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**Urinary Delta-aminolevulinic Acid (ALA) Levels in Childhood Lead Poisoning**

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URINARY DELTA-AMINOLEVULINIC ACID (ALA) LEVELS
IN CHILDHOOD LEAD POISONING

by

Ronald H. Abrahams B.S., R.Ph.

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A Dissertation Submitted to the Faculty of the Graduate
School of Loyola University in Partial
Fulfillment of the Requirements
for the Degree of
Doctor of Philosophy
February
1970
LIFE

Ronald H. Abrahams was born in Chicago, Illinois on November 14, 1942. He attended Senn High School in Chicago and was graduated in June, 1960. After one year of Pre-Pharmacy at the Chicago Branch of the University of Illinois, he attended the University of Illinois College of Pharmacy in Chicago, Illinois where he received the Degree of Bachelor of Science in Pharmacy in June, 1965. The author currently is a Registered Pharmacist in the State of Illinois.

Upon his graduation, the author entered graduate studies in Pharmacology at Loyola University Stritch School of Medicine.

In July, 1969 he was married to the former Miss Carol Marcus of Chicago, Illinois.
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CHAPTER I
INTRODUCTION

A. HISTORY OF LEAD POISONING

Lead and its compounds have been used by man for at least six thousand years (Brunton, 1937). It was among the first metals known to the early Hebrews, Egyptians, and Phoenicians and the symptoms of plumbism were familiar to Greek, Roman, and Arabian physicians before the Christian Era. Hippocrates in 370 B.C. was the first to recognize lead's toxic properties and Nicander in the second century B.C. observed the relationship of constipation, abdominal pain, and pallor to the action of lead on the body. Over the years more has been written about the causation and symptomatology of poisoning by lead than by any other toxic agent (Johnstone, 1966).

Modern interest in lead poisoning dates from the 17th and 18th centuries when outbreaks of lead poisoning occurred throughout western Europe as a result of the addition of lead to wine to promote fermentation and because of its use in the manufacture and storage of cider and in material for cooking-vessels and other household articles (Cantarow and Trumper, 1944). Crato in about 1600 attributed the colic which was common in Moravia to the use of "falsified wine" and Citosis, a physician of Poitiers, in 1616 mentioned bad wine which had been treated with lead as the cause of colic. In 1745 Huxham described a disease appearing in Devonshire, England having
symptoms identical with true lead colic and this was traced by Sir George Baker (1767) to the lead used in the manufacture and storage of cider. A similar type of colic of similar etiology occurred in Spain and was described by Luzuriaga in 1796.

Tanquerel des Planches in 1839 published an epoch-making treatise containing an accurate clinical description of lead poisoning and since Tanquerel's time, little has been added to our clinical knowledge of lead poisoning. This treatise was responsible for the initiation of a period of intensive clinical and experimental investigation of lead poisoning and this research is presently continuing.

Gilfillan (1965) has recently linked lead poisoning with the fall of the Roman Empire. The Roman upper class died out with great rapidity beginning about the 2nd or 1st century B.C. and plumbism is believed to have contributed to this decimation because the upper class commonly used lead-lined pots, lead aqueducts, and lead containing paints, cosmetics, and home prepared concoctions such as wine-mead. The poorer classes could not afford the luxury of using lead and were therefore spared lead's toxic effects.

B. **ABSORPTION OF LEAD**

1. **Respiratory Tract**

Emissions of lead into the atmosphere result from burning of lead-bearing materials, industrial processes in which lead is used, re-entrain-
ment of lead-bearing soil, and combustion of lead-bearing fuels. Gasoline and coal are the two major lead-bearing fuels and coal combustion in the Cincinnati area emits between 54 and 270 pounds of lead into the atmosphere each day, depending on the amount of lead retained in the ash or air cleaning equipment. Combustion of leaded-gasoline in the Cincinnati area causes an emission of 1700 pounds of lead into the atmosphere per day (McCaldin, 1966).

The atmospheric lead concentration of a general urban area ranges from 1 to 3 \( \mu g/m^3 \), while measurements taken near automobile traffic increase to 40 \( \mu g/m^3 \) (U.S.D.H.E.W., 1965). This indicates the importance of combustion of leaded-gasoline as a source of atmospheric lead pollution.

Kehoe (1964a) has calculated that the respiratory intake of lead by a normal adult living in a large city is in the order of 30 to 40 \( \mu g \) per day. Of this amount, 70 to 75% is discharged in the expired air and only 25 to 30%, never more than 50%, is retained in the lungs and absorbed. The respiratory tract thus can account for an absorption of up to 20 \( \mu g \) of lead each day.

A number of factors influence the absorption of lead by the respiratory tract. Among these are particle size and density, rate and depth of breathing, duration of exposure to the lead, and the hygroscopic nature of the lead particle (Tomashefski and Mitchell, 1966). The early studies of Goadby and Goodbody (1909) and of Blumgart (1923-24) have shown that even relatively
insoluble lead compounds can be absorbed by the respiratory tract from the nasopharynx to the pulmonary alveoli. Robinson et al. (cited by Tomashefski and Mitchell, 1966) have found that the particle size of atmospheric lead in the Los Angeles area is of such a magnitude that it reaches the alveoli where deposition, retention, and absorption via the lungs can occur. A total of 60% of the atmospheric lead particles were found to have a diameter less than 0.45 micron, 75% less than 0.90 micron, and 90% less than 1.60 microns.

