled but also by the particle size and chemical species involved.

Absorption depends also on specific host factors such as age, nutrition, and physiological status. In addition, the total quantity ingested in food and water varies greatly from individual to individual, and the total quantity inhaled depends on the size and weight of the individual and on the energy expended in day-to-day activity.

10.2.1 Respiratory Absorption

The International Radiological Protection Commission (IRPC) Task Group on Lung Dynamics[1] developed a model designed to predict the percentage of inhaled aerosols that would be deposited and retained in the lungs. This model predicted that approximately 35 percent of the lead inhaled in general ambient air would be deposited in the airways. Since the aerodynamic diameter of lead particles is generally in the range of 0.1 to 1.0 μm, deposition would occur predominantly in the deeper regions of the lung. Emissions from stationary sources frequently include a significant proportion of larger particles that, when inhaled, would be deposited primarily in the nasopharynx. Because these particles usually fall out of the air rather quickly, exposure to these larger particles is limited primarily to the near vicinity of the emission source. The deposition within the respiratory tract of very small particles (<0.1 μm) apparently occurs chiefly by diffusion,[2] and rates or sites cannot be predicted. The IRPC model predicts a total airway deposition of 40 to 50 percent for 0.5 μm particles, but a study in human volunteers indicated only 6 to 16 percent desposition, depending on the rate and depth of respiration.[3] Chemical composition also affects uptake as does the aging of the aerosol (Chapter 6). This illustrates the difficulty in choosing the appropriate chemical composition[4] for studies on deposition.

Airway clearance of lead aerosols as predicted by the IRPC lung model[1] is even more tenuous than are predictions regarding deposition. The model indicates that the absorption or clearance of lead deposited in the airways would vary greatly depending on the solubility and on the inherent toxicity of the particles to the clearance mechanism (lung macrophages and cilia).

10.2.1.1 HUMAN STUDIES

Actual studies on the fractional deposition of particles in the respiratory tract of man have not been extensive, especially in the case of lead. Using an air

lead level of 150 $\mu g/m^3$, Kehoe[5-7] studied the deposition of combusted tetraethyl lead in human volunteers. The source of lead was combusted tetraethyl lead, which produced lead (III) oxide (Pb_2O_3) in the air. Subjects breathed air containing 150 $\mu g/m^3$ lead; the smaller particles averaged 0.05 μm in diameter and the larger ones averaged 0.9 μm in diameter, as viewed under the electron microscope. This represents a mass median equivalent diameter of approximately 0.26 and 2.9 μm, respectively.[8] Thirty-six percent of the smaller particles and 46 percent of the larger particles were deposited.

Nozaki[9] reported that when lead fumes generated in a high-frequency induction furnace were inhaled at a concentration of 10,000 $\mu g/m^3$ deposition was related to respiration rate and particle size. At 10 respirations per minute, the deposition decreased from 63 to 42 percent as the particle size was reduced from 1.0 to 0.05 μm. At 30 respirations per minute, deposition rates were about halved. The results, which are similar to those of Kehoe,[5-7] are fairly consistent with the IRPC lung deposition model.[1]

These data suggest that a 30 ± 10-percent deposition rate can be expected in individuals breathing ambient air and that deposition may vary considerably, depending on the particle size and the frequency of respiration.

The rate of lung clearance has been studied by means of gamma-ray lung scans following inhalation of ^{212}Pb, but the relevance of the results to the rate of clearance of the chemical and physical forms of lead usually inhaled by man is highly questionable.[10] These studies involved the absorption of ^{212}Pb atoms on carrier aerosol particles; however, desorption under these artificial circumstances may be totally unlike the clearance rate for ambient air lead particles.

Kehoe[5-7] reported a substantial increase in fecal excretion when large-particle lead oxide aerosols were inhaled for many weeks at 105 $\mu g/m^3$; the increase probably resulted from the swallowing of particles trapped in the nasopharynx. When air with a similar lead concentration in small particles was inhaled, only a small rise in fecal lead excretion was observed.

In a recent study, Chamberlain et al.[11] found a 35-percent rate of deposition, at a respiration rate of 15 per minute, when subjects inhaled automobile exhaust fumes containing radioactively labeled tetraethyl lead (^{203}Pb). This compares favorably with Nozaki's previous findings.[9] These authors[11] calculated that under conditions of chronic airborne lead exposure roughly 50 percent of the deposited lead is absorbed. Although alveolar macrophages ingest particles deposited in the lungs, these cells may be damaged by inorganic lead compounds.[12] Such damage has been demonstrated in rats and guinea pigs. It is possible, then, that lung defense mechanisms may be impaired when air contains high lead concentrations.

10.2.1.2 ANIMAL STUDIES

Animal studies by Bingham et al.[13] have demonstrated a pronounced reduction in the number of lung macrophages resulting from inhalation of lead oxide at both 10 and 150 $\mu g/m^3$. Similar results have been reported by others.[12,14,15] This suggests that the lung clearance mechanism may function less effectively when air lead concentrations are high. Thus, Pott and Brockhaus[16] reported that large doses of lead bromide solution or lead oxide suspension administered intratracheally to rats (1.5 mg of lead oxide per dose on 8 successive days) were retained by the body as completely as were intravenous doses. At one-third the dose, however, retention via the intratracheal route was significantly less.

Randall and his coworkers[17] exposed 4 baboons to aerosolized lead (Pb_3O_4) of varying particle size (mass median diameters of 5.9, 3.2, and 2.0 μm, respectively). The air lead concentrations varied from approximately 1 to 4 $\mu g/m^3$. The exposure period lasted 4 weeks, and blood sampling continued for 6 weeks. The rate of absorption of lead into blood was faster and reached a higher level for coarse (mean diameter = 1.6 μm) particles than for fine (mean diameter = 0.8 μm).

10.2.2 **Gastrointestinal Absorption**

10.2.2.1 HUMAN STUDIES

It must be noted at the outset that the absorption of lead from food varies with the physical form of dietary intake. For example, the literature indicates that the percent absorption of lead from beverages is about five to eight times greater than that from solid food.[18-20] Kehoe[5-7] concluded from long-term balance studies that approximately 10 percent of the intake of lead from food and beverages was absorbed from the gastrointestinal tract since this was the amount excreted in the urine. This estimate, however, disregarded the urinary lead that might have come from inhalation, as well as the lead excreted in feces after absorption from the gastrointestinal tract.

Rabinowitz et al.,[21] however, obtained similar

results using orally administered ^{204}Pb incorporated into the diet.

Alexander et al.[22] studied the absorption of lead from the gastrointestinal tract in 8 infants and young children aged 3 months to 8.5 years and concluded that 53 percent of ingested lead was absorbed. Absorption and retention were consistent within the age range studied. This study, however, has been criticized because the values varied greatly.

In a recent study, Ziegler et al.[23] showed that a greater percentage of intake lead was absorbed and retained by infants than by older subjects. In this report, 2 separate series of investigations were conducted. In the first, 3 to 8 balance studies were performed with 9 infants each. In the second, each of 6 infants consumed randomly allocated diets providing low, intermediate, and moderate amounts of lead. When intakes of lead exceeded 5 μg/kg/day, which is a reasonable level given typical dietary patterns, net absorption averaged 42 percent and retention averaged 32 percent of intake. It should also be noted that there was an inverse relationship between calcium intake and blood lead level.

These results are in general agreement with animal studies and to some extent corroborate the findings of Alexander et al.[22] The study of Ziegler et al.[23] appears to be much better designed than that of Alexander et al.[22]

Ingested or dietary lead is often thought of as reaching a subject via a distinctly different route of exposure than inhaled lead. Inhalation of lead may be regarded as direct exposure to airborne lead. Some portion of dietary lead may also be attributed to exposure to airborne lead, but indirect rather than direct, with lead reaching food either by deposition onto aerial edibles or by fallout onto soil and subsequent absorption by root crops. (Internal translocation of lead between roots and aerial parts is apparently small.) In addition, variable fractions of inhaled lead are ingested after deposition in the airways; they are cleared by retrograde movement to the pharynx, where the particles are then swallowed.

Section 7.4.1 cites lead concentrations typically found in various foods, but no research has been brought to light that clearly partitions the origins of food lead. There would seem to be three principal candidates: (1) deposition of airborne lead (on primary food crops and on animal feed crops); (2) absorption of soil lead (much of this lead is often the historical accumulation of airborne fallout); and (3) lead acquired in the processing and canning of foods.

Based on the contrasts between fresh and processed foods (excluding frozen foods), it would appear that processing and canning of certain foods regularly doubles or triples their average lead concentrations (Table 10-1).

TABLE 10-1. LEAD CONTENT OF FRESH, PROCESSED, AND CANNED FOODSTUFFS[24]

Food	Lead concentration (ppm)	Food	Lead concentration (ppm)
Fresh produce		Canned vegetables	
Carrots	0.205	Beets	0.381
Lettuce	0.130	Beans	0.318
Potatoes	0.050	Peas	0.425
Avg.	0.128	Tomatoes	0.710
		Avg.	0.458
Processed foods		Canned juices	
White flour	0.052	Tomato	0.338
Cornmeal	0.143	Vegetable	0.215
Rice	0.104	Orange	0.135
Cereal	0.107	Fruit	0.251
Sugar	0.031	Avg.	0.235
Avg.	0.087		
Processed meats		Canned fruits	
Hot dogs	0.446	Peaches	0.417
Hamburger	0.578	Pineapple	0.402
Avg.	0.512	Applesauce	0.320
		Avg.	0.380
Fresh meats			
Beef	0.120		
Chicken	0.191		
Liver	0.150		
Avg.	0.154		

The data on lead in foods are not comprehensive enough to permit construction of a spectrum of levels ranging from fresh to canned and characterization of the resulting lead exposure. It must suffice to state at this point that some fraction of dietary lead probably is indirectly of airborne origin.

10.2.2.2 THE RELATIONSHIP OF ORAL INTAKE TO BLOOD LEAD LEVELS

It has been demonstrated repeatedly that blood lead levels increase when the oral intake of lead increases, but a quantitative expression of this relationship has not been determined. Studies from various parts of the world, as noted below, have shown that the increase in the blood lead for each 100 μg of lead ingested daily ranges from less than 6 to more than 18 μg/dl.

It is important to point out that the high end of this range was gathered using subject groups whose dietary intake may be of questionable relevance to the general population. Tepper and Levin[25] employed adult females in their study while Coulston et al.[26] used an adult prison population. These findings are not only in contrast to those from earlier U.S. studies but also to the results of a number of European studies based on the general population.[27] The European data are more consistent with a contribution of about 6 μg/dl to the blood lead level per 100 μg daily oral intake of lead; a similar level is reported by Kehoe.[5-7]

Children, particularly infants, absorb a larger percentage of lead than do adults. Consequently, the contribution of dietary lead to blood lead levels probably is less for adults, but definitive data are not available.

10.2.2.3 ANIMAL STUDIES

The absorption of lead from food and changes in absorption with age have been investigated in many animal studies; the usual values found ranged between 5 and 10 percent. However, Kostial et al.[28] demonstrated that 5- to 7-day-old rats absorb at least 55 percent of single oral tracer doses of ^{203}Pb, and Forbes and Reina[29] reported that in rats the gastrointestinal absorption of tracer doses of ^{212}Pb, ^{85}Sr, and ^{59}Fe was high prior to weaning but decreased rapidly thereafter. The absorption rate for lead was 83 percent at 16 days; it then decreased gradually to 74 percent on the day of weaning (22 days) and rapidly thereafter to about 16 percent at 89 days. Although there may be some question about the applicability of these data, they are consistent with results reported from studies of young children.

Kello and Kostial[30] have shown that milk increases lead absorption in 6-week-old rats. Fasting enhances lead absorption in mice.[20] Low dietary levels of calcium, iron, zinc, copper, selenium, and vitamin D have been reported to enhance lead absorption.[31,32] It has also been demonstrated that rats on an iron-deficient diet accumulate more lead in their bodies than do rats on an iron-sufficient diet.[33] Table 10-2 presents the data of Barltrop[34] relating to the effects of various nutritional factors on lead absorption as reflected in blood lead levels. It should be noted that these studies are short term studies obtained over a period of 48 hr.

TABLE 10-2. EFFECT OF DIFFERENT DIETS ON LEAD ABSORPTION EXPRESSED AS THE RATIO OF MEAN RETENTION FOR EXPERIMENTAL AND CONTROL SUBJECTS[34]

	Ratio of mean retention of lead (experimental : control)			
Diet	Blood	Kidneys	Femur	Liver
Low protein	5.1	2.5	2.8	2.2
High protein	1	3.7	2.6	1
Low fat	1	1	1	1
High fat	9.6	7.6	4.8	4.2
Low minerals	17.7	11.9	13.7	8.8
High minerals	0.2	0.2	0.1	0.1
Low fiber	1	1	1	1
High fiber	1	1	1	1
Low vitamins	1	1	1	1
High vitamins	1	1	1	1

The absorption of lead in paint chips has received attention because of the risk for young children who tend to ingest this material. Recent data from rat studies indicate that lead chromate and lead naphthenate incorporated into dried paint films are substantially available for absorption, although the absorption rate is 30 to 50 percent what it is for lead naphthenate in oil or for lead nitrate in aqueous solution.[35,36] The absorption of lead as a function of chemical form is shown in Table 10-3.[37]

TABLE 10-3. PERCENTAGE ABSORPTION OF DIFFERENT LEAD COMPOUNDS RELATIVE TO LEAD ACETATE[37]

Lead compound	Absorption, %
Control (no lead)	4
Metallic lead (180 to 250 μm)	14
Lead chromate	44
Lead octoate	62
Lead naphthenate	64
Lead sulfide	67
Lead thallate	121
Lead carbonate (basic)	164

10.2.3 **Cutaneous Absorption**

Absorption through the skin is of importance only in the case of organic compounds of lead, particularly the lead alkyls and lead naphthenates.[38,39] Soon after tetraethyl lead was introduced into commercial use, Eldridge[39] reported that it was absorbed through the skin with great facility in both dogs and guinea pigs. The presence of gasoline has been said to delay the penetration of tetraethyl lead through the skin,[40] although it has no effect on its uptake by the lungs.[41] In rats, five cutaneous or subcutaneous applications on alternate days of lead acetate or lead naphthenate produced, compared with unexposed animals, a decrease in ALAD in liver, a decrease in liver and body weight, and distribution of lead in assayed body tissues. Lead content was highest in kidney. Lead naphthenate was considered by the investigators to be more toxic than lead acetate because of more pronounced skin reactions, higher lead accumulation in brain, and the occurrence of a paralytic syndrome before death in two animals.[42]

The rate of absorption of tetraethyl lead and inorganic compounds through the skin was studied by Lang and Kunze.[43] They applied solutions of lead acetate, lead orthoarsenate, lead oleate, and tetraethyl lead to the bare skin of a number of rats and measured the amount of lead in the kidney as an index of absorption. In all cases, the amount of lead in the kidney was greater than in controls; tetraethyl lead produced the greatest difference. If the skin was traumatized before the lead solutions were applied, there was a threefold or fourfold increase in renal lead concentration. It is likely that absorption through unabraded skin by various lead compounds is primarily dependent on their relative lipid solubilities.

Because of the difficulty, particularly in tetraethyl-lead-contaminated atmospheres, in attempting to separate cutaneous exposure from respiratory exposure, the role of cutaneous lead absorption in relation to blood lead levels is still unclear.

10.3 **DISTRIBUTION**

When a single dose of lead enters the body, it is distributed initially in accordance with the rate of delivery of blood to the various organs and systems. The material is then redistributed to organs and systems in proportion to their respective affinities for lead. When daily ingestion is consistent for an extended period, a nearly steady state is achieved with respect to intercompartmental distribution. The steady-state condition will be disturbed, however, whenever short-term high levels of lead intake are superimposed on such a long-term ingestion pattern.

10.3.1 **Human Studies**

Autopsy data have shown that lead becomes localized and accumulates in bone. This accumulation begins in fetal life,[44,45] since lead is readily transferred across the placenta. The concentration of lead in the blood of newborn children is similar to that of their mothers,[46,47] and the distribution of lead in fetal tissue is similar to that of adults.[45]

The total content of lead in the body may exceed 200 mg in men aged 60 to 70 years, but in women it is somewhat lower. Calculations by several investigators[48] show that in nonoccupationally exposed adults 94 to 95 percent of the total body burden is in the bones.[44,49,50] These reports not only reaffirm the affinity of bone for lead, but also provide evidence that the concentrations of lead in bones increase at least until middle age (50 to 60 years old).[48,51] On the contrary, neither soft tissues nor blood show age-related changes in lead concentration after age 20.[52,53] Thus, it seems that the skeleton is a repository that reflects the long-term accumulative exposure to lead, whereas body fluids and soft tissues equilibrate rather rapidly and reflect only recent exposures.

The concentration of lead in the blood is utilized as an index of exposure to assess conditions considered to represent a risk to health.[54] Plasma lead concentrations have been shown to be constant at 2 to 3 μg/dl over a range of 10 to 150 μg/dl whole blood.[55] Recent studies have indicated that lead is bound primarily to erythrocyte protein, chiefly hemoglobin, rather than to stroma.[56]

Rabinowitz used a stable lead isotope tracer (^{204}Pb) to determine the rate of equilibration of blood lead with input.[21] He found that in human subjects with a constant daily oral intake of ^{204}Pb, a virtually constant concentration was measured in blood after about 110 days. When lead was removed from the diet, the concentration in the blood disappeared with a half time of approximately 19 days. Tola et al.[57] reported that the concentration of lead in the blood rises fairly rapidly to a new steady-state level in about 60 days when men are introduced into an occupational lead-exposure situation, a situation similar to that cited for exposure chambers in clinical studies.[58]

Although the body burden of lead increases throughout life,[48,50,52] measurements of specific organs and systems show that the total burden is

divided between two general pools within the body. The major portion of the lead is contained in bone. This pool is clearly highly accumulative and, as a consequence, lead accumulates here rather consistently and continuously. The second pool comprises other organs and systems and accumulates much less. The levels of lead in this pool tend to stabilize early in adult life and thereafter demonstrate a turnover rate sufficient to prevent accumulation.

Since the organs and systems that contain the relatively mobile lead pool are of greater toxicological significance, it is clear that a mobilizable or exchangeable lead burden is a more important concept than is total body burden. In this connection, chelatable urinary lead has been shown to provide an index of the mobile portion of the total lead burden.[59,60] Among adults in the general population there are no age-related differences in concentrations of lead in whole blood or in blood serum. Thus, in a general way, the blood lead level is an indicator of the concentration of lead in soft tissues, and the changes in blood lead levels observed when there are changes in exposure levels probably reflect similar changes in some organs and soft tissues.

Lead exposure causes the development of nuclear inclusion bodies containing lead in both man[61-63] and animals. Although they seem to occur most frequently in the kidney, they have been found in other organs as well.

The concentrations of lead in deciduous teeth are of interest because tooth analysis represents a noninvasive technique and because teeth provide a record of long-term lead exposure. The dentine is particularly useful in this respect because it is laid down from the time of eruption to the time the tooth is shed. Concentrations of lead in dentine are reported to be considerably lower in suburban school children than in children residing in areas of high lead exposure.[64]

Primarily because of the relative ease with which hair can be collected, there have been some studies of the possible use of hair lead as an index of exposure. These studies have not been sufficient, however, to provide significant information on the relationship between hair lead concentrations and the amount of exposure. Rabinowitz et al.[65] fed labeled lead (^{204}Pb) to 3 subjects daily for approximately 100 days. Levels of isotope in the blood were immediately elevated but in facial hair there was a much more gradual response, with a delay of approximately 35 days.

10.3.2 **Animal Studies**

Administration of a single dose of lead to rats produces high initial concentrations of lead in soft tissues which then fall rapidly as the result of excretion and transfer to bone.[66] The distribution characteristics of lead within the animals' bodies were found to be independent of the dose over a wide range. Castellino and Aloj[67] described the rate constants for the elimination of lead from various tissues in rats following a single dose. Lead was eliminated from bone much more slowly than from other tissues. Bolanowska et al.[68] reported that the rate of elimination of a single dose of lead from rats became slower with time, reflecting progressively decreasing mobility of the residual body burden.

Goldstein et al.[69] sacrificed 21-day-old rats 24 hr after intravenous injection of various single doses (1, 50, 200 μg) of labeled lead (^{210}Pb) and found that the concentration of radioactivity in the brain was directly proportional to the blood radioactivity. Studies of O'Tuama et al.[70] indicate, however, that this process may not be one of simple passive diffusion of lead into neural tissue. These latter investigators sacrificed guinea pigs at 5, 60, and 240 min. after the intravenous injection of tracer doses of lead (^{210}Pb) in both subacutely intoxicated (155 mg $PbCO_3$/day for 5 days) and control animals. Radioactivity in barrier tissues (such as choroid plexus and meninges) rose rapidly, with concentrations ranging to more than ten times the simultaneous brain radioactivity. Subsequently, there was a fall in the barrier tissue levels, but brain levels remained fairly constant and low throughout the period of the study. The apparent discrepancies may be explained in large part by the differences in the ages of the animals as well as other factors, including species differences, time of sacrifice, or a more complex mechanism of distribution.

Rather striking age-related differences in the distribution and retention of lead in rats have been observed.[71] Elimination of a single tracer dose of ^{203}Pb from the whole body, blood, and kidney occurred more rapidly in adult than in suckling rats. In sucklings there was a slight increase with time in the ^{203}Pb content of the brain following administration of the dose, whereas the content in other soft tissues decreased with time.

The intracellular distribution of lead has been studied in rat tissue, mainly by cell-fractiona-

tion techniques.[72,73] Membranes, especially mitochondria, have shown an affinity for lead. Little lead is found in lysosomes,[73] however, in contrast with the intracellular distribution of many other metals, e.g., mercury, copper, and iron.

There are few studies of target organs in which lead concentrations at the site of the effect have been specifically determined. In particular, direct assessment of lead level in bone marrow is difficult to carry out, although the sensitivity of the hematopoietic system to lead has been extensively investigated. Formation of nuclear inclusion bodies is observed in rats with renal lead concentrations of about 10 mg/kg (wet weight) of kidney.[74] Other effects of lead were found to occur only at higher levels of organ concentration. Death in cattle is associated with lead levels of about 50 mg/kg (wet weight) of kidney cortex.[75]

The concept of estimating the lowest level of metal accumulation that results in adverse effects in a target organ has not been well explored in the case of lead. This is in contrast with cadmium, for which estimates have been made of the minimum concentrations in the kidney cortex at which evidence of renal damage appears.[76]

10.4 ELIMINATION

The major portion of excreted lead appears in urine and feces, but lesser quantities are removed via sweat, hair, nails, and exfoliated skin.

10.4.1 Human Studies

Fecal excretion represents the major route of organic and inorganic lead elimination. The rate of fecal lead excretion has been reported to be 100 times the rate of elimination in urine;[77,78] however, most of the lead in feces represents metal that has not been absorbed.

Rabinowitz et al.[79] studied the excretion of tracer lead from the blood of a nonoccupationally exposed human subject. Urinary and fecal excretion of ^{204}Pb from the blood amounted to 38 and 8 μg/day, accounting for 76 and 16 percent, respectively, of the measured recovery. The crude estimation of lead in hair, nails, and sweat yielded a value of 4 μg (8 percent). The urinary excretion was similar to the average daily lead excretion of 31 μg/day reported by Teisinger and Srbova.[80] Booker et al.[81] administered lead(^{212}Pb) intravenously to 2 human subjects and then recovered 4.4 percent of the dose in urine during the first 24 hr. Lead was not detected in feces. During the second 24 hr, about 1.5 percent was measured in both urine and feces. Thus, gastrointestinal transit appears to play an important role in the rate of excretion in feces of systemically administered lead.

The clearance of lead from the blood of man into urine was found by Vostal[82] to be proportional to the rate of creatinine excretion, with urinary lead extrapolating to zero at zero creatinine.

The characteristics of urinary lead excretion may be affected by the chemical form of lead. Whereas all of the lead in urine of subjects with normal exposure can be precipitated by the addition of agents such as oxalate, phosphate, or carbonate, only one-third to two-thirds of the lead in the urine of lead workers is available for precipitation.[83] These results suggest the presence of a stable lead complex in the urine of exposed workers.

Lead is excreted in sweat as well as urine. Schiels[84] reported that ingestion of lead acetate increased the lead concentration of sweat twofold to fourfold. Schroeder and Nason[85] found the concentration of lead in the sweat of lead-intoxicated subjects to be similar to that in urine.

Since studies of net lead retention suggest that, at low level exposures, higher intakes are followed by higher rates of excretions[5-7,17,59] and that lead excretion appears to be disproportionately low in cases of high-level exposure, there is not yet a predictable relationship between increases in lead exposure and in lead excretion.

10.4.2 Animal Studies

The relative importance of lead excretion from blood into urine and feces varies with the species tested. Within 12 hr, 7.4 percent of an intravenous dose appeared in the feces of rats compared with a recovery of 2.3 percent from urine.[86] In sheep, also, fecal elimination is more rapid than urinary excretion of lead.[87] In contrast, urinary excretion was reported to be two times greater than fecal excretion in baboons.[89]

There are indications that most of the translocated lead (from blood to intestine) is derived from bile. Of the 7.5 percent of an intravenous dose of lead acetate excreted by sheep in the feces within 6 days, 81 percent originated in bile.[87] Similar results were obtained from rats.[86,89] Although species differences in gastrointestinal transit time and the presence of a gallbladder can explain differences in the rate of appearance of a single injected dose of lead in the feces, these factors do not account for differences in the relative amounts of steady-state lead elimination in urine and feces.

Measurements of lead clearance into the urine of

animals, like those in man, require an accurate measurement of the free lead concentration in blood. Since most of the lead in blood is bound to red cells and to plasma proteins, this measurement is virtually impossible.

Whether the renal tubule takes an active part in lead excretion is open to question. More recently, it has been found that the renal tubule cell transports lead into the urine,[90] perhaps because of the presence of lead-binding ligands in the tubular cell. These observations demonstrate that the renal excretion of lead involves more than filtration of the metal at the glomerulus. It is likely that the responses of secretory and reabsorptive processes to increased circulating lead levels contribute to the relative constancy of urinary excretion in humans[5-7] and in laboratory animals[74] that were exposed to high doses of lead.

10.5 ALKYL LEAD METABOLISM

The toxic effects caused by tetraethyl lead and tetramethyl lead are not produced by the tetraalkyl compounds themselves, but rather by the trialkyl derivatives formed by dealkylation in the liver.[86,91] Tetraethyl lead is converted primarily to triethyl lead and partly to inorganic lead.[92] Triethyl lead concentrates in organs and disappears very slowly. Even after several days, there is no significant reduction. Tetramethyl lead is much less toxic than tetraethyl lead, probably because it is dealkylated to the trialkyl toxic form much more slowly than is tetraethyl lead.[93]

Since both these compounds have toxic and biochemical effects unlike those of inorganic lead, the biochemical indices used in assessing inorganic lead exposure would not be expected to have the same significance in assessing exposure to organic lead. Indeed, in cases of severe, acute tetraethyl lead poisoning, urinary coproporphyrins and ALA excretion are not usually elevated, and free erythrocyte porphyrins are only moderately and inconsistently elevated.[94,95] These biochemical tests are therefore of little use in short-term exposure situations. In long-term exposure situations, however, it is possible that some of them may be useful. Indeed, Robinson[96] has shown that in industrial workers exposed to tetraethyl lead, urinary excretion of ALA is increased, but not to the same degree as in workers exposed to inorganic lead who have similar levels of total urinary lead excretion (organic plus inorganic). Bolanowska et al.[97] demonstrated that in three fatal cases of tetraethyl lead poisoning the ratio of inorganic lead to triethyl lead ranged from 67:1 to 18:1 in the urine. This ratio did not reflect the ratio of inorganic to triethyl lead in tissues, including the brain where the ratio was approximately 1:1.

10.6 METABOLIC CONSIDERATIONS IN THE IDENTIFICATION OF SUSCEPTIBLE SUBGROUPS IN THE POPULATION

The discussion on the metabolism of lead has up to now only tangentially specified differences in metabolism between children and adults (Section 10.2.2). There are, however, physiological dynamics of child growth and development that have significant implications for the increased risk of children exposed to lead. There are, as well, differences between children and adults in the intake, desposition, etc., of lead.

Metabolic, physical, and other differences between children and adults that must be considered include: (1) children have considerably less surface area than adults, e.g., a 2-year-old child has one-third the surface area of an adult; this parameter is not known to be directly related to the risks associated with lead exposure;[98] (2) there is greater lead intake by infants on a per-unit-body-weight basis, which is probably related to greater caloric and water requirements; (3) there is greater intake in children as well as net absorption (Section 10.2.2), resulting from greater net respiratory intake along with greater net absorption and retention from the gastrointestinal tract; (4) the rapid growth rate of children may reduce the margin of safety against a variety of stresses, including iron deficiency, etc.; (5) dietary habits of children in some respects[99-101] are quite different from those of adults; normal hand-to-mouth activity such as thumb sucking occurs as well as the habit of retrieving dirt-contaminated foodstuffs; (6) in children the likelihood of protein, calcium, and iron deficiency is so great relative to intake that a negative balance in these factors may exist; (7) in very young children metabolic pathways[99-101] are known to be incompletely developed, e.g., the blood-brain barrier in newborns; and (8) partitioning of lead in the bones of children is different from that of adults.[100,101] Only 60 to 65 percent of the lead body burden is in the bones of children. More important is the possible lability of the bone fraction of lead in children, particularly in the case of coexisting calcium deficiency. Rosen and Wexler[102] find an increasing resorption of lead in rat bone organ culture when calcium in the medium is reduced.

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11. BIOLOGICAL EFFECTS OF LEAD EXPOSURE

11.1 INTRODUCTION

As noted in Chapter 2, air-quality criteria documents present the scientific knowledge of the relationship between pollutant concentrations and their adverse effects on public health and the environment. This chapter addresses the important human health and biological effects of lead exposure.

Section 11.2 treats the biochemical and pathological basis of the various health effects of lead, centering on enzymology and the subcellular and cellular aspects of lead effects on the various organ systems. Section 11.3 presents a brief overview of clinical lead poisoning — that is, lead exposure leading to a constellation of adverse health effects that require medical intervention. It is not the purpose of that subsection to suggest that airborne lead invariably induces clinical lead poisoning as defined in this document; rather, it seeks to state the consequences to health, both immediate and long term, of the upper range of exposure to this pollutant regardless of its source and to state this in a discrete portion of the document.

The respective sections on organ systems have been ordered according to the degree of known vulnerability to lead of each system. The emphasis is not only on the three systems classically considered most sensitive — hematopoietic, nervous, and renal — but also on reproduction and development in view of lead's effects on the fetus, and, therefore, on pregnant women. Some effects can be considered to involve a number of organ systems, and the available data on multisystemic effects are presented in the final subsection.

Subdividing the chapter on the health effects of lead into organ systems was done for the purpose of easier discussion. It must be kept in mind that, in reality, all systems function in delicate concert to preserve the physiological integrity of the whole organism. Furthermore, all systems are interdependent in the organism, so that not only are effects in a critical organ transmitted to other systems but also low-level effects, which may be construed as less important in a single specific system, contribute to the cumulative or additive adverse effects of minimal biological response in a number of systems.

