Pathological Effects of Lead

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I. Introduction—Concern about Lead

Lead is one of man's oldest poisons. Although pathological effects of lead have been recognized for more than 2000 years, questions related to the distribution of this metal in the environment and potential adverse health effects are of present-day concern. The pathological effects of lead may be manifested in several ways (Goyer, 1971a). Traditional episodes of acute and chronic lead intoxication still occur even with abundant public information regarding the toxicity of lead. However, many cases are the result of unwary exposure to sources such as glazes or adulterated whiskey, and will be difficult if not impossible to eliminate entirely. There has been a high incidence of overt lead intoxication among lead industry workers, but protection and surveillance of these workers is undergoing continuing improvement.

A larger and more serious health problem related to lead is the clinical and subclinical intoxication of thousands of young children living in old, dilapidated housing. This risk, once thought to be largely confined to slum areas in the largest cities, may exist in any town with old dwellings (Clark and Hallett, 1971).

The pathological effects of lead that may have the greatest significance are those related to the body budren of lead, that is, the amount of lead present in body stores in all members of society. The body burden of lead and related factors are discussed in Section III (metabolism of lead). The question of greatest concern is whether the body content of lead prevalent in the general population is in any way harmful to health. There is no evidence at present to indicate that lead serves any essential or even useful function in the body. Although, it is clear that lead is a toxic element, it is not known at what level of lead minimal toxic effects occur and how to measure that level.

In this review we shall consider the pathology of lead with emphasis on cellular effects and on factors influencing lead metabolism. Other reviews of various aspects of health effects of lead have recently been published (Committee Report, 1971; Chisolm, 1971; Goyer and Chisolm, 1972; Goyer, 1971c; Hammond, 1969).

II. Lead in the Environment

Current concern for adverse effects of lead on human health stems from an apparent increase in the content of lead in some portions of the environment, which has resulted from increased use of lead. Consumer use of lead has nearly doubled since 1940, and only about one-third represents recycling. The electric storage battery industry is the largest user of lead in the United States, but the greatest increase in use has been in leaded gasoline. More than 20% (250,000 tons) of the total lead consumed per year is for leaded gasoline. On the other hand, use of lead in paint has been steadily declining. Other consumer uses that may have brought on health-related problems include the use of lead for ceramic glazes and of lead arsenate as an insecticide.

Emission from the internal combustion engine totals about 200,000 tons per year and is the largest single factor in the redistribution of lead from natural sources to biological systems. All other sources of emission of lead in the United States equal less than 4000 tons, or less than 2% of the lead emitted from gasoline exhausts (Committee Report, 1971).

A scheme of the ecological paths by which lead enters the metabolism of man is shown in Fig. 1. As far as is known, the form of lead in the environment is inorganic lead. There is no evidence that a lead alkyl or "methyl" lead is synthesized in nature or is produced in a stable form as a product of any industrial process. Organic lead compounds employed as gasoline fuel additives are converted to halides by halogens added to automobile fuels as scavengers to facilitate removal of lead from auto engines. Lead is less active chemically than some other heavy metals, e.g., mercury, and naturally occurring organic forms are unknown. Because of the greater toxicity of organic forms of lead it is important to be able to recognize any such compounds if they do occur naturally.

Lead from industrial and atmospheric sources is eventually deposited in the soil. Inorganic lead is only slightly soluble and fixation by the clay of normal soil converts added lead to insoluble, inactive compounds. Very acid soils increase the solubility of lead compounds (Gilbert, 1957). Soluble lead compounds eventually wash into seawater, where they enter the natural marine cycle. It has been calculated that industrial lead is

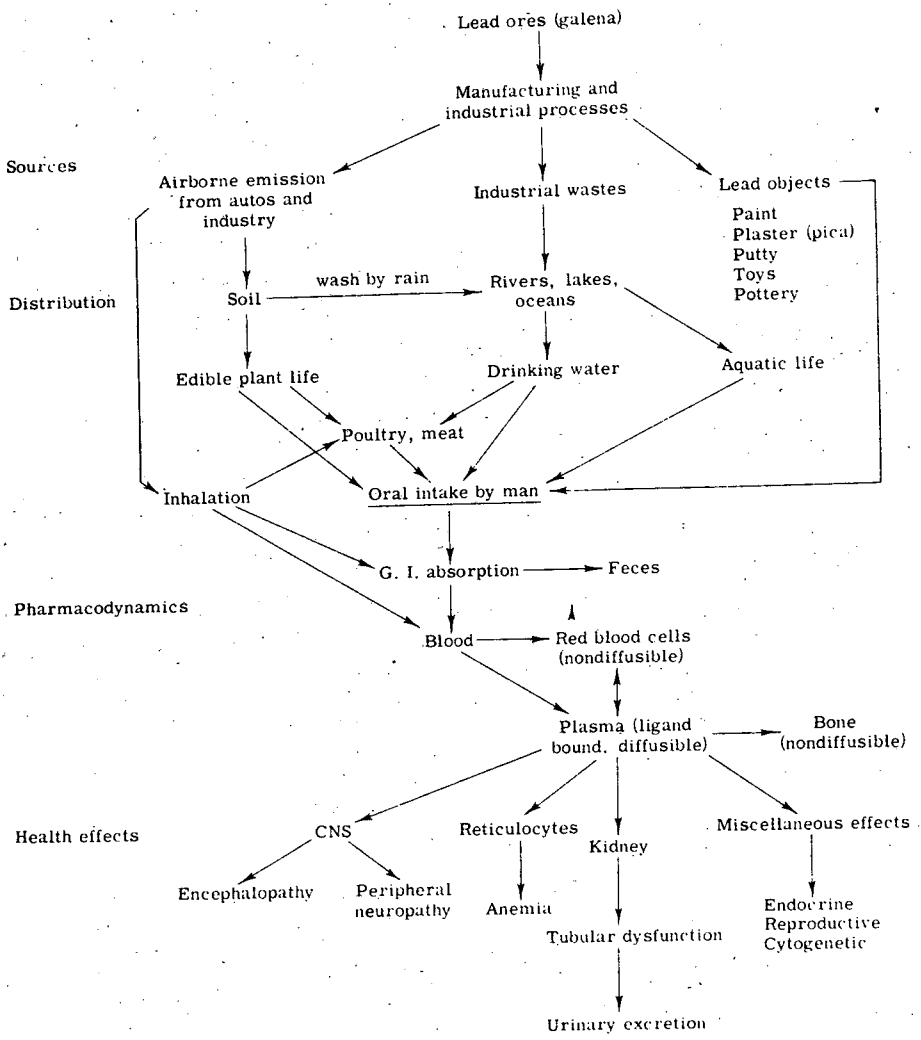


FIG. 1. Ecodiagram showing movement of lead from the environment to man. From Goyer and Chisolm (1972), by permission of Academic Press, New York.

now being added into the oceans at about ten times the rate of introduction by natural weathering (Tatsumoto and Patterson, 1963).

Lead content of arctic snow is also thought to reflect increasing contamination of the atmosphere and water (Murozumi *et al.*, 1969). There has been an increasing lead content of layers of snow deposited during

the last century, a steep incline occurring during the past 20 years (Fig. 2). Concentrations of other trace minerals in samples tested remain constant.

Increase in lead content of plants and lower forms of animal life might be expected as a natural sequel to increased lead in soil and water. Observations of this trend are sparsely documented. Ruhling and Tyler (1968) found chronological increases in lead concentrations in Swedish mosses from 1860 to 1968. These increases are thought to be related to greater burning of coal from 1875 to 1900 and the use of leaded gasoline from 1950 to 1968.

Of particular concern to man is the incorporation of lead in food and vegetables. Approximately 50% of the lead emitted from automobile exhausts is deposited within 30 meters of the roadway (Singer and Hanon, 1969). A number of studies have shown that grasses, garden vegetables, and tree leaves contain from 100 ppm to 3000 ppm (washed speci-

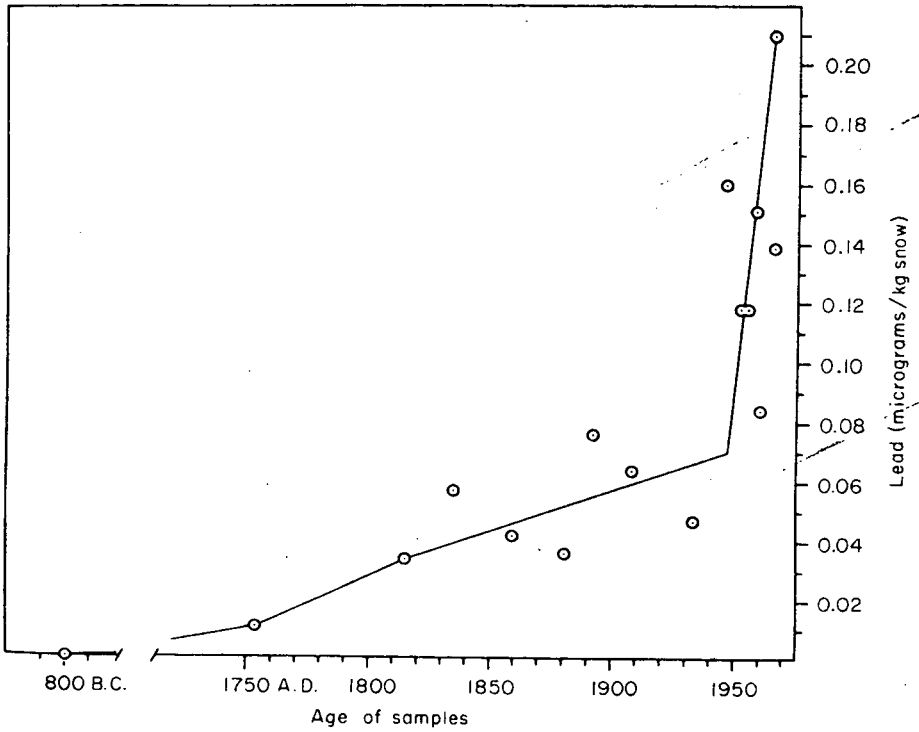


FIG. 2. Increase in lead content of arctic snow with time since 800 B.C. From Murozumi *et al.* (1969). Reproduced from *Geochim. Cosmochim. Acta*, by permission of Pergamon, New York.

mens) (Cannon and Bowles, 1962; Chow, 1970; Connor *et al.*, 1971). Incorporation of lead in plant life in sufficient concentrations may have an adverse effect on the growth of plants (Koeppe and Miller, 1970; Miller and Koeppe, 1971), but more important, lead in food sources contributes to the content of lead in animal life, particularly man.

III. Balance and Retention—Body Burden of Lead

A. Quantitative Aspects of Intake, Output, and Retention

The daily lead intake of healthy adult persons in the United States varies from less than 0.10 mg per day to more than 2 mg per day and may average as little as 0.12 or as much as 0.50 mg per day. From balance studies on normal healthy male volunteers, Kehoe (1961) concluded that daily intake of lead for an individual without excessive exposure to lead was about 330 μg, and that persons with this level of intake were in approximate balance; about 10% was excreted in the urine (30 μg day) and the remainder appeared in the feces (300 μg).

The major source of daily lead intake is food and beverages. Sampling of food and water shows that lead content is extremely variable (Kehoe, 1961; Crawford and Morris, 1967). A recent study of lead content of food items in Germany suggests that there are practically no lead-free foods (Lehnert *et al.*, 1969), and a study from Milan, Italy suggests that lead ingested orally from foods and beverages may equal 400–500 μg per day (Zurlo *et al.*, 1970). Concentration of lead in a number of food items, including beverages, from the United States averages 0.2 μg gm (Patterson, 1965).

Drinking water has long been suspected of being a source of excessive dietary lead. Since lead pipe is no longer used for household plumbing, the lead content of most municipal water supplies measured at the tap is somewhat below the WHO recommended limit of 0.05 μg ml. Nevertheless, assuming the maximum permissible content of lead were present in water and assuming a daily water intake of about 2 liters (including foods and beverages), as much as one-third (100 μg) of daily lead intake may be ingested with water. It is not likely, however, that many people consume more than 1 liter of drinking water per day with a lead content of 20 μg liter; this is equivalent to only 20 μg day of lead from drinking water.

The measurable quantities of lead in body tissues in persons without symptoms of lead toxicity have been referred to as body burden of lead. Total body burden of lead increases with age from less than 2 mg for

children under 10 years of age to over 200 mg for persons in their eighth and ninth decades (Barry and Mossman, 1970). Absorbed lead is distributed in bone and various soft tissues, and estimates of total body burden in adults who are not occupationally exposed range from approximately 100 to 400 mg (Kehoe, 1961; Schroeder and Tipton, 1968; Barry and Mossman, 1970; Thompson, 1971), and average 240 mg for the "standard" 70-kg man (Barry and Mossman, 1970). Asymptomatic, occupationally exposed lead workers have, as expected, higher total body lead content, ranging to just over 500 mg in Barry's study. Thompson (1971) estimates that this level of lead accumulation can be accounted for by a retention of less than 4% of the average daily intake or about 10 μg per day.

A critical question is the extent to which the modern average daily intake of lead may be exceeded without producing symptoms. Kehoe (1961) found that the addition of 1.0–2.0 mg of lead per day to the diet of normal human volunteers will increase blood lead levels to the toxic range in about 9 months. This level of lead exposure, however, is not experienced by nonoccupationally exposed persons, and total daily intake of less than 1 mg will not result in overt lead toxicity in the normal adult. Young children appear to tolerate much smaller levels of lead exposure, although studies directly comparable to Kehoe's experiments with adults have not been performed on children. Nevertheless, an ad hoc committee formed by the Bureau of Community Environment Management (DHEW) has recommended that the daily permissible intake of lead from all sources for children under 3 years of age be limited to 300 μg (King, 1971). Lead intake above this level will not be entirely excreted, and significant accumulation in the body occurs. Total daily ingestion of 300–650 μg will result in increases in blood lead levels[2] above 40 μg/100 gm and may result in increases in urinary *d*-aminolevulinic acid (*d*-ALA) reflecting impairment of heme metabolism.

B. Routes of Absorption and Excretion

1. *Gastrointestinal System*

Net absorption of lead by the gastrointestinal tract in humans is 5–15% or less; the remaining lead is excreted in the feces (Kehoe, 1961). Studies with feeding large doses in rats suggested that percentage of absorption or retention of lead decreases as the dosage of lead increases, but that, nevertheless, increased dosage or exposure to lead does result in increased retention (Goyer *et al.*, 1970b). Little is known about the mechanism for gastrointestinal absorption of lead, but it is likely that the phosphate

[2] Blood lead content will be expressed per 100 gm of whole blood; 100 gm of whole blood is equivalent to 105 ml of whole blood.

content of the diet and perhaps the content of other minerals, such as iron and calcium, may influence efficiency of lead uptake.

2. *Pulmonary Tract*

The contribution of airborne lead to body lead content is dependent on multiple factors and is difficult to measure accurately. The two most important factors with regard to the fate of the inhaled particles appear to be size and distribution in the respiratory tract. Most atmospheric lead in urban environments consists of particulate lead compounds from exhaust emission of automobiles. Lead particle size in urban air varies over a wide range (0.16–0.43 μ) and has an average mass median equivalent diameter (MMED) of 0.25 μ (Robinson and Ludwig, 1967; Lee *et al.*, 1968). A model for estimation of dust deposition in the respiratory tract has been proposed by the Task Group on Lung Dynamics (1966). The respiratory tract may be described as three functional areas: the nasopharnyx (N-P); the tracheobronchial tree including the terminal bronchioles (T-B); and a pulmonary compartment (P) consisting of respiratory bronchioles, alveolar ducts, atria, alveoli, and alveolar sacs. Only a very minor fraction of particles under 0.5 μ in MMED are retained in the (N-P) or (T-B) compartments. The remainder are cleared by ciliary action of respiratory epithelial cells and swallowed. The studies of Nozaki (1966) on the influence of respiratory rate and particle size on deposition of lead in the respiratory tract showed that 22–63% of particles 0.1–1.0 μ in MMED are deposited in the lung. The percentage of particles less than 0.5 μ retained in the lung increases with reduction in particle size. It is estimated that nearly 90% of lead particles in ambient air that are deposited in the lung are small enough to be retained (Committee Report,

TABLE I

BLOOD LEAD LEVELS OF ADULTS: INFLUENCE OF ENVIRONMENT AND GEOGRAPHIC LOCATION

Occupations in U.S.A.[a]	μg/100 ml
Suburban nonsmokers, Philadelphia	11
Suburban smokers, Philadelphia	15
All policemen, Cincinnati	25
Service station attendants, Cincinnati	28
Traffic police, Cincinnati	30
Tunnel employees, Boston	30
Parking lot attendants, Cincinnati	34
Garage mechanics, Cincinnati	38

[a] Hammond, 1969.

1971). Assuming 37% deposition of lead-containing particles from ambient air (average MMED of 0.25 μ) and 24-hour volume of inhaled air to be 15 m^3, airborne lead only contributes 15 μg/day at atmospheric concentrations of lead at 3 $\mu g/m^3$. At atmospheric concentrations of lead below 2–3 $\mu g/m^3$, inhaled lead probably does not contribute significantly to blood lead levels (Committee Report, 1971). There is some evidence, however, that higher concentrations of airborne or atmospheric lead may influence blood lead levels (Table I). Estimates of respiratory inhalation of airborne lead particles by an individual in downtown Cincinnati may be as high as 30–40 μg/day, whereas a person in a rural environment might inhale less than 1 μg/day (Goldsmith and Hexter, 1967).

Retention by the pulmonary compartment may not be equivalent to absorption. Recent experimental evidence indicates that a portion of particles retained in alveoli can be expected to be cleared by pulmonary macrophages (Bingham *et al.*, 1968). However, the quantitative aspects and mechanisms for pulmonary clearance of inhaled lead are not entirely understood.

3. *Renal Excretion*

Renal excretion of lead is presumed to involve two routes, glomerular filtration and transtubular flow or excretion. Experimental evidence supporting these mechanisms is from the study of Vostal and Heller (1968) on the urinary excretion of simultaneously injected radioisotopes of inulin and lead in the renal portal circulation of the chicken. Studies to date give no indication of the relative importance of the two routes; also, there is no knowledge of the extent to which tubular reabsorption of lead influences renal lead excretion.

C. Blood Lead

Whole blood lead levels in children and adults without excessive exposure to lead vary from 15 to 40 μg 100 gm. Whether these levels represent a "normal" or "physiologic" blood level regardless of body burden or absence of environmental exposure to lead is uncertain. Patterson (1965) has calculated that without "contamination" of the environment with lead, a natural blood lead level should be 0.25 μg 100 gm, or about one-hundredth of current levels. On the other hand, present day blood lead levels are only about one-half of levels generally regarded as toxic (50–80 μg 100 gm). The significance of blood lead levels is discussed in Section IX. The significance of the rural–urban blood lead gradient has been debated (Bazell, 1971; Committee Report, 1971; Goldwater and Hoover, 1967; Hammond, 1969).

Lead contained in blood is in two forms. More than 90% is bound to red blood cells and is nondiffusible; the remainder is bound to microligands in the plasma. Since the major fraction of whole blood lead is bound to red blood cells, hematocrit or packed red blood cell volume will greatly influence total blood lead content and must be considered in the interpretation of blood lead levels (Williams, 1966). The manner in which lead binds to red blood cells has been reviewed by several investigators. It was long ago suggested that lead reacts with inorganic phosphate (Aub *et al.*, 1925) or glycerophosphate (Maxwell and Bischoff, 1929) groups in the red blood cell membrane. *In vitro* studies of lead binding in red blood cells by Clarkson and Kench (1958) showed that 95% of lead chloride added to whole blood is rapidly attached to the cells. More than 90% of a lead chloride solution added to plasma (free of red blood cells) remained in solution whereas lead added to Krebs-Ringer solution precipitated. They suggest that lead forms a peptized lead phosphate sol in the plasma. The peptized sol then combines with the red cell by coagulation or flocculation on the cell surface. Competitive binding by other heavy metals such as Cu, Hg, and Zn does not occur. Also, lead is removed from red blood cells *in vitro* only very slowly by chelating agents such as EDTA.

Barltrop and Smith (1971) have separated lead-containing fractions of red blood cells by Sephadex gel filtration and ultracentrifugation. Lead was found bound to cell contents rather than stroma. A lead-containing protein fraction with a mean molecular weight of about 240,000 was isolated by gel filtration. Binding of lead by low-molecular weight fractions has also been suspected.

The binding of lead to a small diffusible or microligand in plasma may be argued from the demonstration that lead does traverse cell membranes (Castellino and Aloj, 1969). This fraction is, of course, most important with respect to lead transport and tissue content of lead.