2. **Gastrointestinal Tract**

Lead is present in all classes of natural foods and water and the average daily intake from the diet is about 0.3 mg per day with a range between 0.1 and 2.0 mg (Kehoe, 1961). Only 10% of the ingested lead is absorbed (Kehoe, 1961) and this absorption occurs mainly from the small intestine, to a lesser extent from the colon, and not at all from the stomach (Harvey, 1965). The major part of the absorbed lead enters the enterohepatic circulation and only a small portion ever reaches the systemic circulation (Cantarow and Trumper, 1944). Thus, even though 90% of the total lead intake is contributed by the diet, the respiratory tract, under normal conditions, is the most important portal of entry of lead.

Kehoe et al. (1943) have shown that none of the variations in milk, calcium, phosphorus, pectin, or ascorbic acid intake affect the absorption of lead. The only factor found to significantly influence this absorption was
the motor activity of the bowel. A decrease in the number of evacuations caused an increased absorption of lead, while an increase in frequency caused a decreased absorption.

3. Skin

Absorption of inorganic lead compounds through the intact skin is of little significance in the development of lead poisoning (Legge and Goadby, 1912; Hamilton, 1912; Oliver, 1914). However, lead salts can be absorbed through abrasions and numerous cases of lead poisoning have been reported to occur following the application of lead containing lotions and ointments to ulcers, burns, skin eruptions, and areas of inflammation (Tanquerel des Planches, 1839). Plumbism has also been reported to occur from the absorption of lead from bullets lodged in tissues (Oliver, 1914; Machle, 1940).

Organic lead compounds such as tetraethyl lead (TEL) have been shown to pass readily through intact skin. Kehoe and Thamann in 1931 demonstrated that pure TEL is rapidly absorbed when applied to the skin of rabbits. However, TEL absorption from gasoline containing 0.1% TEL was found to be inappreciable. Since TEL is eventually converted in the body to inorganic lead, continued absorption of small amounts of TEL can result in the classical syndrome of chronic lead intoxication.

C. TRANSPORTATION OF LEAD IN THE BODY
Once lead is absorbed, it is transported by the blood in which 90 to 95% is bound to the red blood cells (Blumberg and Scott, 1935; Cantarow and Trumper, 1944; Stover, 1959). The form in which it is carried is believed to be either as an albuminate (Oliver, 1914), a dibasic phosphate in colloidal form (Aub et al. 1925), or a diphosphoglycerate (Maxwell and Bischoff, 1929). Witschi (1965) has recently found that lead is bound at least partly to the deeper layers of the plasma protein film on the erythrocytic surface.

The normal range of lead levels in blood is 15 to 40 µg per 100 ml (Goldwater and Hoover, 1967) with the upper limit of normal being 60 to 80 µg per 100 ml. Kehoe (1964b) stated that when the lead concentration in the blood reaches or exceeds the level of 80 µg per 100 g, the individual has absorbed a potentially dangerous quantity of lead. Several investigators, however, believe that a blood lead level of only 60 µg per 100 ml is indicative of an excessive lead absorption (Berman, 1966; Cramer et al., 1966; Weber, 1966).

D. DEPOSITION OF LEAD IN THE BODY

Lead present in the general circulation is at first distributed to the kidneys, liver, lungs, spleen, and muscle. However, within a few days, the lead is re-distributed to the bones where it is deposited as an insoluble tertiary lead phosphate (Aub et al., 1925). Normal individuals have a
higher concentration of lead in the long bones than in the flat bones, however, in cases of lead poisoning the reverse has been found to be true (Kehoe, 1961). Growing bones have the highest concentration of lead in the epiphyseal portion of the long bones and "lead deposits may be detected by X-ray examination as rings of increased density in the ossification centers of the epiphyseal cartilage and as a series of transverse lines in the diaphyses" (Harvey, 1965). These findings are of diagnostic importance in the determination of lead poisoning in children.

Bolanowski et al. (1964) using adult male albino rats injected with a single dose of Pb$^{210}$ divided the organs into three basic compartments differing in lead exchange rates. The first is the rapid exchange pool consisting of blood, heart, lungs, liver, kidneys, spleen, and GI tract. The second compartment is the intermediate pool consisting of the muscles and skin and the third is the slow exchange pool including bone and whole tail.

Factors affecting the metabolism of calcium similarly affect the metabolism of lead (Harvey, 1965). "High phosphate intake favors skeletal storage of lead and a lowering of the lead content in soft tissues." A high calcium intake in the absence of an elevated phosphate intake
causes a mobilization of lead from the bone due to competition with lead for the available phosphate. "Vitamin D tends to promote the deposition of lead in bone provided a sufficient amount of phosphate is available. Parathyroid hormone mobilizes lead from the skeleton and elevates the blood level and urinary excretion." Acidosis promotes the mobilization of lead from bone as do iodides and bicarbonate.

The body of an average 80 kg human has been reported to contain 100 to 400 mg of lead (Kehoe, 1961). The concentration of lead found in the brain, lung, heart, liver, spleen, kidney, muscle, flat bone, and long bone expressed as mean lead mg per 100 g of tissue is 0.04, 0.02, 0.04, 0.12, 0.03, 0.05, 0.03, 0.65, and 1.78, respectively.

E. EXCRETION OF LEAD

The total intake of lead due to food, beverages, and air approximates 0.33 mg per day (Kehoe, 1964a). Of this amount, 0.3 mg per day is eliminated in the feces and 0.03 mg per day is eliminated in the urine. The amount present in the feces consists largely of the lead that had passed through the GI tract without being absorbed, while the amount in the urine reflects that amount that was absorbed and is present in the blood. The normal individual has a urinary lead content of 20 to 65 μg
per liter with an average of 35 (Goldwater and Hoover, 1967). The upper limit of normal has been stated to be 150 µg of lead per liter of urine (Kehoe, 1964b).