11.2 CELLULAR AND SUBCELLULAR EFFECTS OF LEAD

11.2.1 Effects on Enzymes

In general, the effects of lead on enzymes may be manifested in several ways. Lead, in common with a number of other metals, has an affinity for a number of complexing groups resident in the structure of many biomolecular entities, such as imidazole nitrogen, the cysteine sulfhydryl group, and the ϵ-amino group of lysine. An effect may be imparted, therefore, by binding-site competition with the native ion, by perturbation of the structural integrity of enzymes, or by the impediment of substrate-enzyme binding.

Cellular damage caused by lead may also permit the movement of enzymes into the circulatory system, with a resulting elevation of enzyme activity in, for instance, plasma.

The effects of lead on enzymes and enzyme systems have been studied in both animals and exposed human subjects and *in vitro* and *in vivo*. Clearly, many of these studies in the literature are of marginal relevance to this particular document and are briefly summarized for reference reading without evaluation.

On the other hand, a number of other enzyme systems are of such distinct relevance that they are better elaborated in the specific sections on organ effects. For example, the enzymology relating to the heme biosynthetic pathway is discussed in Section 11.4.

Enzymes that have been shown to be affected by lead in animal studies are presented in Table 11-1, and results of studies on enzymes in humans are presented in Table 11-2.

11.2.2 Organellar and Cellular Effects

It is of interest to duscuss briefly the subcellular distribution of lead before further comment is made on cellular effects.

TABLE 11-1. ENZYMES AFFECTED BY LEAD IN ANIMAL STUDIES

Enzyme	Effect on activity	Reference
Lipoamide dehydrogenase	Inhibited	1
DNAase	Enhanced	2.3
Serum glutamic oxaloacetic transaminase (SGOT)	Enhanced and transitory	4-7
Serum glutamic pyruvic transaminase (SGPT)	Enhanced and transitory	4-7
Serum alkaline phosphatase (AP)	Lowered	7
Erythrocyte and liver AP	Variable	7.8
Acid phosphatase	Quenched or markedly inhibited	7,9,10
Catalase	Variable	11-13
Cholinesterase	Markedly inhibited	5.6.14
α-Mannosidase	Increased	15
β-Acetyl glucosaminadase	Increased	15
Succinate oxidase	Decreased	16
Cytochrome c reductase	Decreased	16
Glutamate dehydrogenase	Decreased	16
Cytochrome oxidase	Decreased	16
Rat brain adenyl cyclase	Decreased	17
β-Glucuronidase	Elevated	18
β-Galactosidase	Elevated	18

TABLE 11-2. ENZYMES AFFECTED BY LEAD IN HUMAN STUDIES

Enzyme	Effect on activity	Reference
SGOT and SGPT	Enhanced	19-21
SGOT and SGPT	No effect	22
Serum alkaline phosphatase	Reduced	23
Acid phosphatase	No effect	24.25
Aldolase	Enhanced	26
Cholinesterase	Inhibited	27-29
Glutlathione reductase	Enhanced	30
Glucose-6-phosphate dehydrogenase	Inhibited	30

Castellino and Aloj,[31] using ^{210}Pb, found a decrease in radioactivity over a 24- to 72-hr time interval in the nuclear fraction and an increase of ^{210}Pb in the mitochondria fraction using liver. Over the same time interval, an increase in radioactivity occurred in the kidney in both nuclear and mitochondrial fractions, and there was a decrease in microsomes. Mitochondrial binding was particularly strong.

Similarly, Barltrop et al.[32] measured ^{203}Pb in heart, liver, kidney, and spleen following intraperitoneal (i.p.) administration. Their results indicated that most of the lead accumulated in the mitochondria.

A detailed study of the rat kidney by Goyer et al.[33] showed that over a protracted period the cell nucleus accumulated the highest proportion of lead.

Under lead challenge, a cellular reaction typical of a variety of animal species is the formation of intranuclear inclusion bodies, the early experimental history of which has been reviewed by Goyer and Moore.[34] The presence of considerable lead in these bodies has been verified by X-ray microanalyses;[35] ultrastructural studies show that this entity consists of a rather dense core encapsulated by a fibrillary envelope.

The work of Goyer,[33,36] indicates that these inclusion bodies are a complex of lead and protein, the protein moiety having characteristics of the residual acidic fractions of proteins in normal nuclei. The morphological integrity of these inclusion bodies collapses on treatment *in vitro* with metal chelants such as EDTA. A role for the inclusion body as a cellular protective mechanism during transcellular lead transport has been postulated.[36]

How the localization of lead in nuclear inclusions relates to nuclear function has not been established; however Choie and Richter[37] have shown that i.p.-administered lead enhances DNA synthesis and proliferation of renal tubular cells. The effects of lead on cell division are detailed in Section 11.2.4. Disaggregation occurs in the ribosome in the presence of lead.[1,38]

Animal experiments and human studies, mainly centered on cellular energetics and morphological aberrations, have shown mitochondria to be highly sensitive to lead. Teras and Kakhn[39] showed decreased respiratory rates in mitochondria of rabbit tissue under chronic lead challenge using a-ketoglutarate, succinate, and pyruvate as substrates. Phosphorylation was also retarded.

A marked sensitivity of the pyruvate-NAD reductase system in kidney mitochondria of lead-intoxicated rats is suggested by the work of Goyer and Krall,[40] who note impairment of pyruvate-dependent respiration using ADP/O ratios and respiratory control rates (RCR's) as indices. Succinate-mediated respiration in lead-intoxicated rats, however, was not different from that of control animals.

Rhyne and Goyer[41] state that their observations of decreased oxygen uptake rates for both State III and IV in succinate-dependent respiration in

mitochondria of the kidney from lead-intoxicated animals may be evidence of decreased succino-oxidase enzyme, which would also be consistent with decreased mitochondrial protein.

Walton[42] reported an accumulation of lead in granules produced in isolated rat-liver mitochondria following incubation in media containing lead, with the lower end of the range of the free lead level employed approaching that found in lead-poisoning victims. It was noted that lead-rich granules, unlike those obtained with calcium, were not dispersed after treatment with dinitrophenol. This finding would indicate that lead removal after deposition is difficult. The above observations and other studies prompt the suggestion that lead effects are twofold: (1) energy diversion to the active accumulation of lead would prevent ATP synthesis and the preservation of ionic gradients in the membrane; and (2) the chemical action of lead would promote ATP hydrolysis, and lead would complex with essential SH groups of mitochondrial enzymes and interact with anions. The net result is the inability of cells to maintain themselves structurally and metabolically.

Recent studies by Kimmel et al.[43] on the chronic long-term exposure of rats to lead showed that subcellular effects of lead on the renal system were apparent when comparatively low levels of lead (5, 50, and 250 ppm in drinking water) were administered prenatally and up to 9 months of age postnatally. Light microscopy showed karyomegaly and cytomegaly at all dose levels. Electron microscopic examination indicated swollen mitochondria, numerous dense lysosomes, and intranuclear inclusion bodies at 50 and 250 ppm. Furthermore, considerable alteration in the activity of heme biosynthetic pathway enzymes (δ-ALA synthetase and ferrochelatase) was observed at 50 and 250 ppm. The blood lead values for the different dose regimens ranged from 5 μg/dl for control animals and 10 μg/dl for the 5 ppm group to 25 μg/dl for the 50 ppm group and 70 μg/dl for the 250 ppm animals.

Cramer et al.[44] studied renal biopsy tissue of five workers having varying periods of exposure to lead. Although the typical lead-induced nuclear inclusion bodies were found only in those with short exposure, all subjects showed mitochondrial changes. Mitochondria in the tubular lining cells showed swelling and distortion of cristae, with some of the mitochondria transected by cristae.

An important comment here relates to the indications of impaired mitochondrial function of erythroid tissue in humans that can be assessed by changes in levels of free erythrocyte (actually zinc erythrocyte) protoporphyrin (FEP) and urinary coproporphyrin. The discussion of the hematopoietic system in Section 11.4 includes treatment of this relationship.

In addition, the intramitochondrial stages of heme synthesis have been suggested to have an intermediary role in introcellular metabolism and are probably required for the continued transfer of iron from extracellular sites to normoblasts of reticulocytes.[45,46]

The *in vivo* effects of lead on erythrocytes in humans include: accumulation of lead, increased osmotic resistance, increased mechanical fragility, increased glucose consumption, increased potassium loss in incubation, decreased sodium- and potassium-dependent ATPase activity in membrane fragments, and elevation in the number of immature red cells.[47-49] A more detailed discussion of erythrocyte-lead relationships is given in the hematopoietic section.

Jandl and coworkers[50] demonstrated that the uptake of ^{59}Fe by human reticulocytes was almost completely inhibited by 5×10^{-4} M lead and that its incorporation into hemoglobin was almost entirely prevented. This resulted in an elevated level of iron in the erythrocyte membrane.

The major cellular pathology of concern in the kidney with reference to lead is that of the proximal convoluted tubular cells. Initial atrophy of the epithelial cells in this region is followed by cell regeneration along with an increase in intertubular connective tissue, basement membrane thickening, and round cell proliferation. Tubular cell mitochondria swell and degenerate, as noted before, and glomeruli show increased cellularity.[51] Tubule cells also show the presence of nuclear inclusion bodies, a description of which has been made *(vide supra)*.

In the suckling-rat model for lead encephalopathy employed by Pentschew and Garro[52,53] in which lead exposure of the pups is via milk from mothers fed lead carbonate, epithelial cell dysfunction in brain capillaries is evidenced by abnormal permeability to Trypan Blue and Thorotrast (colloidal thorium dioxide). The lesion possibly centers on interference with an energy-regulating mechanism peculiar to its barrier function.

Schlaepfer[54] has suggested that the neuropathy of lead poisoning may be caused by initial damage to the supporting cells of the nervous system. Dorsal root ganglion capsular cells show a proliferation and accumulation of dense bodies in their cytoplasm that microscopically possess the features of a heavy

metal. It is possible that the metabolism of the capsular cells is impaired, which then causes the degeneration of associated neurons and axons and has a deleterious effect on the ganglion cells. A common site for intoxication in both the capsular cells and the Schwann cells of the peripheral nervous system has been suggested to account for both axonal degeneration and segmental demyelination[55] because these two cells have a common embryological origin.[56]

Moore et al.,[57] in a study of the cardiac effects of lead in the drinking water of rats, found that when rats were exposed to lead in drinking water at a level similar to levels previously found in Glasgow, Scotland, there was a significant inhibition of cardiac ferrochelatase and δ-aminolevulinic acid dehydratase that was maximal after 6 months. Moreover, electron microscopy revealed marked changes in myocardium and myocardial mitochondria.

11.2.3 **Effects of Lead on Chromosomes**

The examination of chromosomes for damage is technically difficult. The evaluation of the relevance of many studies can therefore be equally difficult. Because the appropriate separation of chromosomes into two chromatids and equal redistribution of chromatids during cell division are necessary for the reproduction of stable new cells for the maintenance of healthy tissue, the implications of injury to chromosomal material are profound, and interruption of the processes involved can be serious. Incorrect division of cells by the breakage of the chromatid, the migration of an inappropriate set of chromatids into either portion of a dividing cell, the abnormal reproduction of the complementary new chromatid to complete a viable chromosome in the new cell, and other deviations from the normal process can produce abnormal cells. Such chromosomal aberrations can, therefore, be responsible for the production of such serious consequences as genetic defects in offspring of the affected organism.

In the last few years, a number of reports have been published on the chromosomal effects of excessive exposure to lead in animals[3] and humans.[56-68] Although some of these reports have been essentially negative,[63-65] others have concluded that there is a definite increase in the number of chromatid and chromosome changes in subjects who are occupationally exposed to lead.[56-62] Thus, the literature is controversial in regard to chromosomal abnormalities induced by exposure to lead.

O'Riordan and Evans[63] did not find any significant increase in chromosomal damage in male workers exposed to lead oxide fumes in a shipbreaking yard. These shipbreakers had blood lead values ranging from 40 to over 120 μg/dl. Schmid et al.[64] found no evidence of increased chromosomal aberrations in peripheral lymphocytes, studied both *in vivo* and *in vitro*, in lead manufacturing workers. Furthermore, Bauchinger et al.[65] found no abnormalities in the chromosomes of policemen with elevated blood lead levels (20 to 30 percent above the mean for the control group).

An increase in chromosomal aberrations in people occupationally exposed to lead whose mean blood lead values were 38 to 75 μg/dl has been reported, however, by Forni and Secchi[58] and by Schwanitz et al.[56] Moreover, Deknudt et al.[60] reported chromosomal damage in a group of 14 male workers with signs of lead poisoning. Although the workers were exposed to zinc and cadmium as well as lead, the authors concluded that lead should be considered responsible for the aberrations. The study by Forni and Secchi[58] showed that the rates of chromatid changes were higher in 65 workers with preclinical and clinical signs of lead poisoning but were not significantly raised for workers with past poisoning. Forni et al.[62] also examined 11 subjects before and during initial occupational exposure to lead. The increase in the rate of abnormal chromatid metaphases (the separation of the pair of chromatids during normal cell division) was doubled after 1 month of exposure, was further increased after 2 months, remained in this stage up to 7 months, and then decreased. The fact that most alterations were of the chromatid type, that is, occurring in cell culture after DNA synthesis, indicates that these could be culture-produced aberrations and may not reflect a realistic *in vivo* situation. Also, a number of participants dropped out in the later stages of this study. Thus, the actual biological significance of these results is unknown.

In a recent report, Bauchinger et al.[66] found that chromosomal aberrations were significantly increased in a group of 24 male workers occupied in zinc electrolysis and exposed to zinc, lead, and cadmium. The workers had clearly elevated blood lead and blood cadmium levels in comparison with a control group. The authors emphasized the possibility of a synergistic effect of several metals on the chromosomes. They also pointed out the similarity between this group and the group studied by Deknudt et al.[60] in regard to exposure to a combination of lead, zinc, and cadmium. Referring to studies indicating the mutagenicity of cadmium, Bauchinger

and his colleagues[66] were inclined to consider cadmium as being mainly responsible for aberrations; but Deknudt et al.[60] concluded that the abnormalities found were caused mainly by lead rather than by combinations of the three metals.

The question of whether chromosomal abnormalities occur in humans as a result of lead exposure, either alone or in combination with other pollutants, remains unanswered.[67] Furthermore, the human health significance of chromosomal abnormalities seen in lymphocyte cultures, a method used in some of the studies reported, is not yet known.[68] An assessment of the possible mutagenic effects of lead is further hampered by the technical difficulties that are inherent in the study of chromosomes.

11.2.4 **Carcinogenesis**

Lead salts have been shown to be at least co-carcinogenic in rats and mice.[69] The ultrastructure of experimentally lead-induced renal tumors in animals[70] is characterized by cellular and nuclear hypertrophy, the presence of numerous lysosomes and microbodies, and the absence of the infolding of basal plasma membranes that is normally seen in renal tubular lining cells. These tumor cells do not contain intranuclear inclusion bodies, and the lead content of the tumors is less than that in adjacent renal cortex. Renal adenomas and carcinomas were first observed in rats by Zollinger[71] in 1953 following long-term injections of lead phosphate. Later Kilham et al.[72] reported similar tumors in wild rats believed to have been exposed to lead fumes from burning refuse in a city dump. Lead-induced renal epithelial tumors have since been confirmed by a number of investigators.[70,72,73]

Swiss mice fed diets containing 0.1 percent basic lead acetate [$Pb(C_2H_3O_2)_2 \cdot 2Pb(OH)_2$] developed both benign and malignant renal tumors.[73] The same compound fed to rats at the 0.1 or 1 percent level similarly induced both benign and malignant kidney tumors,[70,74] and the incidence and size were related to the duration of lead feeding.[74] In male Sprague-Dawley rats fed a diet containing 1 percent basic lead acetate, Oyasu et al.[75] observed 2 cerebral gliomas and 13 kidney tumors in 17 animals. Van Esch and Kroes[73] reported renal changes but no neoplasms in 2 groups of 22 and 24 male hamsters fed, for up to 2 years, a standard laboratory diet containing 0.1 or 0.5 percent basic lead acetate.

Renal tumors were observed in rats fed diets containing 1 percent lead acetate [$Pb(C_2H_3O_2)_2 \cdot 3H_2O$],[76] and Goyer and Rhyne[77] reported that 60 to 80 percent of rats on a diet containing 1 percent lead acetate for more than 1 year developed renal adenomas or carcinomas with an increase in both size and incidence of carcinomas related to duration of exposure.

Subcutaneous or intraperitoneal injections of lead phosphate repeated over a period of several months also induced renal tumors. The total doses administered varied between 120 and 680 mg lead in the animals developing the tumors.[71,78]

In addition to renal neoplasms, tumors of the testes, the adrenal, thyroid, pituitary, and prostate glands, and the brain have been reported in Wistar rats fed lead acetate.[79]

The morphologic and co-carcinogenic effects of lead on the respiratory system were studied by Kobayashi and Okamoto.[80] Male and female golden hamsters were given a combination of 1 mg lead oxide and 1 mg benzo[*a*]pyrene intratracheally once weekly for 10 weeks; lung adenomas occurred in 11 of the 26 animals within 60 weeks. One adenocarcinoma of the lung was also observed. Any differences in frequency of occurrence between males and females were not mentioned. Such tumors did not occur in animals given the same dose of lead oxide or benzo[*a*]pyrene alone. It should be noted, however, that because lead compounds are only a small fraction of total particulates in air, there may be enough particulates even without lead for benzo[*a*]pyrene to be adsorbed so as to cause increased carcinogenicity.

Tetraethyl lead (TEL) [$Pb(C_2H_5)_4$] is an important, widely used, antiknock additive for motor fuels. Epstein and Mantel[81] reported that subcutaneous injection of 0.6 mg of TEL given as four equally divided doses to Swiss mice between birth and 21 days of age produced malignant lymphomas in 1 of 26 males and 5 of 41 females, compared with 1 of 39 males and none of 48 female control animals. The tumors were observed 36 to 51 weeks after the first injection in treated females.

No definite relationship between carcinogenicity and occupational exposure to lead has been established from human studies. In 1963, Dingwall-Fordyce and Lane[82] found only marginal evidence for any significant incidence of malignant diseases in their study of 425 persons who had been exposed to lead while working in a battery factory.

A study of the causes of mortality among lead smelter and lead battery workers in 1975 concluded that the incidence of malignant neoplasms, although somewhat greater than expected, was not statistically different from the incidence in the non-exposed population.[83] This seems to support the

conclusion of a Working Group of the International Agency for Research on Cancer (IARC) that there is no evidence suggesting that exposure to lead salts causes cancer in humans.[69] The IARC view is supported by the fact that the comparable level of lead exposure that has been associated with malignant tumors in experiments on rodents is considerably higher than the toxic dose in humans.[69]

11.3 CLINICAL LEAD POISONING

Lead poisoning gives rise to recognized but nonspecific syndromes including acute encephalopathy, chronic encephalopathy, peripheral neuropathy, chronic nephropathy, and anemia.

Encephalopathy is the most severe acute clinical effect of lead poisoning and may emerge rather rapidly with the onset of intractable seizures followed by coma and cardiorespiratory arrest. When the outcome is fatal, death often occurs within 48 hours of the onset of encephalopathy.

In its fulminant form, development of encephalopathy occurs in less than a week. Periods of vomiting and apathy progressing to stupor are interspersed with periods of hyperirritability, poor memory, inability to concentrate, mental depression, persistent headache, and tremor. Several reports[85,86] indicate that children with acute encephalopathy may also incur acute renal injury (Fanconi syndrome), showing hyperaminoaciduria, glycosuria, and hyperphosphaturia.

Pediatric patients with lead poisoning frequently exhibit antisocial behavior and other behavioral disorders, including loss of motor skills and speech. They may also exhibit convulsive disorders; however, there are no clinical features that distinguish lead-induced convulsions from other seizure disorders. These findings are usually associated with blood lead levels in excess of 60 μg/dl and with increased density (in X-rays) at the end of long bones (lead lines). Because the latter indicates prolonged absorption of lead, this clinical picture is termed chronic encephalopathy. The above is very similar to the pattern seen with recurrent episodes of acute lead poisoning with or without acute encephalopathy, and the latter has been described by Byers and Lord[87] as well as Perlstein and Attala.[88] However, there may have been unrecognized episodes of acute encephalopathy at a previous time.

The peripheral neuropathy of lead poisoning centers on motor involvements with little effect on the sensory systems. This involvement may assume three clinical forms: (1) severe pain and tenderness in trunk and extremity muscles giving way to weakness and slow recovery; (2) the more common painless peripheral extensor weakness; and (3) neuropathic and myopathic features that are indistinguishable. This pattern is generally seen in workmen after 5 or more years of chronic exposure.

Patients with a history of one or more episodes of acute lead intoxication develop a nephropathy characterized by progressive and rather irreversible renal insufficiency. Progressive azotemia and sometimes hyperuricemia are noted. Late lead nephropathy generally is recognized at an irreversible stage.

The question of renal sequelae as a result of acute lead poisoning in children has been addressed in several reports. Henderson[89] noted that survivors of childhood lead poisoning in Australia demonstrate a very high frequency of chronic nephritis. Tepper,[90] however, did not confirm this in his Boston studies. Apparently, the length of exposure is of significance, as Tepper's subjects had incurred acute lead poisoning during preschool years whereas the Australian groups may have had exposure to lead for longer periods of time.

The anemia of lead poisoning is hypochromic and sometimes microcytic. It is also associated with shortened red cell life span, reticulocytosis, and the presence of basophilic-stippled cells. Further discussion of the hematopoietic effects is contained in Section 11.4.

Kline studied five patients having chronic lead poisoning.[91] At autopsy, evidence was found for lead encephalopathy in all, as well as evidence for chronic myocarditis. The latter was characterized by interstitial fibrosis with a serous exudate and relatively few inflammatory cells. From these observations, routine electrocardiographic studies and close scrutiny for evidence of myocardial damage was recommended by the author.[91]

Approximately 25 percent of young children who survive an attack of acute encephalopathy sustain severe permanent neurological sequelae.[92-94] Two studies[92,93] have indicated that a pediatric victim of acute encephalopathy has an almost 100 percent chance of severe permanent brain damage when returned to the same environment.

In its most severe form, acute lead encephalopathy may be followed by cortical atrophy, hydrocephalus *ex vacuo,* severe convulsive disorder, mental incompetence, and blindness. These results are becoming rare, however, and subtle neurological deficits and mental impairment are the more common outcomes.

Many children with documented prior attacks of symptomatic lead poisoning develop aggressive,

hostile, and destructive behavior patterns. Although seizure disorder and behavior abnormalities may diminish during adolescence, mental incompetence is permanent.[2,87]

11.4 HEMATOLOGICAL EFFECTS OF LEAD

11.4.1 Anemia

Anemia of varying degree is a manifestation (often the earliest one) of clinical lead intoxication. Classically, the anemia is mildly hypochromic and sometimes microcytic. The anemia is associated with reticulocytosis (because of the shortened red cell survival) and the presence of basophilic stippling. Childhood lead poisoning is most frequently observed in children 1 to 6 years old and of lower socioeconomic status; in both these groups the prevalence of iron deficiency is quite high. A combination of iron deficiency and increased lead intake results in more severe anemia. Anemia, however, is also observed in children with increased lead intake who are not iron deficient. Six and Goyer[95] demonstrated that dietary iron deficiency in rats produced increased lead retention in liver, kidney, and bone with increased urinary δ-ALA excretion. Kaplan et al.[96] showed that the uptake of lead by erythrocytes in the presence of iron was decreased. These findings raised the possibility that iron-deficient children are more susceptible to the toxic effects of lead.

Although it is well known that anemia occurs in severe lead intoxication, the threshold blood lead level at which anemia occurs is not clearly established. In lead workers, Sakurai[97] could not demonstrate any difference in hemoglobin level up to a blood lead level of 50 μg/dl. Tola et al.[98] reported an effect of blood lead level on hemoglobin in a study of 33 workers at the beginning of their exposure to lead in an occupational setting and found that after 100 days of exposure, at the time when the average blood lead level had reached 50 μg/dl, the average hemoglobin level had decreased to 13.4 g/dl from the initial value of 14.4 g/dl ($p = < 0.001$). Pueschel[99] observed a negative correlation between hemoglobin level and blood lead level in 40 children with blood lead levels ranging between 30 and 120 μg/dl. In this study, however, the ages of the individual children are not stated. A number of other studies also bear out the above observation.[100-102] It is known that in children aged 1 to 6 years there is a progressive physiological increase in hemoglobin level and that both iron deficiency and lead intoxication are most frequent in the youngest children.

The mechanism of anemia in lead poisoning appears to be a combination of decreased erythrocyte production as a result of the interference of lead with hemoglobin synthesis and increased destruction as a result of direct damage by lead to the red cell itself. The specific effects of lead at various steps in erythropoiesis are discussed below.

Approximately 90 percent of blood lead travels with the erythrocytes[103,104] as the lead is rapidly transferred from plasma to erythrocytes. Rosen et al.[100] have shown that plasma lead levels are a constant 2 to 3 μg/dl over a range of 10 to 150 μg/dl whole blood. McRoberts,[105] however, has shown that the plasma levels can fluctuate considerably and are associated with the appearance of symptoms in cases of occupational exposure. Kochen[104] has shown that erythrocytes primarily serve as a carrier for blood lead, with a binding capacity well above those lead levels associated with even very heavy exposure. It would appear, then, that the whole blood content of lead is relatively independent of hematocrit.

11.4.2 Effects of Lead on Erythrocyte Morphology and Survival

In lead poisoning, even in absence of iron deficiency, the erythrocytes are microcytic and hypochromic. Basophilic stippling is a frequent but inconstant feature of lead poisoning and has been employed as a method of monitoring workers in the lead industry. This test has the disadvantage of being nonspecific, as basophilic stippling may be observed in the erythrocytes of individuals with thalassemia trait and in several types of hemolytic anemia. Moreover, a good correlation between the amount of stippled erythrocytes and blood lead level has not been observed.[106] Recently Paglia and Valentine[107] have indicated that the basophilic stippling in lead poisoning results from the inhibition of the enzyme pyrimidine-5'-nucleotidase, which under normal conditions plays a prominent role in the cleavage of residual nucleotide chains that persist in the erythrocytes after extrusion of the nucleus. Decreased activity of this enzyme in persons with elevated blood lead levels is observed even when basophilic stippling is not morphologically evident, and it probably contributes to the shortening of the erythrocyte survival. It is known, in fact, that a severe chronic hemolysis is present in people who are genetically defective in pyrimidine-5'-nucleotidase.[108]

Osmotic fragility is decreased in lead poisoning. This is a common feature of many microcytic anemias, as it expresses the increased surface-to-

volume ratio that results from the reduced hemoglobin content of individual erythrocytes. In lead poisoning, however, an increased osmotic resistance also results from a direct effect of lead on the erythrocyte membrane because increased osmotic resistance may be produced by lead *in vitro*.[109] Increased osmotic resistance has been proposed as a screening test for lead poisoning in children.[110] Other evidence of direct damage to the red cell membrane in lead poisoning is the markedly lowered activity of the sodium- and potassium-dependent membrane ATPase, which is indispensably coupled to active cation transport.[111] Shortening of erythrocyte survival has been shown by Hernberg et al.[112] using tritium-labeled difluorophosphonate and by Berk et al.[113] using detailed isotopic studies of a patient with severe acute lead poisoning. Leikin and Eng[114] observed shortened survival time in three out of seven children with lead poisoning and anemia. These studies indicated that hemolysis is not the exclusive mechanism of anemia and that diminished erythrocyte production plays an important role.

An additional factor is a large component of ineffective erythropoiesis. This was demonstrated by the detailed study of the patient of Berk et al.[113] in whom a marked increase of labeled stercobilin was observed after administration of labeled ^{14}C-glycine, a heme precursor. The presence of increased amounts of this heme catabolite in the urine demonstrates altered hemoglobin synthesis as a result of metabolic blockage or premature intramedullary destruction of red cell precursors, or both.

11.4.3 **Effect of Lead on Heme Synthesis**

The effects of lead on heme synthesis are quite well known both because of their prominence and because of the large number of studies in humans and experimental animals. The process of heme synthesis results in the formation of protoporphyrin IX, a complex molecule from small building blocks, glycine and succinate (as succinyl coenzyme A); it culminates with the insertion of iron at the center of the porphyrin ring. The initial and final steps of heme synthesis take place in the mitochondria, whereas most intermediate steps take place in the cytoplasm (Figure 11-1). Heme is formed in the mitochondria, and it is also an essential constituent of the cytochrome system located in the inner crest of the mitochondria themselves and is essential to cell respiration. Besides being a constituent of the cytochrome system and of several other heme proteins in the body, heme is the prosthetic group of

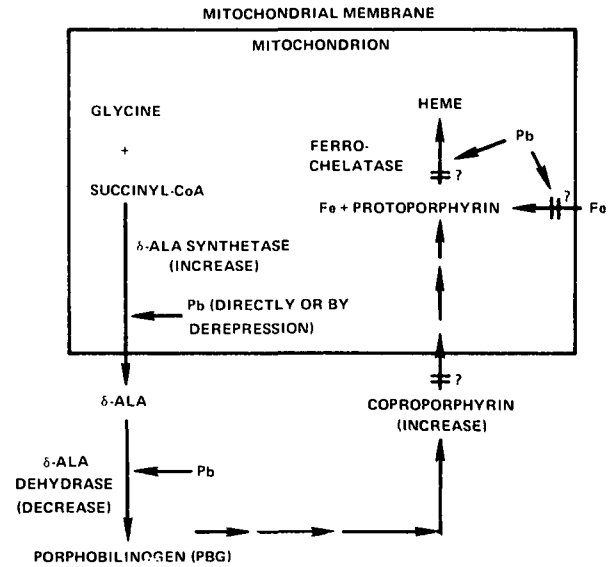

Figure 11-1. Lead effects on heme biosynthesis.

hemoglobin, the protein that transports oxygen from the respiratory system to every cell of the body. Hemoglobin represents 33 percent of the weight of red cells; so a normal 70-kg male with a red cell mass of 3000 ml has 1 kg of hemoglobin, approximately 35 g of which are heme. Therefore, with a red cell life span of 120 days, the daily production of heme for hematopoietic use is only around 300 mg.

Lead interferes with heme synthesis at several points. The two most important steps affected are the condensation of two molecules of δ-aminolevulinic acid (δ-ALA) to form the porphobilinogen ring (at the step catalyzed by the enzyme, δ-aminolevulinic acid dehydratase) and the insertion of iron into protoporphyrin IX (catalyzed by the enzyme, ferrochelatase). Other steps in the heme synthesis are affected by lead, such as δ-ALA-synthetase and coprogenase; these, however, may be affected indirectly through feedback derepression.