D. Organ Content of Lead

Lead content of individual organs in control persons without excessive exposure to lead and persons with lead intoxication is shown in Table II. The largest concentration of lead is usually in the bone, where lead is bound in a nondiffusible form. Only the diffusible lead fraction from plasma passes in and out of capillaries, permeates cell membranes, and enters parenchymal cells of the central nervous system, liver, kidneys, and other organs. The relatively large content of lead in liver and kidney may be related to the excretory function of these two organs, whereas only trace amounts of lead are present in muscle and brain. The major

TABLE II

LEAD CONTENT OF TISSUE FROM 15 PERSONS WITH NO ABNORMAL EXPOSURE TO LEAD (CONTROLS) AND PERSONS DYING FROM INORGANIC AND ORGANIC (TETRAETHYL) LEAD INTOXICATION[a]

Tissue	Controls[b]	Lead intoxication	
		Inorganic[c,d]	Organic[c,e]
Bone	0.67–3.59	5.6–17.6	2.9
Liver	0.04–0.28	1.8–8.0	2.35–3.4
Kidney	0.02–0.16	0.6–5.5	0.79
Spleen	0.01–0.07	1.13	0.29
Heart	0.04	0.2–0.8	—
Brain	0.01–0.09	0.24–1.2	0.74–1.9

[a] Values are milligrams per 100 gm of wet tissue, range or single value from references cited.
[b] Kehoe (1961).
[c] Cumings (1967).
[d] Thienes and Haley (1964).
[e] Cassells and Dodds (1946).

symptoms of lead intoxication are related to the content of lead of soft tissue, particularly the hematopoietic system, the liver, and the kidneys, and factors which enhance lead content of these organs may influence susceptibility to lead toxicity. These factors are discussed in Section X.

As can be seen from Table II, exposure to an organic form of lead is likely to result in rapid accumulation of lead in the tissues most sensitive to the toxic effects of lead, particularly the central nervous system (Cassells and Dodds, 1946; Cumings, 1967). Experimental studies have shown that tetraethyllead is converted to triethyllead and inorganic lead (Cremer, 1959; Cremer and Callaway, 1961). Triethyllead is relatively stable and becomes rapidly distributed between brain, liver, kidney, and blood (Bolanowska, 1968).

IV. Cellular Response to Lead

A. Intranuclear Inclusion Bodies

A characteristic cellular reaction in lead intoxication is the formation of discrete, dense-staining, intranuclear inclusion bodies. These were

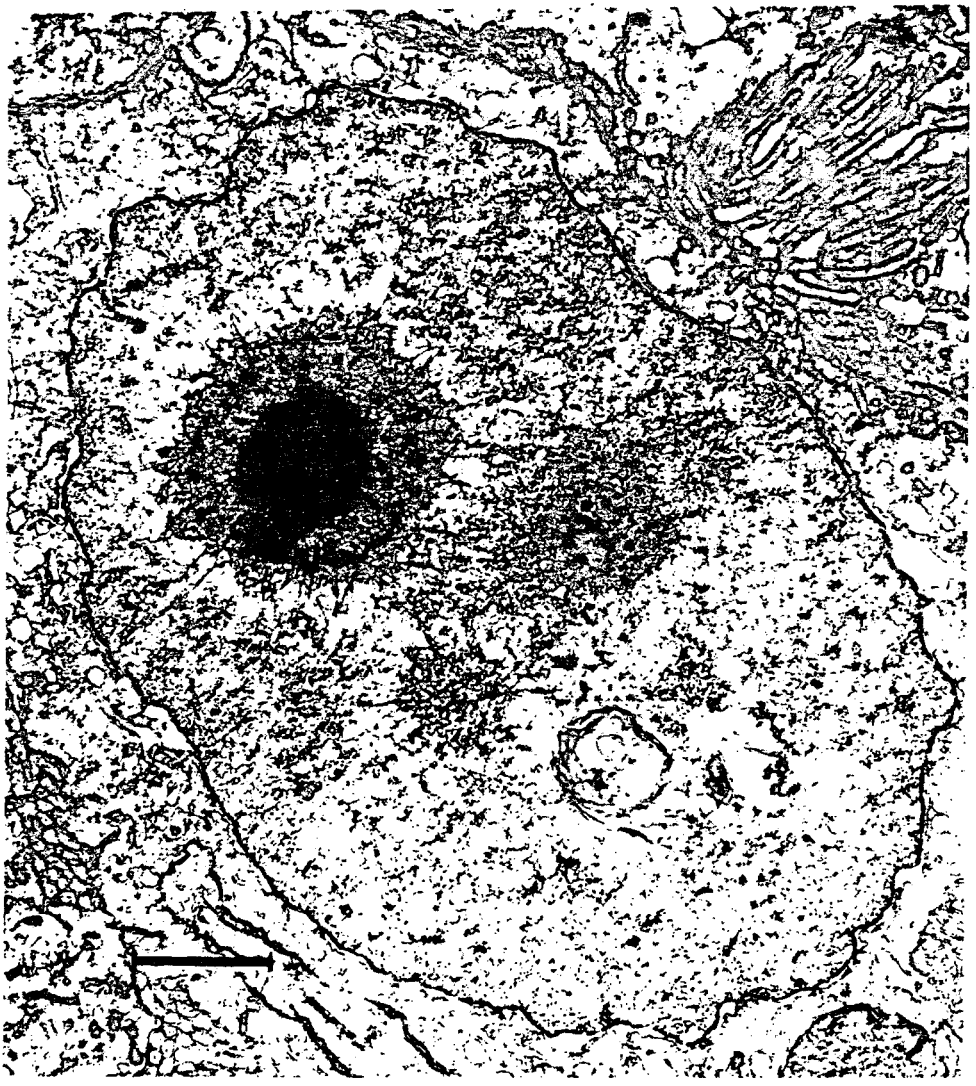


FIG. 3. Nucleus of proximal renal tubular lining cell contains lead-induced inclusion body with dense central core and outer fibrillary zone. Nucleolus is to the right of inclusion body. A small or incipient inclusion body is adjacent to membranous whorls. ×17,000. From Goyer *et al.* (1970c). Reproduced from *Lab. Invest.*

initially observed by Blackman (1936) some 35 years ago in hepatic parenchymal cells and renal tubular lining cells of children dying of acute lead encephalopathy. These findings have been confirmed many times, in man and in animals. Among more recent reports may be cited those of Watrach and Vatter (1962) (liver and renal tubules of swine), of Stowe (1972) (dogs), of Hass *et al.* (1964) (rabbits), of C. F. Simpson *et al.* (1970) (fowl), and of Goyer (1968) (rat kidneys).

The ultrastructure of a typical inclusion body in the nucleus of a renal tubular lining cell of a lead-intoxicated rat is shown in Fig. 3. It consists of a dense central core and an outer fibrillary zone. The inclusions are always independent of nucleoli. Inclusion bodies vary considerably in size and in ratio of core to outer fibrils (Bracken *et al.*, 1958; Beaver, 1961; Richter *et al.*, 1968). Richter *et al.* (1968) estimate the thickness of the fibrils to range from 100 Å to 130 Å, although the size of fibrils varies with fixation and staining procedures. A definite substructure (periodicity) has not been observed in the fibrils. Similar inclusion bodies are found in bismuth intoxication (Pappenheimer and Maechling, 1934), but the inclusion bodies induced by lead have distinguishing characteristics. Wachstein (1949a) has shown that they differ from viral inclusion bodies by being acid-fast when stained with the Ziehl-Neelsen technique. Inclusion bodies associated with ingestion of bismuth are also acid-fast (Wachstein, 1949b), but their ultrastructural appearance differs from the lead-induced inclusion body in that they tend to be spherical and homogeneous and sharply circumscribed (Beaver and Burr, 1963). Also, bismuth-induced bodies appear in mitochondria as well as in nuclei (Goyer and Rhyne, 1972).

The composition of lead-induced intranuclear inclusion bodies has been studied by histochemistry, autoradiography and direct analysis after isolation. Histochemical studies have been summarized by Richter *et al.* (1968). Inclusion bodies do not stain with the Feulgen reaction, although they are sometimes surrounded by Feulgen-positive material. They do not stain with fast green after treatment with trichloroacetic acid, but do stain strongly with mercuric bromophenol blue and with basic fuchsin, which suggests that they contain protein, but probably not histones. The basis of the acid-fast reaction is uncertain; it may simply be a physical phenomenon. Landing and Nakai (1959) showed that the inclusions contain at most small amounts of lipid, and they suggested that the acid-fast properties of these bodies were related to the presence of sulfhydryl groups in the protein.

The presence of lead in the inclusion bodies was suspected but unproved for many years. Finner and Calvery (1939) stained sections of kidneys

FIG. 4. Intranuclear inclusion bodies isolated from kidneys of lead-poisoned rats. ×15,000. From Goyer *et al.* (1970*c*). Reproduced from *Lab. Invest.* Copyright © 1970, William & Wilkins, Baltimore, Maryland.

from lead-poisoned rats with hydrogen sulfide and found particles of lead sulfide occurring in nuclei of tubular epithelia, presumably corresponding to intranuclear inclusion bodies. Other attempts to identify the metal either histochemically or by X-ray diffraction have been unsuccessful (Watrach and Vatter, 1962; Molnar and Gueft, 1964). More recently, lead within the intranuclear inclusion body has been positively demonstrated by autoradiography (Dallenbach, 1965) and electron-probe X-ray microanalysis (Carroll *et al.*, 1970).

The structure and composition of the inclusion bodies have been further studied after isolation by differential centrifugation and sonication, combining the method for isolating nuclei (Chauveau *et al.*, 1956) with the Blobel and Potter (1966) method for isolation of nucleoli (Goyer *et al.*, 1970c). Isolated inclusions appear exactly as they do in the intact cell (Fig. 4). Treatment of inclusions in tissue sections (Richter *et al.*, 1968) or if isolated inclusion bodies (Goyer *et al.*, 1970c) with proteolytic enzymes results in partial digestion, but they are not altered by incubation with

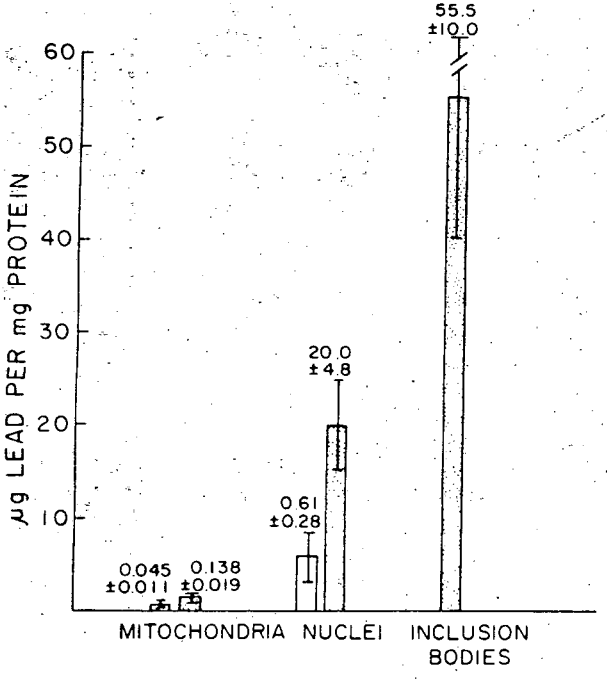


FIG. 5. Lead content of organelles isolated from kidneys of control rats (□) and rats fed diet containing 1% lead acetate for 15–17 weeks (□). From Goyer (1971a), by permission of *Amer. J. Pathol.*, Durham, North Carolina.

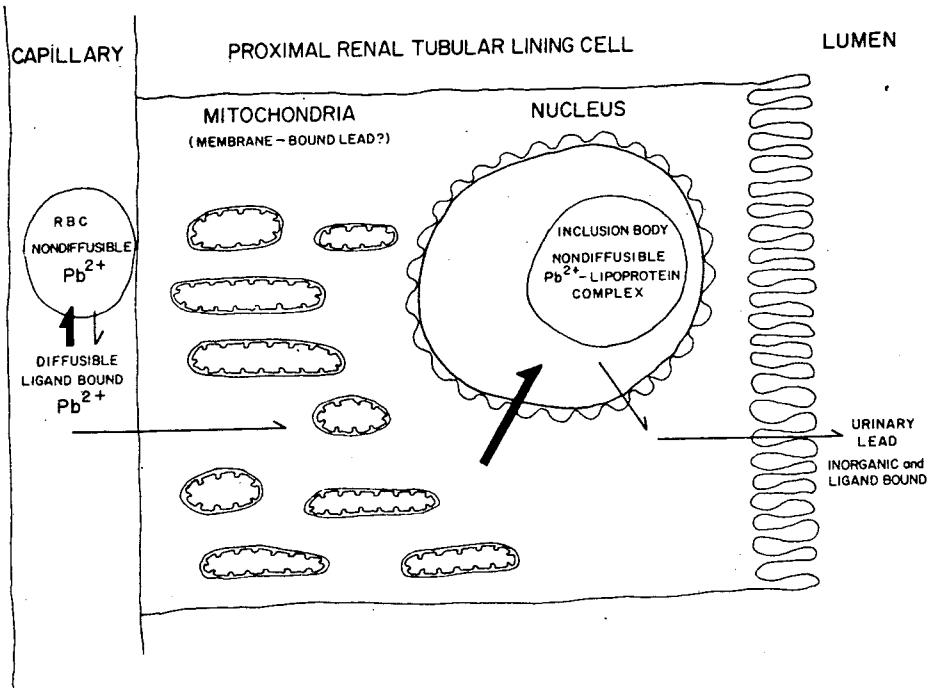


FIG. 6. Scheme for role of intranuclear inclusion body. From Goyer (1971a), by permission of *Amer. J. Pathol.*, Durham, North Carolina.

DNase or RNase. Direct chemical analysis of isolated inclusion bodies confirms that they are composed of a lead–protein complex containing approximately 50 μg of lead per milligram of protein. Lead within the inclusion bodies is 60–100 times more concentrated than in whole kidney (Goyer *et al.*, 1970b; Horn, 1970). Amino acid composition and solubility characteristics of the inclusion body protein resemble those in the residual acidic fraction of proteins in normal nuclei (Goyer *et al.*, 1971).

The lead content of organelles isolated from renal tubular lining cells of control and lead-poisoned rats is shown in Fig. 5. The largest increment occurs in nuclei in which lead is present as part of inclusion bodies. Although intracellular lead becomes bound to mitochondrial membranes and mitochondrial function is sensitive to the toxic effects of lead, the increment in mitochondrial lead in renal tubular lining cells of leaded rats is relatively small when compared to lead of inclusion bodies. Barltrop *et al.* (1971) have shown that most of a single parenteral dose of radioactive lead is bound to mitochondria, little to lysosomes.

A scheme has been proposed for the possible role of the intranuclear

inclusion body in lead poisoning (Fig. 6) (Goyer, 1971a). It is suggested that the inclusion body serves as an adaptive or protective mechanism during transcellular transport of lead. In the course of excretion of lead from liver sinusoids to bile via hepatic cells, or by transtubular flow in renal tubular lining cells, a portion of the lead enters the nucleus where it is bound into a lead–protein complex and becomes no longer diffusible. This mechanism has the effect of maintaining a relatively low cytoplasmic concentration of lead and, therefore, of reducing the toxic effects of lead on sensitive cellular functions, such as mitochondrial respiration and protein synthesis.

Indirect support for this hypothesis is found in experimental studies of the relationship of the formation of intranuclear inclusion bodies and

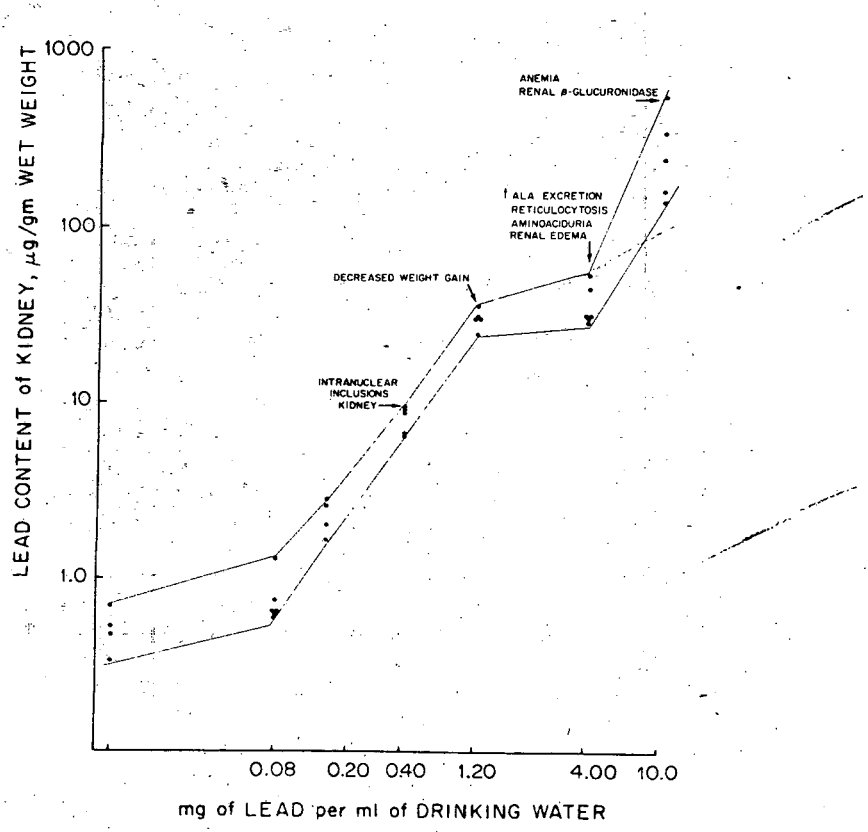
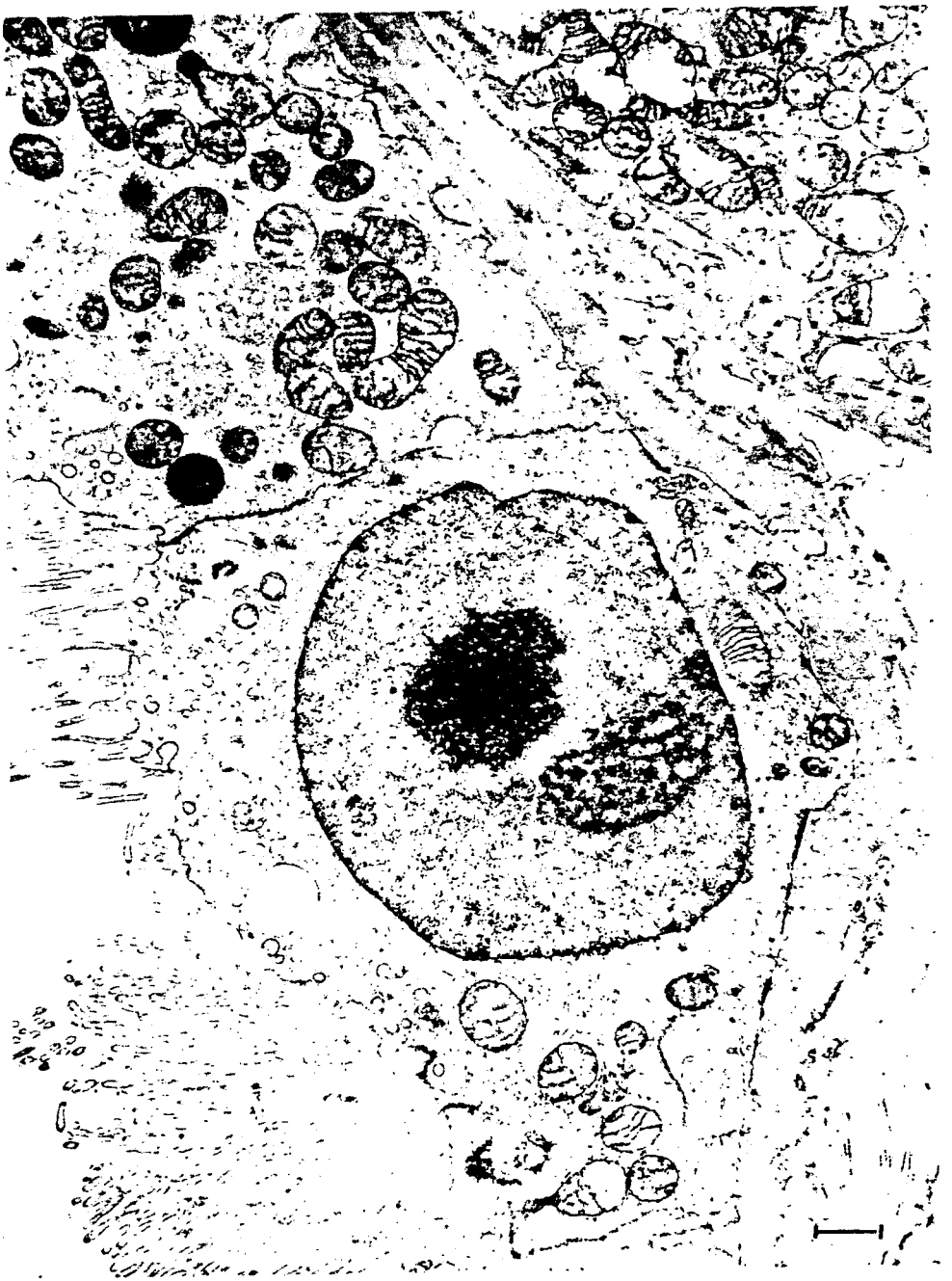


FIG. 7. Correlation of lead content of kidney and different doses of lead fed to rats for 10 weeks with various parameters of lead toxicity. Modified from Goyer *et al.* (1970b).



other renal effects of lead. When groups of rats are given different concentrations of lead in their drinking water, intranuclear inclusion bodies are observed at a lower dose than is any other renal effect of lead—in fact, at a lower dose of lead than that which produced signs and symptoms of lead toxicity (Fig. 7). Inclusion bodies appear to be related to renal lead content and discernible in the rat models when kidney lead concentration is between 10 and 20 μg/gm wet weight.