F. LEAD INTOXICATION

The accumulation of large amounts of body lead constitutes an important health hazard with regard to both children and adults. Lead poisoning in children is often the result of oral ingestion of chips of lead-containing paint, while lead poisoning in adults usually results from accidental exposure to lead in an industrial environment.

1. Signs and Symptoms
   a. Acute lead intoxication

"Acute lead poisoning is quite rare and occurs following the accidental ingestion of lead compounds that dissolve readily in the stomach contents" (Harvey, 1965). The symptoms are a sweet metallic taste, thirst, nausea, vomiting, abdominal pain, and diarrhea or constipation. Acute nervous system symptoms including parasthesias, pain and muscle weakness occur and kidney damage and urinary changes are evident. The shock syndrome may develop and acute hemolytic anemia may be present. Death usually results within 1 to 2 days.
b. *Chronic lead intoxication*

The major clinical features of adult lead intoxication are abdominal pain, constipation, vomiting, nonabdominal pain, muscle weakness, and paraesthesia (Dagg et al., 1965). Abdominal pain is the most prevalent complaint and in Dagg's study occurred in 90% of the patients with lead poisoning. Anemia, stippled erythrocytes, lead line on the gums, and reticulocytosis are also commonly seen in lead-intoxicated patients. Anemia was noted in over 90% of the patients, while stippled red cells were present in 76%.

In children, the microcytic hypochromic anemia associated with chronic lead intoxication is often more pronounced than that seen in adults and is often accompanied with abnormally low serum iron levels (Watson et al., 1958). Often, the pallor due to anemia is the only sign or symptom seen in children with chronic lead poisoning. As in adults, children demonstrate GI disturbances, anorexia, vomiting, and constipation. Children, however, usually develop more severe central nervous system complications including encephalitis and mental retardation. Chisolm Jr. (1965) has stated that the serious sequelae of lead intoxication during early childhood are mental retardation, behavior disturbances,
convulsive disorders, and nephropathy.

i. Stippled erythrocytes

Behrend in 1899 was the first to report that lead causes stippling of the erythrocyte. Today, we recognize that two different types of granulations may be present in the erythrocytes of lead-intoxicated patients and animals (Griggs, 1964). The first type of granulation is due to an accumulation of excessive iron unusable for hemoglobin formation and the second type represents damaged mitochondria that do not contain iron.

The spleen is thought to play an important role in regulating the number of stippled erythrocytes in the general circulation. Stippled erythrocytes are young cells (Baikie and Valtis, 1954) and Bessis and Breton-Gorius (1962) have suggested that the spleen removes these cells from the circulation and causes removal of the granules either by maturing the cell through some unknown mechanism or by a process similar to that seen for the removal of siderocytic granules from red blood cells.

ii. Erythrocytic fragility

The erythrocytes from patients with lead poisoning often demonstrate increased resistance to osmotic lysis and increased susceptibility to
mechanical trauma (Griggs, 1964). Aub et al. (1925) produced similar
effects in vitro by incubating normal red blood cells with lead. Harris
and Greenberg (1954) also demonstrated that the addition of lead chloride
to normal human red blood cells suspended in autologous serum or
isotonic saline is followed by an increase in the mechanical fragility of
the cells and a decrease in susceptibility to osmotic lysis. Waldron (1964),
however, found that red blood cells incubated with lead have only a
slightly greater mechanical fragility index than controls.

Changes in osmotic and mechanical fragility indicate alterations in
the morphology and permeability of the erythrocytic membrane. Min
(1964) has reported that lead chloride induced a loss of potassium from
rabbit erythrocytes and Hasan et al. (1967a) have found that the erythro­
cytes from lead-exposed workers have an enhanced potassium loss. The
activity of the /Na⁺+K⁺/ -ATPase present in the membranes of the red
blood cells from lead-exposed individuals has been reported to be
decreased (Hasan et al., 1967b) and this finding is consistent with an
accelerated loss of intracellular potassium since /Na⁺+K⁺/ -ATPase is
closely associated with active ion transport.

The red blood cell's increased resistance to osmotic lysis seen in
both the in vivo and in vitro experiments indicates that a greater amount
of water could enter the cell before lysis occurs. The decrease in mean corpuscular volume, the increase in specific gravity, and the increase in mean corpuscular hemoglobin concentration indicate that "leaded" cells are shrunken and therefore have a greater capacity for swelling in hypotonic solutions.

iii. Erythrocyte survival

Hernberg (1967) has reported that workers exposed to lead for a mean of six years have a 20% shorter red cell survival time than non-exposed workers and this difference was found to be highly significant. Sheets et al. (1951), using the Ashby technique, studied the red cell survival time from a patient with lead poisoning and found that 50% of the cells had disappeared in approximately 25 days, compared to a normal 50% survival time of 55 to 60 days. Rubino et al. (1958), Crepet et al. (1963), and Griggs (1964) have also reported shortened red blood cell survival times in lead-poisoned adults. Leikin and Eng (1963) have likewise shown that children with chronic lead poisoning have shortened red cell survival times. Those children having a relatively short history of lead intoxication demonstrated the most pronounced shortening.