11.4.3.1 EFFECTS OF LEAD ON δ-AMINOLEVULINIC ACID DEHYDRATASE (δ-ALAD) AND δ-ALA EXCRETION

This enzyme is highly sensitive to the effect of lead and is directly inhibited by chelation of essential SH groups. The inhibition may be completely reversed by reactivation of the SH group *in vitro* by reducing compounds such as mercaptoethenol and dithiothreitol. The observation that δ-ALAD is inhibited by lead was first reported by Nakao et al.[115] and DeBruin[116] in 1968. Hernberg et al.[117] demonstrated that the logarithm of the activity of δ-ALAD was negatively correlated with blood lead level over

a range from 5 μg/dl (the lowest value observed) to 95 μg/dl. In a detailed study of 25 healthy individuals with blood lead levels below 16 μg/dl, the same investigators found a similar correlation even in this lowest range.[118] These data suggested the direct inhibition by lead of δ-ALAD with no threshold effect because the enzyme was 50 percent inactivated at a blood lead level of 16 μg/dl and 90 percent inactivated at a blood lead level of 55 μg/dl. These observations have been confirmed by several other laboratories[119-123] for the general population, for industrial workers, and for children. In a study of 123 subjects, including 44 lead workers and 79 nonexposed persons (blood lead range of 4.5 to 9.3 μg/dl), Wada et al.[124] noticed a similar exponential negative correlation between blood lead and δ-ALAD. In a subsequent investigation,[125] the same author studied δ-ALAD in three groups: (1) 10 families (each including parents and one child) from a village far north of Tokyo with average blood lead of 8.3 μg/dl (range 5 to 10), (2) 10 families from central Tokyo with average blood lead of 12.8 μg/dl (range 9 to 17), and (3) 10 male workers with average blood lead of 26.5 μg/dl (range 14 to 36). In this study, a significant negative correlation between log ALAD and blood lead was found by combining groups 1, 2, and 3 or groups 1 and 2. In the first group, however, no such correlation could be demonstrated. Because this latter group comprised only 10 families from a small village, it is possible that failure to observe any relationship could be caused by the very narrow range of blood lead (5 to 10 μg/dl) and/or by genetic factors.

More recently Granick et al.[126] studied the ratio of δ-ALAD activity before and after reactivation with dithiothreitol in 65 children with blood lead levels between 20 and 90 μg/dl. By regression analysis, they estimated in this series that a ratio of reactivated/nonreactivated δ-ALAD of 1 (corresponding to no inhibition) would occur at a blood lead level of 15 μg/dl. Because of the wide range of variation and the small number of observations, however, the confidence limits of their estimate are quite large. On the other hand, Hernberg et al.[118] have shown a negative correlation in individuals with blood lead levels below 16 μg/dl, whereas the lowest blood lead level studied by Granick et al.[126] was 20 μg/dl. For these reasons, the observations of Granick et al.[126] do not contradict the evidence by Hernberg et al.[118] that δ-ALAD is already inhibited at the lowest levels of blood lead observed in humans in industrialized countries. Because the inhibition of this enzyme is a direct effect of lead in the blood, its correlation with blood lead is not surprising, and δ-ALAD activity may be used to estimate blood lead with a good degree of accuracy. The inhibition of δ-ALAD in erythrocytes reflects a similar effect of lead in body tissues, as shown by the studies of Secchi et al.[120] which demonstrated that, in 26 persons without industrial exposure to lead and with blood lead levels between 12 and 56 μg/dl, there was a clear correlation between erythrocyte and liver δ-ALAD and an expected negative correlation between blood lead and δ-ALAD in erythrocytes. Millar et al.[119] showed that when suckling rats were fed diets containing lead there was a significant and commensurate reduction of δ-ALAD activity not only in erythrocytes but also in liver and brain tissues. In a recent study by Roels et al.,[127] however, changes in tissue ALAD and free tissue protoporphyrin (FTP) were not found following postnatal lead administration in the rat. Lead was administered in the drinking water (0, 1, 10, 100 ppm) from parturition until day 21. In the offspring, an increase in Pb-B and a reduction in ALAD activity were found in the 10 and 100 ppm groups but no differences in hematocrit, hemoglobin, or FEP were observed. Lead storage in the kidney of the 100 ppm group was associated with a marked rise in kidney FTP but no differences were found in either ALAD or FTP in either liver, heart, or brain.

The inhibition of δ-ALAD is reflected in increased levels of its substrate, δ-ALA, in urine. Plasma δ-ALA has been shown[128] to be elevated in children with severe lead poisoning; however, because of the technical cumbersomeness of the techniques for measuring δ-ALA, few data are available on δ-ALA plasma levels at lower blood lead levels. On the other hand, urinary δ-ALA has been used extensively as an indicator of excessive exposure to lead, and it has even been suggested as a screening tool for lead poisoning.[129] Its use for this purpose has, however, been rejected because of the wide range of individual variability in daily excretion observed in some studies[130,131] Industrial use of this technique has been satisfactory, however.

Several studies have indicated that an excellent correlation exists between blood lead level and the logarithm of the level of urinary δ-ALA. Selander and Cramer[132] first described this relationship in 150 lead workers with blood lead ranging between 7 and 92 μg/dl. Their observations have been confirmed by other studies[123,124,133] in which a similar correlation was observed, in one case, even in the lower blood lead level range. Selander and Cramer[132] noticed that if lead workers were divided

into two groups, those with blood lead levels below and above 40 μg/dl, two different linear correlation slopes could be derived, although with a lesser degree of correlation than the exponential relationship derived from the entire group. As cited in the NAS publication,[2] studies from Chisholm's laboratory showed a similar exponential correlation between blood lead level and urinary δ-ALA is 51 children aged 1 to 5 having blood lead levels ranging between 25 and 75 μg/dl. In 55 adolescents with blood lead levels ranging from 8 to 40 μg/dl, however, no clear correlation could be observed. It appears that apart from this last observation (which is restricted to adolescents, the great majority of whom had blood lead levels in a very narrow range: 14 to 24 μg/dl), all other studies reported show a clear exponential increase in δ-ALA urinary excretion with increase in blood lead. These observations parallel the reported exponential inhibition of the enzyme δ-ALAD and indicate that this is one of the earliest effects of lead on heme synthesis.

The urinary excretion of δ-ALA does not exceed the normal range (0.6 μg/dl) until the blood lead level reaches 40 μg/dl. The normal range, however, is derived from values obtained from individuals with blood lead levels up to 40 μg/dl. It is apparent that if δ-ALAD were inhibited by lead without any threshold of concentration and if δ-ALA were similarly affected, the definition of a normal range of δ-ALA excretion for individuals with a blood lead level less than 40 μg/dl would be ambiguous at best. Some of the discrepancies reported in the literature could in part reflect the larger variability of δ-ALA urinary excretion in comparison with erythrocyte δ-ALAD. In a detailed study by Alessio et al.[123] of 169 males with blood lead levels ranging from 5 to 150 μg/dl, the correlation between blood lead and δ-ALAD was much greater than with urinary δ-ALA. For these reasons and because of the uncertainty of defining a normal range, it is generally accepted that urinary δ-ALA becomes clearly abnormal at blood lead levels greater than 40 μg/dl. It has been postulated that there may be an excess of δ-ALAD activity, so that normal δ-ALA metabolism is still sustained by even 50-percent-inhibited enzyme at blood lead levels near 40 μg/dl. Above this value, however, the inhibition results in functional impairment and clear accumulation of δ-ALA, and increased urinary excretion may be observed. A recent study suggests that ALA may, in fact, be toxic systemically. The relative contributions from decreased utilization of ALA, as a result of ALAD inhibition and the derepression of ALA-synthetase, to urinary levels of ALA at blood levels at which excretion is significant cannot be determined at this time.

11.4.3.2 EFFECTS ON IRON INSERTION IN PROTOPORPHYRIN

The accumulation of protoporphyrin in the erythrocytes of humans with lead intoxication has been known since the 1930's.[135] Its use as an indicator of lead body burden, however, has been limited by the technical difficulties associated with the measurement of protoporphyrin by solvent partition and spectrophotometry. In 1972, the development of a simpler and more accurate technique, combining simplified extraction and fluorometry, made the measurement of protoporphyrin a widely used and accessible test.[136] As discussed in Chapter 9, several modifications of this technique have been developed, including an instrument that measures protoporphyrin by direct fluorescence in capillary blood samples without any extraction or manipulation of the blood sample.

Accumulation of protoporphyrin in the erythrocytes is the result of decreased efficiency of iron insertion into protoporphyrin, the final step in heme synthesis, which takes place inside the mitochondria. When this step is blocked by the effect of lead, large amounts of protoporphyrin without iron accumulate in the erythrocyte, occupying the available heme pockets in hemoglobin. Hence, protoporphyrin, rather than heme, is incorporated in the hemoglobin molecule where it remains throughout the erythrocyte life span (120 days).

The accumulation of protoporphyrin in lead poisoning is different from that observed in erythropoietic protoporphyria, a congenital disorder in which excess protoporphyrin is produced after heme synthesis is complete. In that case, the excess of protoporphyrin formed (as a result of a congenital defect in ferrochelatase) is attached to the surface of hemoglobin at a site that bridges the α- and β-chains of hemoglobin.[137,138] Because this type of bond to hemoglobin is very loose in erythropoietic protoporphyria, protoporphyrin diffuses through the plasma into the skin where it induces photosensitivity. In lead intoxication, on the other hand, the protoporphyrin in hemoglobin is bound more firmly to the heme pocket; hence, no diffusion into the plasma occurs and no photosensitivity is observed, despite extremely elevated erythrocyte protoporphyrin levels.

An additional important difference between the increased protoporphyrin level in the erythrocytes

of persons with lead intoxication and erythropoietic protoporphyria is the fact that only in the former is the center of the protoporphyrin molecule occupied by zinc.[139] This difference is probably caused by the different affinity for zinc of protoporphyrin in the heme pocket. In lead intoxication, then, the largely prevalent species is zinc protoporphyrin, whereas in erythropoietic protoporphyria it is an unchelated protoporphyrin base. These two compounds differ in fluorometric spectra and the two conditions may be easily distinguished by spectrofluorometry.[140] Zinc protoporphyrin, attached in the heme pocket of hemoglobin, is also the prevalent species observed in iron deficiency, another condition in which an increased level of protoporphyrin is observed in the erythrocytes.

Accumulation of protoporphyrin in the erythrocytes in lead poisoning indicates a failure of the last step of heme synthesis. This could result either from a direct effect of lead on ferrochelatase itself or from an effect of lead on mitochondrial membranes of erythroid tissue in bone marrow, with consequent failure of iron transport. The latter mechanism would make iron, one of the two substrates of ferrochelatase, less available to enzyme action, with subsequent accumulation of the unutilized substrate, protoporphyrin IX.

Interference by lead with the mitochondrial transport of iron in the normoblast appears to be the most likely mechanism underlying the increased level protoporphyrin in the erythrocytes. Four facts support this statement: (1) iron accumulation within the erythrocyte is diminished by the presence of lead, whereas iron incorporation into heme is completely inhibited; (2) lead is deposited on the mitochondrial membrane, where it produces profound ultrastructural changes; (3) iron transport through the mitochondrial membrane is accomplished by both energy-dependent and energy-independent mechanisms that are impaired by lead;[141] and (4) in iron deficiency (when ferrochelatase activity is normal but iron is scarce) zinc protoporphyrin bound in the heme pocket is accumulated, whereas in erythropoietic protoporphyria (when iron is normal but ferrochelatase activity is decreased[142,143]) free protoporphyrin base loosely attached to the hemoglobin surface is formed.

Experimental evidence from animal studies and epidemiologic human studies, using intact mitochondria, have demonstrated the failure of iron incorporation into protoporphyrin in the presence of lead. These studies cannot clarify whether the effect of lead is exerted on the enzyme itself or on overall mitochondrial function. It is possible that mitochondrial transport of iron and ferrochelatase are both affected by lead.

The effect of lead on iron incorporation into protoporphyrin is not limited to the normoblast and/or to the hematopoietic system. Formation of the heme-containing protein, cytochrome P450, which is an integral part of the liver mixed-function oxidase system, may also be inhibited by lead.[144] Accumulation of protoporphyrin in the presence of lead has been shown to occur also in cultured cells of chick dorsal root ganglion, indicating that inhibition of heme synthesis takes place in the neural tissue as well.[145] These observations, and the fact that lead is known to disrupt mitochondrial structure and function, indicate that the lead effect on heme synthesis is exerted in all body cells, possibly with different dose/response curves holding for effects in different cell types. On the other hand, it must be noted that increased levels of protoporphyrin in the erythrocyte reflect an accumulation of substrate and therefore imply a functional alteration of mitochondrial function in the same way that the increased urinary excretion of urinary δ-ALA implies impairment. In other words, if a reserve activity of ferrochelatase exists, such as has been suggested for δ-ALAD, accumulation of protoporphyrin in the erythrocytes indicates that this has been hampered by the lead effect to the point that the substrate has accumulated. For these reasons, as well as for its implication of the impairment of mitochondrial function, accumulation of protoporphyrin has been taken to indicate physiological impairment relevant to human health.[146]

The elevation of erythrocyte protoporphyrin was shown to be exponentially correlated with blood lead level by Piomelli in a study of 90 children, covering the blood lead level range from 5 to 90 μg/dl.[147] In a later study of 1038 children, 568 of whom had blood lead levels greater than 40 μg/dl,[148] this correlation was confirmed, and it was clearly shown that all children with blood lead levels greater than 60 μg/dl had erythrocyte protoporphyrin greater than 250 μg/dl red blood cells (RBC's). Kamholtz et al.[149] and Sassa et al.[150] also showed a similar degree of correlation and indicated that a value of 140 μg FEP/dl RBC's would appear to be a more appropriate cut-off point for screening children for lead poisoning. This value, also suggested by McLaran et al.,[151] was accepted by Piomelli et al.,[152] who indicated that more than 70 percent of children with a blood lead level of 40 to

49 μg/dl have erythrocyte protoporphyrin in excess of this value. Several additional studies have confirmed the exponential correlation between blood lead and erythrocyte protoporphyrin in children[140,153-156] and in lead workers.[123,133,140,157,158]

Sassa et al.[150] demonstrated that a better correlation was observed between blood lead and erythrocyte protoporphyrin in children with a steady blood lead level. This finding suggested that a significant part of the scatter observed when blood lead is correlated to erythrocyte protoporphyrin on a random basis is the result of fluctuations of lead caused by day to day variation and experimental error.

Lamola et al.[140] demonstrated that the slope of elevation of erythrocyte protoporphyrin versus blood lead is steeper in children than in adult lead workers. This observation was confirmed by Roels et al.[159] who also demonstrated that the slope of elevation is similar in children and females. Reigert et al.[160] and Levi et al.[161] also demonstrated that an elevation of erythrocyte protoporphyrin can predict which children tend to increase their blood lead level and suggested that erythrocyte protoporphyrin is a more valuable indicator of childhood body burden of lead than the blood lead level itself. In adult workers, the elevation of erythrocyte protoporphyrin was shown to correlate with blood lead level, ALAD, ALA-U, and the duration of exposure to lead.[158] Chisholm et al.[162] suggested that in children erythrocyte porphyrin is a better indicator of overexposure to lead than blood lead. In addition to being elevated in lead intoxication, erythrocyte protoporphyrins may also be elevated in iron deficiency, but to a lesser degree. Several studies have indicated that erythrocyte protoporphyrin levels are an excellent indicator of the body iron store[164,165] and that these levels may also be used to discriminate between the microcytic anemia of iron deficiency (where they are elevated) and of thalassemia trait (where they are normal).[166-168]

These observations and the data collected on over 300,000 children screened by both erythrocyte protoporphyrin and blood lead in New York City were the basis for the statement by the Center for Disease Control (CDC) in which an elevation of erythrocyte protoporphyrin above 60 μg/dl of whole blood in the presence of a blood lead level above 30 μg/dl were indicated as cut-off points for the detection of childhood lead poisoning.[146]

Most studies on the relationship between erythrocyte protoporphyrin and blood lead have focused on persons (children or adult workers) with markedly elevated blood lead. Some studies, however, have shed light on the threshold level below which no effect is observed. In a study of children with blood lead levels over the range of 20 to 40 μg/dl, Sassa et al.[150] could not detect any threshold effect. Data from Roels et al.,[159] who studied 143 school children having blood lead levels ranging from 5 to 40 μg/dl, indicate a threshold effect at blood lead levels between 15 and 20 μg/dl. In a study by Piomelli et al.[169] of 1816 children aged 2 to 12 years (median age 4.7 years), the threshold for no effect of blood lead on erythrocyte protoporphyrin was estimated to be 15.5 μg/dl, using both probit analysis and segmental curve-fitting techniques.

Because an elevation of erythrocyte protoporphyrin is caused also by iron deficiency, it is important to take into consideration the iron state of the population under study in any evaluation of the relationship of this hematological index to lead exposure. No information is available with regard to the iron state of the population studied by Sassa et al.[150] In the Roels study,[159] similarly, no direct measurements of the iron status were obtained; however, the children studied ranged in age from 10 to 15 years, a group in which iron-deficiency anemia is uncommon. Moreover, the differences in blood lead were clearly related to living near or away from lead-emitting smelters. There is no reason to believe that the children who live near a smelter should have lower iron stores than the children who live in rural areas. Also, in the same study, it must be noted that the hematocrit of the children living in the rural area was slightly but significantly lower than the hematocrit of the children living near the smelter; therefore, if anything, the prevalence of any iron deficiency may have been greater in the rural children (with the lowest EP) than in the children living near the smelter (with the highest EP). These facts suggest that iron-deficiency anemia was not a factor in the elevation of erythrocyte protoporphyrin observed in this study, but that this EP increase was directly related to lead. In the study of Piomelli et al.,[169] an analysis of children aged 2 to 4 years versus children older than 4 years failed to show any difference in the EP/blood lead relationship. This indicates that iron-deficiency anemia, which is much more prevalent in the younger children, did not influence the EP response. Moreover, in the same study, the iron stores were measured in children with blood lead levels < 15 μg/dl and in children with blood lead levels of 15 to 28 μg/dl and no difference

was observed. It appears, therefore, that the effect of the lead on EP, which is extremely well documented at the much higher blood lead level, occurs also in children with blood lead levels between 15 and 28 μg/dl.

These studies consistently demonstrate that an elevation of erythrocyte protoporphyrin, which indicates physiological impairment of heme synthesis and mitochondrial function, can be detected in children at a blood lead level that is well below levels normally encountered in screening procedures.

11.4.4 **Other Hematological Effects**

The effects of lead on δ-ALAD and on iron incorporation in protoporphyrin are the best known. In lead intoxication, however, other abnormalities of heme synthesis are observed. These include an increased activity of δ-ALA synthetase,[170] which may result by derepression, according to the scheme of negative feedback control proposed by Granick and Levene.[171] *In vivo* inhibition of coproporphyrinogen and uroporphyrinogen decarboxylases in rabbits[172] and inhibition of uroporphyrinogen I synthetase[173] have been reported. On the other hand, no accumulation of porphobilinogen has been observed in humans. An increased excretion of coproporphyrin in the urine of lead workers and children with lead poisoning is well known. Urinary coproporphyrin has been used extensively as a clinical indicator of lead poisoning. It is not known, however, whether this effect results from specific enzyme inhibition, from upstream accumulation of substrate secondary to inhibition of iron incorporation into protoporphyrin, or from both; or, alternatively, whether it is expressed as a disturbance of coproporphyrin transport through the mitochondrial membrane. Similarly, no data are available to establish a threshold blood lead level below which no excess coproporphyrin excretion in the urine takes place.

Besides the effect of lead on heme synthesis, hemoglobin synthesis may also be impaired because of inhibition by lead of the synthesis of globin (the protein moiety of hemoglobin). Kassenar et al.[174] showed impairment of globin synthesis. This work was confirmed by the results of Wada et al.[170] White and Harvey[175] showed a decreased synthesis of α chains compared to β-globin chains. Recently, Ali et al.[176] have shown an effect on globin synthesis *in vitro* on human reticulocytes at lead concentrations as low as 10^{-6} M, which corresponds to a blood lead level of 20 μg/dl.

11.4.5 **Summary of Effects of Lead on the Hematopoietic System**

A number of significant effects on the hematopoietic system in humans have been observed in lead poisoning. These effects are prominent in clinical lead poisoning, but they are still present to a lesser degree even in persons with lower body burdens of lead.

Anemia is a clinical fixture of lead intoxication. It results from both increased erythrocyte destruction and decreased hemoglobin synthesis. Erythrocytes are microcytic and have abnormal osmotic fragility as a result of direct effect of lead on the cell membrane, and show basiphilic stippling caused by the inhibition of pyrimidine-5'-nucleotidase. Erythrocyte survival time is shortened, and this results in hemolysis.

In children, a threshold level for anemia is about 40 μd Pb/dl, whereas the corresponding value for adults is about 50 μg Pb/dl.

Lead interferes with hemoglobin synthesis by inhibiting synthesis of the globin moiety and affecting several steps in the synthesis of the heme molecule. Most sensitive to lead in the heme synthetic pathway is the activity of the enzyme δ-ALAD, a zinc-activated enzyme that mediates the conversion of two molecules of δ-ALA into prophobilinogen. Inhibition of this enzyme results in increased plasma levels and urinary excretion of δ-ALA. Lead also inhibits the last step (incorporation of iron into protoporphyrin), which takes place in the mitochondria, probably by interference with the mitochondrial transport of iron and coproporphyrin. This effect results in the accumulation of coproporphyrin, which is excreted in the urine, and of protoporphyrin, which is retained in the erythrocytes, in the heme molecule. The overall effect of lead is a net decrease in heme synthesis, which in turn derepresses the enzyme involved in the first step of heme synthesis, δ-ALA synthetase.

Inhibition of δ-ALAD occurs at extremely low blood lead levels and has been shown to start at a blood lead level of 10 μg/dl. The resultant increased urinary δ-ALA excretion also starts at a very low blood lead level and becomes pronounced at a blood lead level $\geq$ 40 μg/dl.

The precise threshold for coproporphyrin excretion is not well established. It is probably similar to the threshold for δ-ALA, but it is less specific. An increase in erythrocyte protoporphyrin occurs at a threshold blood lead level of approximately 16 μg/dl in children. In adult females, the threshold is

probably similar. In adult males, the threshold is probably slightly higher (20 to 25 μg/dl). The threshold for increase in δ-ALA synthetase is not established, but increases have been noticed at blood lead levels of ≥40 μg/dl.

Although doubt exists as to the health-effects significance of δ-ALAD inhibition, increased urinary δ-ALA excretion above 40 μg/dl is accepted as an effect probably reflecting physiological impairment.[2] Elevation of erythrocyte protoporphyrin has the same implication of physiological impairment *in vivo* as is found in urinary δ-ALA. Also, because elevation of erythrocytic protoporphyrin indicates impairment of mitochondrial function, it is considered of greater physiological relevance.[177] For these reasons, the consensus of clinicians who participated in the preparation of the statement in 1975 by CDC together with the American Academy of Pediatrics was that this finding should be used as an indicator of a significant and worrisome body burden of lead.

11.5 EFFECTS OF LEAD ON NEUROPHYSIOLOGY AND BEHAVIOR

Neurological and behavioral deficits have long been recognized as some of the more severe consequences of toxic exposure to lead.[178-182] What levels of lead exposure are necessary to produce specific deleterious neurological or behavioral effects and whether such effects are reversible, however, have been controversial medical issues extensively debated since the early 1900's. Much of the impetus for debate on the subject has been generated by progressively increasing medical concern over an evolving scientific literature that has consistently suggested, as more information is gained, that lead exposure levels previously accepted as harmless are actually sufficient to cause significant neurological or behavioral impairments. At present it is generally accepted that, at toxic, high levels of lead exposure that produce blood lead levels greater than 80 to 100 μg/dl, a person is at unacceptable risk for the occurrence of the clinical syndrome of fulminant lead encephalopathy. This syndrome includes neurological and other symptoms of such severity that immediate medical attention and, frequently, hospitalization is demanded in order to avoid irreversible neural damage or death. The risk involved is unacceptable because of the unpredictability of the symptoms observed at high blood lead levels. Based on the literature reviewed below, it now also appears that lower levels of lead exposure, yielding blood levels below 80 μg/dl, produce much less well-defined but medically significant neurobehavioral deficits in apparently asymptomatic adults and children, that is, in the absence of the neurological symptoms or other signs that typify acute lead intoxication requiring immediate clinical treatment.

The range of lead exposures necessary to produce the more subtle, subclinical neurobehavioral deficits is difficult to estimate with certainty and remains a matter of considerable controversy. There is some evidence reviewed below that suggests that such effects may occur at blood lead levels even as low as 30 to 40 μg/dl, whereas certain other negative findings suggest the lack of neurobehavioral effects at blood lead levels less than 80 μg/dl. In an effort to estimate the exposure levels necessary for manifestation of the full range of neurobehavioral effects of lead, the present discussion will critically review the literature dealing with the obviously toxic effects of high level lead exposures and with the more subtle neurobehavioral effects associated with lower exposure levels.

The relevant literature on the neurobehavioral effects of lead has been derived from studies of both humans and other mammalian species. Such effects have been indexed by means of a variety of approaches, including: (1) the assessment of structural neuropathology by classical histological and ultrastructural analyses of morphological damage; (2) the analysis of altered neurochemical parameters or processes by various biochemical assays; (3) the assessment of altered electrophysiological responses in both the central and peripheral nervous system; (4) the assessment of neurobehavioral effects both by neurological examinations and diverse types of behavioral testing methods; and (5) the assessment of alterations in neuropharmacological responses affecting many of the types of variables assessed by the other approaches. The effects of toxic, high-level exposures to lead have been well documented by most of these approaches. At lower-level exposures, however, the demonstration of lead effects by any of the above types of assessments has been complicated by several other methodological considerations that should be noted as a prelude to any critical review of the literature.

Data about lead exposure have been obtained via two basically different methods, epidemiological and experimental. Unfortunately, these methodological techniques are highly correlated with the species studied; that is, epidemiological techniques, with their unique problems, provide most human data, and experimental techniques are

used to provide most nonhuman data. Although epidemiological studies have immediate environmental relevance at the human level, there are often difficult problems associated with interpretation of the findings. With epidemiological studies, the exact parameters of the most recent exposure level, the duration of any level of exposure, and the mode of intake usually cannot be entirely known. Similarly, any previous lead levels and exposure durations usually cannot be definitively established. It is also possible that other variables that were highly correlated with the presence of environmental lead, such as socioeconomic level, previous behavioral or neurological damage, etc., are responsible for the effects observed rather than lead exposure alone. There is a need for appropriate and sensitive measures of exposure effects, especially when testing for low-level effects for which less-than-dramatic neurobehavioral deficits can be expected. Nevertheless, the contribution of lead exposure to any neurobehavioral deficit(s) can be reasonably estimated with proper controls for many of the above extraneous factors.

Obviously such parameters as exposure levels and durations can be defined with much more precision in experimental studies carried out in the laboratory. Unfortunately, however, appropriate experimental designs are frequently lacking. In addition, environmental relevance of experimental laboratory data is limited by two major considerations. The most serious of these is the fact that nonhuman models are, of necessity, typically used for the establishment of dose-response curves, and it is well known that a large species difference exists in sensitivity to lead,[52] so that adequate nonhuman exposure models are difficult to devise. A second problem is that animal experiments frequently use doses of lead much higher than would be expected to occur in the environment. Besides the question of exposure levels, experimenters must attend to proper experimental controls for possibly reduced nutrition levels because of food palatability, if the delivery system is via food or water; effects of altered maternal behavior, if the delivery is via the mother's milk or the placenta, etc. Further, if central nervous system (CNS) alterations are noted, it is often difficult to separate damage caused by direct versus indirect effects on neural tissue. Again, despite the above difficulties, useful data on the neurobehavioral effects of lead have been obtained through animal studies, with potential implications for understanding human exposure effects.

Key variables that have emerged in determining the effects of lead on the nervous system include (1) the duration and intensity of exposure and (2) age at exposure. In reference to age at exposure, evidence exists for greater vulnerability of the developing nervous system in the young than of the fully matured nervous system in adults. Particular attention will, therefore, be accorded to the discussion of the neurobehavioral effects of lead in children as a special group at risk.

11.5.1 **Human Studies**

11.5.1.1 EFFECTS OF HIGH-LEVEL LEAD EXPOSURES

The severely deleterious effects of exposures to high levels of lead, especially for prolonged periods that produce overt signs of acute lead intoxication, are by now well documented in both adults and children. The most profound effects that occur in adults are referred to as the clinical syndrome of lead encephalopathy, described in detail by numerous investigators.[183-186] Early features of the syndrome that may develop within weeks of initial exposure include dullness, restlessness, irritability, poor attention span, headaches, muscular tremor, hallucinations, and loss of memory. These symptoms may progress to delirium, mania, convulsions, paralysis, coma, and death. The onset of such serious symptoms can often be quite abrupt, with convulsions, coma, and even death occurring very rapidly in patients that shortly before were apparently asymptomatic or exhibited much less severe symptoms of acute lead intoxication.[185,187] Symptoms of encephalopathy similar to those that occur in adults have been reported to occur in infants and young children,[85,90,185,188,189] with a markedly higher incidence of severe encephalopathic symptoms and deaths occurring in them than in adults. This may reflect the greater difficulty in recognizing early symptoms in young children that allows intoxication to proceed to a more severe level before treatment is initiated. In regard to the risk of death in children, the mortality rate for prechelation therapy period encephalopathy cases was approximately 65 percent.[190] Various authors have reported the following mortality rates for children experiencing lead encephalopathy since the inception of chelation therapy as the standard treatment approach: Ennis and Harrison,[191] 39 percent; Agerty,[192] 20 to 30 percent; McKhann and Vogt,[189] 24 percent; Mellins and Jenkins,[193] 24 percent; Levinson and Zeldes,[194] 19 percent; Tanis,[195] 18 percent; and Lewis et al.,[19] 5 percent. These data, as well as other data tabulated more recently,[2] indi-

cate that once lead poisoning has progressed to the point of encephalopathy a life-threatening situation clearly exists and, even with medical intervention, is apt to result in a fatal outcome.

The morphological findings in cases of fatal lead encephalopathy vary.[197-199] On macroscopic examination the brains are often found to be edematous and congested. Microscopic findings of cerebral edema, altered capillaries (endothelial hypertrophy and hyperplasia), and a perivascular glial proliferation are often noted. Neuronal damage is variable and may be caused by anoxia. In some cases gross and microscopic changes are minimal.[198] The neuropathologic findings as reported are essentially the same for adults and children. Lead encephalopathy is considered by some to be primarily a vasculopathy, with the encephalopathy reflecting perturbed blood-brain barrier function;[198,199] that is, damage to neuronal elements may be secondary to lead effects on the vascular system. Evidence for such effects has been advanced by Pentschew.[198]

Pentschew[198] described neuropathology findings for 20 cases of acute lead encephalopathy in infants and young children. The most common finding was activation of intracerebral capillaries characterized by dilation of the capillaries with swelling of the endothelial cells. Diffuse astrocytic proliferation in the gray and white matter was also present. According to Pentschew, this proliferation is the earliest morphological response to an increase in permeability of the blood-brain barrier (dysoria).

Concurrent with the dysoric alterations were changes that Pentschew[198] attributed to hemodynamic disorders. These ischemic changes were manifested as cell necrosis, perineuronal incrustations, or neuronophagia (loss of neurons). The isocortex and the basal ganglia were areas of predilection for the ischemic changes. Pentschew concluded that the structural changes in infantile lead encephalopathy are a mixture of dysoric and hemodynamic parenchymal alterations. In the cerebellum, which in a restricted sense is the predilection area of damage, the changes are purely dysoric.