Also consistent with the hypothesis is the finding of intranuclear inclusion bodies in renal biopsies of two lead industry workers with excessive lead exposure (Fig. 8). Both workers had subclinical lead toxicity and complained of weakness, nausea, and some abdominal colic; they had blood lead levels of about 100 μg/100 gm of blood (Goyer and Cramér, 1971).

Inclusion bodies may be useful in the diagnosis of lead poisoning since they can be identified in renal biopsy material (Angevine *et al.*, 1962) of adults or by examination of acid-fast stained smears of urinary sediment in children (Landing and Nakai, 1959). The latter may be a helpful screening technique for identifying the effect of lead on the kidney in persons with excessive exposure to lead.

B. Mitochondrial Effects of Lead

Lead has a strong affinity for mitochondria and mitochondrial membranes. After a single parenteral injection of radioactive lead an equilibrium is established between the lead content of intracellular organelles of liver and kidney within 72 hours (Castellino and Aloj; 1969). Whether binding of lead to mitochondria is confined to mitochondrial membranes, or whether lead penetrates mitochondrial compartments is uncertain. Lead bound to mitochondria resists removal by washing with 0.25 *M* sucrose.

The lead-binding sites on mitochondria are presumed to be the reactive groups on amino acids for which metal binding has been demonstrated *in vitro*. These are believed to include the carboxyl and α-amino groups of lysine, the imidazole group of histidine, the phenoxy group of tyrosine, the sulfhydryl group of cysteine, and the guanidinium group of arginine

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Fig. 8. Proximal renal tubular lining cell in renal biopsy from worker with industrial exposure to lead. Nucleus contains lead-induced inclusion body adjacent to nucleolus. Mitochondria contain distorted cristae. ×8400. (Biopsy material obtained by K. Cramér, M.D., Goteborg, Sweden.)

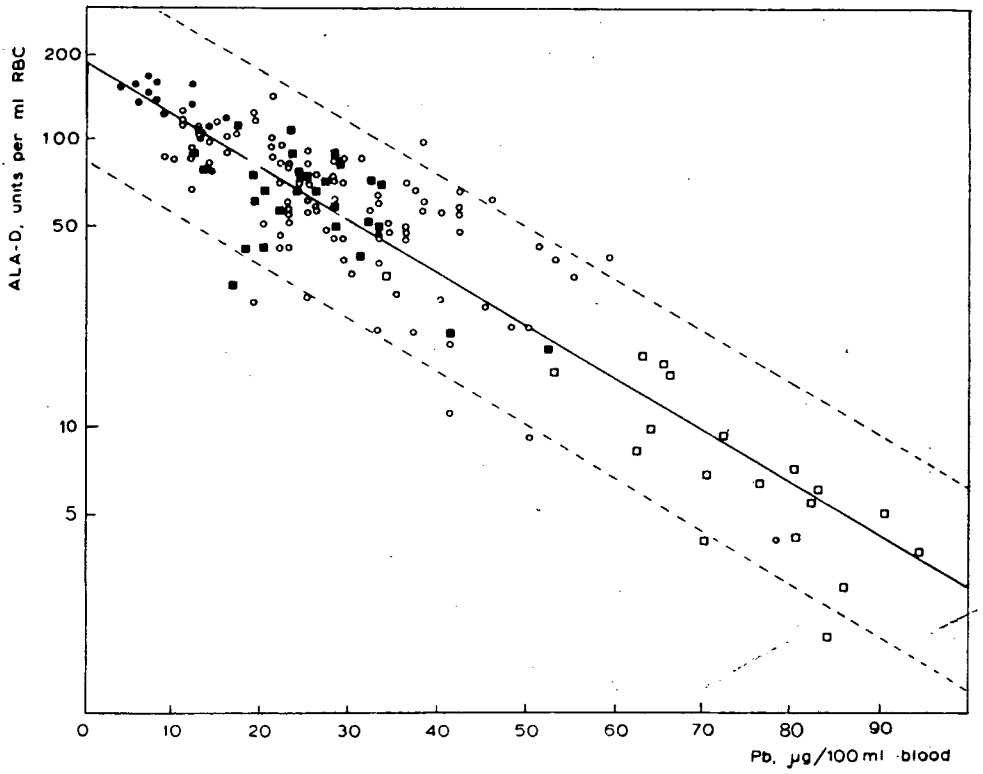


FIG. 27. Correlation between *d*-aminolevulinic acid dehydratase (ALA-D) and blood lead of 158 persons representing different degrees of natural and occupational exposure to lead. Note logarithmic scale on ordinate. ●, medical students; ○, workers in printing shops; ■, automobile repair workers; □, lead smelters and shipscrapers. From Hernberg *et al.* (1970), by permission of the American Medical Association, Chicago, Illinois.

X. Factors Influencing Susceptibility or Dose Response to Lead

Among the most important unanswered questions about pathological effects of lead are: How much lead is harmful? and, Why do specific clinical manifestations of lead poisoning tend to develop in particular clinical circumstances? The recognition of synergistic and antagonistic factors which influence the toxicity of lead is essential to effective control of the consequences of environmental lead on human health. Such understanding must, of course, be predicated on knowledge of the mechanisms of lead toxicity. The extensive literature about lead contains many clues, and impressions of various factors, both adverse and beneficial, which influence

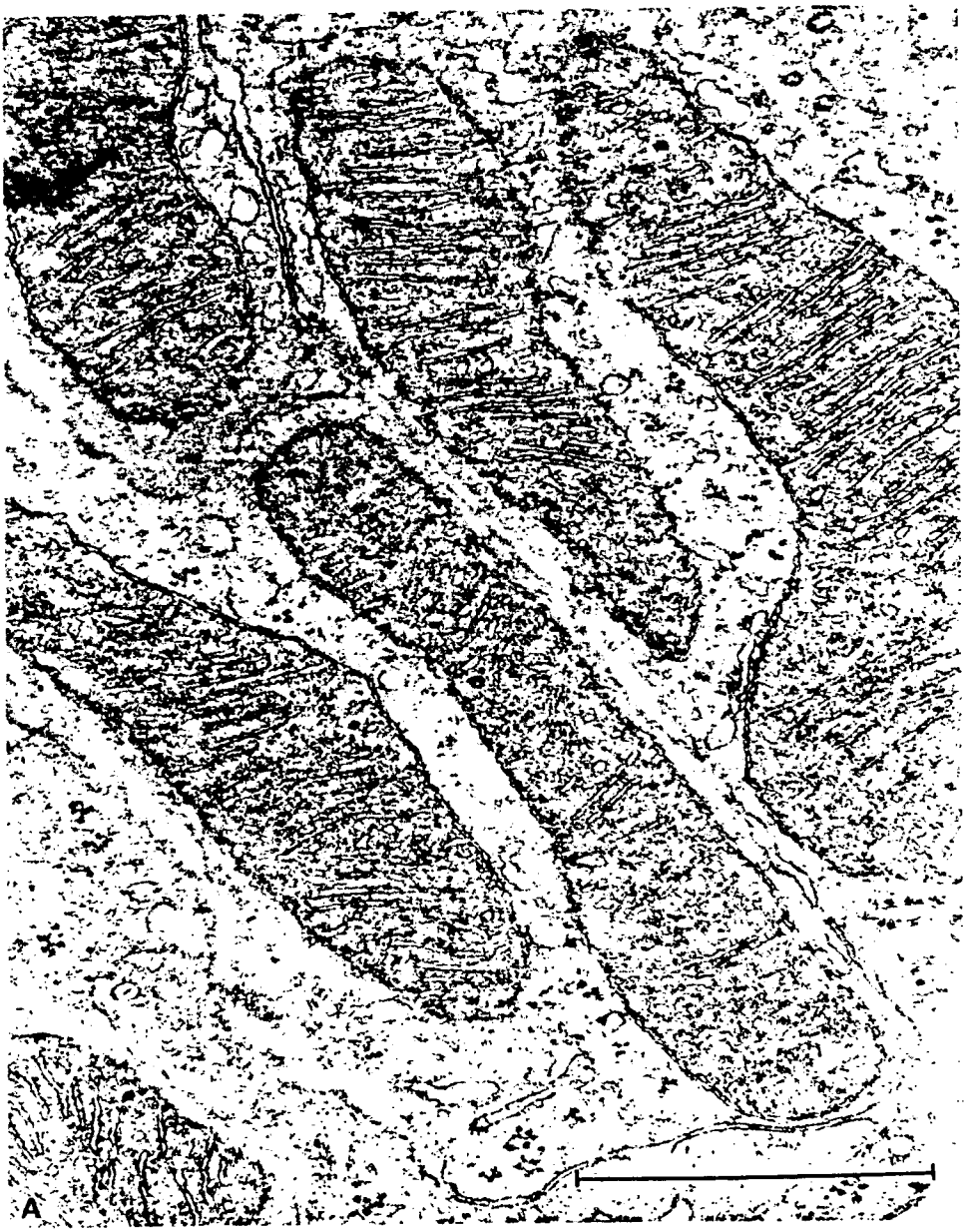


FIG. 9A. Mitochondria in proximal renal tubular cell. Control rat. Mitochondria are oblong and contain numerous cristae. ×46,000. From Goyer and Rhyne (1972), by permission of Academic Press, New York.

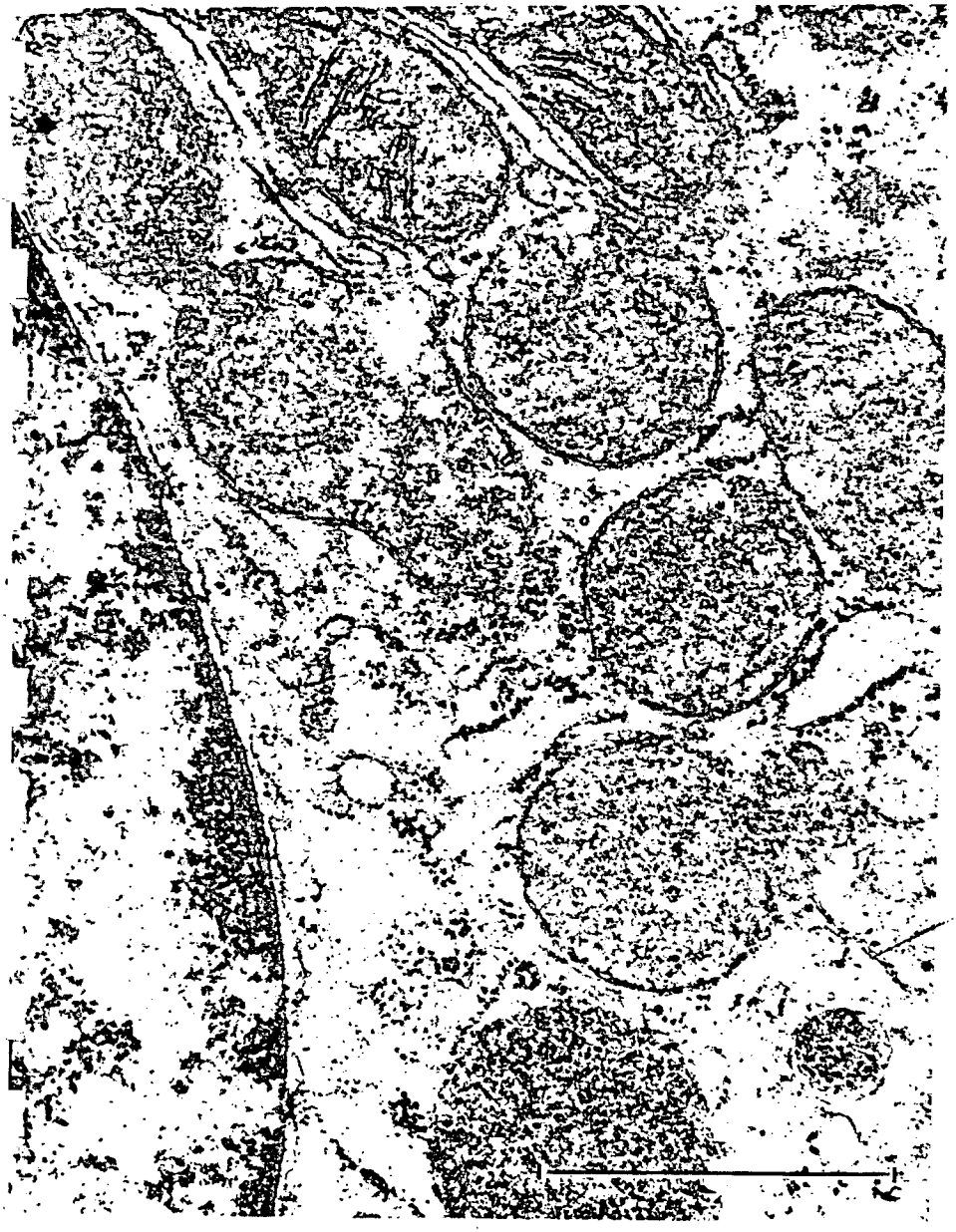


FIG. 9B. Rat fed diet containing 1% lead for 10 weeks. Mitochondria are swollen and contain few cristae. ×46,000. From Goyer and Rhyne (1972), by permission of Academic Press, New York.

(Vallee and Wacker, 1970). If rats are pretreated with subtoxic amounts of lead, mitochondrial binding by radioactive lead is decreased, which suggests that lead binding sites may become saturated (Barltrop *et al.*, 1971).

Binding of lead to mitochondrial membranes as occurs in lead intoxication results in ultrastructural alterations. Mitochondria in proximal renal tubular lining cells of children and adults and of experimental animals with lead poisoning show swelling and dilution of matrical granules (Goyer, 1968) probably secondary to increased membrane permeability (Figs. 9A and B). Such changes are similar to the nonspecific swelling that occurs in the early stages of other forms of cellular injury and is probably reversible. The fate of such mitochondria is uncertain, but increased numbers of myelin figures and autophagocytosis suggest an increased rate of turnover.

Inclusion bodies as observed in nuclei in lead poisoning do not occur in mitochondria. However, an intramitochondrial lamellar-type of crystalloid has been reported in liver parenchymal cells of lead-poisoned swine (Watrach, 1964). These are composed of intersecting bands of fine, closely packed arrays that run parallel and perpendicular to form a lattice pattern. Such lamellar formations are not specific for lead injury, but are characteristic of chronic cellular injury accompanying a number of metabolic abnormalities such as diabetes mellitus, ammonium intoxication, and biliary obstruction as well as experimentally induced hypoxia (Shiraki and Neustein, 1971). Mitochondria containing lamellar formations often show varying degrees of enlargement, alterations in density of matrix, and decrease in number and length of cristae.

Mitochondria isolated from liver and kidneys of lead-intoxicated experimental animals have impaired respiratory and phosphorylative abilities. Teras and Kakhn (1967) found decreased respiratory rates in mitochondria of rabbits treated with daily intraperitoneal injections of lead acetate (10–20 mg/kg body weight) for 7 months. ADP-stimulated respiration in mitochondria from lead-treated rats was depressed, as determined with three different substrates: α-ketoglutarate, pyruvate, and succinate. Phosphorylation was also decreased. Teras and Kakhn suggested that respiratory impairment is most pronounced with pyruvate as substrate.

Studies with mitochondria isolated from kidneys of lead-poisoned rats have shown a specific impairment in respiratory and phosphorylative abilities in pyruvate-dependent respiration as evidenced by altered respiratory control (RCR) and ADP:O ratios (Table III). Slightly higher state IV and lower state III rates of toxic mitochondria in the presence of pyruvate suggest partial uncoupling of phosphorylation. These func-

TABLE III

RESPIRATORY AND PHOSPHORYLATIVE ABILITIES OF MITOCHONDRIA ISOLATED FROM KIDNEYS OF CONTROL RATS AND RATS FED 1% LEAD FOR 10 WEEKS[a]

Mitochondria	Substrate	μAtoms O_2/gm protein per minute			
		State IV	State III	Respiratory control	ADP:O
Control	Pyruvate	43.2	130.3	3.3	2.7
Lead	Pyruvate	46.5	101.8	2.2	2.1
Control	Succinate	76.9	178.4	2.4	1.8
Lead	Succinate	62.4	140.0	2.4[b]	1.8[b]

[a] Modified from Goyer and Krall (1969a).
[b] No significant difference between lead and control mitochondria.

tional parameters which are reflected by RCR and ADP:O ratios are not different from the control mitochondria in succinate-dependent respiration (Goyer and Krall, 1969a, b). Decreased oxygen uptake rate for both state III and state IV in succinate-supported respiration in lead-intoxicated mitochondria may be evidence of decreased succinic oxidase enzyme which correlates with decreased mitochondrial protein (Rhyne and Goyer, 1971). It is suggested that the pyruvate-NAD reductase portion of the transport system is a site sensitive to effects of lead. A recent report indicates that, for NAD-coupled respiration, magnesium restores oxidative ability and dinitrophenol sensitivity to lead-intoxicated mitochondria, but that magnesium has no effect on succinate-supported respiration (Krall and Dougherty, 1971).

Ulmer and Vallee (1969) concluded that lead acts specifically with dithiol bonds in the lipoamide dehydrogenase of the pyruvate dehydrogenase system, but this was validated only by work *in vitro*. This site is sensitive to other metals, such as mercury and cadmium, but it is uncertain whether it is accessible to lead *in vivo*.

It is probable that mitochondria from erythroblastic cells in bone marrow of lead-intoxicated rats are also functionally impaired. However, it has not been possible to isolate from these cells quantities of mitochondria sufficient for direct analysis. Nevertheless, respiratory rates of reticulocytes from lead-intoxicated rats are less than normal (Lessler *et al.*, 1968).

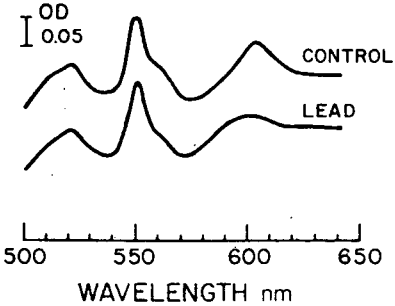


FIG. 10. Difference spectra of cytochromes from mitochondria from control rats and rats fed lead-containing diet for 10 weeks. From Rhyne and Goyer (1971), by permission of Academic Press, New York.

C. EFFECTS OF LEAD ON PROTEIN SYNTHESIS

The effect of lead on cellular content of heme proteins, such as cytochromes, catalase, and peroxidases, has received limited attention. Lead has been found to be a more potent inhibitor of globin and heme synthesis than several other metals, e.g., Sb, Hg, Al, Cu, Au (Borsook *et al.*, 1957). In an attempt to compare effects of lead poisoning on hemoglobin and other cellular porphyrin moieties, Prader and Vannotti measured levels of hemoglobin and cytochrome *c* in rabbit liver (Vannotti, 1955). In contrast to decreased hemoglobin values, the cytochrome *c* level remained constant. Measurement of the total cytochrome content of rat kidney mitochondria by difference spectra showed a selective decrease in cytochrome aa_3 in lead poisoning (Rhyne and Goyer, 1971) (Fig. 10). Whether this decrease results from inhibition of mitochondrial protein synthesis which is reflected by decrease of cytochrome aa_3 or from specific inhibition of the cytochrome aa_3 heme moiety, has not been resolved. The protein moiety as well as the heme moiety differs for each class of cytochromes, so inhibition of either fraction would result in a decrease in the cytochrome.

There is also some information to suggest that lead may affect protein synthesis in a more general way, at the level of the ribosome. Waxman and Rabinovitz (1966) and Ulmer and Vallee (1969) observed that lead causes disaggregation of polyribosomes. The latter workers also reported inhibition by lead of leucine incorporation into tRNA in *Escherichia coli*. Purified RNA is depolymerized by lead (Farkas, 1968).

D. Cytogenetic Effects of Lead

Nuclear polyploidy and abnormalities in mitosis have been noted in bone marrow cells by a number of authors (Waldron, 1966), but chromosomal aberrations in lead intoxication have only recently been recognized. Chromosomes from leukocyte cultures from mice fed a diet containing 1% lead acetate show an increased number of gap-break type aberrations usually involving only single chromatids (Muro and Goyer, 1969). Similar gap-break types are also found in lead workers with blood lead levels ranging from 62 to 88 μg/100 gm. In addition, a variety of nonspecific changes in chromosome morphology was also present, such as adhesions and spiralizing defects. Tetraploid mitoses and the mitotic index were also increased in the workers. Moreover, the percentage of abnormal mitoses correlated very well with urinary *d*-ALA excretion (Schwanitz *et al.*, 1970). Similar studies have not been performed on acutely intoxicated children, nor is the mechanism for this lead effect known. Lead, like other heavy metals, does have an affinity for nucleic acid binding, but whether lead becomes biochemically bound to chromosomes *in vivo* is not known.