iv. Anemia

The precise cause of lead induced anemia is not known at present. Aub et al. (1925) concluded that the anemia was hemolytic and this view was
supported by the work of McFadzean and Davis (1949) and by Pirrie (1952). These latter two groups of investigators demonstrated that the anemia which developed in lead-poisoned animals was rapid in onset and was relieved by splenectomy. More recently Crepet et al. (1963) have reported that the anemia is produced by hyperhemolysis and by defective hemoglobin formation and these factors may act together or individually. Waldron (1966) also stated that the anemia of lead poisoning is due to the effect of lead on the enzymes responsible for heme synthesis. Gajdos et al. (1964) defined the anemia induced in experimental lead poisoning as being due to (1) inhibition of heme synthetase, the enzyme that catalyses incorporation of iron into protoporphyrin, (2) inhibition of erythropoiesis, and (3) partial hemolysis of the red blood cells which shortens their survival time. Inhibition of heme synthetase by lead has also been reported by deBruin and Hoolboom (1967).

v. Encephalopathy in children

The infantile brain is extremely susceptible to the toxic effects of lead and it is common for lead-poisoned children to develop restlessness, irritability, and convulsions. Once encephalopathy occurs, there is a case fatality rate of almost 25% with more than 50% of the survivors having damage to the central nervous system (Smith et al., 1965).

Okazaki et al. (1963) using the brains from lead-poisoned children found that the hippocampal and frontal cortices contained the highest lead
levels, while the occipital white matter and pons had the lowest levels. These investigators suggested that the initial effects of lead poisoning were selectively exerted upon the small caliber blood vessels in the brain causing increased permeability, focal necrosis, occlusion, and the transudation of plasma fluid into the perivascular, interstitial and subpial spaces. Pentschew (1965) reported that the brains from lead-poisoned children had dilated capillaries with swollen endothelial nuclei protruding into the lumen and microglial nodules spreading out over various gray areas and also over the white matter. The molecular layer of the cerebellar cortex contained loose accumulations of microglia cells and this layer was found to suffer the greatest damage in lead encephalopathy. The underlying cause of lead encephalopathy was considered to be a toxic disorder of porphyrin synthesis which resulted in a chronic metabolic hypoxidosis.

vi. Effect of lead on the germ cells

Constantin Paul in 1860 was the first to allege that lead might injure the male germ cell. Based upon his observations of clinical cases, he reported that lead poisoning of either the mother or father often caused stillbirth and that the infant mortality of children born of such parents was particularly high during the first three years of life.

Meillere (1903), Baader (1928), and Fraenkel (1905) have shown that lead-exposed workers have diminished sperm motility, testicular atrophy, and
diminished spermatogenesis. Koinuma (1926) compared the marital life records of workers exposed to lead in Japanese storage battery plants with the histories of those working in nonlead occupations and reported that infertile marriages constituted 24.7% for the lead group as opposed to only 14.8% for the nonlead group. The percentage of pregnancies ending prematurely or in stillbirth was 8.2% for the lead and 0.2% for the control group. Additional evidence that lead can injure the male germ cell was supplied by Weller (1915-16, 1916-17) when he demonstrated that the offspring of lead-poisoned male guinea pigs were underweight and weak. The "leaded" males were usually not sterile, but when mated with a normal female the weight of their young was 18.5% below normal. Similar results have also been reported using rabbits and fowl (Cole and Bachuber, 1914). Puhac et al. (1963) fed male rats lead nitrate for 30 days and found that when they were paired with untreated females no reproduction took place for 45 days.

In women lead is an active abortifacient and an increased frequency of miscarriages, stillbirths, and premature births in female lead workers has been recognized for years. Lead-exposed women may experience transitory periods of sterility with subsequent occurrence of normal pregnancy after withdrawal from exposure and this indicates that lead injures only the germ cells formed during the period of intoxication and that the damage to the gonads is not permanent (Paul, 1860). Women who have had several
miscarriages and stillbirths during periods of lead exposure may subse­quentl y have normal pregnancy after cessation of exposure. If living children are born to women with lead intoxication, they usually are smaller, weaker, slower in development, and have a higher infant mortality (Legge and Goodbody, 1912; Aub et al., 1925) and similar observations have been made in dogs, cats, rabbits, guinea pigs, and fowl. These phenomena have been attributed to (a) placental injury and hemorrhage, (b) degenerative changes in the chorionic epithelium, (c) spasm of the uterine muscle, and (d) direct action of lead on the fetal tissue (Cantarow and Trumper, 1944).

Lead-poisoned female rabbits have shown alterations of the chorion resulting in the death of the embryo and Hamilton (Cited by Moeschlin, 1965) reported that women abort due to lesions of the chorion epithelium or of the fetus itself. Bell (1924) from his studies on the action of lead on malignant cells concluded that lead acts directly on the embryonic cells of the chorion epithelium causing degeneration. Since lead passes from the maternal circulation to the fetus and has been found in all of the fetal organs (Cantarow and Trumper, 1944), it is also possible to speculate that intra­uterine death and abortion are caused by the direct toxic action of lead on fetal tissues. Bell (1924) found that lead inhibits the growth of embryonal and other growing tissues and concluded that the arrest of growth in animal organisms is strictly proportional to the capacity for, or power of, growth.
The fact that children born of mothers with lead poisoning develop more slowly than normal and have a higher mortality during the first year of life indicates that intrauterine exposure to lead has damaging effects.

vii. **Lead nephropathy**

Damage of the kidney has long been associated with lead poisoning and the nature of damage depends upon the type of intoxication (Albahary et al., 1965). Tubular involvement is dominant in the acute form, while diffuse changes occur after prolonged exposure. In chronic lead poisoning, nephrosclerosis develops as a result of damage to the renal vessels.