In addition to producing the above effects on the CNS, lead also clearly causes damage to peripheral nervous systems (PNS) of both man and animals at toxic, high exposure levels. The PNS changes involve predominantly the large myelinated motor fibers.[200] Pathologic changes in the PNS consist of segmental demyelination and in some fibers, axonal degeneration.[200] The lead effect appears to be in the Schwann cell, with concomitant disruption of the myelin membranes.[201] Remyelination has been observed in animal studies, suggesting either that the lead effect may be reversible or that not all of the Schwann cells are affected equally.[201] Reports of *pes cavus* deformities resulting from old peripheral neuropathies in humans,[202] however, suggest that lead-induced neuropathies of sufficient severity could result in permanent peripheral nerve damage. Morphologically, the neuropathy is characteristically detectable only after prolonged or high exposure to lead or both; data from experimental studies indicate that there are distinctly different sensitivities among different species.

Perhaps of even greater concern than the occurrence of fatalities are the neurological sequelae that occur in cases of severe or prolonged nonfatal episodes of lead encephalopathy that are qualitatively quite similar to those seen with many types of traumatic or infectious cerebral injury, with the occurrence of permanent sequelae being more common in children than in adults.[85,90,193] The most severe sequelae in children are cortical atrophy, hydrocephalus, convulsive seizures, and severe mental retardation.[85,90,193] More subtle sequelae also occur, such as impaired motor coordination, altered sensory perception, shortened attention span, and slowed learning. These latter effects have been reported in children with known high exposures to lead but without a history of the life-threatening forms of acute encephalopathy.[85,86,91,203] Of historical interest here in relation to the extremely slow progress in recognizing the full consequences of lead intoxication is the fact that, although many cases of childhood lead poisoning had been reported since the early 1900's,[180-182] it was several decades before the work of McKhann and Vogt[189] and Byers and Lord[85] called attention to the long-term irreversible neurobehavioral sequelae of acute lead intoxication.

Establishing precise threshold values for lead exposures necessary to produce the above acute intoxication symptoms or sequelae in humans is difficult in view of the usual inaccessability of extensive data on environmental lead levels contacted by the victim, the period of exposure, or the body burdens of lead existing prior to the manifestation of clinically significant symptoms. Nevertheless, enough information is available to allow for reasonable estimates to be made regarding the range of blood lead levels needed to produce acute encephalopathic symptoms or death. According to Kehoe[204-206] blood lead levels well in excess of 120 μg/dl are usually necessary to produce such deleterious irreversible effects for adults. Recurrent bouts of lead intoxication in

the absence of acute encephalopathy may also lead to progressive mental deterioration. Other data exist, however,[187] that suggest that acute lead intoxication, including severe gastrointestinal symptoms or signs of encephalopathy or both, can occur in adults at lead levels somewhat less than 100 μg/dl; but ambiguities in these data make interpretation difficult.

The data on threshold levels for children indicate that lower blood lead levels have been associated with the occurrence of acute encephalopathy symptoms and death. Probably the most extensive compilation of information bearing on this point is a summarization[187] of data from the work of Chisholm[84,207] and Chisholm and Harrison.[90] That data compilation relates the occurrence of acute encephalopathy and death in children in Baltimore to blood lead levels determined by the Baltimore City Health Department (dithizone method) between 1930 and 1970. Elevated blood lead levels associated with asymptomatic cases or less severe signs of acute lead poisoning were also tabulated. Asymptomatic increased lead absorption was observed at blood levels ranging from 60 to 300 μg/dl (mean = 105 μg/dl). Acute lead poisoning symptoms, other than signs of encephalopathy, were observed from approximately 60 to 450 μg/dl (mean = 178 μg/dl). Signs of mild encephalopathy (hyperirritability, ataxia, convulsions) and severe encephalopathy (stupor, coma, convulsions repeated over a 24-hr period or longer) were associated with blood lead levels of approximately 90 to 700 or 800 μg/dl, respectively (means = 328 and 336 μg/dl, respectively). The distribution of blood lead levels associated with death (mean = 327 μg/dl) was essentially the same as for levels yielding either mild or severe encephalopathy. These data suggest that threshold blood lead values for death in children are essentially identical to those for acute encephalopathy and that such effects are manifested in children starting at blood lead levels of approximately 100 μg/dl. Other evidence reviewed below, however, suggests that the threshold for acute encephalopathy effects in the most highly susceptible children may, in some rare instances, be somewhat lower than the 100 μg/dl figure arrived at on the basis of the Baltimore data compilation presented above.

Occasionally appearing in the literature since the 1930's are scattered reports of acute lead encephalopathy or death occurring in children at what were formerly considered to be moderately elevated blood lead levels. For example, Cumings[185] listed references to studies on acute lead encephalopathy that appeared from 1938 to 1956. Several of the reports purportedly demonstrated acute encephalopathy in children at blood lead levels even down to 30 to 50 μg/dl. Detailed analyses of the articles referenced, however, indicate that the actual data reported in most did not clearly associate such low-level exposures to the occurrence of acute encephalopathy symptoms. Still, cases in at least some of the referenced articles and in other reports reviewed below suggest that acute encephalopathy occurred in a few children at blood lead levels below 100 μg/dl. Again, the ambiguities in these data regarding confirmation of lead exposure and elimination of alternative etiological factors make interpretation difficult. A further precaution has to do with the analytical methods themselves in terms of using good methods with skilled personnel.

In 1938, Gant[208] reported on five cases of acute lead encephalopathy in children under 2 years of age. Blood lead levels of 60 and 80 μg/dl, as well as 190, 240, and 320 μg/dl, were obtained for the different children upon first admission to the hospital. All five had convulsions. Smith[187] listed a 3-year-old female patient as having a blood lead level of 60 μg/dl at the time of acute lead intoxication from paint ingestion, followed by death attributed to plumbism 2 days after a 70 μg/dl reading was obtained during a period when only mild symptoms of lead poisoning were present. In 1956, Bradley et al.[209] reported that 19 children under 5 years old from a low income area of Baltimore were found to show CNS symptoms that included irritability, lethargy, or convulsions at blood lead levels below, as well as above, 100 μg/dl. Eight children who had been previously classified as asymptomatic and who had blood lead levels of 50 to 80 μg/dl were later hospitalized during the study[210] for treatment of acute lead encephalopathy; blood lead levels at the time of later hospitalization were not reported but were likely further elevated. Other data are reported[211] on 10 children from a low-income area of Providence, Rhode Island who were selected at 4 to 8 years of age for a follow-up investigation of possible long-term neurobehavioral deficits resulting from earlier acute encephalopathy episodes. Blood lead assays obtained at the time of the initial hospitalization of the children because of acute encephalopathy symptoms (4 out of 10 had convulsions, 8 out of 10 had ataxia, 7 out of 10 had drowsiness, and 6 out of 10 had irritability) yielded maximum blood lead values that averaged 88 ± S.D. 41 μg/dl. Because individual cases were not described, however, no clear association between particular lead intoxication symptoms and specific blood lead

levels can be established. Overall, the above reports suggest that at least some children — perhaps especially inner-city children less than 4 years old — may be vulnerable to acute encephalopathy at blood lead levels of 80 to 100 μg/dl.

From the preceding discussion, it can be seen that severity of symptoms varies widely for different adults or children as a function of increasing blood lead levels. Some show irreversible CNS damage or death at levels less than 100 μg/dl, whereas others may not show any of the usual clinical signs of lead intoxication even at blood lead levels in the 100 to 200 μg/dl range. This difference may be caused (1) by individual biological variation in susceptibility to lead effects; (2) by changes in blood lead values from the time of initial damaging intoxication; (3) by better tolerance for a gradually accumulating lead burden; or (4) by any number of other interacting factors, such as nutritional state or inaccurate determinations of blood lead. In any case, in attempting to estimate exposure levels for adverse health effects of lead, the range of exposure levels yielding damaging effects to the most susceptible individuals needs to be emphasized rather than any average level at which such effects are seen. For adults, it would appear that the most susceptible individuals do not exhibit acute encephalopathy symptoms until blood lead levels of 100 μg/dl are reached or, more typically, are substantially exceeded. In regard to children, the majority of cases showing acute encephalopathic symptoms have blood lead levels of 100 μg/dl or more. For a very few cases, levels as low as 80 μg/dl have been reported.

11.5.1.2 EFFECTS OF LOW-LEVEL LEAD EXPOSURES

Also of great relevance for establishing safety limits for exposure to lead is the question of whether exposures lower than those producing symptoms of overt acute intoxication may exert more subtle, subclinical neurobehavioral effects in apparently asymptomatic adults or children. Attention has been focused in particular on whether exposures leading to blood lead levels in the 30 or 40 to 80 μg/dl range may lead to neurobehavioral deficits in the absence of any classical signs of lead encephalopathy. The literature on this subject is somewhat limited and controversial but still allows for certain statements to be made about the possible hazard of low to moderate lead exposure levels.

If such neurobehavioral deficits occurred in adults with great frequency, one might expect this to be reflected by higher rates of absences or reports of neurologically related symptoms among occupationally exposed lead workers. Some recent epidemiological studies have investigated possible relationships between moderately elevated blood lead levels and general health as indexed by records of sick absences that have been certified by physicians. No correlation between elevated blood lead levels and sickness rates or types of symptoms reported were found[212] for groups of workers in a lead storage battery factory from high-, medium-, and low-exposure areas versus control workers in nonexposure areas of the same plant. It should be noted, however, that mean blood lead levels for workers in the three exposure groups were 60, 50, and 42 μg/dl, respectively, compared with 45 μg/dl for the so-called nonexposure control group, rendering the conclusions of the report of dubious value. Similar negative findings were reported by Robinson[213] for tetraethyl-lead (TEL) workers having mean blood lead values of 43 μg/dl and daily urinary excretion of 0.089 mg of lead per liter urine over an 8- to 10-year period (3 to 4 times the rate for control group). Data on sickness rates were based on a retrospective study of records over a 20-year period. Absence or sickness reports, however, are probably not sensitive enough measures to detect subtle neurobehavioral symptoms.

Only a few studies have employed more sensitive psychometric and neurological testing procedures in an effort to demonstrate subclinical lead-induced neurobehavioral effects in adults. For example, Morgan and Repko[214] reported preliminary results of an extensive study of behavioral functions in 190 lead-exposed workers (mean blood lead level = 60.5 ± 17.0 μg/dl). In 68 percent of the subjects, blood lead was <80 μg/dl. The majority of the subjects were exposed between 5 and 20 years. The authors examined 36 nonindependent measures of general performance and obtained 44 measures of sensory, psychomotor, and psychological functions. Initial data analysis suggested that blood lead levels correlated with several reaction-time measures, and δ-ALAD changes correlated with effects on hand-eye coordination. This study, therefore, suggested that below a blood lead level of 80 μg/dl some behavioral changes did occur in adult workers. In addition, variability of performance increased with increasing blood lead level; however, only during period of high-demand performance did a worker's capacity clearly decrease as a result of lead exposure. Unfortunately, aspects of the Morgan and Repko work can be criticized because of methodological problems, including reported ap-

paratus failures during testing of subjects. Also, findings analogous to those reported by Morgan and Repko were not obtained in a similar study[215] that found no differences between control and lead-exposed workers on a number of psychometric and other performance tests.

In addition to the above study[213] suggesting possible CNS dysfunctions, numerous investigations have provided electrophysiological data indicating that peripheral neuropathy symptoms are associated at times with blood lead values < 80 μg/dl. As reviewed by Seppäläinen,[216] reductions in nerve conduction velocities and electromyographic deficits have been observed in patients with known lead poisoning but without clinical neurological symptoms.[217-219] More recently, such peripheral nerve deficits were established by Seppäläinen[220] for lead workers whose blood lead levels were as low as 50 μg/dl and had never exceeded 70 μg/dl during their entire exposure period (mean = 4.6 years), as determined by regular monitoring. Similar results were obtained in a study by Melgaard et al.[221] on automobile mechanics exposed to TEL and other lead compounds in lubricating and high-pressure oils. Results of a multielemental analysis of the worker's blood for lead, chromium, copper, nickel, and manganese indicated a clear association between lead exposure and peripheral nerve damage. Half of the workers (10 of 20) had elevated blood lead levels (60 to 120 μg/dl) and showed definite electromyographic deficits. Mean blood lead level for the control group was 18.6 μg/dl. Melgaard et al.[221] reported additional results on associating lead exposures with polyneuropathy of unknown etiology in 10 cases from the general population. Another study reported recently by Araki et al.[222] provides further confirmation of the Seppäläinen[220] and Melgaard et al.[221] findings in that evidence for peripheral neuropathy effects were reported for lead-industry workers with blood lead values of 29 to 70 μg/dl. The very low blood lead levels, below 50 μg/dl, reported in some of the above studies, however, should probably be viewed with caution until further confirmatory data are reported on samples of larger size using well verified blood assay results.

In summary, the above studies, when taken together, appear to provide reasonably strong evidence that subclinical peripheral neuropathies occur in some adults having blood lead levels in the 50 to 70 μg/dl range. Furthermore, although it could be argued that substantially higher lead body burdens existing before the time of some of the studies were actually responsible for producing the neuropathies, it appears that in at least one case[220] blood levels always below 70 μg/dl were sufficient to cause peripheral nerve dysfunctions. That study by Seppäläinen[220] was also generally methodologically sound, having been well controlled for the possible effects of extraneous factors such as temperature differences at the nerve conduction velocity assessment sites. On the other hand, it should be noted that the data reported for control subjects were obtained at an earlier time (1971 to 1973) than data for the lead exposed subjects (early 1973); and no blood lead levels were reported for the control subjects. Still, when the Seppäläinen[220] results are viewed collectively with the data from other studies reviewed above, strong evidence appears to exist for peripheral neuropathies occurring in adults at blood lead levels of 50 to 70 μg/dl or, possibly, at even lower levels.

In addition to suspected neurobehavioral effects of relatively low-level lead exposures in adults, there is an increasing concern that low-level exposures producing blood lead levels of 40 to 80 μg/dl (or even less) in children may induce subtle neurological damage, especially to the very young developing CNS. This issue has attracted much attention and generated considerable controversy during the past decade. The evidence for and against the occurrence of significant neurobehavioral deficits at relatively low levels of lead exposure is, at this time, quite mixed and largely interpretable only after a thorough critical review of the methodologies employed in each of the various important studies on the subject.

One of the major approaches that has been employed is the retrospective analysis of lead levels existing in populations of apparently asymptomatic children that are then divided into nonexposed control and one or more lead-exposed experimental groups for comparisons of their performance in various neurological and psychometric tests. A few studies have been followed by subsequent further reevaluation of the same children by the same investigators in an effort to assess whether indications of continuing neurobehavioral impairment still existed. Among the major studies that have employed this basic approach and that are widely cited in regard to this issue, those of de la Burde and Choate,[223,224] Perino and Ernhart,[225] Albert et al.,[226] and Landrigan et al.[227] suggest significant effects of asymptomatic, low-level lead exposure. In contrast, the studies of Kotok,[228] Lansdown et al.,[229] and McNeil et al.[230] report generally negative

results. Two other studies, one by Landrigan et al.[231] and one by Kotok et al.,[232] although not reporting clearly statistically significant differences between moderately lead-exposed and control subjects, nevertheless report certain findings that are highly suggestive of a relationship between moderate lead exposure and cognitive impairment.

Among the several studies presenting evidence for CNS deficits being associated with blood levels of less than 80 μg/dl are the work of de la Burde et al.[223,224] and Perino and Ernhart.[225]De la Burde et al.[223] observed dysfunctions of the CNS, fine motor dysfunction, impaired concept formation, and altered behavioral profile in 70 preschool children exhibiting pica and elevated blood lead levels (in all cases above 30 μg/dl, mean = 59 μg/dl) in comparison to matched control subjects not engaging in pica. In a follow-up study on the same children (at 7 to 8 years old), de la Burde[224] reported further confirmation of continuing CNS impairment as assessed by a variety of psychological and neurological tests. This was despite the fact that many of the blood lead levels of the lead-exposed children had by then dropped significantly from the initial study. In general, the de la Burde et al.[223,224] studies appear to be methodologically sound, having many features that strengthen the case for the validity of their findings. For example, there were appreciable numbers of children (67 lead-exposed and 70 controls) whose blood lead values were obtained in preschool years and who were old enough (7 years) during the follow-up study to cooperate adequately for reliable psychological testing. The specific psychometric tests employed were well standardized and accepted as sensitive indicators of minimal brain damage, and the neurobehavioral evaluations were carried out blind, that is, without the evaluators knowing which were control or lead-exposed subjects.

The de la Burde[223,224] studies might be criticized on several points, none of which in the final analysis provide sufficient grounds for rejecting their validity. One difficulty is that blood lead values were not determined for control subjects in the initial study, but the lack of history of pica, as well as tooth lead analyses done later for the follow-up study, render it very improbable that appreciable numbers of lead-exposed subjects might have been wrongly assigned to the control group. Also, results indicating no measurable coproporphyrins in the urine of control subjects at the time of initial testing further help to confirm proper assignment of those children to the nonexposed control group. A second point of criticism addresses the probably inappropriate use of multiple chi-square statistical analyses in the manner employed to analyze the results of the study. Upon recomputation of the statistical significance of observed differences, by means of the more appropriate Fisher's exact probability test and accounting for the number of tests conducted, several measures originally reported to be statistically significant still turn out to be significant at $p < 0.05$ or lower. One last problem relates to ambiguities in subject selection that complicate interpretation of the full meaning of the results obtained. Because it is stated that the lead-exposed group included children with blood lead levels of 40 to 100 μg/dl, or of at least 30 μg/dl with "positive radiographic findings of lead lines in the long bones, metallic deposits in the intestines, or both," the reported deficits might be readily attributed to blood lead levels as low as 30 μg/dl. Other evidence,[101] however, suggests that such a simple interpretation may not be completely accurate. That is, the work of Betts et al.[101] indicates that lead lines are usually not seen unless blood levels exceed 60 μg/dl for most children at some time during exposure, although some (approximately 25 percent) may show lead lines at blood lead levels of 40 to 60 μg/dl. Virtually none have lead lines at levels below 40 μg/dl. In view of this, the de la Burde results probably can be most reasonably interpreted as demonstrating lasting neurobehavioral deficits at blood lead levels in excess of 50 to 60 μg/dl.

Similar conclusions are also warranted on the basis of results of the Perino and Ernhart study,[225] which demonstrated a relationship between neurobehavioral deficits and blood lead levels ranging from 40 to 70 μg/dl in a group of 80 inner-city preschool black children. One of the more interesting aspects of the findings is that the normal correlation of .50 between parent's intelligence and that of their offspring was found to be reduced to only .10 in the lead-exposed group, presumably because of the influence of another factor (lead) that interfered with the normal intellectual development of the lead-exposed children. Many of the methodological virtues of the de la Burde studies[223,224] were also present in the Perino and Ernhart[225] work, and blood lead determinations and statistical analyses appeared sound. About the only alternative explanation for these results might be differences in the educational backgrounds of the parents of the control subjects when compared with lead-exposed subjects, because parental education level was found to be significantly negatively related to the blood lead levels of the children participating in this study.

Parents of children in the lead-exposed group had significantly poorer educational backgrounds than the control group parents. The importance of this point lies in the fact that several other studies[233-235] have demonstrated that the higher the parental education level, the more rapid the development and the higher the intelligence quotients (I.Q.'s) of their children. It is nevertheless interesting that the de la Burde studies and the Perino and Ernhart work point to essentially the same conclusion, i.e., that neurobehavioral deficits occur at blood lead levels possibly as low as 40 μg/dl. Also, in both cases, the children studied were from inner-city, low-income areas.

Two other studies with positive findings had, for the most part, some serious methodological limitations. Albert et al.[226] found that asymptomatic children (5 to 15 years old) whose blood lead levels at an earlier age were elevated (>60 μg/dl) later had significantly more mental disorders and poorer school performance than a control group with lower lead levels in both blood and deciduous teeth. Unfortunately, however, no assay of the lead burden, in either blood or teeth, was done for about one-half of the children in the control group; and no significant effects were reported for children with lead levels $<$ 60 μg/dl. Also, another major criticism is that some children in the control group had relatively high blood lead levels (>40 μg/dl). In another study, Landrigan et al.[227] found that asymptomatic, lead-exposed children living near a smelter scored significantly lower than matched controls on measures of performance I.Q. and finger-wrist tapping. The control children in this study were, however, not well matched by age or sex to the lead-exposed group, although it should be pointed out that results remained statistically significant even after appropriate adjustments were made for age differences.

In another relevant study, presented in a doctoral dissertation by Rummo,[211] significant neurobehavioral deficits were found (hyperactivity, lower scores on McCarthy scales of cognitive function, etc.) for children who had previously experienced high levels of lead exposure that had produced acute lead encephalopathy. Mean maximum blood lead levels for those children at the time of encephalopathy were 88 $\pm$ S.D. 40 μg/dl. Children with moderate degrees of blood lead elevation, however, were not significantly different from controls on any measure of cognitive functioning, psychomotor performance, or hyperactivity. On the other hand, if the data for performance on the McCarthy General Cognitive Index or several McCarthy Subscales are plotted graphically, as in Figures 11-2 and 11-3, then a rather interesting relationship between test performance and levels and duration of lead exposure becomes apparent.

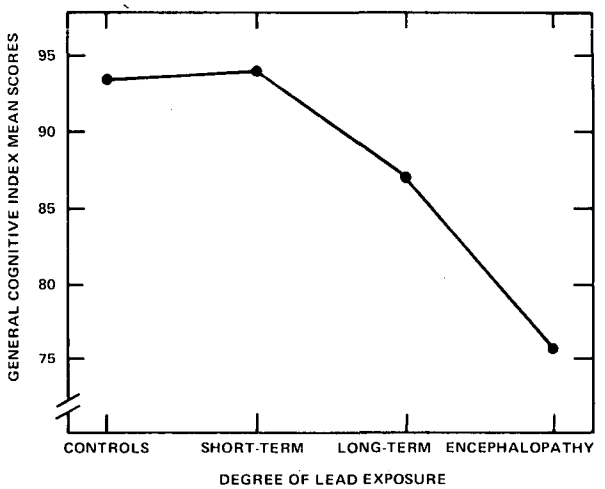

Figure 11-2. McCarthy General Cognitive Index scores as a function of degree of lead exposure.[211]

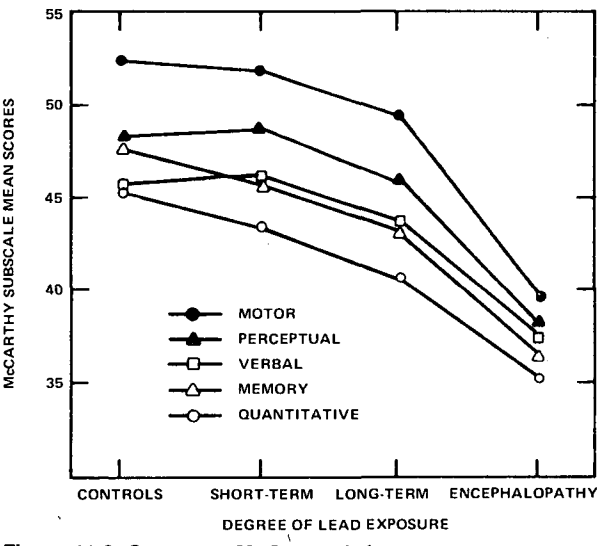

Figure 11-3. Scores on McCarthy Subscales as a function of degree of lead exposure.[211]

Although the scores for short-term moderate-exposure subjects are essentially the same as control values, an interesting aspect is that the scores for long-term moderate-exposure subjects consistently fall below those for control subjects and lie between the latter and the encephalopathy group scores. Thus, it would appear that long-term moderate lead exposure may, in fact, exert subtle neurobehavioral effects. This might be shown to be statistically significant by means of other types of analyses or if larger samples were assessed. It should be noted that (1) the maximum blood lead levels for the short-term and long-term exposure subjects were all >40

μg/dl (means = 61 ± S.D. 7 and 68 ± S.D. 13 μg/dl, respectively), whereas control subjects all had blood lead levels below 40 μg/dl (mean = 23 ± S.D. 8 μg/dl); and (2) the control and lead-exposed subjects were inner-city (Providence, Rhode Island) children well matched for socioeconomic background, parental education levels, incidence of pica, and other pertinent factors.

A somewhat similar pattern of results emerged from a more recent study by Kotok et al.[232] in which 36 Rochester, New York, control group children with blood lead levels < 40 μg/dl were compared with 31 asymptomatic children having distinctly elevated blood lead levels (61 to 200 μg/dl). Both groups were well matched on important background factors, including, notably, their propensity to exhibit pica. Again, no clearly statistically significant ($p < .05$) differences between the two groups were found on a number of different tests of cognitive and sensory functions.

As indicated, however, by test results from the Kotok et al. study[232] presented in Table 11-3, the mean scores of the control-group children were consistently higher than those of the lead-exposed group for all six of the ability classes listed. Also, in one case the level of significance achieved borderline significance ($.10 > p > .05$), a pattern of results that hints at a trend existing toward lower ability levels for the lead group. The authors cautiously stated that "the data do not prove that these children have sustained no neurologic damage by lead" and that "later longitudinal testing may demonstrate cognitive or educational deficiencies." They also indicated that "evaluation of behavior, neurologic, or motor functioning was not carried out" and noted that "subtle cognitive and fine motor changes were demonstrated in an extensive and carefully controlled evaluation of asymptomatic children residing in the vicinity of an El Paso lead smelter."[227] They go on to imply that the earlier exposure to lead in infancy of the El Paso children and their longer period of exposure (mean = 6.6 years versus < 3 years for the Rochester group) might account for the disparity in results between the two studies. Their results, on the other hand, plus the pattern seen in the Rummo[211] study, can be construed as evidence consistent with the findings of the de la Burde studies[223,224] and others reviewed above that report results linking low to moderate levels of lead exposure to significant behavioral impairments.

Other studies have produced mixed or negative results in attempts to determine whether a relationship exists between lead exposure and CNS deficits using various standardized psychometric techniques, neurologic examinations, and ratings by teachers, parents, or experimenters. For example, Kotok[228] reported earlier that developmental deficiencies (using the Denver Development Screening test, which is a somewhat insensitive measure of development) in a group of asymptomatic children having elevated lead levels (58 to 137 μg/dl) were identical to those in a control group similar in age, sex, race, environment, neonatal condition, and presence of pica, but whose blood lead levels were lower (20 to 55 μg/dl). The deficiencies could be correlated with inadequacies in the children's environment. Children in the lead-exposed group, however, had blood lead levels as high as 137 μg/dl, whereas some of the controls had blood lead levels as high as 55 μg/dl. Thus, the study was in effect a comparison of two groups with different degrees of elevation in lead exposure rather than one of lead-exposed versus nonexposed control children.

TABLE 11-3. COMPARISON OF TEST RESULTS IN LEAD AND CONTROL GROUPS[232]

Ability class	Group	Mean I.Q. ± S.D.	Significance
Social maturity	Lead	123.5 ± 22.7	$p > .10$
	Control	126.3 ± 17.7	
Spatial	Lead	92.0 ± 18.0	$.10 > p > .05$
	Control	100.8 ± 18.7	
Spoken vocabulary	Lead	91.7 ± 13.9	$p > .10$
	Control	92.9 ± 13.7	
Information-comprehension	Lead	94.5 ± 14.9	$p > .10$
	Control	96.3 ± 16.6	
Visual attention	Lead	89.7 ± 18.3	$p > .10$
	Control	93.3 ± 23.0	
Auditory memory	Lead	93.0 ± 24.0	$p > .10$
	Control	99.9 ± 32.0	

In several studies of children living in the vicinity of smelters or factories, significant neurobehavioral effects have typically not been found at moderate elevations of blood lead levels. For example, Lansdown et al.[229] found a relationship between blood lead level in children and the distance they lived from lead-processing facilities, but no relationship between blood lead level and mental functioning was found. Only a minority of the lead-exposed sample had blood lead levels over 40 μg/dl, however, one would not expect a striking relationship between mental functioning and lead levels below such a level.

In an extensive, generally thorough study, McNeil et al.[230] found that a sample of children living near a lead smelter in El Paso was comparable medically

and psychologically to matched controls living elsewhere in the same city other than in the direct effects of lead (blood lead level, free erythrocyte protoporphyrin levels, and X-ray findings). Lead-exposed children in the group living near the smelter did, however, have significantly different personality test results, which were ascribed by the authors as being due to recent upheaval in the lives of the lead-exposed children who had been recently forced to move from the vicinity of the smelter. Considerable community unrest existed at the time of both the McNeil et al. study[230] and the work of Landrigan et al.[227] on the El Paso smelter area population, which grew out of circumstances associated with the discovery of the lead exposures and disposition of legal matters surrounding them. The impact of the extraneous unrest on both studies has tended to cloud interpretation of the true meaning of their respective results which in turn have become quite controversial. See Appendix E for more information. The personality test results of the McNeil et al.[230] study could nevertheless have been caused by the effects of lead on the exposed group. Also, it should be noted that a few suggestive trends toward statistical significance for certain interactions between lead and age of subjects ($p < .10$) were reported for some of the cognitive-function test measures.

Based on the above results, the authors of the Lansdown et al.[229] and the McNeil[230] papers concluded that no evidence was found for the occurrence of neurobehavioral effects at subclinical lead exposure levels in their studies. Perhaps that conclusion could be generalized to suggest that no significant CNS deficits typically occur as a result of subclinical, low to moderate lead exposures of children living in the vicinity of smelters or other lead-processing facilities. To the extent that those children may differ in significant ways from the inner-city children shown by de la Burde,[223,224] Perino and Ernhart,[225] and Albert[226] to have neurobehavioral impairments at blood lead levels as low as 40 to 50 μg/dl, ambiguous results from the smelter children are not necessarily contradictory to the better established findings of significant CNS effects for inner-city children. Furthermore, it should be noted that reports of peripheral neuropathies for both populations of children at low to moderate lead exposure levels, as described, may indicate that both groups are at significant risk for at least that type of neural tissue damage.

An additional approach, different from the basic strategy employed in the above studies, has been utilized in other studies in an effort to demonstrate that low or moderate blood lead levels cause significant neurobehavioral deficits. This approach consists of identifying populations of children with diagnosed neurobehavioral deficits of unknown etiology and assaying blood lead or making other assessments in order to link past lead exposures to the children's present neurobehavioral impairments. Thus, for example, efforts have been made to implicate moderate or low level lead exposures as a causative factor in at least some cases of hyperactivity of unknown etiology. The possibility that such low-level lead exposures induce hyperactivity has gained credence through the well documented[87,203] fact that hyperactivity is one of the frequent neurobehavioral sequelae observed in children who survive episodes of acute encephalopathy resulting from high-level lead exposures. The evidence for and against the hypothesis that low-level lead exposures produce hyperactivity has been accumulating at a rapid rate during the past few years and has generated considerable controversy on the subject. Only a few of the more salient findings are viewed below and in the section on animal studies (Section 11.5.2).

In a case-control study, David et al.[236] compared the incidence of elevated blood lead levels in five groups of children: (1) a pure hyperactive group with no apparent cause for hyperactivity; (2) a group of hyperactive children with a highly probable cause of hyperactivity, e.g., prematurity; (3) a group of hyperactive children with a possible cause; (4) a group of children who had recovered from lead poisoning; and (5) a nonhyperactive control group. Pure hyperactive children had statistically significantly higher blood lead levels (mean = 26.2 ± 8 μg/dl) than controls (mean = 22.2 ± 9.6 μg/dl), whereas children with a highly probable cause did not (mean = 22.9 ± 6.6 μg/dl). Similarly, the pure hyperactive children tended to excrete more lead than controls or probable cause hyperactives when given a single dose of penicillamine. Although the causal relationship between lead exposure and hyperactivity cannot be said to be proved by this study, the data of David et al.,[236] when placed with findings of hyperactivity in children known to have recovered from lead poisoning and numerous animal studies demonstrating alterations in motor activity following lead administrations, might be interpreted as supporting the hypothesis that a relationship between moderate lead exposure and altered motor activity exists.