V. Neuropathology of Lead

A. Encephalopathy

The nervous system is particularly sensitive to the toxic effect of lead. Children with lead intoxication who are brought to hospitals or emergency rooms for medical aid invariably manifest some central nervous system effect. Symptomatology varies from ataxia to stupor, coma, and convulsions. Adults with acute lead intoxication may also present with encephalopathy, but less commonly than children. Encephalopathy in adults usually follows acute and heavy overexposure to lead, particularly to fumes or vapor, or to an organic form of lead. More often, adults experience chronic forms of lead intoxication, such as occur with occupational exposure, and develop peripheral neuropathy manifested by weakness and palsy of an upper or lower limb.

In spite of the seriousness of the clinical effects of lead on the central nervous system, less is known about lead-induced lesions of the nervous system than about renal or hematopoietic effects. The most prominent pathologic changes noted in the brain are cerebral edema associated with

an increase in cerebrospinal fluid (CSF) pressure, proliferation and swelling of endothelial cells accompanied by dilatation of capillaries and arterioles, proliferation of glial cells and focal necrosis and neuronal degeneration. These changes have been given different emphasis by various authors. In acute fulminating cases, edema and increase in CSF pressure seem paramount. Aub and co-workers (1925) stressed the importance of the meningeal effects of lead resulting from the passage of lead from blood to CSF, which in turn alter either the mechanisms for secretion or reabsorption of CSF. Inflammatory cells may be observed in the meninges. Severe cases may present as acute communicating hydrocephalus due to inflammation of pia-arachnoid or choroid plexus (Mirando and Ranasinghe, 1970). Elevations in CSF may result from increased stimulation of secretion by choroid plexus or interference with reabsorption by the arachnoid mater. Other reports have stressed the significance of swelling of endothelial cells in capillaries and arterioles of meninges and cerebral and cerebellar cortical tissue (Freifeld, 1928; Blackman, 1937; Popoff *et al.*, 1963; Okazaki *et al.*, 1963; Pentschew, 1965). These vascular effects may contribute to cerebral swelling (Smith *et al.*, 1960). The changes in capillaries resemble the angioblastic response seen in other toxic and inflammatory processes and are sometimes associated with extravasation of red blood cells or perivascular hemorrhages.

Pentschew (1965) believes swelling of capillary endothelial cells to be the most constant change in lead encephalopathy. Electron microscopy of these cells shows cytoplasmic swelling with increase in number and size of intracytoplasmic vesicles (Raimondi *et al.*, 1966). Again, these changes are nonspecific. Cortical blood vessels may contain basophilic deposits (Blackman, 1937). Histochemically, these deposits give a positive reaction for iron (Smith *et al.*, 1960).

Another common feature of lead encephalopathy is a diffuse astrocytic proliferation in the gray and white matter. Pentschew (1965) makes the interesting observation that the astrocytic proliferation and less common microglial proliferations are not related to neuronal degeneration, but may be a primary toxic reaction. Also, in some cases the glial response is focal with formation of astrocytic aggregates and microglial nodules. In autopsied cases the extent of neuronal loss in cortical gray matter is variable but does, of course, provide a morphological basis for the permanent sequelae that follow acute lead encephalopathy. Neuronal loss may also have a patchy distribution and may accompany focal areas of necrosis in cortical gray matter as well as in the thalamus and basal ganglia.

Not all children dying of acute lead encephalopathy have discernible histologic changes. No apparent abnormality could be found by Pentschew (1965) in three of 20 autopsied cases. In such cases cerebral edema must

be the major factor in determining the clinical outcome. Nevertheless, in spite of the detailed descriptions of human autopsy material, the pathogenesis of lead-induced encephalopathy is not well understood. The primary vascular change must account for the cerebral edema and increase in intracerebral pressure. The role of altered CSF dynamics in the cerebral edema is unclear. Although cerebral edema is regarded as a characteristic feature of lead encephalopathy, Pentschew (1965) points out that patients may come to autopsy without significant edema and has suggested that edema may be a consequence of convulsive episodes.

The cause of neuronal damage in lead encephalopathy is not clear. One explanation is that it is secondary to the vascular effects, change in hemodynamics, and hypoxia, but on the other hand, little is known about the role of the direct toxic effect of lead on the central nervous system. Lead concentrations in the brain are not as high as in other organs, such as liver and kidney, which manifest toxic signs. The highest concentrations of lead are in the cortical gray matter and basal ganglia, and there is much less lead in the cortical white matter (Klein *et al.*, 1970). These findings suggest a relationship between the most marked histologic changes and lead concentration, and suggest that lead may, in fact, have a direct toxic effect on neurons as well as stimulate astrocytic and glial responses.

Exact simulation of human lead encephalopathy in experimental animals is not entirely possible. The major criticisms of most experimental models are that very large doses of lead are required to produce central nervous system effects and that there is a paucity or even absence of clinical manifestation of central nervous system toxicity in these models. An added difficulty is that the nature of the pathological lesion differs in different animals. An experimental model closely resembling the morphological alterations occurring in humans with lead toxicity has been described by Pentschew and co-workers in suckling Long-Evans rats when their mothers are fed a diet containing 4% lead carbonate (Pentschew and Garro, 1966; P. Lampert *et al.*, 1967). Paraplegia of hind legs occurs about 20–30 days after birth. The cerebellum of these animals contains numerous small petechial hemorrhages. Abnormal vascular permeability was demonstrated by intravenous injections of Trypan Blue and Thorotrast. Staining of the brain was most striking in the corpus striatum, occipital cortex, the cerebellum, and lower spinal cord. Thorotrast passed between, but not through, endothelial cells and throughout intercellular spaces between glial and neuronal cells and processes. Proliferation of microglial cells and astrocytes as well as endothelial cells was observed. Astrocytes showed an increase of glial filaments, ribosomes, and mitochondria, features thought to be characteristic of reactive, proliferating cells (Fig. 11). Neurons were well preserved but foci of axonal degeneration surrounded by proliferating

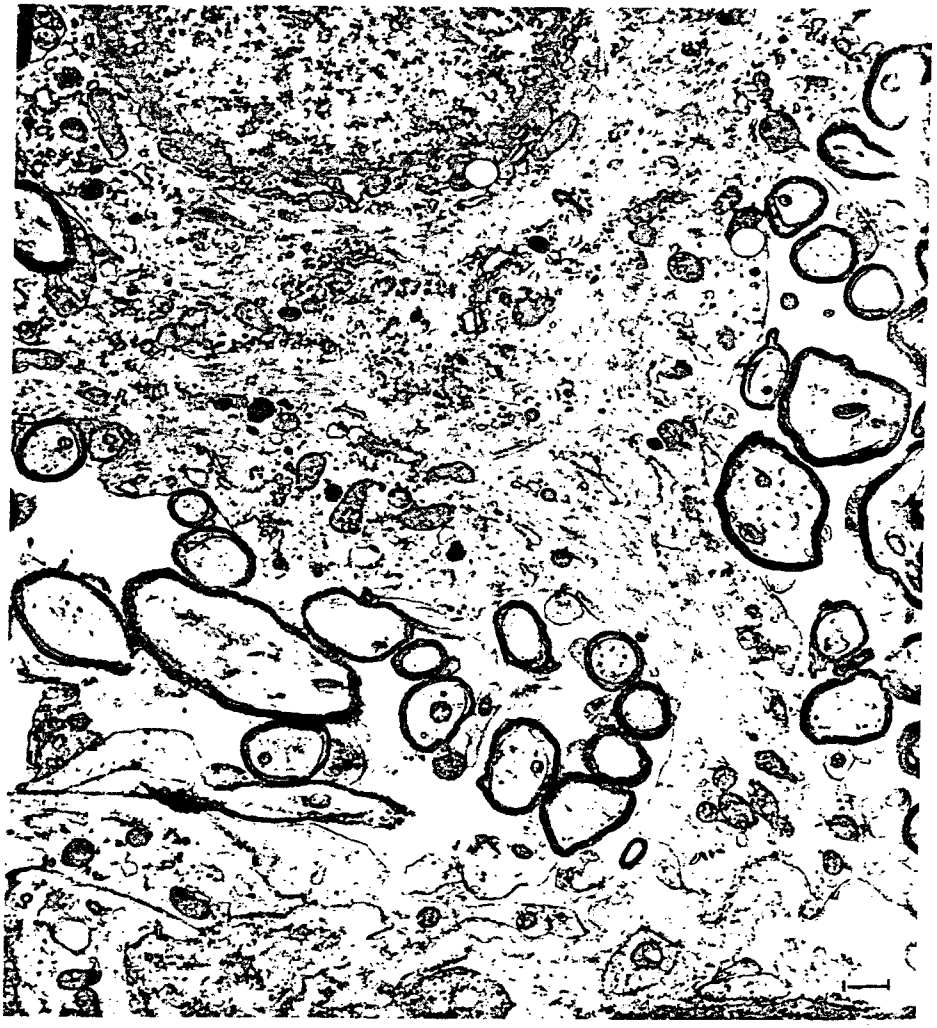


FIG. 11. Fibrous astrocyte filled with glial filaments in cerebellar white matter of lead-poisoned, 19-day-old rat. Extracellular spaces containing myelin sheaths are enlarged. ×7000. From P. Lampert *et al.* (1967), by permission of Springer-Verlag, Berlin and New York.

microglial cells were present. A recent study by electron microscopy of brains from suckling Long-Evans rats fed lead in the manner described by Pentschew and Garro has shown that cortical glial cells adjacent to the surface of the brain contain intranuclear inclusion bodies (Fig. 12, Krigman and Wilson, 1971).

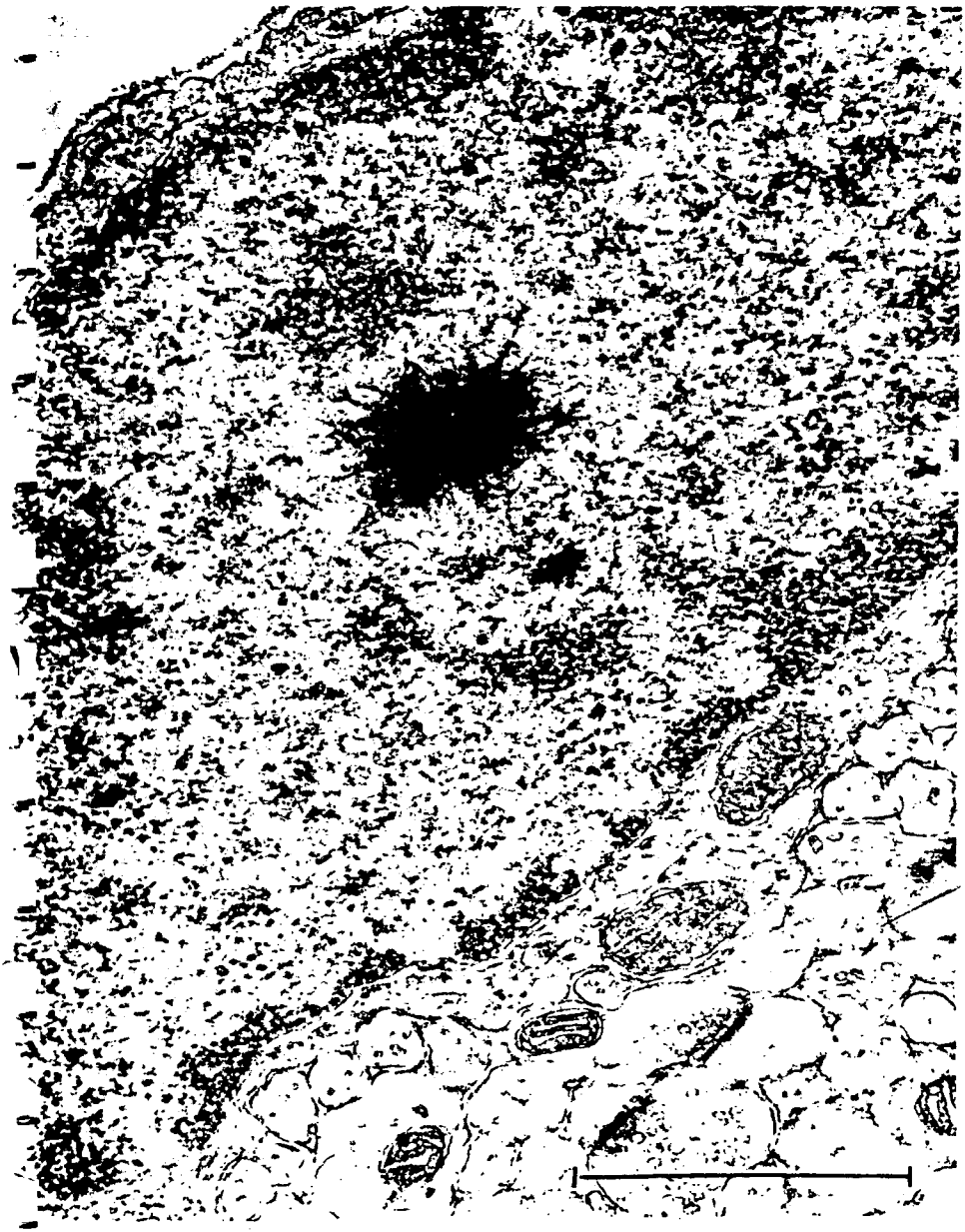


FIG. 12. Nucleus of astrocyte in superficial cerebral cortex of lead-poisoned, 30 day-old rat, containing lead-induced inclusion body. ×43,500. (Courtesy of M. R. Krigman and M. H. Wilson, University of North Carolina.)

Similar induction of lead toxicity in suckling mice results in retardation of growth and development, but clinical neurological effects are sparse except for a broad-based gait and loss of equilibrium and enhanced flexion of hind limbs (Rosenblum and Johnson, 1968). Permeability of blood vessels was only minimally altered, but great numbers of intervascular strands have been described. The significance of these is not known.

In a somewhat different approach, Brun and Brunk (1967) noted a slight reduction in alkaline phosphatase in swollen epithelial cells of capillaries in cerebral cortex of lead-poisoned rat brains. Increase in acid phosphatase in neurons was also present, which presumably reflects a release of this enzyme from lysosomes, a sign of neuronal degeneration. Focal discrete loss of cortical neurons also occurred.

Interest in the potential usefulness of nonhuman primates was recently stimulated by clinical and pathological findings in accidentally lead-poisoned simian primates in Washington's National Zoological Park (Zook, 1971).

Lead was believed to have been ingested from bars and walls of cages that had been painted with material containing lead. Clinical signs included weakness, paralysis, amaurosis, convulsions, and death. Central nervous system lesions included dilated and proliferating capillaries, laminar cortical necrosis and focal neuronal loss.

Hopkins (1970) recently showed that with repeated intratracheal injections of lead carbonate in baboons for periods ranging up to 1 year the animals may have convulsive episodes. Histological changes in the brain were not reported in this study.

B. Peripheral Neuropathy

Lead-induced peripheral neuropathy is manifested by motor weakness and palsy and is one of the more common manifestations of chronic lead toxicity in adults. In an early clinical study of neuropathy in lead-poisoned adults, nearly two-thirds of 55 patients were found to have some degree of muscle weakness, and almost half had wrist drop (Thomas, 1904).

Children may also develop peripheral neuropathy with chronic lead poisoning. It is said that wrist drop is the most common manifestation of lead neuropathy in adults while foot drop and generalized weakness are more common in children (Seto and Freeman, 1964). Whether skeletal muscle is directly affected by lead is debatable. Early investigators suggested that lead produced a primary myopathy (Aub *et al.*, 1925), but lead content of skeletal muscle in lead intoxication is usually not very marked. Also, electromyography of affected patients does not show a myopathic pattern (Preiskel, 1958). Experimentally it has been shown

that lead ions block ganglionic transmission and reduce output of acetylcholine. Addition of calcium ions reverses this effect of lead (Kostial and Vouk, 1957). Whether lead affects ganglionic transmission in man is not known.

An association between motor neuron disease, particularly amyotrophic lateral sclerosis, and chronic lead toxicity has long been suspected. J. A. Simpson *et al.* (1964) noted muscle fasciculations and proximal girdle atrophy similar to that occurring in amyotrophic lateral sclerosis. Livesley and Sissons (1968) have identified lead industry workers with the clinical diagnosis of motor neuron disease. In spite of suggestive clinical evidence

FIG. 13. (A) Longitudinal section of a sciatic nerve from a rat that had been fed lead in the diet for 7 months. The axon is surrounded by a disintegrating myelin sheath. The cells engulfing the disrupted myelin penetrate the sheath from both ends of the internodal segment. The arrow points to an area where the processes of the invading cells have insinuated themselves beneath the myelin. Paraphenylenediamine. ×350. AFIP Neg. 67-11563. From P. W. Lampert and Schochet (1968).

(B) Longitudinal section of a sciatic nerve from rat that had been on lead diet for 7 months. The upper axon shows a completely demyelinated segment that is surrounded by several Schwann cells, while at the right, myelin disintegration is still in progress. The lower axon shows short remyelinated segments that are separated by nodes of Ranvier (arrows). Paraphenylenediamine. ×350. AFIP Neg. 67-11564. From P. W. Lampert and Schochet (1968). (A) and (B) reproduced by permission of *J. Neuropathol. Exp. Neurol.*, New York.

(Campbell and Williams, 1968), a cause and effect relationship between lead and motor neuron disease has not been established (Currier and Haerer, 1968).

Knowledge of the lesions in lead-induced peripheral neuropathy has evolved from study of experimental models beginning with the observations that guinea pigs with chronic lead intoxication develop a peripheral neuropathy characterized by segmental degeneration of myelin sheaths (Gombault, 1880). Recent extension of these experiments by Fullerton (1966) has confirmed the frequent occurrence of segmental demyelination and of axonal degeneration. Lampert and Schochet (1968) and Schlaepfer (1968, 1969) have shown that in rats segmental demyelination and remyelination are related to Schwann cell degeneration and proliferation (Figs. 13A, B and 14A, B). Schlaepfer (1969) has also demonstrated

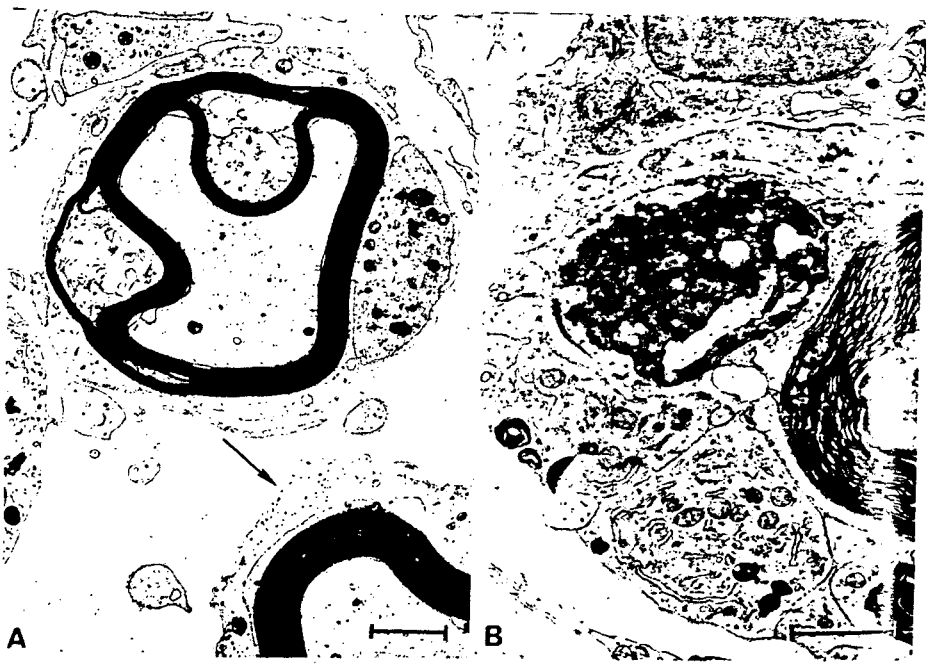


FIG. 14. (A) Myelinated axons from rat that had been on lead diet for 7 months. The myelin sheath of the upper axon shows wide disruptions and vesicular breakdown of lamellae. Both sheaths are surrounded by proliferated Schwann cell processes, some of which are degenerating (arrow). ×8000. From P. W. Lampert and Schochet (1968).

(B) Myelinated axon from rat that had been on lead diet for 7 months. The degenerating Schwann cell and sheath are surrounded by multiple cytoplasmic processes. The ruffled appearance of the myelin is artifact. ×12,000. From P. W. Lampert and Schochet (1968).