Oliguria, albuminuria, and cylinduria have been reported to occur in lead-exposed workers (Legge and Goadby, 1912; Oliver, 1914). Dreessen et al. (1941) found that albuminuria occurs twice as frequently in lead-exposed workers with other evidence of lead intoxication as in nonaffected workers or in workers in other industries, the incidence and severity of albuminuria increasing with duration of exposure and increasing concentrations of lead in the air to which the subjects were exposed. Following the intravenous injection of colloidal lead preparations to human subjects, Bell et al. (1925) observed oliguria, albuminuria, and edema, with marked degenerative changes in the renal tubular epithelium. Cases of lead intoxication have also been reported with glycosuria, aminoaciduria (Wilson et al., 1953) and hyperphosphaturia (Chisolm Jr. et al., 1955; Chisolm Jr. and Leahy, 1962).
The renal tubular cell linings of rats fed a 1% lead acetate diet were found to contain intranuclear inclusions after eight weeks on the diet, cytoplasmic vacuoles after one week, and swollen mitochondria with disrupted cristae after three weeks (Goyer et al., 1965). Galle and Morel-Maroger (1965) reported that in both man and rats lead selectively affects the proximal tubular cells and produces intranuclear inclusions, iron containing intracytoplasmic inclusions, and marked mitochondrial lesions. Intranuclear acidophilic inclusion bodies were also found by Blackman (1936) in the renal tubular epithelium of 21 children with lead encephalopathy. Some of the affected cells were enlarged, others flattened and basophilic in appearance, some contained fat and some were necrotic. Some of the tubules were atrophied and there was also evidence of mild chronic inflammation and fibrosis.

Henderson (1954) has reported follow-up studies on children in Queensland, Australia who had acquired lead poisoning from the ingestion of powdered white lead paint. He noted that many of these children died later in life due to chronic nephritis, and of the 187 who could be located alive, 17 had hypertension and albuminuria. Studies by Croll (1929), Nye (1933), and Murray (1939) have shown that chronic renal disease was unusually frequent in persons under forty years of age in Queensland. This high incidence was related to the occurrence of lead poisoning in infancy and early childhood due to the ingestion of paint from the veranda railings of the homes. Nye has concluded that every child who has had lead poisoning is a
potential subject for chronic renal disease later in life.

2. **Heme Biosynthesis**

a. **Normal heme biosynthesis**

The biosynthetic sequence of heme has been elucidated as a result of the research conducted mainly in the laboratories of Shemin and Granick in the United States and Neuberger and Rimington in Great Britain. As early as 1948 it was found that avian red blood cells can synthesize heme *in vitro* (Shemin et al., 1948) and soon after, cell free extracts of duck erythrocytes were found to accomplish heme synthesis from the proper substrates (Shemin et al., 1955). When chicken cells, rabbit reticulocytes, or human red cells from erythroblastosis fetalis are hemolyzed, a number of the enzymes of the heme biosynthetic pathway are found to be soluble and can be separated from each other by zone electrophoresis on starch (Granick and Mauzerall, 1958). These soluble enzymes are localized in the cytoplasm and include delta-aminolevulinic acid (ALA) dehydrase, porphobilinogen (PBG) deaminase, uroporphyrinogen (UPG) isomerase, and uroporphyrinogen decarboxylase. The insoluble enzymes are localized in the mitochondria and include ALA synthetase, coproporphyrinogen (CPG) oxidase, and heme synthetase (Sano and Granick, 1961).

The first step in heme synthesis is the condensation of succinyl CoA with glycine (Shemin et al., 1955; Gibson et al., 1958; Kikuchi et al., 1958) to form \( \alpha \)-amino-\( \beta \)-keto adipic acid which readily decarboxylates yielding delta-aminolevulinic acid (Shemin et al., 1955; Neuberger, 1961). This
reaction is catalyzed by the enzyme ALA synthetase and requires pyridoxal phosphate as a co-factor (Lascelles, 1957; Schulman and Richert, 1957; Kikuchi et al., 1958). The enzyme ALA dehydrase condenses two molecules

A = acetic acid; P = propionic acid; M = methyl; V = vinyl

GENERAL SCHEME OF HEME BIOSYNTHESIS
of ALA forming one molecule of porphobilinogen and water (Gibson et al., 1955). Three molecules of porphobilinogen then combine under the influence of PBG deaminase to form tripyreryl methane and this in the presence of UPG isomerase and PBG is converted to uroporphyrinogen III (Bogorad, 1958a, b; Cornford, 1964). The four acetic acid side chains of uroporphyrinogen III are then decarboxylated by UPG decarboxylase to produce coproporphyrinogen III (Mauzerall and Granick, 1958). Two propionic acid side chains of coproporphyrinogen III are oxidatively decarboxylated to form vinyl groups by CPG oxidase and this results in the formation of protoporphyrinogen IX (Sano and Granick, 1961). Protoporphyrinogen IX is then autooxidized to form protoporphyrin IX (Sano and Granick, 1961) and heme synthetase inserts one ferrous ion into each molecule of protoporphyrin IX forming heme (Goldberg et al., 1956; Labbe and Hubbard, 1960).