On the other hand, several other points argue against acceptance of such a thesis at this time. For one thing, the David et al.[236] study itself and the

author's conclusions can be questioned on several bases. In that study, for example, the closeness of the match of subjects in the five groups on variables other than age and sex is not clearly specified by the authors. Also, the interpretation of differences in blood lead levels in the 7-to-8-year-old children is fraught with numerous problems, not the least of which is the fact that such levels are probably not very accurate indices of long-past lead exposures that presumably occurred during preschool years. Many factors in the interim between presumed lead exposure and assay for lead could affect the results, including possible differentially higher incidences of pica in the hyperactive children than in control subjects. Klein et al.[237] have noted that pica may be part of certain behavioral syndromes that exist even in the absence of lead exposure, but that would predispose the affected child toward more lead ingestion by virtue of the habit's presence. Indeed, there is evidence that among mentally subnormal children whose mental deficiency can be definitely attributed to etiologies other than lead poisoning that there is both a high incidence of pica and moderately elevated blood lead.[238] Last, it should be noted that a number of other investigators,[211,227,229,230,239] who expressly looked for evidence of lead-induced hyperactivity as part of their screening for neurobehavioral deficits associated with blood lead levels as low as 40 μg/dl, failed to find any significant effects that support the thesis that low-level lead exposures induce hyperactivity. Thus, even though the hypothesis is intriguing and certainly worthy of further investigation, it cannot be stated at this time that sufficient evidence exists to establish hyperactivity as a neurobehavioral deficit clearly associated with low or moderate lead exposures.

In addition to the above data bearing on possible links between subclinical lead exposures and the induction of hyperactivity, certain recently reported data[240] provide evidence implicating increased heavy metal absorption, including lead uptake, in the etiology of learning disabilities. More specifically, children identified for other classification purposes as having learning disabilities were found to have significantly elevated levels of lead, as well as cadmium and some other metals, in their hair when compared with control children not classed as learning disabled. In fact, a discriminant function analysis yielded 98 percent accuracy in classifying children as normal or learning disabled based on a combined factor of cadmium, cobalt, manganese, chromium, and lithium levels. Lead was not included in this five-metal discriminant function, since its predictive value was well served by cobalt and cadmium because of a significant negative correlation between lead and cobalt ($r = .67; p < .01$) and a significant positive correlation between lead and cadmium ($r = + .53; p < .01$). Unfortunately for present purposes, no blood lead levels or possible past exposure histories were provided for the children in the above study.[240]

Other recent studies analogous in basic approach to that employed by David et al.[236] and Pihl and Parkes[240] have provided intriguing new information tending to link prenatal lead exposures to the later development of mental retardation. For example, Beattie et al.[241] identified 77 retarded children and 77 normal children matched on age, sex, and geography. The residence during the gestation of the subject was identified, and a first-flush morning sample of tap water was obtained from the residence. Of 64 matched pairs, no normal children were found to come from homes served with water containing high lead levels (>800 μg/liter), whereas 11 of the 64 retarded children came from homes served with water containing high lead levels. The authors conclude that pregnancy in a home with high lead in the water supply increases by a factor of 1.7 the risk of bearing a retarded child.

In a follow-up to the Beattie study, Moore et al.[242] obtained lead values from blood samples drawn during the second week of life and stored on filter paper. These samples had been obtained as part of a routine phenylketonuria screening study and were kept on file. Blood samples were available for 41 of the retarded and 36 of the normal children in the original study by Beattie. Blood lead concentrations in the retarded children were significantly higher than values measured in normal children. Mean blood lead for retardates was 1.23 ± 0.43 μMol/liter (25.5 ± 8.9 μg/dl) and for normals was 1.0 ± 0.38 μMol/liter (20.9 ± 7.9 μg/dl). The difference in lead concentrations were significant ($p = 0.0189$) by the Mann-Whitney test.

These two studies suggest that lead exposure to the fetus during the critical period of brain development may cause perturbations in brain organization that are expressed later in mental retardation syndromes, and they raise for careful scrutiny the risks of intrauterine exposure to lead. Insufficient information exists, however, to allow estimation of the levels of lead exposure of pregnant women that might cause those prenatal effects in the fetus that may result in later neurobehavioral impairments.

One last adverse effect of lead on neural function in children remains to be considered, and that is the

possible induction of peripheral neuropathies by low-to-moderate lead exposures. It is generally accepted that lead-induced peripheral neuropathies, although frequently seen in adults after prolonged exposures, are extremely rare in children. Several articles[243-245] in the literature, however, do describe case histories that confirm the occurrence of lead-induced peripheral neuropathies, as indexed by electromyography, assessments of nerve conduction velocity, and observations of other overt neurological signs, such as tremor, wrist and foot drop, etc. Some of these frank neuropathic effects have been observed for several cases at blood lead levels of 60 to 80 μg/dl,[245] and in other cases peripheral neuropathy was associated with blood lead values of 30 μg/dl; however, in the latter cases, lead lines in long bones suggest probable past exposures leading to prior blood lead levels at least as high as 40 to 60 μg/dl and probably in excess of 60 μg/dl (based on the data of Betts et al.[101]). In each of the present case studies, there was reported some, if not complete, recovery of affected motor functions after treatment for lead poisoning. Further, it should be noted that a tentative association has been hypothesized between the existence of sickle cell disease and increased risk of peripheral neuropathy as a consequence of childhood lead exposure. Most of the cases reported involved inner-city black children, several with sickle cell trait. In summary, it appears that (1) evidence for frank peripheral neuropathy in children certainly exists; (2) such neuropathy can be associated rather well with blood lead levels at least as low as 60 μg/dl; and (3) evidence suggests that inner-city children with sickle cell disease may be at special risk.

Further evidence for lead-induced peripheral neuropathies in children is provided by the data of Landrigan et al.[231] derived from a study of children living in close proximity to a smelter in Idaho. The nerve conduction velocity results from this study are presented in Figure 11-4 in the form of a scatter diagram relating peroneal nerve conduction velocities (NCV) to blood lead levels in the children studied. No clearly pathologic conduction velocities were observed, although a statistically significant negative correlation was found between peroneal NCV and blood lead levels ($r = 0.38$, $p < .02$ by one-tailed t test). These results, therefore, provide evidence for significant slowing of nerve conduction velocity and, presumably, advancing peripheral neuropathy as a function of increased blood lead levels. The data do not allow for clear statements to be made regarding any threshold for a pathologic slowing of NCV.

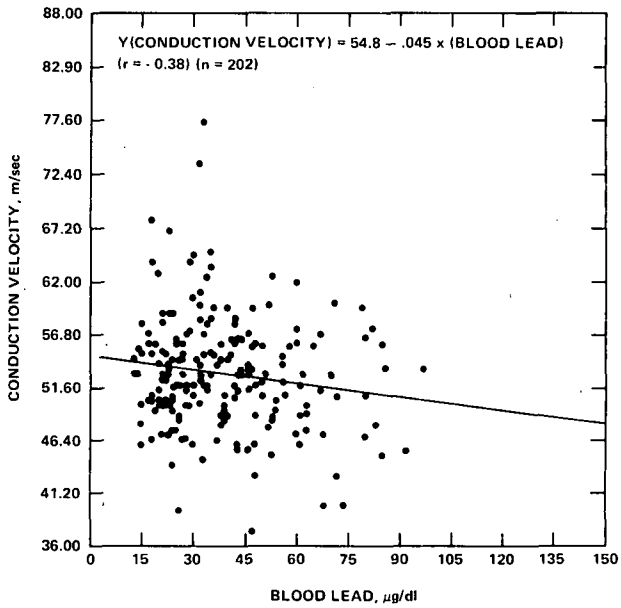

Figure 11-4. Peroneal nerve conduction velocity versus blood lead level, Idaho, 1974.[231]

11.5.1.3 SUMMARY AND CONCLUSIONS FOR HUMAN STUDIES

Rather than simply recapitulating in briefer form the findings reviewed above, an attempt will be made here to integrate information derived from the review and to focus on certain key issues concerning the impact of lead on human neurobehavioral functions. Among the key points to be addressed are: (1) the internal exposure levels, as indexed by blood lead levels, at which various adverse neurobehavioral effects occur; (2) the reversibility of such deleterious effects; and (3) the population(s) that appear to be most susceptible to neural damage.

Regarding the first issue, it would appear from data reviewed above that surprisingly low levels of blood lead can, at times, be associated with the most extreme effects of lead poisoning, including severe, irreversible brain damage as indexed by the occurrence of acute or chronic encephalopathy symptoms or death or both. For most adults, such damage does not occur until blood lead levels well in excess of 120 μg/dl are reached. Evidence does exist, however, for the occurrence of acute encephalopathy and death in some adults at blood lead levels somewhat below 120 μg/dl. For children, the effective blood lead levels for producing encephalopathy or death are lower, typically starting at approximately 100 μg/dl. Again, however, good evidence exists for the occurrence of encephalopathy in some at lower levels, i.e., 80 to 100 μg/dl.

It should be emphasized that once encephalopathy occurs death is not at all an improbable outcome, regardless of the quality of medical treatment available at the time of any acute crisis. In fact, certain diagnostic or treatment procedures themselves tend to exacerbate matters and push the outcome toward fatality if the nature and severity of the problem are not fully recognized or are misdiagnosed. It is also crucial to note the rapidity with which acute encephalopathy symptoms or death can develop in apparently asymptomatic individuals or in those only apparently mildly affected by elevated body burdens of lead. It is not unusual for rapid deterioration to occur, with convulsions or coma suddenly appearing and progressing to death within 48 hr. This strongly suggests that even in apparently asymptomatic individuals rather severe neural damage probably does exist at high blood lead levels even though it is not yet overtly manifested in obvious encephalopathic symptoms. This tends to be borne out by studies showing that children having high blood lead levels (over 80 to 100 μg/dl), but not observed to manifest acute encephalopathy symptoms, are permanently, cognitively impaired, as are individuals who survive acute episodes of lead encephalopathy.

Other evidence tends to confirm that some type of neural damage does exist in asymptomatic children, and not necessarily only at very high levels of blood lead. The body of studies on low- or moderate-level lead effects on neurobehavioral functions, as summarized in Table 11-4, present overall a rather impressive array of data pointing to that conclusion. Several well-controlled studies have found effects that are clearly statistically significant, whereas others have found nonsignificant but borderline effects. Even some studies reporting generally nonsignificant findings at times contain data confirming statistically significant effects, which the authors attribute to various extraneous factors. Another way to look at the situation is to consider that elevated blood lead level is the single common factor extant in all of the groups showing significant behavioral deficits in the different studies. It should also be noted that, given the likely subtle nature of some of the behavioral or neural effects probable at low levels of lead exposure, one would not expect to find striking differences in every instance. The blood lead levels associated with neurobehavioral deficits in asymptomatic children appear to be in excess of 50 to 60 μg/dl. Great uncertainties remain, however, as to whether these exposure levels (observed blood lead levels) represent the levels that were responsible for the behavioral deficits observed. Monitoring of lead exposures in the subjects has in all cases been highly intermittent during the period of life preceding the behavioral assessment. In most cases, only one or two blood lead values are provided per subject.

11.5.2 **Animal Studies**

Pentschew and Garro[52] initially described an animal model of lead encephalopathy in which morphological changes occurred similar to those reported in children. Neonatal rats were exposed to lead by feeding mothers a diet containing 4 percent lead carbonate. The lead was then transmitted to the suckling young via the mothers' milk. Between 23 and 29 days of age, 90 percent of the animals developed paraplegia lasting no longer than 2 weeks. Eighty-five to 90 percent of the paraplegic animals died during this period.

Neuropathological examination of these animals revealed capillary activation, glial proliferation, areas of transudation, and spotty hemorrhages, primarily in the cerebellum and the striatum. The white matter of the cerebral hemispheres, especially the corpus collosum, was also involved, though to a lesser extent. Ischemic neuronal changes in focal distribution were rare and were localized in the cerebral cortex. Pentschew and Garro[52] concluded that the lead encephalopathy of the suckling rat was caused by a disorder in the permeability of the capillaries, resulting in dysoric encephalopathy. The suckling rat therefore differs from the human in that the latter shows a mixture of both dysoric and hemodynamic alterations. Further verification of the dysoric nature of lead encephalopathy in the suckling rat was provided by Lampert et al.,[53] who used either colloidal thorium dioxide (Thorotrast) or Trypan Blue, neither of which normally penetrates the blood-brain barrier. In suckling rats poisoned with 4 percent lead carbonate, however, both Thorotrast and Trypan Blue were found to penetrate the striatum and cerebellum.

Since the initial description by Pentschew and Garro,[52] lead encephalopathy in the rat has been replicated by Thomas et al.[246] Michaelson and Sauerhoff,[247] and Krigman and Hogan.[248]

Clasen et al.[249] reported lead encephalopathy in juvenile Rhesus monkeys exposed to 0.5 g of lead per day for 6 to 18 weeks. They reported morphological changes similar to those occurring in humans, which consisted of edematous changes in the cerebellar and subcortical white matter. Diffuse

TABLE 11-4. SUMMARY OF RESULTS OF HUMAN STUDIES ON NEUROBEHAVIORAL EFFECTS AT MODERATE BLOOD LEAD LEVELS

Reference	Population studied	N/group	Age at testing, yr	Blood lead, μg/dl	Psychometric tests employed	Summary of results (C= control; Pb= lead)[a]	Levels of significance[b]
De la Burde and Choate (1972)[223]	Inner city (Richmond, VA)	Control= 72 Lead= 70	4 4	Not assayed[c] 40-100[d]	Stanford-Binet IQ Other measures	C= 94 Pb= 89 C > Pb on 3/4 tests	$p < .05$ N.S.-$P < .01$
De la Burde and Choate (1974)[224]	Follow-up same subjects	Control= 67 Lead= 70	7 7	See above[e] See above[e]	WISC Full Scale IQ Neurologic exam Other measures	C= 90 Pb= 87 C better than Pb C > Pb on 9/10 tests	$p < .01$ $p < .01$ N.S.-$p < .001$
Perino and Ernhart (1974)[225]	Inner city (New York, NY)	Control= 50 Lead= 30	3-6 3-6	10-30 40-70	McCarthy General Cognitive McCarthy Subscales	C= 90 Pb= 80 C > Pb on 5/5 scales	$p < .01$ N.S.-$p < .01$
Pueschel et al. (1972)[99]	Inner city (Boston, MA)	Control= 56 Lead= 56	? ?	<40 >40	Stanford-Binet IQ Neurologic exam	C= ? Pb= 86 C better than Pb	— $p < .001$
Landrigan et al. (1975)[227]	Smelter area (El Paso, TX)	Control= 46 Lead= 78	3-15 ($\bar{x}$= 9.3) 3-15 ($\bar{x}$= 8.3)	<40 40-68	WISC Full Scale IQ[f] WPPSI Full Scale IQ[g] WISC + WPPSI Combined WISC + WPPI Subscales Neurologic testing	C= 93 Pb= 87 C= 91 Pb= 86 C= 93 Pb= 88 C > Pb on 13/14 scales C > Pb on 4/4 tests	N.S. N.S. $p < .01$ N.S.-$p < .01$ N.S.-$p < .001$
Rummo (1974)[211]	Inner city (Providence, RI)	Control Ss= 45 Short Pb Ss= 15 Long Pb Ss= 20 Post enceph Pb= 10	4-8 ($\bar{x}$= 5.8) 4-8 ($\bar{x}$= 5.6) 4-8 ($\bar{x}$ = 5.6) 4-8 ($\bar{x}$= 5.3)	$\bar{x}$= 23± 8 $\bar{x}$= 61± 7 $\bar{x}$= 68± 13 $\bar{x}$= 88± 40	McCarthy General Cognitive McCarthy Subscales Neurologic exam rating Objective neurologic tests	C= 93; S= 94; L= 88; P= 77 C+ S > L > P on 5/5 tests C+ S L > P on ratings C+ S > L > P on 3/12 tests	N.S.-$p < .01$ (p vs C) N.S.-$p < .01$ (p vs C) N.S. N.S.-$p < .01$ (p vs C)
Kotok et al. (1977)[232]	Inner city (Rochester, NY)	Control= 36 Lead= 31	1.9-5.6 ($\bar{x}$= 3.6) 1.7-5.4 ($\bar{x}$= 3.6)	11-40 61-200	IQE for six ability classes: Social maturity; Spatial relations; Spoken vocab; Info. comprehension; Visual attention; Auditory memory	IQ Equivalent for each: C= 126 Pb= 124 C= 101 Pb= 92; C= 93 Pb= 92; C= 96 Pb= 95; C= 93 Pb= 90 C= 100 Pb= 93	$p < .10$ for spatial $p > .10$ for all other ability classes
Kotok (1972)[228]	Inner city (New Haven, CT)	Control= 25 Lead= 24	1.1-5.5 ($\bar{x}$= 2.7) 1.0-5.8 ($\bar{x}$= 2.8)	20-55 58-137	Denver Developmental Scale	C > Pb on 1/3 Subscales	N.S.
Lansdown et al. (1974)[229]	Inner city (London, England)	Control= 172 Lead= 43	6-16 6-16	<40 40-60+	WISC WAIS	C= 100 Pb= 101-105	N.S.
McNeil et al. (1975)[230]	Smelter area (El Paso, TX)	Control= 61-152 Lead= 23-161	1.5-1.8(Mdn= 9) 1.5-1.8(Mdn= 9)	<40 >40	McCarthy General Cognitive WISC-WAIS Full Scale IQ Oseretsky Motor Level California Personality Frostig Perceptual Quotient Finger-Thumb Apposition	C= 82 Pb= 81 C= 89 Pb= 87 C= 101 Pb= 97 C= 80 Pb= 72 C= 100 Pb= 103 C= 27 Pb= 29	N.S. N.S. N.S. N.S. N.S. N.S.

[a] Mean test scores obtained for control children are indicated by C = $\bar{x}$; mean scores for respective lead-exposed groups are indicated by Pb = $\bar{x}$ (except for Rummo[211] study where C = control, S = short-term lead-exposed subjects; L = long term lead-exposed group and P = post-encephalopathy lead group).

[b] N.S. = non-significant, i.e. $p > .05$. Note exception of $p < .05$. Note exception of $p < .10$ listed for spatial ability results in Kotok et al.[232] study.

[c] Urinary coproporphyrin levels were not elated.

[d] Or 30 μg/dl or above with positive radiologic findings. The latter suggest earlier exposure in excess of 50-60 μg/dl.

[e] Assays for lead in teeth showed the Pb-exposed group to be approximately twice as high as controls (202 μg/g vs. 112 μg/g, respectively).

[f] Used for children over 5 years of age.

[g] Used for children under 5 years of age.

astrocytosis and glial nodules were found in white matter along with perivascular exudate.

Lead encephalopathy in the mouse was first described by Rosenblum and Johnson[250] who, like Pentschew and Garro, used the suckling animal. In contrast to the rat brain, the striatum and cerebellum of mice fed either 0.5 or 1 percent lead carbonate displayed only faint staining with Trypan Blue (except for a single animal with darkly stained cerebellum) and only occasional paraplegia. The most striking vascular change in the poisoned mice was the appearance of intervascular strands throughout the brain, especially in the hippocampus and basal ganglia. Rosenblum and Johnson[250] concluded that no cerebral edema and focal destructive lesions were found in the mouse brains. Therefore, the histological response of the suckling mouse exposed to lead differs from that seen in the suckling rat. These differences are probably not attributable to different exposure levels (4 percent carbonate in the rat versus 1 percent in the mouse), because the food consumption per unit body weight is three to four times greater in the mouse than in the rat, making the external exposures roughly equivalent.

Wells et al.[251] have recently reported on experimental lead encephalopathy in calves. Four Jersey bull calves were exposed to 20 mg/kg/day of lead acetate, beginning at 3 months of age and lasting for 8 to 273 days. Histological evaluation of the brains of exposed animals revealed focal vacuolation of the neuropil, neuronal necrosis, and changes in the capillary walls in the cerebral cortex and in subcortical areas. Lesions in these cattle were similar to those seen in children with lead encephalopathy in that both show not only vascular changes in the brain with edema but also areas of neuronal necrosis.

In summary, the histopathological changes associated with lead encephalopathy vary among species and are characterized by their relative involvement of neuronal degeneration and vasculopathy. The fact that lead encephalopathy is reported to occur in the absence of cerebral edema in man,[198] mouse,[250] and guinea pig[252] is argument for a direct neuronal involvement of lead. Recent studies have, in fact, suggested direct neuronal alterations by lead. Bull et al.[253] reported that lead interferes with potassium-stimulated respiration of rat cerebral cortex slices, and Nathanson et al.[17] reported that lead inhibited brain adenyl cyclase *in vitro*. No distinction can be made in these studies, however, between neuronal and other cell types. Thus, a direct biochemical effect of lead on the neuron cannot be definitively concluded from these studies.

The relative involvement of the capillary bed in the lead encephalopathy may depend on the degree of maturation at the time of exposure. Bouldin et al.[252] for example, were able to produce lead encephalopathy in adult guinea pigs with no cerebral edema or increased capillary permeability, whereas the suckling rat shows these effects almost exclusively.

Since the initial description of lead encephalopathy in the rat by Pentschew and Garro,[52] considerable effort has been made to define more closely the extent of CNS involvement at subencephalopathic levels of lead exposure. This experimental effort has focused almost exclusively on the developing organism. The interpretation of a large number of experiments dealing with early exposure to lead have, however, been confounded by a number of flaws in experimental design.

Perhaps the most notable of these experimental shortcomings has been the presence of undernutrition in experimental animals. Changes in nutritional status during early brain development are known to produce changes in behavior.[254,255] Castellano and Oliverio,[256] for example, reported a marked delay in neurological development, an increase in exploratory locomotor activity, and a lowered avoidance performance in mice that were undernourished during early development by being reared in large litters. Neurochemical processes have also been shown to be affected by early undernutrition. Eckhert et al.[257] reported changes in cholinergic enzyme activities when rats were placed on protein-deficient diets during various periods of development. Their results indicate that "the relationship between the activity of individual cholinergic enzymes, nutritional status and developmental age is complex and is not the same for different brain regions or even the same brain region exposed to undernutrition during different periods of development." Therefore, in reviewing the animal literature concerned with the neurotoxicology of lead exposure, the possible contribution of undernutrition must be considered. Furthermore, the possibility also exists that lead and undernutrition may be having a synergistic effect on nervous system development. In this case, the methods of pair-feeding currently employed in some studies[247,258] may not provide adequate control for this undernutrition. Examples of dietary factors known to affect susceptibility of lead toxicity have been reviewed by Goyer and Mahaffey.[259]

Animals fed diets containing lower than recom-

mended concentrations of nutrients generally retain higher concentrations of lead in tissues than animals on normal diets.[259] However, almost nothing is known about the effects of elevating nutrient intake above recommended levels — which is the case with most commercial laboratory chows — on the toxicity of lead. Additional research is needed in this area, and until further data are available on the influence of varying degrees of overnutrition, variation in nutrient intake must be suspected of altering the toxicity of lead.

11.5.2.1 DEVELOPMENT

Several laboratories have reported on the effects of perinatal lead exposure on physical, reproductive, and neurological development.[247,250,260,261] Unfortunately, in a number of studies either no control was provided for undernutrition,[250,260,262] or undernourished pair-fed controls showed similar developmental delays,[247] thus masking any direct effects of lead.

Maker et al.[263] also examined the effects of lead exposure on brain development in mice. Two methods were used in an attempt to control for the effects of early undernutrition. In the first experiment, two pair-fed litters were used. Pair-feeding was accomplished by allowing a litter access to an amount of normal chow equivalent to that consumed by a litter on a 0.8 percent lead diet. Both pair-fed litters showed a reduction in brain weight at 30 days of age, as did the lead-treated animals. The two pair-fed litters, however, differed in the relative reduction in brain weight, one showing an identical response to the lead-treated groups and one showing brain weights intermediate between controls and lead-treated animals. A second attempt to alter the nutritional state of the animals was accomplished by altering litter sizes (3 pups versus 6 pups). Body weights of control animals of the two litter size groups were equivalent, however, so that varying the litter size over this range did not effectively produce undernutrition. Therefore, the conclusion of Maker et al.[263] that underconsumption of food alone does not account for the slow development of litters on a lead diet is not supported by their data.

Reiter et al.[264] examined development in rats exposed to lead both prenatally and during lactation via the mothers' milk. They reported a delay in both the age at eye opening and the age at development of the air righting reflex in the 50 ppm treatment group. This exposure level was shown to produce no depression in growth, which suggested a direct effect of lead on nervous system development. No difference in the development of the acoustic startle response was observed. Kimmel et al.,[265] using a similar experimental design, also reported delays in both surface and air righting in rats exposed to 50 or 250 ppm lead; and no differences were found in either auditory startle, pinna detachment, eye opening, ear opening, or incisor eruption.

Sexual maturation appears to be one aspect of development that is quite sensitive to disruption by lead exposure. Kimmel et al.[265] reported a dose-related delay in vaginal opening in female rats exposed to 25, 50, or 250 ppm lead acetate in the drinking water starting at conception. In the group exposed to 25 ppm lead, no differences in growth rates were observed. This suggests a direct effect of lead on sexual maturation rather than a change secondary to body weight changes.

Der et al.[266] reported on the combined effect of parenteral administration of lead acetate and low protein diet (100 μg subcutaneously daily, ages 20 to 61 days) on sexual development in the Sesco rat. Lead significantly delayed the age at which vaginal opening occurred in animals on the control diet. Females given lead in combination with low protein diets did not exhibit vaginal opening through 61 days of age. The authors interpret these data on the basis of a lead/protein-deficiency interaction. However, since animals were given 100 μg of lead per day regardless of body weights and since their body weights were at 26 percent of control, the dosage of lead per unit body weight was 400 percent greater than lead-treated animals on a control diet, which may account for the additional delay in maturation.

Gray and Reiter[261] studied the effects of lead administration (5 mg/ml in the drinking water at parturition) on sexual maturation in the mouse. Vaginal opening was delayed about 4 days in lead-treated animals. No delay in development was seen in pair-fed controls, further suggesting a primary effect of lead on sexual maturation. Furthermore, no delay in sexual maturation was observed in animals when lead was discontinued at weaning. Therefore, the presence of lead at the time of maturation appears essential for the lead-induced delay and may be related to its effects on circulating hormones at the time of puberty.

11.5.2.2 LOCOMOTOR ACTIVITY

In the animal model, the most commonly employed behavioral index of lead toxicity has been locomotor activity. As with other behavior, locomotor activity is influenced by a variety of factors that

include sex, age, time and duration of testing, type of measurement, etc. The relative influence of these factors on the observed activity will vary with the experimental method employed. The endpoint being measured is activity (not necessarily ambulation), and the nervous-system processes responsible for this activity and their relative contributions may be different. Tapp,[267] for example, compared seven different measures of activity in the rat and found virtually no intercorrelation. These results suggest that the tests he employed were not measuring the same behavior. Capobianco and Hamilton[268] examined the effects of various brain lesions on ambulation as measured by three different methods: open-field, stabilimeter, and activity wheels. These different measures of activity were affected differently by a given brain lesion. Lesions of the diagonal band, for example, produced increased activity in the running wheel, decreased activity in a stabilimeter, and no change in activity in an open field.

Not only is the type of activity-measuring device important, but also of importance is the length of time over which the activity is measured. Short-term measurements of activity in a novel environment have been termed exploratory activity or locomotor reactivity and primarily reflect the animal's reaction to the novel environment. This reactivity in turn will be affected by the structure of the environment. In order to determine spontaneous or basal activity levels, an animal must reside in an environment over long periods of time. Once the animal is established in the environment, the activity levels of the animal, especially the rodent, will be highly dependent on the time of day.

Therefore, in reviewing the lead literature, it must be remembered that locomotor activity measurements do not represent a unitary behavior; careful consideration must be given to the particular experimental methods employed. In addition, attempts to extrapolate the results of an activity measurement in animals directly to the clinical situation are unwarranted. A brief summary of pertinent studies is given in Table 11-5.

TABLE 11-5. EFFECTS OF LEAD EXPOSURE ON LOCOMOTOR ACTIVITY IN LABORATORY ANIMALS

Reference	Species	Exposure conditions	Lead concentration, μg %		Test conditions, in order of presentation: 1. Method 2. Length of testing 3. Group size 4. Age	Nutritional status	Results
			Blood	Brain			
Allen et al.[269]	Monkey	0.5-9 mg/kg/day for 12 weeks	160-400		Observed locomotor activity	Normal	Hyperactivity (qualitative measures)
Bornschein et al.[270]	Mouse, (Charles River) CD-1	5 mg/ml lead acetate in drinking water, starting at parturition	120-190	200-306	Proximity counter 3 hours Individual 35 days	Undernourished	Normal
Brown[271]	Rat (Bar F - Rabbitry)	35 mg/kg P.O. to dams from parturition to 21 days			Photoactometer 20 min Individual 49 days		Normal
Driscoll and Stegner[272]	Rat (Simonsen)	10^{-4} and 10^{-2} M lead acetate in drinking water from conception			Open-field 2 min Individual 31 days	Undernourished	Hypoactive (61% of control)
Gray and Reiter[261]	Mouse, (Charles River) CD-1	5 mg/ml lead acetate in drinking water, starting at parturition			Residential maze 90-240 min Individual 30, 50, 130 days	Undernourished	Hypoactive (75-80% of control)
Hastings et al.[273]	Rat, (Long-Evans, Charles River)	0.2 or 1.0 mg/ml from parturition to 21 days	Control = 11± 4 0.2 mg/ml = 29± 5 1.0 mg/ml = 42± 4		Running wheel 3 weeks Individual 30-51 days	Normal	Normal
Kostas et al.[274]	Rat (Long-Evans) Blue Spruce	0.05-5.0% lead acetate in mother's chow from parturition until 21 days. Continued in offspring at 0.25-25 ppm in chow until 35 days			Shuttle box, activity wheel 1 hr Individual 75-77 days; 90-93 days	Normal - 0.05% group undernourished in 5-5.0%	Hyperactivity in shuttle box (182% of control). Normal in activity wheel.

(continued)

TABLE 11-5 (continued).