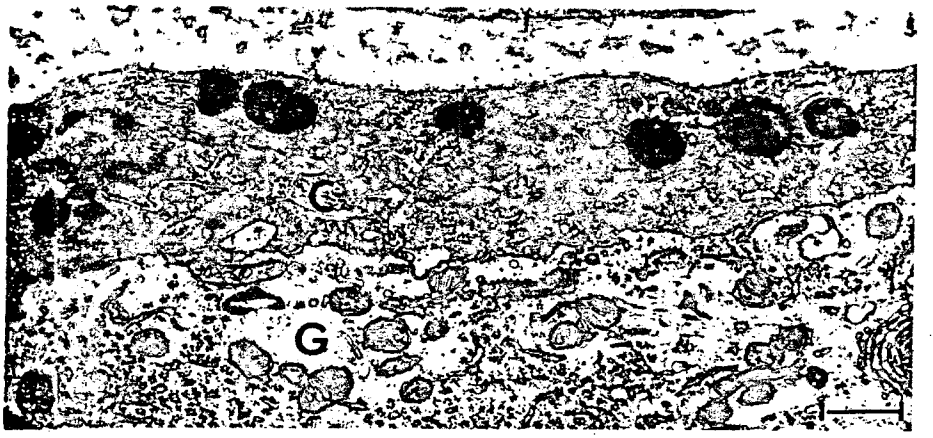


FIG. 15. A ganglion cell (G) surrounded by a thickened capsular (C) investment from dorsal nerve root of rat fed 1% lead acetate. Capsular cell contains numerous dense bodies as well as an increase of mitochondria and ribosomes. ×9000. From Schlaepfer (1969), by permission of *J. Neuropathol. Exp. Neurol.*, New York.

Wallerian degeneration of posterior nerve roots of sciatic and tibial nerves, which suggests a cellular basis for lead-induced paresthesia and sensory nerve loss. Ganglion cells showed no consistent pathological alterations, but surrounding capsular cells contained increased numbers of organelles and dense bodies (Fig. 15).

An effect of lead on anterior horn cells of rats, consistent with lead-induced motor neuron disease in man has not been found experimentally (Schlaepfer, 1969).

C. Neurological Sequelae of Lead Toxicity

The pathological changes associated with acute lead encephalopathy may not be completely reversible. The risk of permanent neurological complications increases with repeated episodes of acute intoxication or with chronic excessive exposure to lead (Chisolm and Harrison, 1956; Byers, 1959). A clinical estimate of permanent neurological effects of lead poisoning in 425 children between the ages of 9 months and 8 years has been reported by Perlstein and Attala (1966). The incidence and nature of sequelae are related to the mode of onset of symptoms as shown in Table IV. The likelihood of sequelae is related to severity of mode of onset; over 80% of children presenting with encephalopathy manifested by signs of increased intracranial pressure experienced some permanent

TABLE IV

NEUROLOGICAL SEQUELAE OF LEAD INTOXICATION[a,b]

Sequelae	Total, 425 (%)	Mode of onset: Encephalopathy 59 (%)	Seizures, 43 (%)	Ataxia, 17 (%)	Gastrointestinal, 232 (%)	Febrile, 16 (%)	Asymptomatic, 58 (%)
None	61	18	33	41	69	81	91
Mental retardation	22	38	33	29	19	19	9
Seizures	20	54	39	35	13	0	0
Cerebral palsy	2	13	0	6	0	0	0
Optic atrophy	1	6	0	6	0	0	0

[a] Modified from Perlstein and Attala (1966).

[b] Percentage total may exceed 100% because of multiple sequelae in the same patient.

neurological deficit, recurrent seizures and mental retardation being most common. Byers (1959) commented that these sequelae do not differ qualitatively from those which follow any diffuse cerebral disease during childhood, such as viral or bacterial encephalitis or meningitis. Morphological descriptions of the central nervous system in children that have survived lead intoxication and have developed permanent neurological abnormalities are not available. Neuronal loss is to be expected. Also, the proliferation of astrocytes and microglial cells noted in experimental lead encephalopathy suggests that some degree of sclerosis may be likely. That lead toxicity may, in fact, result in a form of diffuse sclerosis, has been suggested in several reports that implicate lead as an etiologic agent of multiple sclerosis in adults (Cone *et al.*, 1934; Campbell *et al.*, 1950) and of diffuse sclerosis in an infant (Verhaart, 1941).

Optic atrophy may also occur in adults with chronic lead intoxication (Baghdassarian, 1968). Although not documented in the study of Perlstein and Attala (1966), auditory defects and vertigo may follow lead poisoning (Ciurlo and Ottoboni, 1956). A recent experimental study of eighth nerve changes in lead-intoxicated guinea pigs showed segmental demyelination similar to that described in other peripheral nerves in lead poisoning. The sensory cells of the inner ear and the spiral and vestibular ganglion cells were normal (Gozdzik-Zolnierkiewicz and Moszynski, 1969).

D. Possible Neurological Effects in Persons with Subclinical or Asymptomatic Lead Poisoning

It has been suggested that subtle neurological effects of lead might occur in the course of chronic exposure to lead without accompanying signs and symptoms of overt lead poisoning. The success of studies to detect such effects, like the recognition of subclinical biochemical effects of lead, is dependent upon the sensitivity of the screening technique employed. Fullerton and Harrison (1969) studied lateral popliteal nerve conduction in 19 lead battery factory workers. Exposure to lead was from 5 months to 13 years. Thirteen of the men had blood lead levels over 80 μg/100 gm; some had anemia. A minimal but significant decrease in nerve impulse amplitude was found in workers exposed to lead when compared to a control group. There was some correlation between length of exposure to lead and severity of conduction defect but the number of persons studied was too small to obtain statistical correlation with severity of exposure to lead (Fullerton and Harrison, 1969; Catton *et al.*, 1970).

Apart from functional neurologic sequelae, it has been questioned to what degree lead affects mental development in lead-poisoned children. For the present at least, the question does not seem to be answerable (Wiener, 1970). A difficulty with this type of study is that children who are retarded or have brain damage are more prone to have pica and lead poisoning than intellectually and emotionally normal children. Of considerable interest is the observation of Perlstein and Attala (1966) that 9% of asymptomatic siblings of children with clinical lead intoxication had, in these authors' opinion, mental retardation related to excessive exposure to lead (Table IV). However, Rennert and co-workers (1970) pointed out the difficulties in assaying intellectual effects of asymptomatic levels of lead in the absence of intelligence measurement prior to exposure. In view of the apparent great sensitivity of the central nervous system of children to the toxic effects of lead, more information is needed about the sequelae of chronic low level exposure.

VI. Hematological Effects of Lead

Anemia is an early manifestation of acute or chronic lead intoxication. It is nearly always present when other symptoms of lead toxicity occur, and it may be the only clinical feature of chronic exposure to low levels of lead.

A. Morphology of Erythrocytes

In lead-induced anemia, red blood cells are microcytic and hypochromic, as in iron deficiency, and usually reticulocytosis and basophilic stippling are also observed. Iron deficiency may be a coincident factor, but microcytic hypochromic anemia is seen in children with lead poisoning even when serum iron is normal or elevated (Leikin and Eng, 1963).

Basophilic stippling of red blood cells has long been recognized as a feature of lead-induced anemia and has been employed as a method of monitoring workers in lead industry (McCord *et al.*, 1935), but this test has the disadvantages of being nonspecific and probably does not correlate well with levels of lead exposure (Griggs, 1964). Stippling is more common in erythroblastic cells in bone marrow than in cells in peripheral blood (Waldron, 1966).

The nature of the basophilic stippling has received considerable attention. Stipples are thought to represent clustered ribosomes (Jensen *et al.*, 1965). They may or may not stain for iron (Waldron, 1966).

Large concentrations of lead in blood *in vivo* (25–100 mg Pb^2 given intravenously) or added *in vitro* (20 μg per milliliter of blood) produce red cell shrinkage, distortion, and wrinkling of the membrane (Waldron, 1966).

B. Functional Effects on Erythrocytes

A number of reports following the early studies of Aub and his associates (1925) have shown that the osmotic fragility of red blood cells from lead-intoxicated people or of cells exposed to lead *in vitro* is decreased or altered. However, osmotic fragility may be increased after sterile incubation for 24 hours. These studies are reviewed by Waldron (1966). On the other hand, the mechanical fragility of leaded cells is increased. Whether this effect is related to lead-binding phosphate as suggested by Aub and associates (1925) or to other functional effects of lead on the red cell membrane is unclear. Prankerd (1961) has suggested that decrease in red cell membrane integrity follows impairment by lead of glycolytic enzyme activity. Incubation of red blood cells from workers exposed to lead results in excessive potassium loss (Hasan *et al.*, 1967a), and may be related to inhibition by lead of sodium- and potassium-dependent ATPase's (Hasan *et al.*, 1967b; Hernberg *et al.*, 1967).

C. Hemolytic Effect of Lead

The anemia that occurs in lead poisoning results from two basic defects, shortened erythrocyte life-span and impairment of heme synthesis.

Whether shortened erythrocyte survival is due to an effect on the developing red cells in bone marrow or on the membrane of the circulating cell is debated (Waldron, 1966). A recent study of heme and porphyrin metabolism in an adult with lead poisoning (Berk *et al.*, 1970) has shown that there is a direct hemolytic effect of lead on mature red blood cells which is independent of effects on heme biosynthesis. Previous workers who postulated decreased erythrocyte survival on the basis of shortened half-life of ^{51}Cr-labeled erythrocytes in lead poisoning, were misled by the fact that lead increases the rate of ^{51}Cr elution from tagged cells (Berk *et al.*, 1970). This conclusion was based on the demonstration of an early excretion of ^{14}C-labeled stercobilin and on erythrokinetic studies with multiple tracers.

D. Inhibition of Heme Synthesis

A schematic presentation of the effect of lead on heme synthesis is shown in Fig. 16. At least three steps in heme synthesis may be affected by lead. *d*-Aminolevulinic acid dehydratase (ALA-D) is probably the enzyme in the heme pathway that is most sensitive to lead. Inhibition of this enzyme results in a block in utilization of *d*-ALA and in subsequent decline in heme synthesis. Second, in the scheme of negative feedback

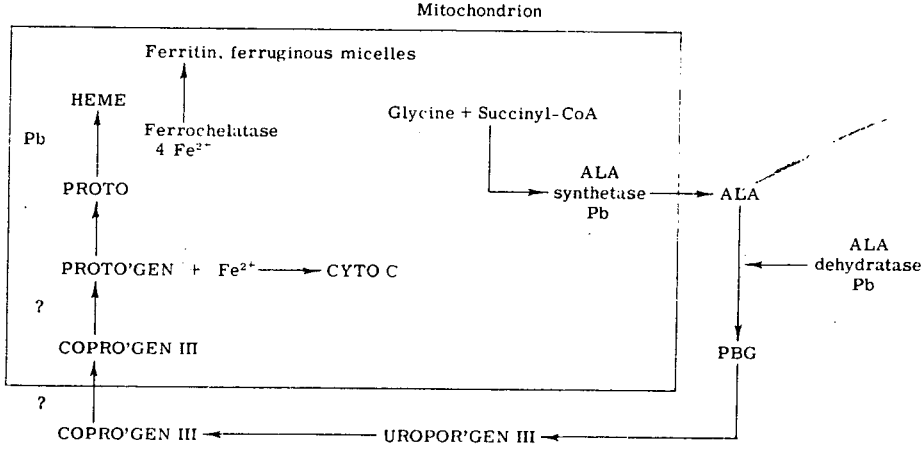


FIG. 16. Scheme of heme synthesis showing sites of lead effect. PBG, porphobilinogen; UROPOR III, uroporphyrinogen III; COPRO III, coproporphyrinogen III; PROTO, protoporphyrin; CoA, coenzyme A; ALA, aminolevulinic acid; CYTO C, cytochrome *c*.

control of heme synthesis proposed by Granick and Levere (1964), *d*-ALA synthetase activity is derepressed, which results in increased activity of the enzyme and increased synthesis of *d*-ALA. Since utilization of *d*-ALA is blocked, urinary excretion of *d*-ALA is greatly increased, serves as a sensitive biochemical index of lead toxicity and is widely used as such (Haeger-Aronsen, 1960).

A third abnormality of heme synthesis in lead intoxication is inhibition of the enzyme ferrochelatase (Ortzonsek, 1967), which is located on the inner membrane of mitochondria (Jones and Jones, 1968). This effect is particularly interesting because of associated ultrastructural changes in the mitochondria. Ferrochelatase catalyzes the incorporation of the ferrous ion into the prophyrin ring structure. Bessis and Jensen (1965) have shown that iron in the form of apoferritin and ferruginous micelles may accumulate in mitochondria of bone marrow reticulocytes from lead-poisoned rats.

Other steps in the biosynthetic pathway of heme may also be abnormal in lead toxicity, but the evidence for this is incomplete. Increase in urinary excretion of coproporphyrin, the degradative product of coproporphyrinogen III, is a sensitive reflection of lead toxicity. Metabolism of porphobilinogen to coproporphyrinogen proceeds unimpaired. However, since ALA-D is an extramitochondrial enzyme, and later steps in heme synthesis are intramitochondrial, coproporphyrinogen must reenter the mitochondrion to be metabolized further. It has been suggested that transport of metabolites like coproporphyrinogen into the mitochondrial matrix might be impaired in the presence of altered inner membrane permeability and reduction in oxidation and phosphorylation (Haeger-Aronsen *et al.*, 1968). Whether the increased urinary coporporphyrinogen occurring in lead poisoning is a reflection of a nonspecific alteration in mitochondrial membranes or reflects a more specific effect of lead on the intramitochondrial enzyme, coproporphyrinogenase, is not known.

VII. Renal Effects of Lead

A. Lead Nephropathy in Human Beings

1. *Acute or Early Effects on the Kidney*

Acute effects of lead on the kidney were distinguished from lead-induced chronic nephropathy more than 50 years ago by the English toxicologist Thomas Oliver (1914). 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. There is little evidence that the glomerulus is affected in acute lead poisoning, although a recent report suggests that ultrastructural changes in the glomerular basement membrane may occur (Macadam, 1969). These consist of complete fusion of epithelial cell foot processes and of increased cytoplasmic density of epithelial cells adjacent to a normal looking basement membrane. As long ago as 1928, Pejic 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. These have been discussed in Section IV.

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 co-workers in 1953. 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 (1955) in England, and Chisolm (1962) found that 9 of 23 children with lead encephalopathy had aminoaciduria, glycosuria, and hypophosphatemia. The aminoaciduria was generalized in that the amino acids excreted in greatest amounts were those normally present in urine, and it was related to severity of clinical toxicity, most marked in children with encephalopathy. The aminoaciduria disappears after treatment with chelating agents and clinical remission of other symptoms of lead toxicity (Chisolm, 1962, 1968). This is an important observation relative to the long-term or chronic effects of lead on the kidney. Restoration of the functional integrity of renal tubular lining cells following treatment of acute lead poisoning implies restoration of normal morphology, but this has not been confirmed experimentally.

2. *Chronic Lead Nephropathy in Man*

The occurrence of a chronic form of renal disease in man is controversial. There are numerous reports in the medical literature of the past

century which describe a form of end-stage renal disease and renal failure in humans which is said to follow many years of excessive exposure to lead. The pathogenesis of chronic lead nephropathy expressed by Charcot and Gombault in 1881 (cited by Aub *et al.*, 1925) relates renal effects of lead on the tubular lining cells resulting in diffuse renal fibrosis characterized by "epithelial cirrhosis of the kidney." Later descriptions by Oliver (1914) and Aub *et al.* (1925) are consistent with this hypothesis, and emphasize tubular atrophy and dilatation with interstitial fibrosis, but with minimal inflammatory cell infiltration. There is progressive contraction of kidney size with subsequent sclerosis of glomeruli. These descriptions have no specific pathological feature, so that the role of lead in the pathogenesis of diffuse chronic nephropathy in a particular person has always been uncertain. The implication of lead as etiological agent of chronic nephropathy is largely the result of association of chronic renal disease with chronic exposure to lead and signs and symptoms of chronic lead poisoning, such as abdominal colic and peripheral neuropathy.

a. Lead Intoxication in Childhood and Chronic Lead Nephropathy in Adulthood. A series of reports from Queensland, Australia, points to a strong association between severe lead poisoning in childhood associated with central nervous system symptoms and chronic nephritis in early adulthood. Henderson (1954) followed up 401 children who had been diagnosed as having lead poisoning in Brisbane between 1915 and 1935. Of these 165 had died, 108 from nephritis or hypertension. This is greatly in excess of expectation. Information was obtained from 101 of the 187 living survivors, and of these 17 had hypertension and/or albuminuria. In a more recent study, Emmerson (1963) presented criteria 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 childhood lead poisoning showed increased excretion of lead. Only 4 of 19 patients with chronic renal disease not attributable to lead poisoning had similarly elevated excretion of lead. A photomicrograph of part of a kidney from a 26-year-old male with a childhood history of lead poisoning is shown in Fig. 17. The presence of intranuclear inclusion bodies is very helpful 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 (1963) found no evidence of chronic renal disease in 42 persons with a well documented history of childhood plumbism 20–35 years previously at the Boston Children's Hospital. Likewise, Chisolm (1970) found no evidence of renal disease in 62 ado-

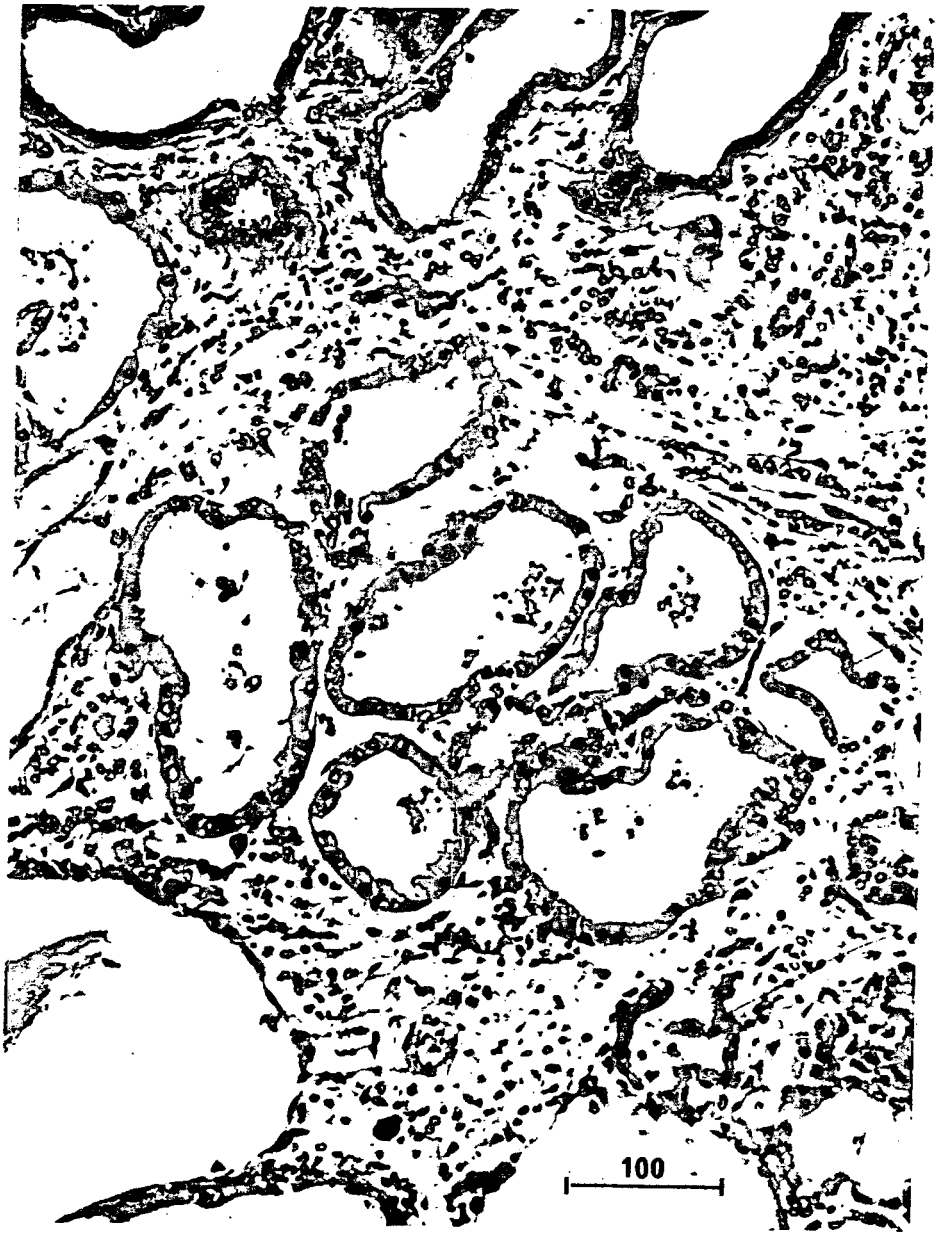


FIG. 17. Renal cortex from a 26-year-old male with history of childhood lead poisoning and chronic lead nephropathy. The patient was hypertensive and died in uremia. At autopsy the kidney was small and fibrosed. Bone lead was high. The histological picture is a mixture of interstitial fibrosis and dilated tubules. Some tubules are atrophic (lower left). Intranuclear inclusion bodies are not present. ×210. (Slide furnished by J. A. Inglis, M.D., Department of Pathology, University of Queensland, Australia.)

lescents known to have had intoxication 11–16 years earlier. An important distinction between the Australian group and American patients was that none of Chisolm's (1970) subjects showed evidence of increased residual body lead burden following the EDTA mobilization test. This difference has suggested to Chisolm (1970) 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 American children suffer from lead toxicity early in childhood, between the ages of one and four, the source being oral ingestion of flecks of wall paint and plaster containing lead. Australian children, at least at the time of the Nye (1929) and Henderson (1955) studies, ingested lead from powder or chalky paint on veranda rails of their homes while playing. They tended to be older than the American children, and had a more chronic form of lead poisoning.

b. Occupational Exposure to Lead—Renal Hypertension and Chronic Lead Nephropathy. Several studies of workers in lead industries in the early part of this century showed an increased incidence of hypertension which appeared to correlate with exposure to lead (cited by Cantarow and Trumper, 1944). However, a relationship of hypertension to renal disease was not established. A retrospective study (Dingwall-Fordyce and Lane, 1963) reviews causes of death among men eligible for pension (65 years of age) who had worked in an accumulator factory in England between 1926 and 1960. The incidence of deaths from cerebral hemorrhage and from thrombosis or arteriosclerosis was higher than expected. All affected persons were employed for not less than 25 years. Again, a renal basis for the hypertension was not demonstrated.