Shemin (1957) demonstrated that labelled glycine, when fed to humans, was incorporated into the heme of newly formed erythrocytes and this led to the finding that glycine and succinyl CoA are the elementary building blocks of heme. Most of the succinyl CoA required for condensation with glycine originates from the oxidation of α-ketoglutarate through the operation of the tricarboxylic acid cycle, although some is generated from succinate and CoA in the presence of a nucleoside triphosphate by the enzyme succinyl CoA synthetase (Brown, 1958; Shemin and Kumin, 1952). The
mitochondria seem to be the principal site of ALA synthesis because succinyl CoA is formed via $\alpha$-ketoglutarate oxidase which is a mitochondrial enzyme complex and because ALA synthetase is present in mitochondria. As the mitochondria degenerate in maturing red blood cells, the ability to synthesize ALA decreases and in mature erythrocytes only the enzyme activities that convert ALA to coproporphyrinogen can be detected (Granick and Levere, 1964).

b. **Effect of lead on heme synthesis**

Lead has been found to exert an inhibitory effect on several of the enzymes necessary for heme synthesis e.g. ALA dehydrase, CPG oxidase, and heme synthetase. ALA dehydrase (Gibson et al., 1955) and heme synthetase (Schwartz et al., 1959) both contain essential sulfhydryl groups and a blockage of such groups by lead may be the cause of the inhibition of their activity.

i. **ALA dehydrase**

Several authors have reported that the activity of this enzyme is decreased in erythrocytes from lead-intoxicated patients (Lichtman and Feldman, 1963; Bonsignore, 1966; deBruin and Hoolboom, 1967). Bonsignore et al., (1965a) injected rabbits subcutaneously with lead acetate and found that the activity of ALA dehydrase in the blood was decreased 1 hour after injection. The activity returned to normal between the first and sixth day
after injection, but then decreased again and remained low to the twentieth
day when further observations were discontinued. In man, ALA dehydrase
has been found to remain inhibited for up to three years after cessation of
lead exposure (Saita and Moreo, 1964).

Bonsignore et al. (1965b) have found that the inhibition of the enzyme
ALA dehydrase is competitive and can partially be removed by adminis­
tering sulfhydryl compounds as cysteine and glutathione. By using in vitro
techniques, a maximum reactivation of 60% of the ALA dehydrase inhibited
by a solution of \(10^{-5}\)M lead acetate was produced by cysteine in a concentra­
tion of \(2.5 \times 10^{-2}\)M.

ii. CPG oxidase

An increased urinary excretion of coproporphyrin from individuals
with lead intoxication has been known for years. Coproporphyrinogen III is
the compound that is excreted in the urine, however, it is rapidly oxidized
to coproporphyrin III (Watson et al., 1951; Weatherall, 1952). The increased
urinary excretion of coproporphyrin III in lead poisoning has been postulated
to be due to destruction in the bone marrow of young red blood cells con­
taining large amounts of coproporphyrinogen III (Schmid et al., 1950). This
increased amount of coproporphyrinogen III in the young red blood cells is a
result of the inhibition of the enzyme CPG oxidase by lead.

iii. Heme synthetase
Erythrocyte protoporphyrin IX levels have been found by Rubino et al. (1958) to be elevated in 21 of 22 patients with lead poisoning. Increased amounts of protoporphyrin IX in the erythrocytes of lead poisoned patients have also been found by Vigliani and Waldenstrom (1937). Whitaker and Vietti (1958) have shown that children with lead intoxication also have increased amounts of protoporphyrin IX in the erythrocytes. Lead inhibits the in vitro synthesis of heme from protoporphyrin IX and iron of chicken, rabbit, and human erythrocytes (Rimington, 1951; Jandl et al., 1959) and it has been reported that in humans, elevated levels of protoporphyrin IX persist in the erythrocytes up to nine years after cessation of lead exposure (Saita and Moreo, 1964).

3. Treatment
   
a. Acute lead poisoning

   Gastric lavage with a 3% sodium sulfate solution combined with ample amounts of animal charcoal should be administered immediately. This converts the soluble lead into the slightly soluble lead sulfate and causes adsorption of the lead to the charcoal. Magnesium sulfate can be given as a cathartic in order to rush the ingested lead through the intestinal tract with as little absorption as possible. Calcium disodium edetate (CaNa₂ EDTA) is then administered.

b. Chronic lead poisoning
i. **Specific therapy**

Dimercaprol (2, 3-dimercaptopropanol) has been reported to be effective in the treatment of chronic lead poisoning (Ryder and Kehoe, 1947; Harvey, 1965; Moeschlin, 1965). Even though it initially causes an increased urinary excretion of lead, this effect is temporary and the amount of lead actually mobilized is negligible. It appears that dimercaprol is only able to chelate the lead in blood and that "lead in bone and soft tissue is too firmly bound to be mobilized."

Calcium disodium edetate, an ion exchanger, was discovered in 1953 to be effective in the treatment of lead intoxication (Rubin et al., 1953) and is now the drug of choice. Lead displaces calcium from the calcium disodium edetate and forms a water-soluble lead chelate which is eliminated by the kidneys. Several investigators have reported that lead excretion increases up to ten fold after initiation of calcium disodium edetate therapy (Hernberg and Laamanen, 1964; Westerman et al., 1965; Selander, 1967).

The use of calcium disodium edetate, however, has certain disadvantages among which are its nephrotoxicity (Foreman et al., 1956; Vogt and Cottier, 1957); and the necessity of it being administered intravenously to obtain maximal efficiency. Opinions on the efficacy of oral calcium disodium edetate vary. Some authors maintain that it is effective (Manville and Moser, 1955; Bell et al., 1956), whereas others consider oral administration to be contraindicated because it increases the absorption of lead from the gut and
may precipitate or increase the symptoms of poisoning (Kehoe, 1955; Byers, 1959). Selander (1967) reported that oral calcium disodium edetate was not effective in causing an increased lead excretion and stated that this was due to its poor absorption from the gastrointestinal tract.