Reference	Species	Exposure conditions	Lead concentration, μg % Blood	Brain	Test conditions, in order of presentation: 1. Method 2. Length of testing 3. Group size 4. Age	Nutritional status	Results
Overman[275]	Rat, (Long-Evans Charles River)	10, 30, 90/mg/kg/day by intubation from 3-21 days of age	21 days 90 mg/kg = 226± 21 35 days 90 mg/kg = 56± 5		Jiggle platform Four days Individual 22-65	Normal	Hyperactive
Reiter[258] Reiter et al.[276]	Rat, Sprague Dawley, Charles River	5% lead carbonate in mother's chow from parturition to 16 days, 50 ppm in drinking water for remainder of experiment			Jiggle cage; Residential maze 4 min; 14 days Individual; group of 3 13-44 days; 120-160 days	Undernourished	Transient hyperactivity (200% of control at 13 days). Normal at 44 days. Normal levels in adults; but disrupted ultradian rhythms.
Reiter et al.[264] Cahill et al.[277]	Rat, Sprague Dawley, Blue Spruce	5,50 ppm in drinking water. 40 day pretreatment of parents. Continued from conception through adulthood	180 day males 0= 5± 0.5 5= 6± 0.4 50= 10± 0.6	180 day males 0= 18± 1 50= 20± 2 50= 27± 2	Residential maze 5 days Groups of 3 120 days	Normal	Hypoactivity (53-78% of controls)
Reiter et al.[264]	Mouse, Charles River, CD-1	5 mg/ml lead acetate in drinking water from parturition until 45 days			Residential maze 2 days Individual 100 days	Undernourished	Normal
Sauerhoff and Michaelson[278] Sauerhoff[279]	Rat, Sprague Dawley, CO	4% lead carbonate in mothers' chow from parturition to 16 days post-partum, 40 ppm in drinking water		29 days 88± 1.1	Selective activity meter 24 hours Groups of 6 26-29, 50	Undernourished	Hyperactive at 29 days (140-190% of control) Normal at 50 days
Silbergeld and Goldberg[262,280,281]	Mouse, Charles River, CD-1	2, 5, 10 mg/ml lead acetate in drinking water starting at parturition			Proximity counter 3 hours Individual 30-150 days	Undernourished	Hyperactivity (300-400% of control)
Sobotka and Cook[282] and Sobotka et al.[283]	Rat, Sprague Dawley (Charles River)	8-91 mg/kg/day by intubation from 3-21 days of age	22 days 81 mg/kg = 71± 12 35 days 81 mg/kg = 23± 1.4		Photoactometer 30 min Individual 24-28 days	Normal	Normal
Sobotka et al.[284]	Dog (Beagle)	1 or 4 mg/kg/day orally from 2 weeks to 5 months			Open field — Individual 3-4 months	Not indicated	Hypoactive
Winneke et al.[285]	Rat (Wistar)	1.38 g lead acetate/kg of chow (745 ppm Pb) for 60 days pretreatment to mothers. Continued in offspring from conception to testing	16 days 26.6 190 days 28.5		Open field 3 min, on consecutive days Individual 90-140 days	Normal	Hyperactive (129% of control)
Zenick et al.[286]	Rat, Sprague	750 or 1000 mg/kg/day to females on restricted watering schedule from 21-99 days of age. Exposure continued through gestation and weaning.			Open field 3 min, on 10 consecutive days Individual 22-32 days	Undernourished	Initial hypoactivity in 100 mg/kg group (days 1 and 2) Hyperactive by the 7th test day.

The data to be reviewed here suggest that perinatal lead exposure produces an altered reactivity of an animal to a novel environment. Reactivity is increased in the young animal, but this increased reactivity disappears as the animal matures. In the adult animal, on the other hand, the lead exposure results in a reduced reactivity. As will be seen, the exact nature of the change in locomotor activity brought about by this altered responsiveness will depend heavily on the structure of the test environment.

Sauerhoff and Michaelson[278] and Sauerhoff[279] exposed lactating females to lead using a modification of the Pentschew and Garro[52] exposure regimen. Litter mates were tested as a group at 25 to 28 days of age in a test case similar to the home cage. The data were collected in four blocks of 3 hr and one block of 12 hr, extended over 4 days. Although they are presented as counts per hour for a 24-hr period, data represent reactivity attributable to multiple short-term exposures to a novel environment, and, therefore, are not comparable with data obtained by continuous sampling over a 24-hr period. Offspring of a lead-exposed mother exhibited elevated activity levels. Since only one group (n = 6 pups) was tested from each treatment, however, no statistical test can be applied to these data.

Using a similar exposure regimen, Reiter[258] and Reiter et al.[276] exposed animals to 5 percent lead carbonate in the chow starting at parturition. Animals were repeatedly tested at various ages, beginning at 13 days of age, using a 4-min jiggle platform activity measurement. This measuring device detects both locomotor activity and stationary body movements. They reported an increased activity (200 percent of control) in 13-day-old animals. This elevated activity declined with age and returned to control levels by 44 days of age. Comparisons were made with pair-fed controls since this exposure resulted in a significant growth impairment. Whether this return to normal levels was caused by maturation or by repeated testing was not determined in this study. Animals were also tested as adults (120 days) in a residential maze, which allows continuous measurement of activity over extended periods of time; this test showed no differences in the activity levels as a result of treatment. However, the ultradian rhythms of activity seen in control animals during the nocturnal period (short-term, 4/hr oscillation in activity) were absent in lead-exposed animals.

Overmann[275] used a similar jiggle platform to measure activity in 22- to 65-day-old rats. As in the Sauerhoff and Michaelson study,[278] animals were rotated between testing cages, and, therefore, the observed increase in activity was consistent with a lead-induced change in locomotor reactivity. In this experiment, pups were directly exposed by daily intubation ranging from 10 to 90 mg/kg/day. This exposure is similar to that used by Sobotka and Cook,[282] who employed intubation levels of 9 to 81 mg/kg/day, but who reported no differences in activity in a photoactometer.

One striking difference between these two experiments was in the reported blood lead levels, shown in Table 11-6. Therefore, the higher internal exposure seen in Overmann's experiment may have accounted for the difference in the observed behavioral effects, although differences in experimental protocol may also have accounted for differences in the behavioral effects.

TABLE 11-6. LEAD EXPOSURE AND RESULTING BLOOD LEAD LEVELS IN EXPERIMENTS MEASURING LOCOMOTOR ACTIVITY IN RATS

Experiment	Blood lead, μg/dl	
	21 to 22 days	35 days
Sobotka and Cook [282] and Sobotka et al.[283] (81 mg/kg/day)	71 ± 12.4	23 ± 1.4
Overmann[275] (90 mg/kg/day)	226.1 ± 21.1	56 ± 4.6

Two different laboratories have reported on the effects of lead administration on open-field activity in 30- to 40-day-old rats. Driscoll and Stegner[272] reported a decreased activity in animals tested for 2 min. Zenick et al.[286] tested animals for 3 min in an open field on 10 consecutive days. On the first 2 days of testing, animals from the high exposure group (1000 mg/kg/day in the drinking water of the mother) showed a decreased activity similar to that reported by Driscoll and Stegner.[272] By the seventh day of testing, however, these animals were ambulating at a higher level than controls. These data can also be interpreted as an increased locomotor reactivity in lead-treated animals that is initially manifested in an open field as decreased ambulation. With repeated exposure, the animals show an elevated activity similar to that in previously reported studies. The difference in the direction of lead-induced change in initial activity levels in these open field experiments as compared to the jiggle platform experiments[258,275] is probably a result of the differences in the size of the test environments. The larger open field results in decreased activity in lead-exposed hyperreactive animals, whereas in the

smaller jiggle platform the animals show increased activity.

Finally, Hastings et al.[273] reported no differences in running-wheel activity of 30-day-old animals exposed to lead by suckling with mothers receiving 1 mg/ml lead in the drinking water. This exposure resulted in blood lead values of 42 μg/dl at weaning. Since animals do not normally show much running activity upon initial exposure to the running wheel, the effects of this lead exposure on reactivity cannot be adequately tested. These results do demonstrate that long-term running wheel activity (3 weeks) was not disrupted in these young, lead-exposed animals.

At or about the time of sexual maturation, lead exposure has not been shown to alter activity levels in the rat. As previously indicated, Sauerhoff and Michaelson[278] and Reiter et al.[276] found no differences in activity in animals tested between 44 and 50 days of age. These were animals which were reported to have increased activity at a younger age. Brown[271] also reported no differences in locomotor activity in animals tested at 49 days of age either in an photoactometer or in an open field.

Available data also suggest that perinatal exposure to lead may produce a decreased reactivity in the adult animals. Kostas et al.[274] measured locomotor activity in adult rats exposed to either 0.05, 0.5, or 5.0 percent lead carbonate in the chow from parturition until weaning and to 0.25, 2.5, or 25 ppm from weaning until 35 days of age. Two measurements of activity were employed. Animals tested for 1 hr in a shuttle box activity cage were found to have increased activity, whereas animals tested in running wheels showed no difference from controls. This lack of sensitivity of the running wheel to lead-induced changes in activity is consistent with the finding of Hastings et al.[273] Again, the nature of the change in shuttle box activity may be interpreted in terms of a lead-induced decrease in reactivity toward a novel environment, since rats made more reactive would tend to freeze in this environment, thus causing decreased ambulation. Winneke et al.[285] found a similar change in reactivity as indicated by open-field activity scores over 5 successive days of testing. Lead-treated rats had significantly elevated activity on the first 3 days of testing, and activity had returned to normal on days 4 and 5.

Reiter et al.[264] reported a lead-induced decrease in activity in adult animals tested in a residential maze. This test system allowed for measurement of various components of the animals' activity, including exploratory, diurnal, and nocturnal activity. Exploratory activity was initially suppressed in lead-treated animals, but this difference disappeared as the animals became established in the environment. On the other hand, activity levels remained suppressed during the nocturnal period.

In a second study, Reiter et al.[276] reported no difference in residential-maze activity of adult lead-treated animals. They speculated that the lack of a lead effect on locomotor activity in the second experiment may have been the result of the choice of animal supplier (Charles River). Since the Charles River animals were normally less active in the maze, it may have been difficult to lower their activity further with treatment. Differences in experimental protocol, i.e., differences in dose and period of exposure, however, also may have accounted for the differences in observed activity.

In summary, data on the rat suggest that perinatal exposure to lead may produce an increased reactivity which disappears as the animal matures. This effect could result from a delay in normal maturation of forebrain inhibitory systems.[287] As the lead-treated animals mature, they pass through a period of normal reactivity which then progresses to a decreased reactivity in the adult animal. These data would be consistent with a maturational lag seen in children[288] and pose an interesting hypothesis that requires further testing.

Sobotka et al.[284] have reported decreased ambulation in young dogs exposed to lead and tested in a 7- by 7-ft open field. Allen et al.[269] exposed infant monkeys to lead via their formula and reported hyperactivity from 3 to 5 months of age. These data and the data of Sobotka et al.[284] are consistent with the lead-induced increase in reactivity seen in the young rat. The data of Allen et al.[269] must be qualified, however, since no quantitative measure of activity was made.

In a series of often cited papers, Silbergeld and Goldberg[262,280,281] reported lead-induced hyperactivity in mice. Lactating females were exposed to lead acetate in the drinking water, starting at parturition, in concentrations of either 2, 5, or 10 mg/ml. The authors reported a 300- to 400-percent increase in locomotor activity that extended from 30 to 150 days of age in the offspring. The lack of a control for the growth retardation found in the lead-treated groups makes interpretation of these data difficult. As previously indicated, Castellano and Oliverio[256] reported that early undernutrition produces hyperactivity in mice. Therefore, the observed hyperactivity may have resulted from the undernutrition, independent of a lead effect. Greater concern, however, stems from the subsequent failure of

two different laboratories[261,270,289] to replicate the findings of Silbergeld and Goldberg, using the same strain of mouse and the same exposure regimen.

Bornschein et al.[270] exposed lactating mice to 5 mg/ml lead acetate and tested offspring in activity chambers identical to those employed by Silbergeld and Goldberg.[262,280,281] They were unable to verify lead-induced hyperactivity in mice even though undernutrition was also present in their animals.

Gray and Reiter[261] were also unable to demonstrate hyperactivity in lead-exposed mice, using a residential maze to measure activity. Furthermore, Reiter et al.[289] were unable to find differences in the activity of mice experimentally exposed to lead from birth to 45 days in Goldberg's laboratory and then transported to the author's laboratory for behavioral testing.

Silbergeld and Goldberg[280,281] also studied the locomotor response of lead-exposed mice to various drugs that are used in the treatment and diagnosis of minimal brain dysfunction in children. Most notably, they reported that their lead-treated, hyperactive mice responded paradoxically to the stimulants amphetamine and methylphenidate. Examination of the activity data following administration of methylphenidate[281] raises questions as to the exact nature of the paradoxical response. Both control and lead-treated animals responded with increased locomotor activity following 40 mg/kg of methylphenidate; however, the response of the lead-treated mice was markedly attenuated. Within 90 to 120 min, animals were below their predrug level. This time course in the response would be expected if the lead-treated animals were entering into stereotypic behavior (a behavioral pattern characteristically seen following high doses of amphetamine-like compounds).[290] Although the authors state that no stereotypic behavior occurred in lead-treated animals, no quantitation of this behavior was made. Furthermore, this stereotypic behavior has been observed in lead-treated mice by other investigators.[270] Again, the possible contribution of early undernutrition to these results must be considered. Bornschein et al.[270] found this paradoxical lowering of activity in undernourished mice following 10 mg/kg of d-amphetamine, but only during the second hour following drug administration. In the first hour, activity showed the expected increase, although to a lesser extent than in controls. These authors postulated that early undernutrition shifts the dose-response curve to the left such that animals given high levels of amphetamine (10 mg/kg) enter into stereotyped behavior sooner than controls, which prevents the occurrence and the recording of locomotor activity.

Reiter[258] examined the dose-response relationship to amphetamine in control, undernourished, and lead-exposed rats. He found, as did Bornschein et al.,[270] that undernutrition shifted the dose-response curve to the left. Lead treatment, on the other hand, shifted the dose-response curve to the right. Thus, under the appropriate conditions, lead exposure per se can be shown to produce an attenuated response to amphetamine. The occurrence of a true paradoxical response, however, is questionable.

The lead-induced attenuated response to amphetamine has been observed regardless of whether the reported predrug activity levels were elevated,[280,281] normal,[258,282] or depressed.[264] The determination of the exact nature of this altered response, i.e., altered CNS sensitivity versus altered absorption, distribution, and metabolism, requires further study.

11.5.2.3 LEARNING ABILITY

There is little doubt that acute, high-level lead exposure in young children can produce overt manifestations of neurotoxicity.[182,203] Mental retardation is an established sequela of lead-induced encephalopathy in children.[85,86] The extent and nature of lead-induced neurotoxicity following long-term low-level lead exposure during the developmental years is also of continuing interest. As indicated previously, restrospective studies in children are generally equivocal and are compromised by serious experimental design limitations, e.g., unsatisfactory documentation of lead-exposure history prior to behavioral testing and inappropriate or unsatisfactory control groups.[291]

In an attempt to overcome some of these limitations, investigators have turned to animal models of chronic, low-level exposure. The major portion of this work has been carried out in rats. Table 11-7 provides a summary of the pertinent studies, including exposure conditions, testing conditions, and results. In an attempt to structure this literature and facilitate evaluation, the organization shown in Table 11-8 has also been developed. It separates the data along two lines: (1) tasks that reportedly are, or are not, sensitive to changes arising from the exposure conditions and (2) the stage of learning during which effects are, or are not, demonstrable. The acquisition column indicates investigations of the rate at which animals form associations between stimulus and reinforcement conditions. Measures

utilized to quantify the process are numbers of days and trials or problem presentations required to reach some predetermined level of performance. The percentage of the test population attaining the defined criterion per unit time is also a common measure. The performance column includes studies of the quantitative nature of the behavior after the desired criterion has been attained. The reversal/extinction column contains studies of behavior observed following the removal of (extinction) or alteration of (reversal) the conditions that produce reinforcement.

TABLE 11-7. PROTOCOLS USED FOR THE STUDY OF ANIMAL LEARNING

		Behavioral task			Lead exposure		Number			Blood lead, μg/dl		
Reference	Species	Apparatus	Reward	Task[a]	Period[b]	Level[c]	Litter per test group	Subjects per test group	Growth[d] rate	Peak	At test	Learning performance
Brady et al.[292]	Rat	water T-maze	neg.	Simult. B.D.	PG,G,L	500 GAV[e]	4	17	N	?	?	Impaired
Brown, D.[271]	Rat	T-maze	pos.	Succ. B.D.	L	100 W[f]	?	6	N	?	?	Impaired
		T-maze	pos.	Succ. B.D.	L	25 GAV[e]	?	?	N	?	?	Impaired
		T-maze	pos.	Succ. B.D.	L(1st 10d)	35 GAV[e]	?	5-6	N	46	?	Impaired
		T-maze	pos.	Succ. B.D.	L(last 11d)	35 GAV[3]	?	5-6	N	20	?	No effect
		T-maze	pos.	Succ. B.D.	L(last 11d)	70 GAV[e]	?	5-6	N	19	?	No effect
		T-maze	pos.	Succ. B.D.	L(last 11d)	140 GAV[e]	?	5-6	A	?	?	Impaired
		T-maze	pos.	Succ. B.D.	Birth to day 10	5 IP[e]	?	7	N	288	23	Impaired
Brown, S. et al.[293]	Rat	water T-maze	neg.	Spatial discrim.	Various (8 days to 5 weeks)	100 IP[e]	?	66	?	?	?	No effect
		Shuttle-box	neg.	2-way	5 weeks	100 IP[e]	?	8	?	?	?	No effect
Driscoll and Stegner[272]	Rat	Y-maze	pos.	Simult. B.D.	PG,G,L,PW	2070 DT[f]	?	4	A	?	?	Impaired
		Shuttle-box	neg.	2-way	PG,G,L,PW	2070 DT[f]	?	5	A	?	?	Improved
		Shuttle-box	neg.	2-way	PG,G,L,PW	2070 DT[f]	?	12	N	?	?	Impaired
		Shuttle-box	neg.	2-way	PG,G,L,PW	2070 DT[f]	?	12	A	?	?	Improved
Overmann[294]	Rat	E-maze	pos.	Spatial discrim.	L	5,16,49 GAV[f]	?	?	N	33,174 226	15,23, 56	No effect
		E-maze	pos.	Tactile discrim.	L	5,16,49 GAV[f]	?	?	N	33,174 226	?	Impaired on reversal
		E-maze	pos.	Visual discrim.	L	5,16,49 GAV[f]	?	?	N	33,174 226	?	No effect No effect
		2-compartment chamber	neg.	1-way	L	5,16,49 GAV[f]	?	?	N	33,174 226	?	Impaired
		2-compartment chamber	neg.	Passive avoid	L	5,16,49 GAV[f]	?	?	N	33,174 226	?	No effect
		Operant	pos.	Temporal disc.	L	5,16,49 GAV[f]	?	?	N	33,174 226	?	Impaired
Snowdon[295]	Rat	CF maze	pos.	Maze learning	Adult exposure and PW	2.7,4.4 6.6 IP[e]	?	8-10	A	?	?	No effect
		CF maze	pos.	Maze learning	L	4.4 IP[e]	17	36	A	?	?	Impaired
Sobotka et al.[283]	Rat	Shuttle-box	neg.	2-way	3-21 days post-partum	5.15,44 GAV	6-7	28-34	?	11 21 23	?	Impaired

(continued)

TABLE 11-7 (continued).

Reference	Species	Behavioral task: Apparatus	Reward	Task[a]	Lead exposure: Period[b]	Level[c]	Number: Litter per test group	Number: Subjects per test group	Growth rate	Blood lead, μg/dl: Peak	Blood lead, μg/dl: At test	Learning performance
Sobotka and Cook[282]	Rat	2-compartment chamber	neg.	passive avoid	3-21 days post-partum	5,15,44[e] GAV	?	5-8	N	18,61,71	?	No effect
		Shuttle-box	neg.	1-way	—	—	?	5-8	N	18,61,71	?	No effect
		Shuttle-box	neg.	2-way	—	—	?	5-8	N	18,61,71	?	Impaired
		Operant	neg.	Spatial discrim.			?	5-8	N	18,61,71	?	Impaired reversal
Slecta[296]	Rat	Operant	pos.	FI schedule	PW (day 22-57)	50,300 1000 W[f]	—	3	?	9,28,40	?	Altered
Shapiro et al.[297]	Rat	Operant	pos.	VI 30 schedule	PW (day 90-126)	.09 - 52 IP[e]	—	7	?	?	?	Altered
Hastings et al.[273]	Rat	Shock grid	neg.	Flinch	L	109, 546 W[f]	3	10	N	29,42	5,9	No effect
		Shock grid	neg.	SEA	L	109, 546 W[f]	3	10	N	29,42	5,9	Less aggressive
		Operant	pos.	Succ. B.D.	L	109, 546 W[f]	3	10	N	29,42	5,9	No effect
Winneke et al.[285]	Rat	Lashley jumping stand	pos.	Simult. PD & SD	PG,G,L PW	745 DT[f]	—	10	N	29	29	Impaired
Bornschein[298]	Mouse	Operant	pos.	Simult. B.D.	L	103,546 2730 W[f]	5	15	A	79 -190	29,120	Impaired
Bornschein[299]	Mouse	Operant	pos.	Simult. B.D.	L	109,546 W[f]	7	14	N	80 180	29	Impaired reversal
Sobotka et al.[284]	Dog	T-maze	pos.	Simult. R.D.	2-week-5 month post-partum	1.4 W[f]	—	10	N	85	—	Impaired
VanGelder et al.[300]	Sheep	Operant	pos.	Auditory discrim.	Adult exposure 9 weeks	100 GAV[e]	—	4	?	?	?	Impaired
		Operant	pos.	FI schedule		1000 DT[f]	—	5	?	30	30	No effect
		Operant	pos.	FI schedule		530 DT[f]	—	5	?	17	17	No effect
Carson et al.[301,302] and VanGelder et al.[300]	Sheep	CF maze	pos.	Maze learning	G	2.3,4.5[e] DT	—	6-8	?	17-25	9-14	No effect
		CF maze	pos.	Maze learning	5 days-12 weeks post partum	2,4,8,16[e] DT	—	4	?	57,81 123,162	—	No effect
		Operant	pos.	Simult. FD & SD	G	2,3,4,5[e] DT	—	6-8	?	17,25	9,14	Impaired
Bowman and Bushnell[303]	Monkey	WGTA	pos.	—	L,PL	0.3,1.0	—	4	—	50,85	50,85	Impaired reversal

[a]Succ. B.D. = Successive brightness discrimination.
Simult. B.D. = Simultaneous brightness discrimination.
1-way = One-way avoidance.
2-way = Two-way avoidance.
FD = Form discrimination.
SD = Size discrimination.
Flinch = Tail-flinch test.
SEA = Shock elicited aggression.
CF maze = Close-field maze.
Operant = Operant chamber.
WGTA = Wisconsin General Test Apparatus.

[b]PG= Pregestation period, external exposure to dam.
G= Gestation period, external exposure to dam.
L= Lactation period, external exposure to dam.
PW= Post weaning period, external exposure to pup.

[c]DT= Diet
GAV= Gavage
IP= Intraperitoneal
W= Drinking water

[d]N= Normal
A= Altered

[e]mg/kg/day

[f]ppm

A review of the studies listed in Table 11-7 leads to the following general critique. Few animal studies adequately simulate the lead exposure conditions found in young children either with respect to the levels of exposure or the timing of the exposure. There is often an inadequate or nonexistent history of lead exposure with respect to both the external and internal dose. In regard to the general experimental designs, several design deficiencies frequently occur. These include: (1) limited sample size or larger samples derived from a few litters — the latter permits genetic effects to have an inordinate influence on the results — and (2) inappropriate application of statistical methods and confounding of variables, e.g., maternal undernutrition or neonatal growth retardation, which results in an inability to determine specific causes for demonstrated effects. The selection of behavioral tasks is often made with little or no apparent rationale that would aid in the formulation and testing of hypotheses and interpretation of data. Finally, most investigators do not provide adequate documentation of the relative sensitivity of the behavioral tasks being used which could be accomplished with the use of a standard reference compound. Since this has not been done, failure to observe a disruption in task acquisition or performance could be taken to mean that (1) the task is not sensitive enough to detect the deficit or (2) the neural systems which mediate the behavior on that task are not affected by lead at the exposure levels examined.

In spite of the extensive methodological differences in these studies, several conclusions can be drawn from the data shown in Tables 11-7 and 11-8.

TABLE 11-8. STAGE OF LEARNING

Treatment effects	Acquisition	Performance	Reversal 'extinction
Present	Brady et al.[292.a] Brown[304.b] Carson et al.[301.b] Driscoll and Stegner[272.a.b] Hastings et al.[273.b] Overmann[294.a] Snowdon[295.b] Sobotka et al.[283.a] Winneke et al.[285.b] Miller et al.[305.b]	Shapiro et al.[297.b] Slecta[296.b] Bornschein et al.[298.b]	Overmann[294.a.b] Sobotka et al.[283.a] Bornschein et al.[298.b] Bowman[303.b]
Absent	Bornschein et al.[298.b] Carson et al.[300.b] Hastings et al.[273.b] Overmann[291.a.b] Sobotka et al.[283.a] Winneke et al.[285.b]		Overmann[294.a] Sobotka et al.[283.a] Miller et al.[305.b]

[a]Negative reinforcer.
[b]Positive reinforcer.

Although learning paradigms are being used to demonstrate effects of lead on CNS function, the present data do not permit a clear distinction between the effects of lead exposure on cognitive function (learning/memory) and effects on sensory-motor function, arousal, or motivation, which in turn can produce performance differences. Therefore, some of the studies appearing in the column titled "Acquisition" (Table 11-8) may, in fact, belong in the "Performance" column. This is especially true of studies that report large group differences on the first day of acquisition (cf. 292, 299). New studies specifically designed to test for this distinction must be conducted.

Tasks that use both positive and negative reinforcers appear to be equally sensitive to disruption following lead exposure (Table 11-8). Therefore, lead exposure does not appear to have a selective effect on a specific motivational system.

Treatment effects have been reported both by those investigators using manual testing procedures which require a high degree of experimenter-subject interaction (cf. 285, 292, 304) and by those using automated operant chambers with minimal experimenter-subject interaction (cf. 283, 294, 306). Thus, reports of significant treatment effects cannot be ascribed to experimenter bias.

The effects appear to persist beyond the immediate exposure period with behavioral disruption

demonstrable in animals with normal blood lead levels (cf. 283, 285, 304, 306).

Procedures have been inadequate for reproducing qualitative and quantitative changes in behavior. This has limited the ability to test hypotheses pertaining to the site of action of lead-induced behavioral changes.

It is not yet clear whether the observed effects are direct effects of lead on the developing nervous system or whether the effects are indirectly mediated through treatment-induced alterations in maternal behavior, maternal milk, or one of several potential peripheral target systems in the neonate.

Some types of learning problems appear to be more sensitive to lead-related disruption than others. For example, in the passive avoidance paradigm, acquisition and extinction are reportedly not sensitive.[282,294] Simple pattern discrimination is also apparently insensitive to lead exposure.[285,294,301] Other forms of visual discrimination such as size[285,301] and brightness[272,292,304,306] appear to be particularly sensitive. The two reports of altered size discrimination[285,301] may in fact be special cases of brightness discrimination since the different size stimuli (white pattern on a black background) also reflect different amounts of light. Further testing will be necessary to resolve this issue. Active-avoidance tasks are also being used successfully to examine effects obtained following lead exposure. Both one-way avoidance[294,306] and two-way, shuttle-avoidance tasks[272,283] reflect a disruption in normal behavior. The one exception is a negative finding by Sobotka et al.[283] using a one-way avoidance task. This negative effect may be related to the fact that shock was terminated automatically after 5 seconds, independent of the animals' behavior. This is not the usual procedure, and its effect cannot be evaluated since no data were presented for this particular task.

Deficits in task performance do not appear to be species specific since they are reported for rats,[272,283,294,304] mice,[298] dogs,[305] sheep,[301] and monkeys.[303] Furthermore, recent studies suggest that behavioral alterations may be present in rats exposed to lead following weaning.[296,297] These reports are contrary to the generally held opinion that adult or post-weaning rats are insensitive to lead exposure.[293,295,301] Since the number of reported studies using adult exposure protocols is extremely limited, however, it is not possible to rule out the suggestion that these conflicting data merely reflect differences in task sensitivity. More research using adult exposure protocols and more sophisticated behavioral testing paradigms will be necessary to resolve the conflict.

11.5.2.4 EFFECTS OF LEAD ON AGGRESSIVE BEHAVIOR

Two reports appeared in the literature in 1973 which suggested that lead exposure produced an increased aggressiveness. Silbergeld and Goldberg[262] reported that mice exposed to either 5 or 10 mg/ml of lead had a "heightened frequency of fighting as determined by the incidence of bite frequencies observed on litter-mate males housed together." Sauerhoff and Michaelson[278] also referred to an increased aggressiveness in lead-exposed rats during the fourth week of development. In neither report, however, was there an attempt to quantitate these observations of increased aggression.

Hastings et al.[306] exposed lactating rats to lead (0, 109, or 545 ppm) from parturition to 21 days. This lead treatment produced no change in growth in the offspring. Individual pairs of male offspring (from the same treatment groups) were tested at 60 days of age for shock-elicited aggression. Lead-exposed groups showed significantly less aggressive behavior than the control group. There were no significant differences among the groups in the flinch/jump thresholds to shock. This latter finding suggests that the differences seen in the shock-elicited aggression were not caused by differences in shock threshold.

Gray and Reiter[261] reported on the aggressive behavior of mice exposed to 5 mg/ml lead acetate from parturition. Aggressive behavior was measured by introducing an adult male intruder into the home cage of an individual experimental male. Control males wounded the intruder 85 times on the average during a 14-hour test period, whereas intruders to lead-treated and pair-fed male cages had means of 32 and 35, respectively. Therefore, the reduced aggressive behavior seen in these experiments cannot be explained by lead exposure alone, because similar reductions were observed in pair-fed controls. Nevertheless, in both the rat and the mouse a quantitative examination of aggressive behavior suggests that lead can cause a decrease rather than an increase in aggressive behavior.

11.5.2.5 NEUROCHEMISTRY

The effects of *in vivo* lead exposure on a variety of neurochemical substances and processes have been studied in the past several years (see Table 11-7). Perhaps most notable are the investigations of lead effects on both putative neurotransmitter systems and on energy metabolism in the central nervous

system. Much of the work, by its nature, has required the use of experimental animal models for broad screening for possible effects across many different transmitter systems or for testing specific hypotheses of altered transmitter functions. Unfortunately, these studies on the neurochemical effects of lead exposure are hindered by the same problems in experimental design as those discussed in the section on behavioral studies.

Research on neurotransmitter systems has concentrated primarily on the effects of lead exposure on cholinergic and monoaminergic functions, probably because of the extensive background literature that exists on the basic neurochemistry of those transmitters and because of the documentation extant on the neurophysiological and behavioral roles played by these transmitters. The approaches employed in these studies have included: (1) biochemical assays of steady-state levels of transmitter substances in brain tissue;[277,283,288,307-315] (2) assessment of synthesis and turnover rates;[309,315] (3) measurement of the activity of enzymes responsible for transmitter synthesis or degradation;[283,309,311,313,316-318] (4) assessment of transport processes involved in synaptic uptake of transmitters or their precursors;[308,309,316] and (5) assessment of synaptic release mechanisms[308,309,315,316] (see Table 11-9).