Control of occupational exposure to lead has undergone continued improvement during the past 50 years, which may be responsible for the failure of other investigators (Belknap, 1936; R. E. Lane, 1949) to find an increase in frequency of hypertension and chronic renal disease. More recently, Cramér and Dahlberg (1966) did not find an excessive incidence of hypertension among workers in a Swedish accumulator factory in which 265 workers were employed for more than 10 years. On the other hand, in those parts of the world where occupational exposure to lead is not closely controlled, there continued to be a high incidence of chronic nephropathy and renal failure. In Yugoslavia, in a study of 53 patients with chronic exposure to lead (2 months to 35 years) and with clinically manifest lead poisoning, Radoševič *et al.* (1961) concluded that lead may induce functional and anatomical lesions of the kidneys. Studies of renal function in 102 patients admitted to the Occupational Diseases Clinic in Bucharest, Rumania, during a 10-year period (1957–1967) were reported by Lilis and co-workers (1968). Renal failure was found in 17

patients who had had several episodes of abdominal colic and occupational exposure to lead for more than 10 years. Thirteen of these patients had arterial hypertension and evidence of renal disease preceding the rise in blood pressure by several years. The authors related the progressive development of renal failure to prolonged exposure to lead, with repeated episodes of lead-induced abdominal colic.

c. Illicit Whiskey and Renal Disease. Contamination with lead, intentional or accidental, is a long-recognized risk attending the consumption of "home brew" or illegally produced alcoholic beverages. The Romans are said to have added lead to wine to improve flavor (Gilfillan, 1965). Devonshire colic, an endemic disease in the County of Devonshire, England, in the middle of the 18th century, was found to be a symptom of lead intoxication caused by drinking cider contaminated with lead (Anonymous, 1968). Homemade wine may be contaminated by lead glaze in earthenware crocks (Whitehead and Prior, 1960; C. R. Lane and Lawrence, 1961).

Contamination of illicit whiskey by lead has been a problem of major proportions in the southeastern region of the United States. Lead is leached into the distilling alcohol from lead-soldered pipe joints. Hammack (Owen *et al.*, 1967) has recorded that over 250 patients were admitted to the Birmingham Alabama Veterans Administration Hospital for intoxication with lead in illicitly distilled whiskey This number of acutely ill patients suggests that many others must be affected with subclinical or chronic lead toxicity and continue to go unrecognized. Morgan and coworkers (1966) have described renal biopsies in 13 of such patients, aged 35–50, with early renal failure, anemia, and increased urinary excretion of lead with or without provocation by EDTA. Two of the patients had fixed hypertension. The biopsies from all cases showed interstitial fibrosis with few inflammatory cells. Some glomeruli were sclerosed; others showed a mild increase in basement membrane thickness. All cases were found to have intranuclear inclusion bodies.

At the Veterans Administration Hospital in Nashville, Tennessee, Sandstead *et al.* (1970) studied the renin–aldosterone response to salt deprivation in 9 men with subclinical or "occult" lead poisoning. All nine had elevated body burdens of lead demonstrated by increased urinary excretion of lead following administration of EDTA. Plasma renin activity and aldosterone secretory rate were measured after administration of the diuretic furosemide and ingestion of a low sodium diet for 5 days. Neither of the measured parameters increased to expected levels in most of the men. Lead toxicity is believed to be responsible for abnormal sodium-conserving functions of the renal tubule.

It cannot be proved unequivocally that lead in alcoholic beverages was

responsible for the renal disease in either of these studies. Increased body burdens of lead were demonstrated in all the patients. Also, the finding of intranuclear inclusion bodies is further evidence of excessive renal lead.

It is also possible that lead and alcohol have a synergistic toxic effect on the kidney. Alcoholic beverages have long been thought to enhance lead toxicity (Oliver, 1914), and avoidance of alcohol is recommended for workmen in lead industries (Cramér, 1966). Lead and alcohol have some similar toxic effects at the cellular level. The basis for this presumption is discussed in Section X in terms of alcohol as a factor influencing susceptibility to lead toxicity. Even though the molecular mechanisms underlying the toxicity of alcohol and lead probably differ, the fact that they affect the same organelle, the mitochondrion, may result in a mutual enhancement of cellular injury. It is also possible that illicitly distilled whiskey contains other impurities that are toxic to kidneys, although none have been documented.

d. Gout and Lead Nephropathy. Because of the common association of chronic renal disease with gout among lead industry workers, industrial physicians and toxicologists in 19th Century believed strongly in a relationship between gout and chronic lead nephropathy. Several case reports of the 19th century linking lead with gout are summarized by Ludwig (1957). Even Sir Alfred Barring Garrod, who is credited with first recognizing elevated blood uric acid levels as an etiological factor in gout, emphasized the relationship of lead poisoning with gout (cited by Ludwig, 1957). A typical early study was Lorimer's (1886) of 107 cases of gout and lead poisoning. Lorimer distinguished saturnine gout as occurring earlier in life than ordinary gout. In addition, there was absence of familial predisposition, and the anemia of lead poisoning was always present. Arthritis was of early onset. But even in this report, the question was raised whether joint involvement could be due to coexistent "rheumatism." Fewer cases have been seen in recent years and the review by Aub *et al.* (1925) questions the lead–gout relationship.

The problem of gout and lead-induced nephropathy has recently been reopened. Among the 33 individuals in Emmerson's (1968) study of patients with lead nephropathy, 16 suffered from acute attacks of gout. Emmerson found that his patients differed from those with idiopathic gout in that more women were afflicted, and often before the menopause. There was often a history of lead poisoning in childhood. Also, the lower limbs were more commonly affected, and attacks of gouty arthritis were less frequent than in patients with idiopathic gout.

Many patients with chronic nephropathy associated with ingestion of illicit whiskey contaminated by lead also have hyperuricemia and gout

(Morgan *et al.*, 1966). In each case, lead nephropathy preceded the onset of gout.

From a review of all admissions for gout at the Birmingham Alabama Veterans Hospital, Ball and Sorensen (1969) were able to classify 33 of 43 patients with gout as secondary to prolonged consumption of "moonshine" contaminated with lead. Kinetic studies of uric acid on three of these patients confirmed the role of the kidneys in the pathogenesis of the hyperuricemia. There was no evidence of overproduction of uric acid. These findings suggest that lead impairs renal tubular secretion of uric acid. However, in another recent study of urate excretion in lead nephropathy, Emmerson (personal communication) measured renal tubular reabsorption and secretion of urate separately by observing the effect on renal deposition of urate of the drug pyrazinamide which blocks active tubular secretion of urate. Tubular reabsorption of urate was found to be greater than expected whereas active tubular secretion of urate was within the range predicted from the plasma urate concentration. These results suggest that lead-induced gout is mediated by excessive tubular reabsorption of urate.

Klinenberg (1969) has noted that few patients with chronic renal disease per se develop gouty arthritis, whereas patients with saturnine gout, despite prolonged exposure to lead, have only moderate impairment of glomerular infiltration rate and are not azotemic. Also, patients with lead gout have normal triglyceride and cholesterol levels; patients with primary gout have a high incidence of hypertriglyceridemia (Emmerson and Knowles, 1971).

B. Experimental Lead Nephropathy

Questions about effects of lead on the kidneys, based on observation of patients with lead poisoning, are difficult if not impossible to investigate in man. The sequence of pathological events leading to a chronic nephropathy induced by lead requires a major portion of the lifetime of an individual. Continuity of study for such a long period is difficult if not impossible. Also, in human studies there is no control of lead dosage or of other (intercurrent) renal disease. And finally, it is not possible to obtain renal tissue at the periodic intervals necessary to document such a chronic, progressive pathological process in man. It is important, therefore, to study progressive effects of lead on the kidney in experimental animals, and to correlate the findings with stages of lead nephropathy seen in man. The rat and the rabbit serve as good models for this purpose

since these animals tolerate large doses of lead for long periods of time and since they both develop progressive structural and functional renal changes in response to lead. Dose-effect relationships can also be studied in animals.

The progressive morphological and functional changes induced by lead in rat kidneys may be divided into three stages—primarily, for the purpose of observing the development of lead effects and, secondarily, for making comparisons to observations in man.

1. *Stage I—Tubular Effects, Reversible*

The first stage of lead nephropathy in rats fed a diet containing 1% lead as lead acetate continues for about 20 weeks and is characterized by the development of intranuclear inclusion bodies after about 4 or 5 weeks. No other changes are apparent by light microscopy. An electron micrograph of a nucleus of a proximal tubular lining cell containing an inclusion body is shown in Fig. 3. Swelling of organelles and mitochondria is also seen by electron microscopy, as shown in Fig. 9B. Impairment of respiratory and phosphorylative abilities of mitochondria isolated from kidneys of rats fed a 1% lead acetate diet for 10 weeks are discussed in Section IV, B. The mitochondrial changes may be related to the triad of functional defects in proximal renal tubules, that is, to aminoaciduria, glycosuria, and hyperphosphaturia, as noted in children with acute lead poisoning.

Excessive aminoaciduria also occurs during stage I nephropathy in rats. After about 10 weeks on the experimental diet, nearly all amino acids are excreted in greater amounts than in control rats. The aminoaciduria is "generalized," with greatest increases occurring in amino acids normally present in urine in largest amounts. These are the small neutral monoamino, monocarboxylic acids, e.g., glycine, serine, and alanine. Renal clearances for most individual amino acids (Fig. 18) increase approximately 1–3 times. Exceptions include a decreased clearance of tyrosine, little change in the clearance of glycine and valine, and a very large increase in the clearance of histidine. Plasma levels of nearly all amino acids from lead-fed rats are either the same or less than plasma levels of control rats. Exceptions are increases in threonine, glycine, and lysine. Renal clearance of glycine is normally considerably larger than that of other amino acids, but it does not increase appreciably in the lead-fed rats. This suggests a prerenal factor in the excessive glycinuria. The large increase in histidine clearance and the disproportionate increases in uninary excretion of other amino acids may also reflect complexing of lead with amino acids (Goyer, 1971b; Goyer *et al.*, 1970a).

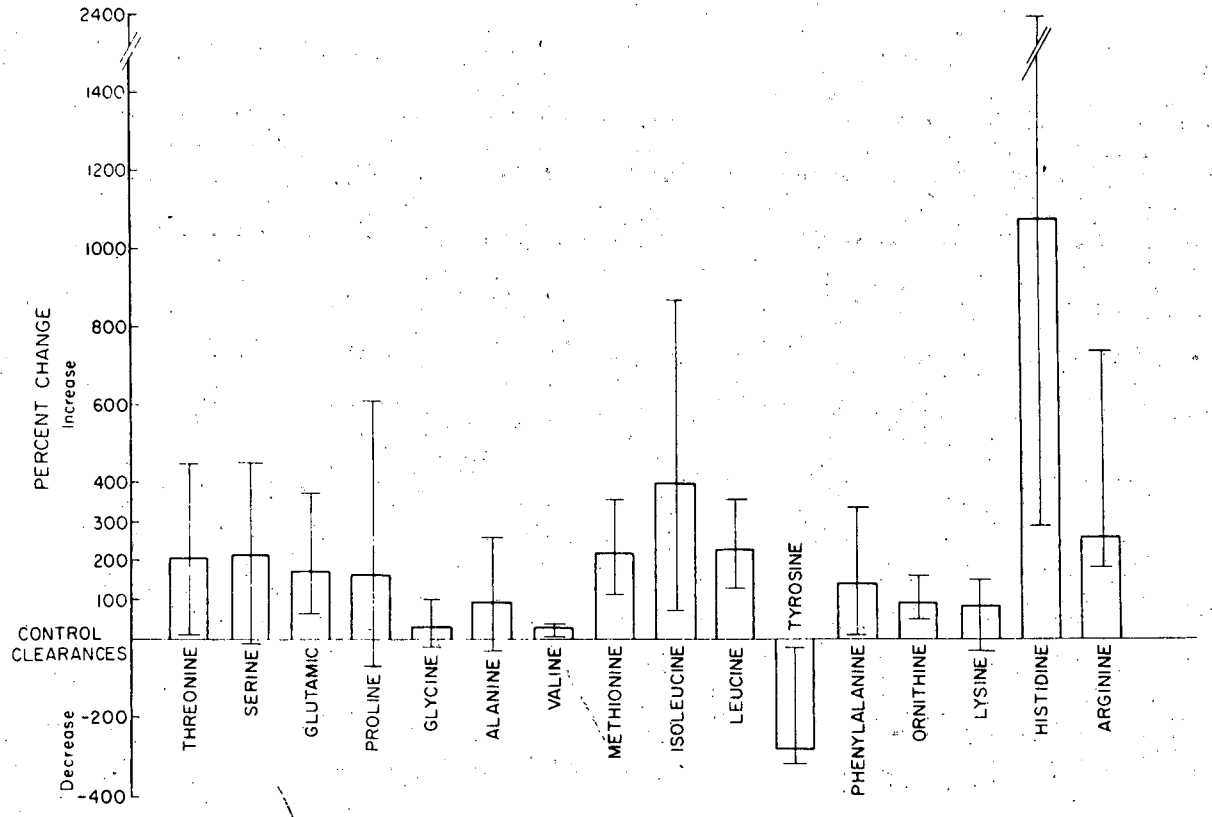


FIG. 18. Change in renal clearance of individual amino acids from three rats fed lead for 10 weeks and three controls pair-fed for 1 week prior to collection of samples. Bars indicate mean and range of percent change. From Goyer *et al.* (1970a), by permission of Academic Press, New York.

Effects of lead on morphology and function of the distal tubules in rat kidneys are less dramatic than those on proximal tubules, and they have not been studied as intensively. Pardoe and Weatherall (1952) showed a number of years ago that tubular excretory activity, as measured by *p*-aminohippuric acid excretion, progressively increases during lead intoxication. Other experiments by these authors suggest that water diuresis is slightly increased, with reduced sensitivity to vasopressin. These functional changes are reversible on reduction of lead dosage. Such studies have not been repeated by others.

Absence of effects of lead on glomeruli is emphasized by Pardoe (1952), who showed normal creatinine clearances in rats fed lead for as long as 6 months, but a later study (Macadam, 1969) suggests that ultrastructural changes in glomerular basement membranes may occur.

Rabbits fed lead subacetate (0.5–1.0%) in their diets also develop intranuclear inclusion bodies (Hass *et al.*, 1964) and hyperaminoaciduria (von Studnitz and Haeger-Aronsen, 1962) in 8–12 weeks. These early lead-induced renal changes progress to stage II nephropathy which is characterized by the development of permanent scarring and tubular changes after about 20 weeks.

2. *Stage II—Chronic, Irreversible Nephropathy*

Continued feeding of lead results in atrophy of tubular lining cells in some tubules, with dilatation and eventual cystic change (Fig. 19) (Goyer, 1971c). The cellular lining of some tubules becomes hyperplastic. There is a progressive increase in interstitial fibrous tissue with few inflammatory cells. The number of intranuclear inclusion bodies becomes maximal between stage I and II and decreases as scarring and tubular atrophy progress. Hyperplastic tubular lining cells do not contain intranuclear inclusions, but many of these cells have hyperchromatic nuclei and are atypical in appearance, which suggests premalignancy. Similar progressive renal effects in rats fed lead for several months have been previously reported by others (Chiodi and Sammartino, 1947; Pardoe, 1952; Murphy *et al.*, 1964), and also occur in kidneys of lead-fed rabbits (Hass *et al.*, 1964).

Greater deposition of lead in renal tubular lining cells may be induced by giving 13 successive intraperitoneal injections with 1 ml of 1% lead acetate solution once in 2 weeks over a period of 26 weeks. In addition to the already described intranuclear inclusion bodies, dense concretions appear in the cytoplasm (Fig. 20). The concretions stain with hydrogen sulfide, suggesting that they contain lead (Murakami, 1971).

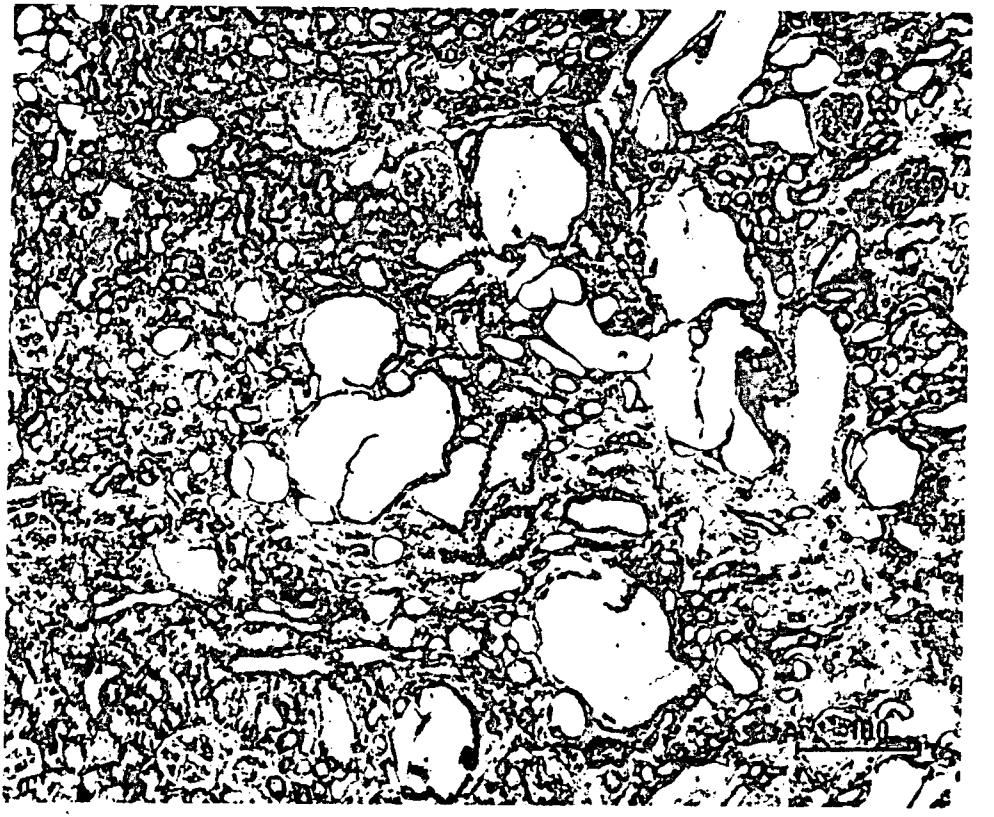


FIG. 19. Renal cortex of rat fed a diet containing 1% lead acetate for 9 weeks. Many tubules, particularly in the deep cortex, are dilated and lined with atrophic epithelium. Cells lining other tubules are hyperplastic and contain hyperchromatic nuclei. Interstitial fibrosis is present, but inflammatory cells are sparse. ×150. From Goyer (1971c), by permission of Springer-Verlag, Berlin and New York.

3. *Stage III—Renal Failure and Cancer*

Rats fed 1% lead as lead acetate in their diets for more than one year have progression of interstitial scarring and fibrosis, sclerotic glomeruli, and frequently develop renal failure and adenocarcinoma. Blood urea levels of six rats after 84 weeks of ingesting a lead-containing diet are shown in Fig. 21A. Elevation of blood uric acid also occurs (Fig. 21B) and may be related to the renal failure, although in these 6 rats there was no clear relationship between the hyperuricemia and uremia. On the other hand, the elevated blood uric acids levels may be analogous to the hyperuricemia seen in human lead nephropathy (Section VII, A, 2, d).

FIG. 20. Tubular epithelial cell with nucleus containing a typical lead-induced inclusion body (IB) and a cytoplasmic concretion (CON). ×8700. From Murakami (1971), by permission of *Sangyo Igaku* (*Jap. J. Ind. Health*), Tokyo.