Penicillamine administered orally has been shown to be effective in the treatment of lead poisoning (Boulding and Baker, 1957; Ohlsson, 1962; Selander et al., 1966). Calcium disodium edetate is preferable in the treatment of severe cases of lead intoxication due to its ability to mobilize 2 to 8 times more urinary lead than penicillamine (Gaultier et al., 1966). However, penicillamine's use in the treatment of lead intoxication is assured because of its effectiveness when given orally and its less severe side effects. Penicillamine has also been recommended as a prophylactic agent against lead poisoning (Albahary and Guillaume, 1966).

ii. Spasmolytics

Chlorpromazine, papaverine, extract of belladonna, and calcium gluconate are the most effective drugs for the treatment of lead colic (Moeschlin, 1965). Hot compresses and hot baths may also help relieve the cramps associated with the colic.

iii. Sedatives

Chlorpromazine and various forms of barbiturates are used for the treatment of excitement, mania, and convulsions. Pentothal-sodium
administered intravenously is used for the treatment of lead encephalopathy.

iv. **Purgatives**

Kehoe (1943) has found that the motor activity of the bowel influences the absorption of lead. A decrease in the number of evacuations causes an increased absorption of lead, while an increase in the frequency causes a decreased absorption. Sodium sulfate is the drug of choice for causing regular evacuation of the bowel (Moeschlin, 1965).

4. **Laboratory Tests Used for the Early Detection of Increased Lead Exposure**

a. **Number of basophilic stippled cells in peripheral blood**

As mentioned previously, there usually is an increased number of stippled erythrocytes in the peripheral blood of lead-exposed individuals. Stippling, however, is generally considered an unreliable criterion of lead intoxication because it also occurs in a large number of blood diseases such as various types of hemolytic anemia, post hemorrhagic anemia, leukemia, and after exposure to benzene, aniline, carbon monoxide, arsenic, copper, and bismuth (Haeger-Aronsen, 1960). The upper limit of normal is approximate and the values cited vary considerably, ranging from 1 per 10,000 RBC (Schmidt, 1919) to 90 per 10,000 (Sanders, 1943).

b. **Determination of coproporphyrin III in the urine**
Coproporphyrin III excretion in the urine has been shown to be elevated in lead poisoning (Wyllie, 1955; Holecek, 1957; Benson and Chisolm, 1960; Haeger-Aronsen, 1960). However, coproporphyrin levels are also increased in various forms of anemia, particularly hemolytic anemias, in jaundice whether due to diffuse liver damage or to biliary obstruction, in some infectious diseases such as pneumonia and acute poliomyelitis, and in several types of intoxication including mercury, bismuth, sulfonal, and sulfonamide poisoning (Haeger-Aronsen, 1960). The upper limit of normal for urinary coproporphyrin is 15 μg per 100 ml of urine.

c. Determination of the concentration of lead in hair

Kopito et al. (1967) have recently described a procedure for the analysis of lead in hair. This procedure involves first segmenting the hair, then washing each segment, and finally digesting the washed segments in nitric and perchloric acids. The lead concentration of each segment is then obtained by use of an atomic absorption spectrophotometer.

Using the procedure described above, Kopito et al. have shown that patients with recent lead ingestion have a high lead concentration in hair near the scalp, while patients with chronic lead ingestion have elevated lead concentrations in all hair segments. The lead levels in hair were reported to have a good correlation with the radiographic findings of increased bone density and with other laboratory findings.
The laboratory procedures used for determining the concentration of lead in hair, however, are time consuming and require skilled laboratory assistants. Therefore, the practicality of using lead in hair as a screening test for early detection of increased lead absorption is questionable. This procedure is also not suitable for diagnosing acute cases of lead intoxication. Still another limitation for the use of this method is the fact that the hair of Negro children grows at a very slow rate and this makes it extremely difficult to separate their hair into enough segments for the performance of a proper lead analysis.

d. Determination of lead in blood and urine

Most investigators agree that an increased level of lead in the blood and urine is the most specific sign of increased lead absorbance. It must be remembered, however, that the blood is rapidly cleared of lead and blood lead levels are considered of value only in acute cases of lead intoxication. Berman (1966) has reported that the blood lead levels in chronic cases of lead poisoning are not remarkable.

The drawing of blood samples and the analytical procedures for determining the lead levels in blood and urine are time consuming processes requiring skilled personnel and it therefore appears that the determination of lead in the blood and urine is not practical as a screening test for early detection of increased lead absorption.
Mauzerall and Granick in 1956 developed a sensitive colorimetric method for the quantitative determination of urinary ALA. Using this method, a 1.0 ml aliquot of urine (pH 5 to 7) containing porphobilinogen, urea, and ALA is placed on a column of Dowex 2 resin in the acetate form. The column is washed with two 2 ml portions of water and porphobilinogen is retained on the Dowex 2 column, while urea and ALA are washed off. The washings from the first column are then passed through a column of Dowex 50 resin in the acid form. This Dowex 50 column containing ALA and urea is then washed with 16 ml of water to remove the urea. A 3 ml aliquot of 0.5 M sodium acetate is added to the column and after draining, the ALA is eluted from the column by the addition of 7 ml of 0.5 M sodium acetate. This eluate is collected in a 10 ml volumetric flask, 0.2 ml of acetylacetone is added, and the solution is diluted to the 10 ml mark with acetate buffer at pH 4.6. The flask is stoppered and is placed in boiling water for 10 minutes, then cooled to room temperature. To a 2.0 ml aliquot of this solution is added 2.0 ml of modified Ehrlich's reagent. The resulting solution is mixed and after 15 minutes its optical density at 553 mµ is read against a blank. The blank consists of water treated in the same manner as the Dowex 50 eluate.
Haeger in 1957 using Mauzerall and Granick's technique, reported that workers exposed to inorganic lead have considerably elevated ALA levels in urine. Since then, numerous other investigators have shown elevated urinary ALA levels in cases of clinical adult plumbism and in adult workers exposed to lead (Haeger-Aronsen, 1960; de Kretzer and Waldron, 1963; Kleinstein et al., 1963; Efe, 1964; Cramer and Selander, 1965; Cramer et al., 1966; deBruin and Hoolboom, 1967).