TABLE 11-9. *IN VIVO* EFFECTS OF LEAD EXPOSURE ON NEUROCHEMISTRY

Reference	Species	Exposure	Nutritional status	Lead concentration: Blood, μg/dl	Lead concentration: Brain, μg/g	Neurochemical parameter	Results
Silbergeld and Goldberg[308]	Mouse	5 mg/ml lead acetate in drinking water starting at parturition	Under-nourished	?	?	1) High affinity transport of phenylalanine, glycine, leucine, NE, 5HT, GABA, DA, choline, and tyrosine	1) Decreased high affinity transport of dopamine and choline Increased high affinity transport of tyrosine
						2) Steady-state ACh NE DA	2) Increased steady state levels of NE
Silbergeld et al.[309]	Mouse	5 mg/ml lead acetate in drinking water starting at parturition	Under-nourished	?	?	1) ACh release 2) Steady state NE, HVA, VMA 3) MAO 4) Choline transport	1) Decreased 40% 2) Increased 27, 41, 15% respectively 3) Increased 20% 4) Decreased 50%
Carroll et al.[316]	Mouse	2, 5, 10 mg/ml lead acetate in drinking water starting at parturition	Under-nourished	?	?	1) K+ -induced release of ACh and choline 2) Spontaneous release of ACh 3) Steady-state levels of ACh, choline 4) CAT CBK AChE	1) Inhibited K+ -induced release of choline and ACh 2) Increased spontaneous ACh release 3) No change in steady state levels 4) No change
Brown et al.[318]	Rat	7.5 mg/kg (IP) from birth to 10 days of age	Normal	?	?	AChE (regional brain analysis)	No change in medulla oblongata, corpus striatum, cerebellum, cerebrum or hippocampus. Inhibited in midbrain (16%)
Golter and Michaelson[307]	Rat	5% lead acetate in mother's diet from parturition to 16 days post partum, 40 ppm in drinking water Intubated with 1.0 mg/day from parturition to 16 days post partum, 40 ppm in drinking water	Under-nourished	?	?	Steady-state NE, DA	Increased NE at 33 days of age (13%); no change in DA
Michaelson and Sauerhoff[314]	Rat	5% lead acetate in mother's diet from parturition until day 16, 25 ppm in drinking water	Under-nourished	?	Control = 0.1 4% = 0.88	1) Steady-state DA 2) Steady-state 5HT, GABA, and NE	1) Decreased DA (20%) 2) No change
Sauerhoff and Michaelson[278]	Rat	4% lead carbonate in mother's diet from parturition until 16 days, 40 ppm in diet	Under-nourished			Steady-state NE, DA	Decreased DA (20%)
Grant et al.[310]	Rat	0, 25, 100 or 200 mg/kg/day by gavage on postnatal days 3-25	?	0 = 16 25 = 26 100 = 43 200 = 63	0.15 0.38 0.51 0.68	Steady-state NE, DA (regional brain analysis)	No change

(continued)

TABLE 11-9 (continued).

Reference	Species	Exposure	Nutritional status	Lead concentration: Blood, μg/dl	Lead concentration: Brain, μg/g	Neurochemical parameter	Results
Hrdina[313]	Rat	0.2 and 1.0 mg/kg IP (100g) for 45 days	?	?	?	Cerebro-corticol ACh, AChE	ACh = 32-48% increase AChE - No significant change
				Brain stem NE, 5HT		NE - 20-27% decrease 5HT - No change	
Modak et al.[311]	Rat	1% lead acetate in drinking water at parturition	Under-nourished	Control = 0 1% = 245	?	1) Steady-state ACh 2) CAT 3) AChE	1) Increased ACh in diencephalon (12%) 2) Increased in medulla-pons, hippicampus and cerebral cortex (11-12%) 3) Lower in medulla-pons, midbrain and diencephalon (10-20%)
Sobotka et al.[283]	Rat	8-91 mg/kg/day by intubation from 3-21 days of age	Normal	8-71	—	1) Steady-state NE, DA, 5HT 2) AChE	1) No change 2) Decreased (marginal)
Shih and Hanin[315]	Rat	4% lead carbonate in mother's chow from parturition to 21 days, 40 ppm in diet	Under-nourished	?	?	1) Steady-state ACh, choline 2) ACh turnover	1) No change in ACh, increased choline 2) Decreased ACh turnover (33-51%)
Cahill et al.[277]	Rat	5, 50 ppm in drinking water. 40 day pretreatment of parents continued from conception through adulthood	Normal	180 day 0 = 5 5 = 6 50 = 10	180 day 0 = 18 5 = 20 50 = 27	Steady-state NE, DA	Decreased DA at 28 days of age Increased NE at 180 days of age
Silbergeld and Chisolm[319]	Mouse	5 mg/ml lead acetate in drinking water starting at parturition	Under-nourished	?	?	Brain HVA and VMA	Increased 33 and 48%
Gerber et al.[312]	Mouse	0.1-1000 mg/l lead acetate in drinking water for one year	?	?	?	Steady-state 5HT	No change
Bhatnagar[317]	Rat (200-250 g)	0, 1, 2% lead acetate for 70 days	?	?	?	Tyrosinase activity	No effect
Bull et al.[253]	Rat (200-400 g)	1) 67 μM *in vitro* lead chloride	—	—	—	K+ stimulated respiration of cerebral slices	Inhibition
		2) 3, 12, 60 mg/Pb/Kg total dose over 2 weeks	Impaired growth in 60 mg/kg group	Control= 0.08 3= 13.2 12= 72.6 60= 380	0.06 0.17 0.41 1.02	K+ stimulated respiration of cerebral slices	Inhibited at 12 and 60 mg/kg exposure level
Holtzman and Hsu[320]	Rat	4% lead carbonate at 2 weeks postpartum	Under-nourished, reduced brain weights	?	?	Cerebral and cerebellar mitochondrial respiration	Impaired respiration after 2 weeks of treatment

Abbreviations:
ACh - Acetylcholine
CAT - Choline acetyltranferase
AChE - Acetylcholinesterase
NE - Norepinephrine
DA - Dopamine
5HT - 5-hydroxytryptamine (serotonin)
GABA - Gamma aminobutyric acid
CPK - Choline phosphokinase

Several studies utilizing high levels of lead exposure have reported inhibition of cholinergic function. In a series of experiments on the mouse, Silbergeld et al.[308,309] and Carroll et al.[316] reported decreased potassium-induced release of acetylcholine (ACh) and decreased high-affinity transport of choline. Unfortunately, no control for growth retardation was provided in these experiments and relatively high external exposures to lead were required to produce the effect. This was also true in the rat studies reported by Modak et al.[311] and Shih and Hanin.[315] At lower levels of external exposure, with no accompanying growth retardation, no consistent effects on cholinergic function have been reported (cf. 283 and 318). Thus, if impaired cholinergic function during *in vivo* lead exposure is also found in the absence of undernutrition, and/or growth retardation, which is probable in view of the *in vivo* work reported later in this section, then its relevance to behavioral effects seen at lower exposure levels will need examination.

The effects of lead exposure on catecholamine function have also been extensively studied. Findings have been reported of increased steady-state levels of norepinephrine,[277,307,308] increased activity of monamine oxidase (MAO),[309] increased synaptosomal transport of the precursor tyrosine,[308] and increased amounts of the norepinephrine metabolite, vanillyl mandelic acid, and homovanillic acid in the brain.[319] In studies on steady-state levels of norepinephrine, changes have been reported either in the absence of undernutrition[227] or when values have been compared to pair-fed controls.[307] However, inconsistencies within a given laboratory (cf. 278 vs. 307), absence of similar findings in different laboratories (cf. 283 and 310), and findings of decreased steady-state levels[313] make any conclusions regarding lead-induced changes in noradrenergic systems equivocal. This uncertainty is also found in the reports on dopamine changes following lead exposure (cf. 277, 278, 314 vs. 283, 307, 310).

Human studies attempting to relate subclinical lead exposures to signs of altered brain monoamine function have been initiated utilizing urinary levels of monoamine neurotransmitter metabolites as indices of CNS monoamine turnover rates. Although initial studies on catecholamine excretion have suggested a lead effect, an appended note by Silbergeld and Chisholm[319] indicated difficulty in finding one of the earlier reported effects in subsequent studies. This clinical study again emphasizes some of the problems and uncertainties that have beset investigations of low-level toxicity. Also, as indicated by Wender et al.,[321] urinary metabolites reflect primarily peripheral nervous system activity. In another study[322] altered levels of 5-hydroxyindole acetic acid (5-HIAA) were reported in the urine of occupationally lead-exposed battery factory workers, suggesting possible lead effects on serotonin (5-hydroxytryptamine) systems. No parallel supportive evidence from animal studies has been advanced for such an effect, however, and in fact most reports claim negative findings for any type of measurements of brain serotonin function.[283,308,312-314]

The reasons for the inconsistencies of lead-induced changes in monaminergic and cholinergic functions may be the result in part of interlaboratory differences in dosing regimens and other variations in experimental protocol. One note of caution, however, is appropriate here in that highly variable results are seen within different laboratories, even with the same exposure regimens, assay procedures, etc., from experiment to experiment. This might suggest that some subpopulations of rats or mice might be resistant to lead effects on monoamine transmitters whereas others are more vulnerable, possibly because of genetic factors, subtle variations in diet, etc. More carefully controlled studies in the future that explicity manipulate such variables (genetics, nutrition, etc.) may reveal lead effects even at low exposure levels, given the right circumstances or population segment tested.

Finally, the recent report of Nathanson and Bloom[323] indicated that *in vitro* lead exposure inhibits adenyl cyclase activity (I_{50} = 2.4 μM). This enzyme is responsible for the synthesis of cyclic adenosine 3',5'-monophosphate (c-AMP) which has been shown to play an important role in the mechanism of action of a number of hormones, including neurotransmitters. The effects of lead exposure on adenyl cyclase and the resultant effects on neurotransmitter systems warrant further investigation.

Several recent reports have dealt with the effects of lead exposure on brain-energy metabolism. Bull et al.[253] reported that both *in vivo* and *in vitro* lead exposure inhibited potassium-stimulated respiration. Of interest was the finding that lower brain levels of lead (approximately 1/30th) were required to inhibit respiration *in vivo* than *in vitro*. Also, these results were seen in animals showing normal growth (12 mg/kg group). Similar findings of inhibited respiration using isolated mitochondria were reported by Holtzman and Hsu[320] and by Brierley.[324]

In contrast to the general lack of consistent effects on steady-state levels of cholinergic substances and

associated enzymes, consistent evidence for effects of lead on cholinergic synaptic uptake and release mechanisms have been reported.

More specifically, lead resembles other divalent cations in that it appears to interfere with chemically mediated synaptic transmission as demonstrated by studies of peripheral neural functions. Kostial and Vouk[325] reported that *in vitro* perfusion of the cat superior cervical ganglion with 4.8 μM lead nitrate depressed or blocked nerve transmission. Contraction of the nictitating membrane during acetylcholine perfusion was unaltered. Also, perfusion of the ganglion with excess calcium restored acetylcholine release and thus reversed the lead blockade. From these findings Kostial and Vouk concluded that lead depressed synaptic transmission by impairing acetylcholine release from the presynaptic terminals.

Manalis and Cooper[326] and Cooper and Manalis[327] showed that lead can influence both pre- and postsynaptic events. Using the frog (*Rana ippiens*) sciatic-nerve/sartorius-muscle preparation *in vitro,* they demonstrated that the principal effect of lead was on presynaptic transmitter release, although lead had a weak, curare-like effect on the postsynaptic response to applied acetylcholine. They confirmed the findings of Kostial and Vouk[325] that lead depresses the phasic release of transmitter evoked by nerve stimulation. They further observed that lead increases spontaneous release of acetylcholine as evidenced by increased miniature end-plate potentials (MEPP's). These MEPP's represent the response of the postsynaptic membrane to released acetylcholine in quantities that are insufficient to depolarize the membrane to threshold levels. In a subsequent experiment Kober and Cooper[328] demonstrated that in the frog, lead blocks synaptic transmission in the sympathetic ganglion by competitive antagonism of spike-evoked entry of calcium into the presynaptic nerve terminals with a resultant reduction in acetylcholine release.

Experiments by Silbergeld et al.[329,330] indicated a similar blockade of transmitter release by lead in the rat. Furthermore, they reported a reduced force of contraction in the phrenic-nerve/diaphragm preparation from mice exposed to lead from birth through 60 days of age. The nerve-muscle preparation from these lead-exposed animals showed a reduction in force of contraction with nerve stimulation. Also, a reduced force of contraction was reported upon direct stimulation of the muscle. This observation agrees with the weak postsynaptic effects of lead reported by Manalis and Cooper;[326] but unfortunately, no data were presented by Silbergeld et al.[329,330] on the muscle contraction following electrical stimulation, so the relative importance of this finding cannot be evaluated. Finally, Cooper and Steinberg[331] demonstrated that lead is also capable of blocking neural transmission at the adrenergic synapse. They measured the contraction force of the rabbit saphenous artery following stimulation of the sympathetic nerve endings. Again, the results indicated that lead blocks muscle contraction by an effect on the nerve terminals rather than an effect on the muscle. Since the response recovered when calcium concentration was increased in the bathing solution, it was concluded that lead does not deplete transmitter stores in the nerve terminals but more likely blocks norepinephrine release.

In summary, *in vitro* experiments have demonstrated that lead interferes with synaptic transmission in the peripheral nervous system. This effect appears to be related to a competitive inhibition of calcium-mediated, evoked release of the neurotransmitters. Further, lead was shown to increase the spontaneous release of transmitter from some synapses.

The effects of lead on synapses within the CNS have not been extensively studied. Carroll et al.,[316] however, reported a decrease in potassium-induced release of both choline and acetylcholine from cortical minces of mice chronically exposed to lead. These changes in acetylcholine metabolism suggest that, as is the case of the peripheral nervous system, the central cholinergic function may be depressed by lead. This is further supported by a report of Shih and Hanin[315] that lead exposure decreased *in vivo* acetylcholine turnover rate in cortex, hippocampus, midbrain, and striatum (35, 54, 51, and 33 percent decreases, respectively) in rat brain after neonatal lead exposures. Along with the *in vitro* findings, this provides additional evidence supportive of lead-induced dysfunctions of cholinergic synaptic uptake and release mechanisms. Unfortunately, these results and many from the above peripheral function studies were obtained at rather high exposure levels and most were performed on undernourished or growth-retarded animals.

One final note concerns the relationship between levels of lead in blood and brain. Several studies on rodents have reported simultaneous lead values for blood and brain resulting from lead exposures of various durations. Bornschein et al.[270] exposed mice to 5 mg/ml lead acetate starting at parturition. Brain/blood ratios showed a steady increase from 20

to 100 days of age. Ratios of 1.05, 2.55, and 4.08 were reported for mice at 20, 40, and 100 days of age, respectively. This increase in the brain/blood ratios resulted from both a steady increase in brain lead levels (increasing from 200 μg % at 20 days of age to 584 μg % at 100 days of age) and a decrease in blood lead levels (decreasing from 190 ppm at 20 days to 143 ppm at 100 days).

Cahill et al.[277] reported blood and brain levels of lead in rats exposed from conception to either 0, 5, or 50 ppm lead in the drinking water. At parturition, brain/blood ratios of offspring were 0.91 and 0.5 for exposure levels of 5 and 50 ppm, respectively. At 180 days of age, these ratios were 3.3 and 2.7, respectively. A ratio of approximately 1 was also reported by Grant et al.[310] in 30-day-old rats exposed to various levels of lead from 3 to 25 days of age. These data are consistent with the results of Bornschein et al.[270] and indicate that initially brain/blood ratios are approximately unity but that with continued exposure the ratios steadily increase.

11.5.2.6 SUMMARY AND CONCLUSIONS

Data obtained in laboratory animals, such as that reported in the rat by Pentschew and Garro,[52] indicate that encephalopathy is produced by high-level perinatal exposure to lead. This encephalopathy occurs to varying degrees in different species and is characterized by the relative involvement of neuronal degeneration and vasculopathy.

It seems clear that with regard to CNS toxicity the developing organism represents the population at greatest risk. Whether this increased risk is attributable to a greater sensitivity or to a greater susceptibility of the developing organism will require further testing. That is, with a given external exposure, the CNS of the developing organism reaches a higher concentration of lead and is therefore more susceptible to poisoning. Whether the threshold for a given effect of lead is lower in the immature nervous system versus the adult will need to be determined.

There is also good evidence that perinatal exposure to lead even at moderate exposure levels will produce delays in both neurological and sexual development. Because these effects have been demonstrated to occur in the absence of either undernutrition or growth retardation, it has been suggested that they represent direct effects of lead in the respective organ systems.

In the animal studies, locomotor activity has been the most commonly used behavioral index of lead toxicity. The data reviewed here suggest that perinatal exposure to lead produces an increase in the animal's behavioral reactivity. If the test conditions are appropriate, this increased reactivity will be manifested as increased locomotor activity, although some text situations will show reduced activity. Therefore, the altered activity levels per se are merely reflective of a more basic nervous system dysfunction. Close scrutiny of the available data would also suggest that altered locomotor activity in young animals occurs only at moderately high exposure levels. A comparison of the data of Sobotka and Cook[282] and Overmann[275] are of interest in this regard (see Table 11-4). Using gastric intubation, Overmann reported hyperactivity in young rats whose 21-day blood lead levels were 226 μg/dl. Sobotka and Cook, using a similar exposure regimen, found normal activity levels with 22-day blood lead levels of 71 μg/dl. It appears, therefore, that this early change in reactivity occurs at fairly high blood lead levels. It may be, then, that the changes in activity currently reported in laboratory animals are more representative of a post-encephalopathic hyperactivity than of subclinical effects as has been suggested.

On the other hand, the reactivity changes reported in older animals with lifetime exposure occur at much lower blood levels (cf. 264, 277, and 285).

Finally, reports on the effects of lead exposure on the acquisition and/or performance of operant responses indicate that perinatal exposure to moderate and low levels of lead may disrupt this behavior. Thus, external exposures that result in blood lead levels ranging from 30 to 80 μg/dl have been reported to disrupt cognitive function (see Table 11-5). As is true with the clinical data, this area requires further investigation.

It has been repeatedly indicated that serious methodological problems exist in the animal literature which make it difficult to interpret many of the available data. Future research in this area would benefit from more tightly controlled experiments including:

1. Better documentation of internal exposure, not only with respect to lead levels but also with respect to correlative indices of lead toxicity, e.g., ALAD, protoporphyrin, etc.
2. The use of exposure protocols that do not produce confounding variables such as undernutrition, differences in both litter size and number of litters per treatment group, etc.
3. Validation of behavioral tests using both positive control substances and cross corre-

lation with other physiological indices. This would include research aimed at more closely defining both the relative sensitivity of the CNS compared to other organ systems as well as the contribution of other indirect actions of lead on the resulting behavioral changes.

11.6 EFFECT OF LEAD ON THE RENAL SYSTEM

11.6.1 Acute Effects

More than 60 years ago an English toxicologist, Thomas Oliver,[332] distinguished acute effects of lead on the kidney from lead-induced chronic nephropathy. Acute renal effects of lead are seen in persons dying of acute lead poisoning or suffering from lead-induced anemia and/or encephalopathy and are usually restricted to nonspecific degenerative changes in renal tubular lining cells, usually cloudy swelling, and some degree of cellular necrosis. Cells of the proximal convoluted tubules are most severely affected. As long ago as 1928, Pejie[333] emphasized that the degenerative changes in proximal tubules, rather than the vascular changes often referred to in earlier studies, are primary evidence of injury to the kidney in lead poisoning. Many subsequent studies have shown at least three pathological alterations in the renal tubule with onset during the early or the acute phase of lead intoxication in the kidney. These include the formation of inclusion bodies in nuclei of proximal tubular lining cells and the development of functional as well as ultrastructural changes in renal tubular mitochondria.

Dysfunction of proximal renal tubules (Fanconi's syndrome) is manifested by aminoaciduria, glycosuria, and hyperphosphaturia, and was first noted in acute lead poisoning by Wilson and coworkers in 1953.[334] Plasma amino acids were normal, which suggested that the aminoaciduria and other functional abnormalities were of renal origin. Subsequently, aminoaciduria in children with acute lead poisoning was observed by Marsden and Wilson[335] in England, and Chisholm[85,86] found that 9 of 23 children with lead encephalopathy had aminoaciduria, glycosuria, and hypophosphatemia. Aminoaciduria was seen more consistently in Chisolm's studies than the other two manifestations of tubular damage. Thus, the amino acid transport system is probably more sensitive to the toxic actions of lead than the transport systems for glucose and phosphate. The aminoaciduria was generalized in that the amino acids excreted in greatest amounts were those normally present in urine. The condition was related to severity of clinical toxicity and was most marked in children with encephalopathy. The aminoaciduria disappears after treatment with chelating agents and clinical remission of other symptoms of lead toxicity.[85] This is an important observation relative to the long-term or chronic effects of lead on the kidney.

In a group of children with slight lead-related neurological signs, generalized aminoaciduria was found in 8 of 43 children with blood lead levels of 40 to 120 μg/dl.[99] It should be noted that the children reported to have aminoaciduria in the study of Pueschel[99] were not specifically identified as to their lead exposure. Thus, it is not possible to state what level of lead exposure within the blood lead range of 40 to 120 μg/dl was associated with the effects. A similar renal tubular syndrome has been reported to occur in industrially exposed adults.[336]

11.6.2 Chronic Effects

There is convincing evidence in the literature that prolonged lead exposure in humans[337] can result in chronic lead nephropathy. Cramer et al.[337] in 1974 reported on a group of 7 lead-exposed workers who had been exposed up to 20 years. Aminoaciduria was not found, and inulin clearance and renal blood flow were also reported normal. The average blood lead level was 100 μg/dl, the minimum was 71 μg/dl, and all had strikingly high urinary ALA excretion. Some with very long exposures were reported to have interstitial and peritubular fibrosis, determined by renal biopsy. This pathological finding is commonly referred to as chronic lead nephropathy, which is characterized by slow development of contracted kidneys with pronounced arteriosclerotic changes, fibrosis, glomerular atrophy, and hyaline degeneration of these vessels. This is a progressive disease, sometimes resulting in renal failure. It seems to occur sporadically, primarily in industrially exposed workers and in older adults who have been diagnosed as having lead poisoning early in life. There is also some evidence that it occurs in long-time drinkers of lead-contaminated whiskey, as reported by Morris et al.[338] in the cases of 16 adults treated over a 10-year period. This study reported lead poisoning manifested by the same symptoms found in children. The most specific pathological change reported was the presence of large numbers of acid-fast intranuclear inclusions within the cells of the kidney tubules and liver. Ball and Jorenson[339] reported a high frequency of saturnine gout resulting from the consumption of lead-contaminated whiskey, convincingly demonstrating reduced renal uric acid clearance associated with plumbism.

In a series of 102 cases of lead poisoning studied by Lilis et al.,[340] 18 cases of clinically verified chronic nephropathy were found. For the whole series, the mean blood lead level was approximately 80 μg/dl, with a range of 42 to 141 μg/dl. Nephropathy was more common among patients who had been exposed to lead for more than 10 years than among those who had been exposed for less than 10 years. In both studies, reduced urea clearance preceded reduced creatinine clearance.

In the Danilovic study,[341] 7 of 23 cases had blood lead levels of about 100 to 200 μg/dl. In the studies of Albahary et al.,[342] blood lead levels were not reported but exposure levels must have been quite high because the mean ALA excretion was about 37 mg/24 hr for 29 workers. These studies indicate that the nature of the effect is glomerulovascular, with reduction in clearance of urea and, in more protracted exposures, also of endogenous creatinine. Also, reduced clearance of uric acid was observed in the study of Albahary et al.[342]

In the recently reported studies of Wedeen et al.,[343] eight subjects suspected of excessive occupational exposure were given detailed examinations for renal function. Four of the subjects showed signs of abnormal renal function. In one subject with asymptomatic renal failure, chelation therapy increased the glomerular filtration rate, the *p*-amino hippurate (PAH) extraction, and the maximal PAH excretion rate, and improved the proximal tubule ultrastructure, despite decreased renal plasma flow. Three of the subjects showed proximal tubule abnormalities via biopsy. In eight subjects, lead-induced nephropathy was established by exclusion. The blood lead values of the individuals ranged from 48 μg/dl, for the subject having asymptomatic renal failure, to 98 μg/dl. The lead levels of the other two subjects in the preclinical renal dysfunction category were 51 and 66 μg/dl. All subjects showed glomerular filtration rates of less than 87 ml/min/1.73m^2. The authors suggest on the basis of these studies that lead nephropathy may be an important occupational hazard in the U.S. lead industry.

A series of reports from Queensland, Australia,[344] points to a strong association between severe lead poisoning in childhood including central nervous system symptoms and chronic nephritis in early adulthood. Henderson[345] followed up 401 children who had been diagnosed as having lead poisoning in Brisbane between 1915 and 1935. Of these 401 subjects, 165 had died, 108 from nephritis or hypertension. This is greatly in excess of expectation. Information was obtained from 101 of the 187 survivors, and 17 of these had hypertension and/or albuminuria. In a more recent study, Emmerson[346] presented a criterion for implicating lead as an etiological factor in such patients: the patients should have an excessive urinary excretion of lead following administration of calcium EDTA. In his study, 32 patients with chronic renal disease attributable to lead poisoning had similarly elevated excretion of lead. The presence of intranuclear inclusion bodies is very helpful in establishing a relationship between renal lesions and lead toxicity, but inclusion bodies are not always present in persons with chronic lead nephropathy.

Attempts to confirm the relationship between childhood lead intoxication and chronic nephropathy have not been successful in at least two studies in the United States. Tepper[90] found no evidence of chronic renal disease in 42 persons with a well-documented history of childhood plumbism 20 to 35 years earlier at the Boston Children's Hospital. Likewise, Chisolm[347] found no evidence of renal disease in 62 adolescents known to have had lead intoxication 11 to 16 years earlier. An important distinction between the Australian group and patients in the United States was that none of Chisholm's[347] subjects showed evidence of increased residual body lead burden following the EDTA mobilization test. This difference has suggested to Chisholm that lead toxicity in the Australian children must have been of a different type, with a more protracted course than that experienced by the American children. Most children in the United States who suffer from lead toxicity do so early in childhood, between the ages of 1 and 4, the source usually being oral ingestion of flecks of wall paint and plaster containing lead.

11.7 REPRODUCTION AND DEVELOPMENT

As reviewed thus far in the present chapter, the adverse effects of lead on the hematopoietic, nervous, and renal systems have been well documented across a wide range of exposure levels and represent a triad of symptoms classically associated with lead poisoning. Extensive evidence for adverse effects of lead on reproduction and development has also been accumulating in the literature for many years and has become a matter of increasing medical concern. Data from both human and animal studies indicate that lead exerts gametotoxic, embryotoxic, and, possibly, teratogenic effects that impact on the pre- and postnatal survival and development of the fetus and newborn, respectively. In addition, it appears that the viability and development of the fetus may also be markedly affected by lead indirectly via ad-

verse effects on various health parameters, e.g., nutritional state or blood chemistry, of the expectant mother. The vulnerability of the fetus to such lead effects while *in utero* has contributed to concern that pregnant women may be a special group at risk for lead poisoning. Certain information on adverse lead effects on male reproductive functions, it should also be noted, has led to additional concern regarding the impact of lead on men.

11.7.1 Human Studies

Data suggesting that lead exerts adverse effects on human reproductive functions have existed in the literature since before the turn of the century. For example, Legge,[348] in summarizing the reports of 11 English factory inspectors in 1897, found that of 212 pregnancies in 77 female lead workers only 61 living children were produced. Fifteen workers had never become pregnant. There were 21 stillborns, miscarriages occurred 90 times, and, of 101 children born, 40 died in the first year. Legge also noted that when pregnant animals were fed lead they always aborted. He concluded that maternal exposure to lead resulted in a direct action of the element on the fetus.

In 1911, Oliver[180] published statistics in Britain on the effect of lead on pregnancy (Table 11-10) which showed that the miscarriage rate was elevated among women employed in industries in which they were exposed to lead.

TABLE 11-10. STATISTICS ON THE EFFECT OF LEAD ON PREGNANCY[180]

Sample	Number of abortions and stillbirths per 1000 females	Number of neonatal deaths (first year) per 1000 females
Housewives	43.2	150
Female workers (mill work)	47.6	214
Females exposed to lead premaritally	86.0	157
Females exposed to lead after marriage	133.5	271

Since the time of the above studies, women have been largely excluded from occupational exposure to lead. Even before the effects of industrial lead exposure on pregnancy were documented, however, lead compounds were known for their embryotoxic properties and were often used to induce criminal abortion.[349] In a study by Lane,[350] women exposed to lead levels of 75 $\mu g/m^3$ were examined for effects on reproduction. Longitudinal data on 15 pregnancies indicated an increase in the number of stillbirths and abortions. No data were given on urinary lead in women, but men in this sample had urinary levels of 75 to 100 μg/liter.

In a more recent study[351] of the pregnancies of 104 Japanese women married to lead workers before and after their husbands began lead work, miscarriages increased to 84.2/1000 pregnancies from a prelead rate of 45.6/1000. The miscarriage rate for 75 women not exposed to lead was 59.1/1000.

Another recent study by Fahim et al.[352] in humans suggests that subtoxic lead absorption during pregnancy may be associated with an increased incidence of preterm delivery and early membrane rupture: 253 women delivered in Rolla, Missouri (Region I), which is 60 to 80 miles from lead smelters, and 249 women delivered in Columbia, Missouri (Region II), where there is no lead industry. The incidence of term pregnancies with early membrane rupture was 17 percent in Region I and 0.41 percent in Region II. The incidence of premature deliveries was 13.04 and 3 percent, respectively. A high correlation was found between lead concentrations in maternal and fetal blood: both were significantly higher in the cases of preterm pregnancies and early membrane ruptures than in term pregnancies.

Pregnancy is a stress that may place a woman at higher risk for lead exposure. Both iron deficiency and calcium deficiency increase the susceptibility of lead toxicity, and women have an increased risk of both deficiencies during pregnancy and postpartum. The cause of the increased perinatal mortality may be a mutagenic or teratogenic effect of lead.[353]

The above studies clearly demonstrate an adverse effect of lead on human reproductive functions, ranging from reduced pregnancy rates to increased incidence of miscarriages, premature deliveries, and stillbirths. The mechanisms underlying these effects are unknown at this time. Many factors could contribute to the above results, ranging from lead effects on maternal nutrition or hormonal state before or during pregnancy to more direct gametotoxic, embryotoxic, or teratogenic effects that could affect fertility or fetal viability during gestation. Efforts have been made to define more precisely the points at which lead may affect reproductive functions both in the human female and male, and in other animals, as reviewed below.

In regard to potential lead effects on ovarian function in human females, Panova[354] reported a study of 140 women working in a printing plant for less than 1 year (1 to 12 months) where ambient air levels were <7 μg lead/m^3. Using a classification of various age groups (20 to 25, 26 to 35, and 36 to 40) and type of ovarian cycle (normal, anovular, and disturbed lutein phase), Panova claimed that statistically significant differences existed between

the lead-exposed and control groups in the age range of 20 to 25 years. It should be noted that the report does not show the age distribution, the level of significance, or the data on specificity of his method of classification. Also, Zielhuis and Wibowo,[355] in a critical review of the above study, concluded that study design and presentation of data are such that it is difficult to evaluate the author's conclusion that chronic exposure to low lead in air leads to a disturbed function of ovaries. It should be noted that no consideration was given to the dust levels of lead, an important factor in print shops.