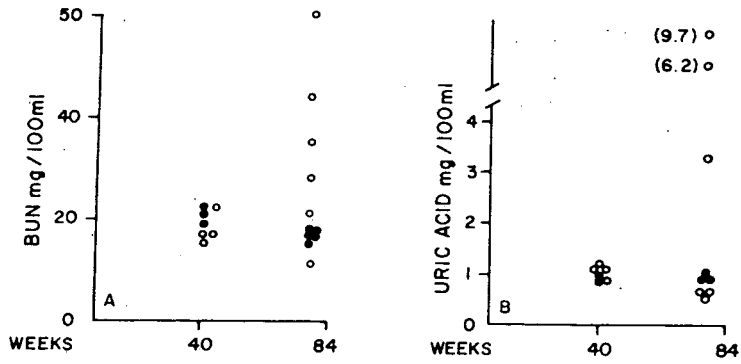


FIG. 21. Blood urea (A) and uric acid (B) from control (●) and lead-poisoned (○) rats sacrificed after ingestion of diet containing 1% lead for 10, 40, and 84 weeks. From Goyer (1971c), by permission of Springer-Verlag, Berlin and New York.

Since hypertension may occur in persons with chronic lead nephropathy, there have been several studies of blood pressure levels in experimental lead poisoning. The results are inconsistent. Several years ago, Fouts and Page (1942) tried unsuccessfully to produce hypertension in dogs by chronic lead poisoning. Griffith and Lindauer (1944) showed a progressive increase in systolic blood pressure of lead-poisoned rats over a 2-month period, but these results were not confirmed in similar studies by Pardoe (1952) and by Padilla *et al.* (1969).

Renal adenomas or carcinomas develop in 60–80% of rats fed for more than one year a diet containing lead. Incidence and size of the tumors are related to duration of lead feeding (Fig. 22). By light microscopy, the tumors are adenocarcinomas, presumably arising from focal areas of hyperplasia of renal tubular lining cells (Fig. 23A).

The ultrastructure of the tumors (Mao and Molnar, 1967) is characterized by cellular and nuclear hypertrophy, numerous lysosomes and microbodies, and absence of infolding of basal plasma membranes as normally seen in renal tubular lining cells. Tumor cells do not contain intranuclear inclusion bodies, and lead content of the tumors is less than that of adjacent renal cortex. In our investigations, about one-fourth of the rats with tumors had metastatic foci in the lungs, but not in other organs (Fig. 23B) (Goyer, 1971c). Renal tumors in lead poisoning were first observed in rats by Zollinger (1953) following long-term injections of lead phosphate, and later by Kilham *et al.* (1962) in wild rats believed to have been exposed to lead fumes in burning refuse in a city dump. Lead-induced renal epithelial tumors have since been studied by a number of investigators (Boyland *et al.*, 1962; Van Esch *et al.*, 1962; Hass

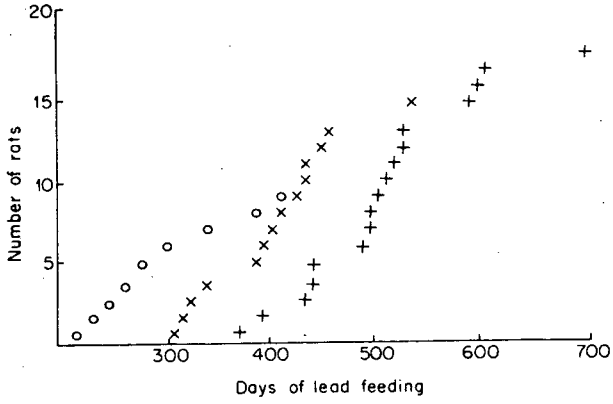


FIG. 22. Correlation of incidence of renal tumors with duration of lead feeding. Open circles indicate rats without tumors (9); ×, rats with microscopic tumors (14); +, rats with gross tumors (17). From Mao and Molnar (1967), by permission of *Amer. J. Pathol.*, Durham, North Carolina.

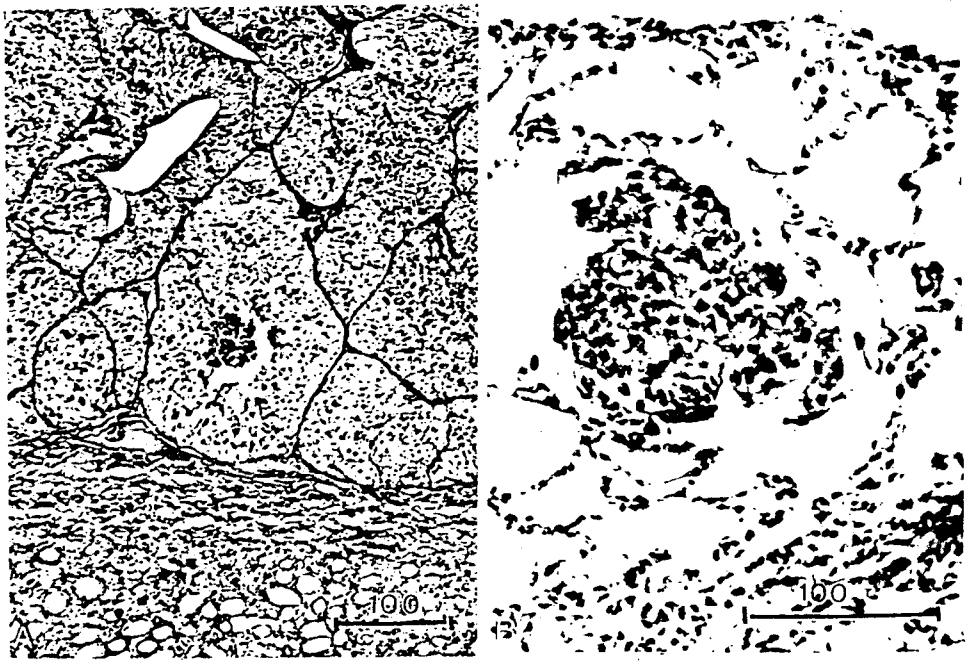


FIG. 23. (A) Renal adenocarcinoma in cortex of kidney of rat fed 1% lead acetate diet for 84 weeks. The tumor appears well demarcated from adjacent cortex, but the cells are poorly differentiated. From Goyer (1971c). ×150. (B) Pulmonary metastases occur as small nests of cells in the periphery of the lung parenchyma. ×250. From Goyer (1971c), by permission of Springer-Verlag, Berlin and New York.

et al., 1967). They have not been noted in lead-intoxicated experimental animals other than rats and mice, less frequently in the latter (Van Esch and Kroes, 1969). The tumors do not appear to have any obvious or immediate relevance to man, since renal tumors have not been noted in industrial workers with chronic exposure to lead. Moreover, the incidence of carcinoma of any type in such workers is not greater than expected in the general population (Dingwall-Fordyce and Lane, 1963).

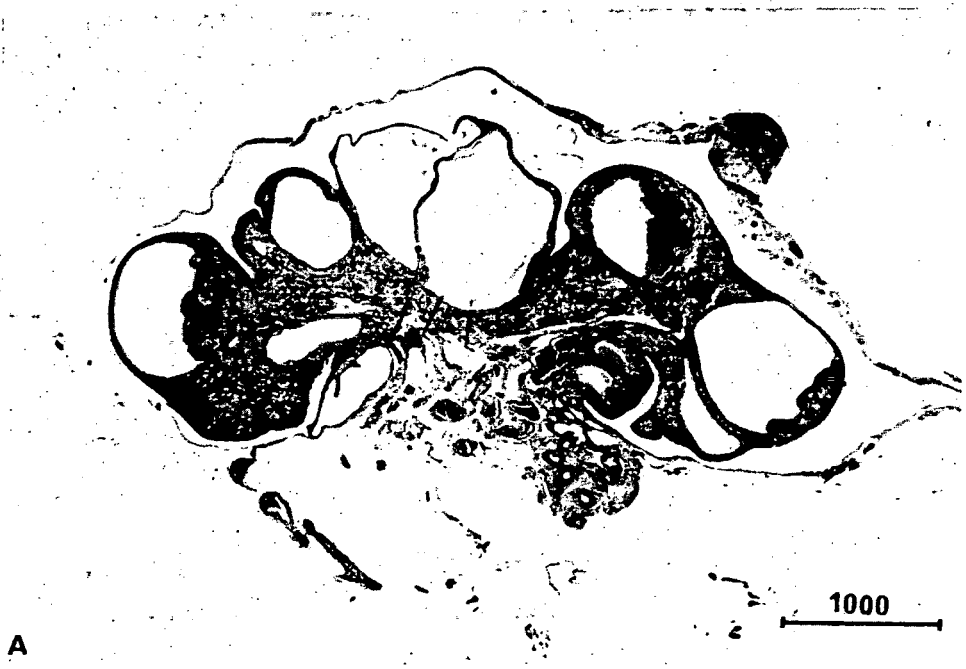
4. *Comparison of Experimental and Human Lead Nephropathy*

Morphological and functional reactions of kidneys in experimental animals fed lead orally and in people, particularly children with acute lead toxicity, have features which are comparable. In both instances, proximal tubular lining cells are affected, intranuclear inclusion bodies are formed, and there is an associated aminoaciduria. Impairment of mitochondrial function has not been demonstrated in man, but may be inferred from morphological changes in human biopsy material (Section IV, A). Recovery from the lead-induced acute tubular lesion in man certainly occurs. Excessive aminoaciduria returns to normal a few weeks after chelation therapy, but intranuclear inclusion bodies may persist for several years after acute lead poisoning (Galle and Morel-Maroger, 1965). Continued formation of inclusion bodies probably reflects a certain body burden of lead.

In man, progression of acute effects of lead on renal tubular epithelial cells to chronic lead nephropathy has been less clearly documented. Morphological changes observed in kidneys of persons with histories of excessive exposure to lead are similar to the changes seen in kidneys of experimental animals. The problem is that in the absence of intranuclear inclusion bodies, the pathology of chronic lead nephropathy, like that of many other forms of chronic renal disease, lacks specificity. Hyperuricemia occurs in rats with lead poisoning and in persons with chronic lead nephropathy. A major difference between the renal reaction to lead in man and in the rat is that rats develop renal adenocarcinoma. There is no evidence that lead enhances malignancy of any type in man.

A problem in the understanding of the progression of acute renal effects in man to chronic renal disease is the paucity of renal biopsy material from persons with excessive exposure to lead.

It appears, however, from reports of studies on man and on experimental animals that chronic effects of lead on the kidney are dependent on a particular renal content of lead during a prolonged period of time, and that lead is certainly capable of inducing a chronic nephropathy. More information is needed about factors that influence susceptibility of the kidneys to effects of lead.



A

B

VIII. Effects of Lead on Other Organ Systems

A. Effects on the Reproductive System

Severe lead intoxication has long been associated with sterility, abortion, stillbirths and neonatal deaths in man (Nishimura, 1964; Gillet, 1955; Potter, 1961), but evidence that lead poisoning affects birth rate or causes injury to the fetus is not conclusive. Studies with rabbits, guinea pigs, and rats indicate that ingestion of lead by either parent may have an effect on reproductive performance. Both size and number of offspring are reduced (Cole and Bachhuber, 1914; Weller, 1915; Morris *et al.*, 1938; Dalldorf and Williams, 1945, Puhač *et al.*, 1963). A recent study by Stowe and Goyer (1971) has shown that ingestion of lead by offspring (F_1 generation) of lead-burdened parents results in decreased reproductive fitness. Both paternal and maternal effects were recognized.

Paternal ingestion of lead appears to result in retardation of embryonic growth of the offspring and in a reduction in the number of weaned pups per litter. These observations may indicate a defect in the spermatozoa of lead-intoxicated males which fertilize the normal ova. No morphological or functional (motility) difference between sperm from control and lead-fed parents was found.

The maternal effects of lead are exhibited by reduced litter size, retardation of fetal development, and impaired postnatal survival. These effects may be classified as gametotoxic, intrauterine, and extrauterine. The combined male and female effects of lead toxicity on overall reproductive ability were greater when both parents had increased lead burdens, which suggests different effects in both parents as well as additive effects. The ovaries of lead-burdened mothers show a reduced number of developing follicles (Fig. 24). Similar morphological changes have been observed in ovaries of rhesus monkeys with lead intoxication (Vermande-van Eck and Meigs, 1960). The offspring in these experiments did not have any congenital malformations.

A teratogenic effect of lead has been demonstrated experimentally in the golden hamster (Ferm and Carpenter, 1967), and chromosome aberrations occur in leukocyte cultures of lead-poisoned mice (Muro and

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FIG. 24. (A) Cross section of ovary from a normal rat 22 days postpartum. Numerous developing follicles are present. ×20. From Stowe and Goyer (1971). (B) Cross section of ovary from an F_1 lead-intoxicated rat, 22 days postpartum. The ovary consists almost entirely of corpora lutea with few developing follicles. ×20. From Stowe and Goyer (1971). Reproduced from *Fert. Steril.*

Goyer, 1969). Both experiments employed relatively large dosages of lead and the teratogenic effect of lead is species specific.

B. Endocrine Effects

Endocrine effects of lead are poorly defined at the present time. Lead decreases thyroid function in man and experimental animals. Conversion of iodine to protein-bound iodine is impaired in lead-intoxicated rats (Sandstead, 1967). Uptake of ^{131}I is sometimes decreased in men with lead poisoning and can be offset by treatment with thyroid-stimulating hormone (Sandstead *et al.*, 1969). The uptake of iodine by thyroid glands in rats poisoned with lead is decreased, and the uptake of iodine by thyroid slices from leaded rats is also decreased (Slingerland, 1955)

C. Myocardial Effects

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. There is sparse mention of direct cardiac effects of lead in the older literature. However, a report of degenerative changes in heart muscle of five children dying of acute lead toxicity has brought attention to this possibility (Kline, 1960). More recently, Silver and Rodriguez-Torres (1968) noted abnormal electrocardiograms in 70% of 30 children with symptoms of lead toxicity. After chelation therapy, the electrocardiograms remained abnormal in only four (13%) of the patients. Electron microscopy of myocardium of lead-intoxicated rats has shown diffuse degenerative changes (Asokan *et al.*, 1971).

D. Changes in the Immune Mechanism Induced by Lead

Although little is known at present about the effects of acute or chronic lead poisoning on the immune mechanism and on susceptibility to infectious disease, recent studies suggest such a relationship. Mice exposed to low doses of lead nitrate for 30 days showed greater susceptibility to challenge with *Salmonella typhimurium* than controls which received no lead (Hemphill *et al.*, 1971). Possible mechanisms for this effect include interference with polymorphonuclear cell function and binding of immune proteins (including antibodies and perhaps complement) by lead. Further understanding will come from additional study, but relevance to lead

toxicity in man may be difficult to establish. Children most frequently affected by lead poisoning live in underprivileged urban groups and frequently have anemia. They may be high-risk candidates for contracting infectious disorders for reasons other than lead toxicity.

IX. Recognition of Lead Toxicity

For the recognition of lead toxicity one must distinguish between body burden of lead (body content) and adverse biological effects of lead which may be regarded as toxic effects. There is no particular level of total body lead content above or below which one is afflicted or not afflicted with lead intoxication. Symptoms of toxicity or cellular effects of lead correlate with tissue content of diffusible or mobile lead as discussed in Section III, D. This concept explains at least in part why one individual with a relatively small exposure to lead and body burden of lead may have clinical symptoms of lead toxicity whereas a lead industry worker with large body stores of lead, largely in the form of fixed or nondiffusible lead in bone, may not have symptoms. Moreover the onset of lead toxicity, even acute toxicity, is not a sharply defined event. Rather, it involves a continuum of change from normalcy to ill health (Goyer and Chisolm, 1972). One published "Statement" intended as a guide for the diagnosis of lead toxicity in adults includes four classes of lead effect (R. E. Lane *et al.*, 1968). Regardless of the number of levels of toxicity one wishes to define, it is important conceptually, to recognize a transitional state between no effect and clinical illness, a state that may be regarded as a compensatory or asymptomatic phase of lead poisoning.

A simple scheme for the diagnosis of lead toxicity is presented in Fig 25.

Sign	No effect	Adaptive or subclinical	Clinical toxicity
Blood lead, μg/100 ml	< 40	40 – 80	> 80
Urine ALA and CP	Normal	Slight increase	> 5-Fold
Anemia	None	Reticulocytosis	Usually
Nervous system effects	None	↓ Nerve conduction?	Ataxia, coma, convulsions
Renal effects	None	None, inclusion bodies?	Fanconi syndrome, chronic nephropathy

FIG. 25. Relationship of various parameters of lead toxicity with stage of lead effect. ALA, aminolevulinic acid; CP, coproporphyrin.

Diagnostic criteria are divided into three phases, no effect phase, an adaptive phase, and a phase of overt or clinical toxicity. Blood lead concentration is probably the single most useful measure of lead toxicity since increased blood lead concentrations reflect soft tissue concentrations which relate to adverse functional or metabolic effects. It is uncommon to find subjects with blood lead concentrations greater than 60 μg/100 gm of whole blood who have not had excessive exposure to lead. Few persons in the general population, adults or children, have blood levels of 40 μg/100 gm without a history of excessive exposure to lead. The "compensated" lead industry worker may have blood lead levels near 80 or 100 μg/100 gm without overt symptoms of lead toxicity. On the other hand, children with blood lead levels greater than 60 μg/100 gm must be regarded as experiencing lead intoxication. Blood lead concentration in itself is not a measure of pathological effects of lead but must be correlated with other biochemical, functional, or clinical manifestations of lead toxicity.

d-ALA and coproporphyrin excretion reflects lead effects on heme synthesis, and these two porphyrin intermediates are the most sensitive indicators of an adverse biochemical effect of lead (Editorial, 1970). The relationship between urinary *d*-ALA and blood lead is shown in Fig. 26. For persons with blood lead levels above 40 μg/100 gm urinary *d*-ALA excretion does relate to blood lead levels although variation is great, and there may possibly be no increase in *d*-ALA with blood lead levels below 80 μg/100 gm. This range corresponds to the adaptive or subclinical phase of lead intoxication. Increase in urinary *d*-ALA is exponential with respect to lead concentration in the blood. Blood lead above 80 μg/100 gm is nearly always associated with increased urinary *d*-ALA excretion and is usually accompanied by discernible clinical effects such as lead-related anemia, with or without effects on the nervous system. Urinary coproporphyrin concentrations similarly reflect a lead effect on heme metabolism and may be even more sensitive than urinary *d*-ALA, but they lack specificity. Nevertheless, measurement of urinary coproporphyrin concentration may be a useful screening test. Anemia is a less sensitive index of lead toxicity than detection of heme products in the urine. During the early or adaptive phase of increased exposure to lead, increased synthesis of hemoglobin and production of erythrocytes may compensate for loss of erythrocytes due to decreased survival or due to inhibitory effects of lead on heme synthesis (Section VI, C), but reticulocytes and basophilic stippling of red blood cells are present nevertheless.

Effects of lead on the nervous system and the kidneys are usually recognizable only during the overt phase of toxicity. Children with blood lead concentrations above 120 μg/100 gm nearly always have lead encephalopathy, and adults with blood lead levels in this range are regarded

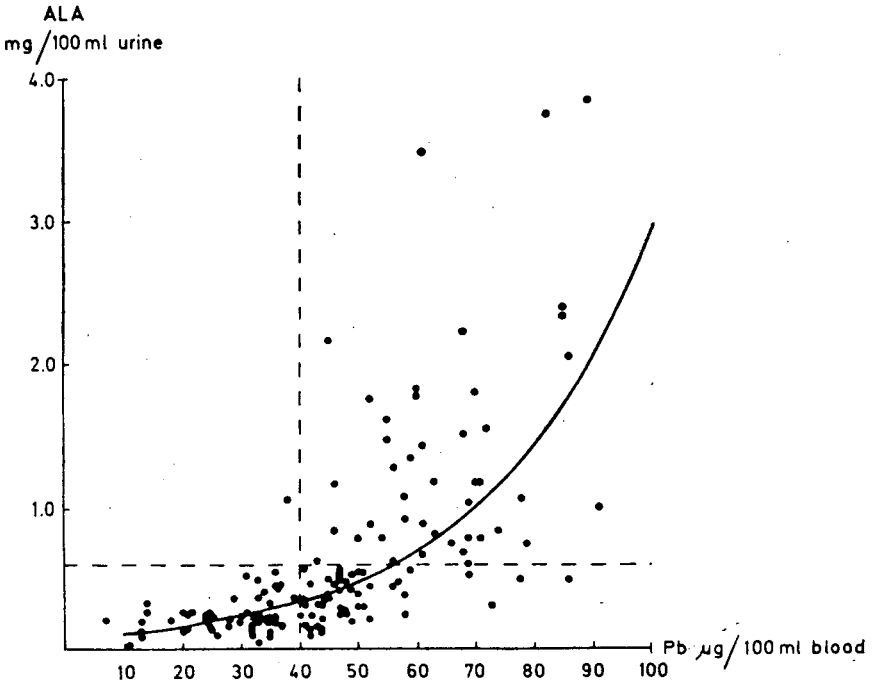

FIG. 26. Values for aminolevulinic acid (ALA) in urine plotted against those for lead in blood. The dotted lines mark the upper normal limits. From Selander and Cramer. (1970), by permission of *Brit. Med. J.*, London.

as having "dangerous" levels of exposure with accompanying acute symptoms and long-term sequelae. Nerve conduction, as expressed by a decrease in nerve impulse amplitude, may be decreased in the adaptive phase of lead toxicity (Fullerton and Harrison, 1969) in workers with excessive exposure to lead (See Section V, D).

Clinically demonstrable renal effects of lead toxicity, namely renal tubular dysfunction, or the Fanconi syndrome, occur only in association with other signs of lead toxicity. Industrial workers with excessive exposure to lead and increased urinary excretion of lead, but without clinical signs of lead toxicity have only minor increases in urinary amino acid excretion (Clarkson and Kench, 1956; Goyer *et al.*, 1972). It is unlikely, therefore, that renal tubular function is impaired to a measurable degree during the adaptive phase. Nevertheless, intranuclear inclusion bodies occur in rats at a lower dosage of lead than is needed to produce functional signs of lead toxicity. Also, inclusion bodies are present in renal biopsy specimens of lead

industry workers with minimal signs of lead toxicity (Section IV, A). It is suggested, therefore, that inclusion body formation involving the binding of renal lead in a nondiffusible lead–protein complex probably occurs during the adaptive phase of increased exposure to lead.

Overt clinical toxicity may be further subdivided into an early, reversible phase and irreversible effects. Reference to reversibility is more meaningful than use of the terms acute or chronic since these terms imply time as a factor. A child with acute lead intoxication may have more significant nonreversible, central nervous system sequelae than an individual with a more prolonged but less severe exposure to lead.

Estimates of body burden of lead in the absence of clinical symptoms are difficult to make and must be somewhat conjectural. In children, X-rays of long bones may reveal excessive storage of lead in the form of bands of increased density at the metaphysis. In adults (Henderson and Inglis, 1957; Westerman *et al.*, 1965), iliac bone biopsies obtained while performing bone marrow aspiration may be useful to measure bone lead. Urine lead level may be normal even with increased body stores, but the increase in urinary lead which follows a provocative dose of EDTA is another useful approach to estimating the body burden of lead (Emmerson, 1963). And, finally, lead content of hair and fingernail clippings may reflect increase in body lead stores (Kopito *et al.*, 1967; Hammer *et al.*, 1971).

Although blood lead levels in the upper range of normal (40–60 μg/100 gm) or in the early adaptive phase are not usually associated with clinical or even biochemical evidence of toxicity, it is not necessarily true that there is no harmful effect of marginal increases in body lead burden or blood lead levels. There is obvious need for more sensitive tests of subclinical effects of lead. A very sensitive biochemical reflection of minimal increases in blood lead is the *in vitro* assay of ALA-D located in the stroma of circulating red blood cells. Hernberg and co-workers (1970) have recently shown that decrease in ALA-D activity correlates with increase in blood lead levels (Fig. 27). The activity of the enzyme may decline to about one-third of its normal level before heme synthesis is affected, resulting in elevated urinary *d*-ALA (Haeger-Aronsen *et al.*, 1971). At higher levels of blood lead the activity of the enzyme is too low to be useful clinically. Whether a decrease in ALA-D in erythrocyte stroma of persons with normal or marginally elevated blood lead levels indicates any adverse effects on health is unknown. It must be kept in mind that this test measures the *in vitro* activity of ALA-D in blood hemolyzate and may not necessarily reflect any impairment of this enzyme in the intact red blood cell *in vivo*. Because of the extreme sensitivity of this test, evaluation of its possible clinical significance must be continued.

the toxicity of lead. It is apparent from a review of the proposed factors that few of them have been subjected to the rigors of experimental confirmation.

A. Age

Children with acute lead poisoning brought to out-patient clinics and emergency rooms of large metropolitan medical centers are usually between the ages of 2 and 5 years (Christian *et al.*, 1964; Ingalls *et al.*, 1961; Rennert *et al.*, 1970). Recent screening studies suggest that the total number of urban children with evidence of subclinical lead poisoning is many times that seen in the medical centers. These numbers contrast with sporadic occurrence of lead poisoning in adults. The latter is usually associated with identifiable episodes of exposure to large amounts of lead.

This difference in incidence of lead poisoning between children and adults is not, however, proof of greater biological susceptibility of children to the toxicity of lead. It may only mean that children are more prone to over-exposure to lead and that the smaller body size of children makes children more susceptible to a particular dose of lead. The opportunities for ingestion of lead contribute to the relative frequency of lead poisoning in young children, and pica probably accounts for most of the incidence of lead poisoning in early childhood.

Greater incidence of lead poisoning must, however, be distinguished from greater biological susceptibility. There are many reasons why the young might be expected to be more susceptible to lead. Many of these have been reviewed by Hardy (1966) and include the greater vulnerability of young growing tissue and greater variation in intestinal acidity or alkalinity, i.e., pH ranges that may facilitate absorption of lead. Also, shifts of lead into and out of the growing bone of a child may influence biological effects. On the basis of Kehoe's (1961) data, which show that blood lead concentrations rise to toxic levels upon chronic feeding of daily supplements of 1–3 mg of lead per day to young, healthy adults, ingestion of 6 mg of lead per day is almost certain to result in clinical toxicity in adults. Whether an adult will develop lead encephalopathy as observed in children is difficult to answer. Encephalopathy is rare in adults except as the result of very great exposure to lead vapors or organic compounds of lead. Dose-response relationships for large amounts of lead are not documented well enough in adults to allow of answering this question. Also, the greater incidence of lead encephalopathy in children may reflect inherently greater sensitivity of the nervous system. Alternatively, adults may have a greater capacity to store lead in an inactive form in bone or as lead-protein complexes or nuclear inclusion bodies. On the other hand, Hardy (1966) has suggested

that workers in lead industry may be more vulnerable to clinical lead toxicity after a particular dose of lead at any given time because of the likelihood of a high level of stored lead or nondiffusible lead in such persons.

B. Seasonal Variation

Clinically, lead poisoning is seen most frequently in the summer months although most affected children are exposed to lead contained in indoor paint, and such exposure is unlikely to vary seasonally. Kehoe's (1961) data suggest that urinary lead excretion in a person voluntarily ingesting supplemental lead is greater in the summer. It would seem therefore that this phenomenon must result from some seasonal metabolic difference. Two explanations cited by Baetjer (1959) include increased vitamin D from the sun's ultraviolet radiation, and increased environmental temperature. The latter notion is supported by experimental studies showing that lead-poisoned rabbits subjected to 37°C die in about 4 days, whereas lead-poisoned rabbits kept at room temperature survive. Mice exposed to high temperature and injected intraperitoneally with lead nitrite die more rapidly and have a higher mortality rate than similarly injected mice kept at room temperature. The added burden of dehydration further lessens the survival of mice injected with lead (Baetjer), although it is unlikely that dehydration is clinically relevant in lead-poisoned children (Chisolm, 1959). Horton points out that seasonal metabolic cycles might explain increased susceptibility to infectious disease and also may influence nutritional and metabolic abnormalities (Horton, 1971). These ideas have been reviewed by Sargent and Sargent (1950), but have not been related to the problems of lead toxicity.

C. Calcium and Phosphorus

The absorption of lead from the gastrointestinal tract as well as the partitioning of lead in various body compartments appears to be regulated by the same physiological mechanisms which control the metabolism of calcium and phosphorus. Interest in the relationship of these minerals to lead metabolism has been directed toward their usefulness in the treatment of lead poisoning. Many of the early studies are summarized in papers by Lederer and Bing (1940) and Shields and Mitchell (1941). Absorption of lead from the gastrointestinal tract is impaired by amounts of dietary calcium and phosphorus above certain low limits. Drinking of large amounts of milk has been practiced as prophylaxis to lead poisoning, but the effectiveness of this custom has been questioned (Longley, 1967). However,

TABLE V

INFLUENCE OF DIETARY CALCIUM ON LEAD EFFECT IN RAT FED NORMAL CALCIUM (0.7%), LOW CALCIUM (0.1%), AND LEAD (200 PPM OF DRINKING WATER) DIETS FOR 10 WEEKS[a]

	Animal groups				Level of significance due to Pb, Ca, and interaction of Pb and Ca		
	Normal Ca	Low Ca	Normal Ca + Pb	Low Ca + Pb	Pb	Ca	Pb × Ca
Number of animals	8	7	7	6			
Blood Pb (μg/100 ml ± 2 SE)	<10	<10	50±20	190±40	0.0001	0.0001	0.0001
Hematocrit (± 2 SE)	45.2±0.8	44.6±2.0	41.9±1.6	38.6±1.2	0.0001	NS	NS
Urinary *d*-ALA (μg/24 hours ±2 SE)	35.9±7.6	35.8±10.8	49.8±20.2	569.3±98.6	0.0001	0.0001	0.0001
Urinary NH_2N_2 (μmoles/24 hours ± 2 SE)	56.3±31	57.9±38	46.8±13	184.6±142	—	—	—
Kidney Pb (μg/gm ± 2 SE)	2.6±1.0	4.4±0.6	29.6±7.0	691±203	0.0001	0.0001	0.0001
Femur Pb	2.2±1.0	9.7±2.2	73.4±25	202±22	0.0001	0.0001	0.0001

[a] Modified from Six and Goyer (1970).

Kostial and co-workers (1971) have shown that calcium and phosphate additives to cow's milk reduce body burden of lead in newborn rats. Greater retention of trace amounts of dietary lead-203 was observed in rats 5–7 days old fed cows milk than in rats given cows milk supplemented with calcium and phosphate.

Shields and Mitchell (1941) concluded that low dietary calcium, phosphorus, or both, induce a higher retention of lead in the body by comparison to diets containing higher levels of these minerals. Attempts to partition the increase in retained lead between bone and soft tissues were limited to a few experiments. More recently, Six and Goyer (1970) have shown that in rats given 200 μg of lead per milliliter of drinking water low dietary calcium greatly enhances the severity of anemia, the blood lead levels, the urinary *d*-ALA excretion and the aminoaciduria by comparison to controls given the same amount of lead but adequate amounts of dietary calcium, (Table V). Partitioning of lead between bone and a specific soft tissue compartment is altered; a greater percentage of the lead increment is in soft tissue. This experiment also emphasizes the correlation between soft tissue lead and severity of clinical toxicity.

D. Protein

Dietary protein may influence lead intoxication. An early paper on this subject is that of Baernstein and Grand (1942). Young rats were fed lead chloride (1.5%) in diets containing 6, 13, or 20% protein (casein). Decrease in weight gain and mortality diminished when diets containing higher protein levels were given. Addition of cystine or methionine to the 6% casein diet decreased mortality and improved weight gain in the rats fed lead as well as in control rats. More recently Gontzea and co-workers (1964) observed that pair-fed rats on a 9% protein diet were more susceptible to lead intoxication than rats fed an 18% casein diet, as judged by the lead content of liver, kidney, and blood.

E. Vitamins

1. *Vitamin D*

Vitamin D may enhance lead poisoning in experimental animals. Lead concentration in blood and bones is greater in rats receiving supplementary vitamin D than in those not receiving it (Sobel *et al.*, 1938). Presumably, vitamin D enhances gastrointestinal absorption of lead as well as of calcium. Whether increased bone deposition of lead during administration

of vitamin D reflects a specific effect on bone metabolism, or merely reflects increased blood levels of lead, is not clear.

2. *Ascorbic Acid*

The addition of large amounts of ascorbic acid to the diet of industrial workers was suggested as a means of alleviating such symptoms of lead intoxication as basophilic stippling of erythrocytes (Holmes *et al.*, 1939). Pillemer and co-workers (1940) found that lead-poisoned guinea pigs treated with a scorbutic diet developed neurological symptoms more readily than did lead-poisoned guinea pigs fed adequate amounts of ascorbic acid. Other investigators have found ascorbic acid to be without effect in lead toxicity (Evans *et al.*, 1943; Dannenberg *et al.*, 1940).

3. *Nicotinic Acid*

A number of experimental studies suggest that nicotinic acid synthesis from tryptophan is impaired in experimental lead poisoning (Pecora *et al.*, 1966). Administration of nicotinic acid may relieve some of the clinical manifestations of experimental lead poisoning (Sales Vazquez, 1943), and it reduces porphyrinuria in lead-poisoned rabbits (Pecora *et al.*, 1966; Benko, 1942). This finding was not confirmed in similar studies with rats (Acocella, 1966).

Others have found reduced nicotinic acid levels in blood and urine in lead poisoning along with increased urinary excretion of xanthurenic acid, which suggests impaired tryptophan metabolism (Pecora *et al.*, 1966). However, on the basis of tryptophan load tests, Tenconi and Acocella (1966) concluded that lead intoxication in rats did not cause changes in tryptophan metabolism similar to those seen in pyridoxine-deficient states.

F. Alcohol

It has long been believed that alcoholism increases susceptibility to the toxic effects of lead, a relationship briefly referred to in Section VII, A, 2 c. Little is known about the basis for the apparent synergism between alcohol and lead. If the cellular pathology of lead and alcohol are compared, similarities can be noted. Both lead and alcohol produce mitochondrial injury. *In vitro* studies of mitochondria from ethanol-treated rats show decreased oxidative properties and increased membrane permeability (French and Todoroff, 1970).

Another explanation of the apparent synergism of lead and alcohol is that the introduction of nutritional deficiencies by alcoholism enhances the

TABLE VI

INFLUENCE OF IRON DEFICIENCY ON TISSUE LEAD CONTENT AND HEMATOCRIT AND URINARY *d*-AMINOLEVULINIC ACID (*d*-ALA) IN RATS FED NORMAL IRON AND LOW IRON WITH AND WITHOUT LEAD (200 μG/ML DRINKING WATER)[a]

	Animal groups				Level of significance due to Pb, Fe, and interaction of Pb and Fe		
	Normal Fe	Low Fe	Normal Fe + Pb	Low Fe + Pb	Pb	Fe	Pb × Fe
Number of Animals	9	8	8	8			
Hematocrit (% ± SD)	45.7±1.2	42.6±1.9	44.2±1.7	37.8±1.8	0.001	0.001	0.05
Urinary *d*-ALA (μg/24 hours)	16.3±8.5	16.3±8.5	180.2±95	356.8±167	0.001	N.S.	0.050
Kidney Pb (μg/gm wet tissue ± 2 SD)	1.0±0.2	1.9±0.8	14.5+3.2	38.7±9.5	0.001	0.001	0.001
Femur Pb (μg/gm wet tissue)	5.6±2.7	10.6±6.0	75.2±26.2	225.2±30.3	0.001	0.001	0.001

[a] Modified from Six and Goyer (1972).

toxicity of lead. These deficiencies include insufficient intake of calcium, of protein, and of vitamins.

G. Iron Deficiency

Children with lead poisoning often have iron deficiency anemia, and either lead poisoning or iron deficiency result in a microcytic anemia (Section VI, A). A synergism between the two conditions has been suspected. The tendency for children to have pica, a factor in childhood lead poisoning, may be one level of interaction between these two conditions. Experimental iron deficiency, enhances tissue concentrations of lead in rats and results in toxic effects when doses of lead are given that are subtoxic to control rats (Six and Goyer, 1972) (Table VI).

H. Synergism with Other Metals

It might be expected that the metabolism of different heavy metals is similar enough to have overlapping or similar toxic effects. There are few clear examples of such synergism. Several of the heavy metals bind *in vivo* to red blood cells. However, the attachment of lead to the red blood cell membrane is not influenced (*in vitro*) by the presence of other heavy metals, including cadmium, mercury, zinc, and aluminum; this suggests that lead may be metabolized independent of other metals (Clarkson and Kench, 1958).

It has been learned recently that cadmium is elevated along with lead in the blood of children with suspected lead poisoning, and the possible toxic synergism of these metals is being explored clinically (Challop, 1971). A synergism of the teratogenic effects of lead and cadmium in experimental animals has been demonstrated recently (Ferm, 1969).

I. Influence of Coexisting Disease

Preexisting disease of major organ systems may enhance the vulnerability of affected persons to the toxic effects of lead, but documentation of this type of synergism is limited. Several reports from Europe point out the increased susceptibility of persons with hemoglobin anomalies, such as hemoglobin S and C disease and thalassemia, to toxic substances which,

like lead, affect erythrocyte metabolism. Carriers of such defects must be recognized and protected from exposure to lead (Gaultier *et al.*, 1968).

Individuals deficient in glucose-6-phosphate dehydrogenase show increased susceptibility to lead and should be identified by a screening test before they are employed in a lead industry (Stokinger and Mountain, 1967).

The kidney has a key role in lead metabolism, and chronic renal disease from any cause must reduce the capacity of an individual to excrete lead. Studies in rats suggest that the immature kidney is more susceptible than the adult kidney, and reduced renal excretory capacity, such as occurs after unilateral nephrectomy, enhances the toxicity of lead (Tange *et al.*, 1965).

XI. Summary

The pathological effects of lead are most prominent in three organ systems: the nervous system, hematopoietic system, and kidneys. Acute encephalopathy is characterized by cerebral edema, proliferation and swelling of endothelial cells, dilatation of capillaries and arterioles, proliferation of glial cells, focal necrosis and neuronal degeneration. The greatest concentration of lead in the brain is in cortical and cerebellar gray matter. The proliferation of glial cells may reflect a direct reaction to lead since lead-induced inclusion bodies may be found in nuclei of astrocytes near the cortical surface of the brain. The sequelae of lead encephalopathy include convulsions, mental retardation, and, less commonly, optic atrophy. Peripheral lead neuropathy is characterized by segmental demyelination and axonal degeneration which are accompanied by Schwann cell degeneration.

Lead produces a microcytic hypochromic anemia. The anemia results from two types of effects, a reduction in red blood cell survival and impairment of heme synthesis. The shortened life-span of erythrocytes may result from a direct effect of lead on mature red blood cells. Impairment of heme synthesis by lead has been investigated in detail, and at least three alterations have been defined: inhibition of ALA-D, derepression of *d*-ALA synthetase, and impairment of incorporation of iron into heme, probably due to inhibition of ferrochelatase activity. Inhibition of ALA-D results in increased urinary *d*-ALA excretion, a sensitive indicator of biochemical effects of lead.

The renal effects of lead consist of acute, reversible tubular dysfunction characterized by the Fanconi syndrome, and of chronic nephropathy with nonspecific changes including interstitial fibrosis and cystic dilatation of atrophic tubules. Chronic lead nephropathy is often accompanied by hyperuricemia and gout. Nephropathy only results from long-term,

continuous exposure to high levels of lead and is presumably uncommon today. In rats, lead-induced nephropathy results in hyperplasia of renal tubular lining cells and in adenocarcinomas. There is no evidence that lead produces cancer in man.

Other pathological effects of lead include a possible reduction in reproductive fitness, altered endocrine function, particularly hypothyroidism, and possibly myocardial changes in persons with acute lead intoxication. It is possible that lead also reduces the effectiveness of immune responses to injections.

The cellular response to lead involves the formation of intranuclear inclusion bodies in cells of organs with the greatest exposure to lead, i.e., the cells lining proximal convoluted tubules in the kidneys, and hepatocytes. Intranuclear inclusions have recently also been demonstrated experimentally in astrocytes of the superficial cerebral cortex of suckling rats. These bodies are composed of a lead–protein complex and may permit cellular function to continue in the presence of increased amounts of intracellular lead by binding the lead in a nondiffusible complex. The formation of inclusion bodies may be important during adaptation to excessive exposure to lead.

Membranes of mitochondria appear to be particularly sensitive to intracellular lead. Mitochondrial lesions include decreased respiratory and phosphorylative abilities. Such lesions may contribute to the dysfunction of renal tubules observed in acute lead poisoning. Since some of the heme-containing enzymes are located within mitochondria, the effect of lead on these organelles in erythroblastic cells is related to the anemia of lead toxicity. Effects of lead on protein synthesis are less well understood. Lead interferes *in vitro* with ribosomal aggregation and may also affect mitochondrial protein synthesis.

To recognize lead toxicity, one must take into account at least three phases: a covert phase, an adaptive or subclinical phase, and a phase of overt clinical toxicity. Blood lead levels are a useful measure of lead toxicity since lead concentration in the blood is in equilibrium with soft tissue content of lead, which in turn is related to adverse functional or metabolic effects. Increase in urinary excretion of *d*-ALA and coproporphyrinuria reflect early effects of lead on heme synthesis. The most sensitive biological assay of blood lead levels presently available is the decreasing activity of the enzyme ALA-D in hemolyzates of whole blood with increasing blood lead levels. Enzyme activity decreases with blood lead levels even in the normal range and is decreased to one-third normal activity before symptoms of lead toxicity can be ascertained. Whether depressed *in vitro* activity of this enzyme reflects an adverse effect of lead on health *in vivo* is not known.

Concern for potential toxic effects of lead has arisen from growing awareness of the relatively large amounts of lead in the environment. There is no question that excessive exposure to lead results in clinical toxicity. There is uncertainty, however, regarding the potential harmful effects of low levels of lead that do not produce overt toxicity. Clarification of this problem will only be achieved with greater knowledge of the metabolism and cellular pathology of lead.

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