Unfortunately, little else besides the above report exists in the literature in regard to assessing lead effects on ovarian function or other factors affecting human female fertility. Nor are there many studies offering firm data on maternal variables, e.g., hormonal state, that are known to affect the ability of the pregnant woman to carry the fetus full term. In addition, there are no studies that demonstrate conclusively a direct lead-induced teratogenic effect on the human fetus, although the transfer of lead across the human placenta and its potential threat to the conceptus have been recognized for more than a century.[356] Nevertheless, documentation of placental transfer of lead to the fetus and data on relevant parameters, e.g., fetal blood lead levels resulting from such transfer, help to build the case for a potential, but as yet not clearly defined, threat for subtle teratogenic and other deleterious health effects.

The placental transfer of lead has been established, in part, by various studies that have disclosed measurable quantities of lead in human fetuses or newborns. An analysis of human fetal tissues by Barltrop[357] demonstrated that placental transfer of lead began as early as the 12th week of gestation and that total lead content increased throughout fetal development, with the highest concentrations occurring in bone, kidney, and liver, and lesser, but significant, amounts occurring in blood, brain, and heart. Barltrop has also pointed out that the distribution of lead within the fetus at different stages of development is probably more important than the total amount present at birth.

Of interest in this regard are the data of Schroeder and Tipton,[358] who showed that the mean lead level in brains of stillborn U.S. children (n = 22) was 10 ppm (dry ash), but that there was an undetectable level for normal infants, young children, and teens (0 to 19 years; n = 23). In this study, however, the levels of detection are not stated, so that the relative increase in level cannot be assessed. Also, it is not clear to what extent fetal tissue preparation and preservation were controlled for contamination.

Wibberly and coworkers[359] have recently found that placental lead levels in the case of stillbirth or neonatal death were significantly higher than in the case of normal births. Placental levels were greater than 1.5 μg/g in only 7 percent of the normal births, whereas levels were greater than this in 61 percent of the stillbirths or neonatal deaths. This does not mean that lead is a causal factor in such deaths and could indicate that lead accumulates in the placenta in times of fetal stress.

There are a number of recent studies on the passage of lead through the placental barrier as assessed by lead levels in cord blood and/or maternal blood. For example, in the study of Gershanik et al.,[360,361] 98 cord-blood samples matched with maternal blood samples showed a high correlation between lead levels in infants (mean = 10.1 μg/dl) and their mothers (mean = 10.3 μg/dl), with a product moment correlation coefficient of 0.6377. This suggests that infants may be born with blood lead levels that essentially match those of their mothers. In regard to assessing groups at risk for such prenatal exposure, these authors also studied a group of 218 cord-blood samples (170 urban, 48 rural) and observed that the mean urban value (9.7 μg/dl) was significantly different ($p < 0.05$) from the mean rural value (8.3 μg/dl), suggesting a higher risk of urban newborns for prenatal lead exposure. Similarly, Scanlon[362] sampled cord-blood randomly from normal infants whose mothers had suburban (n = 15) or urban (n = 13) residences. The average urban value was 22.1 μg/dl (10 to 37 μg/dl), whereas the corresponding suburban level was 18.3 μg/dl. Smoking was without significant effect on these levels. These results tend to confirm the findings of Gershanik et al.[361] Harris and Holley,[363] on the other hand, surveyed cord and maternal blood in 24 pairs (11 suburban and 13 urban) and found a mean cord-blood value of 12.3 μg/dl and a mean maternal blood level of 13.2 μg/dl. No significant difference was therefore seen in cord-blood levels as a function of maternal residence, though a larger sample might have yielded significant effects since the ones found were in the same direction as those found in other studies.

That the prenatal exposure of the fetus to lead, even in the absence of teratogenic effects, may be of consequence in regard to adverse health effects is demonstrated by studies relating fetal and cord-blood levels to some changes in fetal heme synthesis and claimed incidences of premature births. Haas et al.[364] examined 294 mother-infant pairs for blood

lead levels as well as for the corresponding urinary ALA levels. The maternal blood mean was 16.89 μg/dl and the fetal blood mean was 14.98, with a correlation of 0.538 ($p < 0.001$). In the infants, the levels of blood lead and urinary ALA were positively correlated ($r = 0.1877$, $p < 0.01$). Whether a biological significance exists here, however, is not clear. According to the authors,[364] the positive correlation between lead in blood and urinary ALA for the group as a whole indicated there was already an effect at lower blood lead levels, i.e., increased susceptibility of heme synthesis.

In a study of Fahim[365] on cord-blood lead levels, blood lead values in pregnant women having preterm delivery and premature membrane rupture, and residing in a lead belt area (mining and smelting area), had significantly higher blood lead levels than women delivering at full term. A confusing aspect of this study, however, is the similarity of blood lead levels in women in the nonlead and lead belt areas. Though a number of other problems may be seen with the analytical aspects of this study, it must be noted that among the 249 pregnant women in the control group outside the lead belt area the percentage of women having preterm deliveries and premature rupture were 3 and 0.4 percent, respectively, whereas the corresponding values for the lead area ($n = 253$) were 13.04 and 16.99 percent, respectively.

With reference to more subtle prenatal effects, Palmisano et al.[366] noted failure to thrive and neurological deficits in a 10-week-old infant whose mother had lead poisoning concomitant with alcoholism during pregnancy. When this infant was challenged with a chelating agent, an abnormal urinary excretion of lead was observed, indicating intrauterine exposure. Postnatal exposure in this case was ruled out. Other, more controlled laboratory studies on animals (discussed later) also suggest that teratogenic effects occur, but usually only at very high lead exposure levels.

A report[367] on fatal birth defects in children conceived during a period of time when their father was lead poisoned hints at important effects of lead on the fetus being mediated via human males as well as females. Certain other studies[369,370] demonstrated likely lead effects on various aspects of male reproductive functions.

Lancranjan et al.[368] have reported that moderately increased lead absorption (blood lead mean = 52.8 μg/dl) resulted in gonadal impairment. The effects on the testes were shown to be direct in that tests for hypothalamopituitary influence were negative. A group of 150 workmen who had long-term exposure to lead in varying degrees was studied. Clinical and toxicological criteria were used to categorize the men into four groups: lead-poisoned workmen (74.5 μg/dl) and those showing moderate (52.8 μg/dl), slight (41 μg/dl), and physiologic (23 μg/dl) absorption of lead. Semen analysis revealed asthenospermia, hypospermia, and teratospermia in lead-poisoned workers and those with moderately increased absorption of lead (blood lead levels = 50 to 80 μg/dl for the latter). The abnormal spermatozoa included binucleated, bicephalus, amorphous, and tapered forms. In contrast, slightly increased or physiologic absorption of lead had no effects on the reproductive ability of workmen.

In the review of Stöfen,[369] data from the work of Neskov in the USSR were reported involving 66 workers exposed chiefly to lead-containing gasoline (organic lead). In 58 men there was a decrease or disappearance of erection, in 41 there was early ejaculation, and in 44 there was a diminished number of spermatocytes.

The literature reviewed here on lead effects on human reproduction and development leaves little doubt as to the fact that lead does, in fact, exert significant adverse health effects on reproductive functions. Most studies, however, have typically looked at the effects of prolonged moderate-to-high exposures to lead, e.g., those encountered in industrial situations, and many reports do not provide definite information on external exposure levels or blood lead levels at which specific effects are observed.

11.7.2 **Animal Studies**

Animal experiments have demonstrated that levels of lead that are compatible with life have interfered with normal reproduction. Many studies assessed the effects of lead exposure of both parents on reproduction. Schroeder and Mitchener,[370] for example, showed a reduction in the number of offspring of rats and mice that were given drinking water containing lead in a concentration of 25 ppm. In a subsequent report,[371] however, it was noted that animals in the earlier study were chromium deficient. No effects were found in animals with normal diets. The combined effect of maternal and paternal oral lead intoxication upon reproductive performance was studied in rats by Morris et al.[372] who reported significant reduction in weaning percentage among offspring of rats fed 512 ppm lead. Stowe and Goyer[373] assessed the relative paternal and maternal

effects of lead as measured by the progeny of F_1 lead-toxic rats. Sprague-Dawley female rats being fed laboratory chow with and without 1 percent lead acetate were bred to normal, mature, Sprague-Dawley males. The pregnant rats were continued on their respective rations with and without lead throughout gestation and lactation. Offspring of these matings, the F_1 generation, were fed the rations of their dams and were mated in combinations as follows: control female to control male (CF-CM), control female to lead-toxic male (CF-PbM), lead-toxic female to control male (PbF-CM), and lead-toxic female to lead-toxic male (PbF-PbM). The results identifying specific deleterious paternal and maternal effects of lead toxicity upon rat reproduction are shown in Table 11-11.

TABLE 11-11. REPRODUCTIVE PERFORMANCE OF F_1 LEAD-TOXIC RATS[373]

Parameter	Type of mating			
	CF-CM	CF-PbM	PbF-CM	PbF-PbM
Litters observed	22	24	36	16
Pups/litter	11.90 ± 0.40[a]	10.10 ± 0.50	8.78 ± 0.30[b]	7.75 ± 0.50[c]
Pup birth weight, g	6.74 ± 0.15	5.92 ± 0.13[c]	5.44 ± 0.13[c,d]	4.80 ± 0.19[c,d,e]
Weaned rats/litter	9.84 ± 0.50	7.04 ± 0.77[c]	5.41 ± 0.74[c,d]	2.72 ± 0.70[c,d,e]
Survival rate, %	89.80 ± 3.20	73.70 ± 7.90	52.60 ± 7.20	30.00 ± 8.20[c,d,f]
Litter birth weight/Dam breeding weight, %	28.04 ± 1.30	22.30 ± 0.90[c]	19.35 ± 1.00[c]	15.38 ± 1.10[c,d,f]
Litter birth weight/Dam whelping weight, %	19.09 ± 0.80	15.97 ± 0.58[c]	14.28 ± 0.66[c]	11.58 ± 0.78[c,d,f]
Gestation gain/Pups per litter, g	11.54 ± 0.60	11.20 ± 0.74	11.17 ± 0.54	12.34 ± 1.24
Nonfetal gestational/Gain/fetus, g	3.93 ± 0.38	4.83 ± 0.47	4.15 ± 0.42	3.96 ± 0.46

[a]Mean ± S.E.M.
[b]Significantly ($p < 0.05$) less than mean for CF-CM.
[c]Significantly ($p < 0.01$) less than mean for CF-CM.
[d]Significantly ($p < 0.01$) less than mean for CF-PbM.
[e]Significantly ($p < 0.01$) less than mean for PbF-CM.
[f]Significantly ($p < 0.05$) less than mean for PbF-CM.

The paternal effects of lead included a 15 percent reduction in the number of pups born per litter, a 12 percent reduction in the mean pup birth weight, and an 18 percent reduction in pup survival rate. The maternal effects of lead included a 26 percent reduction in litter size, 19 percent reduction in mean pup birth weights, and 41 percent reduction in pup survival. The combined male and female effects of lead toxicity resulted in 35 percent reduction in the number of pups per litter, 29 percent reduction in the pup birth weights, and 67 percent reduction in pup survival to weaning. Stowe and Goyer[373] classified the effect of lead upon reproduction as gametotoxic, intrauterine, and extrauterine. The gametotoxic effects of lead appear to be irreversible and had additive male and female components. The intrauterine effects resulted from the transmammary passage of lead from the dam to the suckling pup adding insult to the gametotoxic and uterine environmental effects.

The effects of lead on the reproduction of sexually mature male and female Sesco rats were reported by Hildebrand et al.[374] The animals were orally fed lead acetate at doses of 5 and 100 μg for 30 days. Control females possessed the same levels of lead concentration in their blood as those for male controls. However, for the treated animals the blood lead levels for the females were higher than those for the males: 30 μg/dl versus 19 μg/dl at 5 μg lead acetate, and 53 μg/dl versus 30 μg/dl at 100 μg lead acetate. They noted impotence and prostatic hyperplasia in the males at the lower dose, progressing to testicular damage with inhibition of spermatogenesis in those reaching blood levels of 50 μg/dl. In the females, they noted irregularity of the estrus cycle at both doses. When lead levels reached 50 μg/dl, the female rats developed ovarian follicular cysts with a reduction in the number of corpora lutea. A subsequent study employing Sprague-Dawley rats was unable to replicate these findings.[375]

A number of other studies have focused more specifically on either maternal or paternal lead exposure effects. For example, in reference to maternal effects, histopathological changes in ovaries in lead-poisoned Rhesus monkeys have been demon-

strated.[376] Most other animal studies have utilized rodents.

Kennedy et al.[377] administered an aqueous solution of lead to mice (days 5 to 15 of gestation) and to rats from days 6 to 16 of gestation. At dosage levels of 7.14, 71.4, and 714 mg/kg body weight there were no observed effects on the number of fetuses resorbed or the number of viable fetuses. No teratogenic effects on gross examination were seen, and an effect on body weight was observed only at the highest level employed (714 mg/kg).

Hubermont et al.[378] exposed female rats to lead in drinking water (0.1, 1, and 10 ppm) for 3 weeks before mating, during pregnancy, and 3 weeks after delivery. In the highest exposure group (10 ppm), maternal and newborn blood and kidney lead values were elevated. Inhibition of δ-ALAD and elevation of FEP in tissues were also noted.

Maisin et al.[379] exposed female mice to lead in the diet (0.1 and 0.5 percent) from the day of vaginal plug to 18 days afterwards. The number of pregnancies decreased and the number of embryos succumbing after implantation increased.

Similarly, Jacquet[380] exposed female mice via lead in diet (0.125, 0.25, and 0.50 percent) from vaginal plug to 16 to 18 days afterwards. At the middle dosage, pregnancy incidence decreased, the number of embryos dying before implantation increased, and the number of corpora lutea showed a decrease. At the highest dosage, the number of embryos dying after implantation increased, whereas decreases in body weight of surviving embryos were seen.

Other studies have focused on lead effects on paternal reproductive functions. For example, the data from studies of rabbits,[381] guinea pigs,[382] and rats[373,383] indicate that paternally transmitted effects from lead can occur, including reductions in litter size, in weights of offspring, and in survival rate.

Cole and Bachhuber,[381] using rabbits, were the first to confirm experimentally the paternal effects of lead intoxication. The litters of dams sired by lead-toxic male rabbits were smaller than those sired by control males. Weller[382] similarly demonstrated reduced birth weights and survival among offspring of lead-toxic male guinea pigs.

Verma et al.[384] fed a 2-percent aqueous solution of lead subacetate in drinking water to 14 male Swiss mice for 4 weeks. The total mean intake of lead amounted to 1.65 g. They placed the male with 3 virgin untreated females for 1 week. The overall incidence of pregnancy, indicative of fertility, was 52.7 percent in the control group as compared to 27.6 percent in the treated group. The fertility of the treated males was reduced to 50 percent. They calculated the mutagenicity index (number of early fetal deaths/total implants) to be 10.4 for lead-treated mice versus 2.9 for controls ($X^2 = 10.4$, $p \leq 0.05$).

In the study of Maisin et al.,[379] male mice received 0.1 and 1 percent lead, as the acetate, in the diet. The percentage of abnormal spermatozoa increased with increasing exposure. Ultrastructural changes were present.

In the review of Stöfen,[369] several studies from Russian laboratories were evaluated. As cited by Stöfen, Egorova et al., for example, injected lead at a dose of 2 μg/kg 6 times over a 10-day period and observed damage to testes and spermatozoa. Stöfen also reported that Golubova et al. found morphological changes in testes of rats that received 2 mg lead/kg but not in rats receiving 0.2 mg/kg.

Lead appears to be teratogenic in some species, at least at high exposure levels. McClain and Becker,[385] for example, administered single doses of 25 to 70 mg/kg of lead nitrate intravenously to pregnant rats on days 8 through 17 of gestation. A urorectocaudal syndrom of malformations was produced when lead was administered on the 9th day of gestation. The lead nitrate was increasingly embryo- and fetotoxic when administered on later days of gestation (days 10 to 15) but not teratogenic. Ferm and Carpenter[386] as well as Ferm and Ferm[387] reported increased embryonic resorption and malformation rates when various lead salts were administered to pregnant hamsters on the 8th day of gestation. The teratogenic effect of lead was almost completely restricted to the tail region. Malformations of the sacral and caudal vertebrae, resulting in absent or stunted tails, were observed.

The reasons for the localization of the teratogenic effects of lead are unknown at this time. Ferm and Ferm[387] have suggested that the specificity could be explained by an interference with specific enzymatic events during early development. Lead alters mitochondrial function and enhances or inhibits a variety of enzymes,[388] any or all of which could interfere with normal development. Ferm[389] has also reported that in the presence of cadmium the teratogenic effect of lead in hamsters is potentiated.

Studies by Giliani[390,391] show that lead is teratogenic to chick embryos. When 2-day-old embryos were given varying doses of lead acetate (0.005 to 0.08 mg/egg) and were examined on the

8th day of incubation, congenital cardiac anomalies were demonstrated.[390] The incidence of cardiac anomalies rose with increasing doses of lead. Other important anomalies were reduced body size, micromelia, shortened neck, microophthalmia, ruptured brain, shortened beak, twisted neck and limbs, and everted viscera.[391] The most common developmental anomalies were retarded growth and neck abnormalities. It should be noted that in these studies high, acute doses of lead were administered.

There is a paucity of information regarding the teratogenicity and developmental toxicity of chronic lead exposure. Kimmel et al.[265] exposed female rats chronically to lead acetate via drinking water (0.5, 5, 50, and 250 μg/g) from weaning through mating, gestation, and lactation. No teratogenic effects were observed, although exposure to 250 μg/g lead acetate caused a slight but nonsignificant increase in fetal resorptions. The lead-treated animals produced litters of normal numbers, but the offspring from the 50- and 250-μg/g groups weighed less at weaning and showed delays in physical development. Reiter et al.[264] have also observed delays in the development of the nervous system in offspring exposed to 50 μg/g lead throughout gestation and lactation. Whether these delays in development result from a direct effect of lead on the nervous system of the pups or reflect secondary changes (resulting from malnutrition, hormonal inbalance, etc.) is not clear. Whatever the mechanisms involved, these studies suggest that low-level, chronic exposure to lead may induce postnatal developmental delays in rats.

It should be noted that the above reports on normal developmental delays might be analogous to certain suggested neurobehavioral effects of lead from *in utero* exposures of humans (Section 11.5). In addition, it has been demonstrated[392] that concentrations of lead of approximately 170 μg/dl whole blood can inhibit δ-ALAD activity in both blood and brain of suckling rats. Although brain tissue was not purged of residual blood, ALAD contribution to brain ALAD activity would not be expected to be significant. It is possible that δ-ALAD activity might be diminished *in utero* at these lead levels and that lead at these levels might have harmful consequences on neurological development of the fetus. There is need for more critical research to evaluate the possible subtle toxic effects of lead to the fetus. This overall evaluation in the offspring may need to be correlated with the possible additive effects of paternal lead burden. At this time, however, insufficient evidence exists to allow for firm statements on exposure levels at which any such effects on the fetus from maternal or paternal lead burdens might be observed.

11.8 THE ENDOCRINE SYSTEM

The endocrine effects of lead are not well defined at the present time. Lead is known, however, to decrease the thyroid function in man and experimental animals. Porritt[393] suggested in 1931 that lead dissolved from lead pipes by soft water was the cause of hypothyroidism in individuals living in southwest England. Later, Kremer and Frank[394] reported the simultaneous occurrence of myxedema and plumbism in a house painter. Monaenkova[395] in 1957 observed impaired concentration of ^{131}I by thyroids in 10 out of 41 patients with industrial plumbism. Subsequently, Zel'tser[396] showed that *in vivo* ^{131}I uptake and thyroxine synthesis by rat thyroid were decreased by lead when doses of 2 and 5 percent lead acetate solution were administered. Uptake of ^{131}I, sometimes decreased in men with lead poisoning, can be offset by treatment with thyroid-stimulating hormone (TSH).[397,398] Lead may act to depress thyroid function by inhibiting SH groups or by displacing iodine in a protein sulfonyl iodine carrier,[397] and the results suggest that excessive lead may act at both the pituitary and the thyroid gland itself to impair thyroid function.

Sandstead et al.[399] studied the effects of lead intoxication on the pituitary and adrenal function in man. There was a decrease in secretion of pituitary gonadotrophic hormones. Their data suggested that lead may interfere with pituitary function in man and may produce clinically significant hypopituitarism in some. Its effects on adrenal function were less consistent, but some of the patients showed a decreased responsiveness to an inhibitor (metapyrone) of 11-beta-hydroxylation in the synthesis of cortisol.

Excessive oral ingestion of lead in man has resulted in pathological changes in the pituitary-adrenal axis as indicated by decreased metapyrone responsiveness, a depressed pituitary reserve, and decreased immunoreactive ACTH.[400,401] These same events may also affect adrenal gland function inasmuch as decreased urinary excretion of 17-hydroxycorticosteroids was observed in these patients.

Suppression of responsiveness to exogenous ACTH in the zona fasciculata of the adrenal cortex has been reported in lead-poisoned subjects,[402] and impairment of the zona glomerulosa of the adrenal cortex has also been suggested.[403]

There also is some evidence suggesting that lead

may cause a derangement in serotonin metabolism or utilization. Tryptophan is the precursor of the neuroendocrine regulatory amine, serotonin. An effect of lead on serotonin synthesis or utilization is inferred in part from the observation of Urbanowicz et al.[404] who reported a rise in 5-hydroxyindole acetic acid (5-HIAA) excretion in the urine of workers heavily exposed to lead. This rise preceded the rise in urinary δ-ALA and coproporphyrin. A similar rise in 5-HIAA excretion was noted in moderately lead-exposed workers.[322] More recently, however, Schiele et al.,[405] using a different analytical method, were unable to find any significant elevation in 5-HIAA excretion.

11.9 THE HEPATIC SYSTEM

The effect of lead poisoning on liver function has not been extensively studied. In a laboratory study of 301 workers in a lead smelting and refining facility, Cooper et al.[406] found serum glutamic oxalacetic transaminase (SGOT) activity at an increased value of 11.5 percent in subjects with blood lead levels below 70 μg/dl, 20 percent in those with a blood lead level of about 70 μg/dl, and 50 percent in workers with a blood lead level of about 100 μg/dl. The correlation between blood lead levels and SGOT was not statistically significant. In the absence of information on the possible influence of diet, infection, or personal habits, however, the authors were unable to draw any definite conclusions concerning the etiology of these changes.

The liver is the major organ for the detoxification of drugs. In acute lead poisoning, the mixed-function oxidase system of liver endoplasmic reticulum is impaired.[407] The activity of this enzyme system, involved in the hepatic biotransformation of medicaments, hormones, and many environmental chemicals, is closely related to the availability of the microsomal hemoprotein, cytochrome P-450.[408] It has been shown that in rats lead induces inhibition of heme synthesis and, therefore, causes a reduction in cytochrome P-450 levels, with consequent impairment of the mixed-function oxidase system.[409] Drug-metabolizing activities were significantly decreased in the lead-poisoned animals. Intensity and duration of these changes were dose dependent. *In vivo* experiments, based on the duration of pentobarbital sleeping time, provided further evidence for the inhibition of drug metabolism in lead-poisoned rats. These data would suggest than an enhanced sensitivity to xenobiotics (drugs, pesticides, food additives, etc.) should be expected to occur in lead-poisoned animals. Alvarez et al.[410] studied the effect of lead exposure on drug metabolism in children and adults. There were no differences between two normal children and eight lead-poisoned children in their capacities to metabolize two test drugs, antipyrine and phenylbutazone. This might suggest that low plasma concentrations of lead do not have an effect on the hepatic cytochrome P-450-dependent enzymatic activities in children. In 2 acutely poisoned children, in whom plasma levels of lead exceeded 60 μg/dl, antipyrine half-lives were significantly longer than normal, and therapy with EDTA led to biochemical remission of the disease and restoration of deranged drug metabolism toward normal.

Hepatic drug metabolism in eight adult patients showing marked effects of chronic lead intoxication on the erythropoietic system was studied by Alvarez et al.[411] The plasma elimination rate of antipyrine, which, as noted above, is a drug primarily metabolized by hepatic microsomal enzymes, was determined in eight subjects prior to and following chelation therapy. In seven of eight subjects, chelation therapy shortened the antipyrine half-lives, but the effect was minimal. The two authors concluded that chronic lead exposure results in significant inhibition of the heme biosynthetic pathway without causing significant changes in hepatic cytochrome P-450-associated enzymatic activities.

11.10 THE CARDIOVASCULAR SYSTEM

Under conditions of long-term exposure at high levels, arteriosclerotic changes have been demonstrated in the kidney. In 1963, Dingwall-Fordyce and Lane[82] reported a marked increase in the cerebrovascular mortality rate among heavily exposed lead workers as compared with the expected rate. These workers were exposed to lead during the first quarter of this century when working conditions were quite bad. There was no similar increase in the mortality rate for men employed more recently.

Hypertension is an important element in the etiology of cerebrovascular deaths. Tabershaw and Cooper[412] did an epidemiological study of 1267 workers who had been exposed to lead as a result of their occupation in either the battery or lead smelting industry between 1947 and 1970. Many were found to have blood lead concentrations in excess of 80 μg/dl. The authors concluded that there was excess mortality associated with only two categories of illness, chronic nephritis and hypertension. The increased incidence of hypertension in lead workers has also been reported by Monaenkova and Glotova[413] and Vigdortchik.[414] On the other hand,

Cramer and Dahlberg[415] studied the incidence of hypertension in a population of 364 industrially exposed men, 273 of whom had a long-term exposure to lead. They subdivided the workers into lead-affected and nonlead-affected groups based on the urinary coproporphyrin test. There was no statistically significant difference between the groups nor was the incidence higher than that expected for nonexposed men in the general population. Other reports on the question do not show hypertension to be unduly prevalent among lead workers.[350,416] It is not clear, therefore, whether the vascular effects of lead in man are direct effects on blood vessels or whether the effects are secondary to renal effects.

There are conflicting reports regarding whether lead can cause atherosclerosis in experimental animals. Sroczynski et al.[417] observed increased serum lipoprotein and cholesterol, and cholesterol deposits in the aortas of rats and rabbits receiving large doses of lead. On the other hand, Prerovska,[418] using similar doses of lead given over an even longer period of time, did not produce atherosclerotic lesions in rabbits.

Structural and functional changes of the myocardium have been noted in children with acute lead poisoning, but, to date, the extent of such studies has been very limited. Cases have been described in adults and in children, always with clinical signs of poisoning. There is, of course, the possibility that the coexistence of lead poisoning and myocarditis is coincidental. In many cases in which encephalopathy is present, the electrocardiographic abnormalities disappeared with chelation therapy, suggesting that lead may have been the original etiological factor.[419-421] Silver and Rodriguez-Torres[421] noted abnormal electrocardiograms in 21 of 30 children (70 percent) having symptoms of lead toxicity. After chelation therapy, the electrocardiograms remained abnormal in only four (13 percent) of the patients. Electron microscopy of the myocardium of lead-intoxicated rats has shown diffuse degenerative changes.[422] In a review of five fatal cases of lead poisoning in young children, degenerative changes in heart muscle were reported to be the proximate cause of death.[91] It is not clear that such morphological changes are a specific response to lead intoxication. Kosmider and Petelnz[423] examined 38 adults over 46 years of age with chronic lead poisoning. They found that 66 percent had electrocardiographic changes, which was 4 times the expected rate for that age group.

Makasev and Krivdina[424] observed a two-phase change in the permeability of blood vessels (first, increased permeability; second, decreased permeability) in rats, rabbits, and dogs that received a solution of lead acetate. A phase change in the content of catecholamines in the myocardium and in the blood vessels was observed in subacute lead poisoning in dogs.[425] This effect appears to be a link in the complex mechanism of the cardiovascular pathology of lead poisoning.

11.11 THE IMMUNOLOGIC SYSTEM

Recent reports suggest that exposure to lead may interfere with normal susceptibility to infection. Hemphill et al.[426] found that mice injected with subclinical doses of lead nitrate for 30 days showed greater susceptibility to challenge with *Salmonella typhimurium* than controls that received a saline injection containing no lead. Selye et al.[427] found that rats injected with lead acetate (minimal effective dose of 1 mg/100 g body weight) were susceptible to a variety of bacterial endotoxins (toxins produced by the bacteria themselves) to which this species is ordinarily resistant. Administration of lead acetate in drinking water to male mice from 4 weeks of age to sacrifice at 9 to 12 weeks old increased the toxic response of the mice to 5 classes of viruses against which it was tested.[428,429] These viruses were an RNA picornavirus (encephalomyocarditis), a DNA herpesvirus (pseudorabies), an RNA leukemia virus (Rauscher leukemia), and RNA arbovirus B (St. Louis encephalitis), and an RNA arbovirus A (western encephalitis).

Among the factors that may be involved in producing this decreased resistance to infection is the decreased production of antibodies. Williams et al.[430] reported that lead binds antibodies *in vitro* and could potentially do so *in vivo*. Chronic exposure to mice of lead acetate in drinking water produced a significant decrease in antibody synthesis, particularly gamma globulins.[431]

Phagocytosis (ingestion of foreign material by a cell specialized for that purpose) by alveolar macrophages is believed to be an important step in the removal of dust particles and bacteria from the respiratory tract. Consequently, the activities of alveolar macrophages are important aspects of pulmonary defense. Bingham et al.[432] found that the continuous inhalation of lead sesquioxide aerosol (10 $\mu g/m^3$ to 150 $\mu g/m^3$) by rats for 3 to 12 months significantly reduced the number of alveolar macrophages. Electron microscopic examination of the lungs of rats that had inhaled particulate lead oxide (200 $\mu g/m^3$) for 14 days revealed ultrastruc-

tural damage (mitochondria and endoplasmic reticulum) to the alveolar macrophages and the type I alveolar epithelial cells. Biochemically, a considerable loss in the activity of the benzopyrene hydroxylating enzyme in the alveolar macrophages was observed by Bruch et al.[433]

Few studies have been made of the effects of lead on the immunologic system in man. Reigart and Graber[434] studied 12 preschool children having elevated free erythrocyte protoporphyrin and blood levels $\geq$ 40 μg/dl and seven nonlead-burdened children for evidence of impairment of their immunological responses. They found no differences between the control group and the lead-exposed group with reference to complement levels, to immunoglobulins, or to anamnestic response to the tetanus toxoid antigen.

Hicks[435] points out that there is a need for systematic epidemiological studies on the effects of elevated lead levels on the incidence of infectious diseases in man. The paucity of information cannot support the formulation of any dose-response relationship at this time.

11.12 THE GASTROINTESTINAL SYSTEM

Colic is usually a consistent early symptom of lead poisoning, warning of much more serious effects that are likely to occur with continued and prolonged lead exposure. Although most commonly seen in industrial exposure cases, colic is also a lead poisoning symptom present in infants and young children.

Beritic[436] reported on the cases of 13 of 64 men exposed on their jobs to occupational levels of lead. The 13 had colic, probably lead related, and constipation. They had blood lead levels ranging from a little less than 40 to 80 μg/dl as determined by polarography, a technique which tends to yield values lower than the actual blood levels. The diagnosis of lead-caused colic was supported by findings of high urinary coproporphyrin, excessive basophilic stippling, reticulocytosis, and some degree of anemia, all of which are other clinical signs of lead poisoning.

Although these symptoms are well documented in the literature, there are insufficient data by which to establish a dose-response relationship for an effect of lead on the gastrointestinal system.

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