Bonsignore et al. (1965) and deBruin and Hoolboom (1967) have demonstrated an early decrease in the activity of ALA dehydrase in the blood of both adult workers and rabbits exposed to lead and it appears likely that the accumulation of ALA in the urine of individuals exposed to lead is due to an inhibition of the ALA dehydrase located in red blood cells. Additional evidence that the increased excretion of ALA is due to an inhibition of ALA dehydrase rather than a stimulation of ALA synthetase has been supplied by Gajdos and Gajdos-Torok (1964) and by Pecora et al. (1964). These investigators studied the activity of ALA synthetase in mitochondria isolated from hepatic tissue of lead-poisoned rabbits, guinea pigs, and rats and reported that the activity of this enzyme was not measureable in the lead-poisoned animals nor in the controls. Rimington (1961) has shown that the livers of both lead-poisoned and control rabbits have similar ALA contents.
Urinary ALA levels have been reported to have a positive correlation with blood lead levels (Selander et al., 1966), urinary lead excretion (Haeger-Aronsen, 1960; Cramer and Selander, 1965), and clinical impression of lead intoxication (Cramer and Selander, 1965). Raised ALA levels in the urine have also been found to be closely correlated to raised ALA levels in serum (Saita and Moreo, 1961; Chiesura and Brugnone, 1963). Blood and urinary ALA levels remain significantly increased for up to three years after cessation of lead exposure (Saita and Moreo, 1964).

An increased ALA excretion has been reported to be an earlier sign of lead exposure than an increased coproporphyrin excretion (Singerman, 1964; Djuric et al., 1966) and several investigators have stated that an increased ALA excretion is the earliest sign of an increased lead absorbance (Efe, 1964; Djuric et al., 1966). An increased urinary ALA level also appears to be pathognomonic of elevated lead absorption since the only other conditions known to result in an increased ALA excretion are acute intermittent porphyria and certain liver diseases. Haeger-Aronsen (1960) determined the urinary ALA content of patients with either biliary disease, acute hepatitis, cirrhosis of the liver,
hemolytic anemia, pernicious anemia, malignant tumor, leukemia, diabetes mellitus, nephropathy, or drug intoxication. The concentration of ALA in the urine was normal in all cases except three. Two of these were patients with acute hepatitis and one was a man with bronchial cancer. Schlenker et al. (1964) determined the urinary ALA content of patients with Hodgkin's disease, cirrhosis, leukemia, multiple myeloma, sickle cell anemia, or cancer and the only patient showing an elevated urinary ALA level was the one with cirrhosis. Griggs (1964) compared the urinary ALA levels of patients with lead toxicity, acute intermittent porphyria, iron deficiency anemia, hemolytic anemia, pernicious anemia, or liver disease and found that elevated levels are seen only in patients with lead poisoning, acute intermittent porphyria, and occasionally liver disease.

Dantchev et al. (1964) have reported that adenosine-5'-monophosphoric acid (AMP) and inosine protect red blood cells against destruction by lead. Brugnone and Galzigna (1963) administered AMP to lead-poisoned rabbits and observed a decreased urinary excretion of ALA. Lead-poisoned rabbits were also treated with AMP plus pyridoxal phosphate and the decrease in urinary ALA excretion from these animals was more pro-
nounced than from those treated with AMP alone. The ALA in the blood was found to follow the same pattern as the urinary ALA. Koziolowa-Lipska and Gutniakowa (1966) administered AMP to patients with chronic lead poisoning but could not demonstrate any significant decrease in urinary ALA excretion.

Haeger-Aronsen (1960) studied the influence of temperature, pH, calcium disodium edetate, and penicillamine on the stability of ALA in human urine and reported that at pH levels between 4 and 7, urine can be stored at +4°C in the dark for at least three weeks without any decrease in the concentration of ALA. At a higher pH, the concentration of ALA was found to fall. The concentration of ALA in urine was found to remain unchanged after the addition of either calcium disodium edetate or penicillamine. Bossenmaier and Cardinal (1968) also studied the stability of ALA in human urine and reported that urine having pH levels between 1 and 9 can be stored at -20°C for at least four weeks without any appreciable decrease in the concentration of ALA. The stability of ALA in frozen urine thus makes it possible to collect and store large quantities of urine samples prior to the determination of their ALA content and this would be a definite advantage if a screening program using urinary ALA levels for the detection of early lead exposure would be implemented.
G. PORPHYRIA

The porphyrias are metabolic disorders, mostly of hereditary nature, characterized by an increased excretion of porphyrins and of their precursors. In the erythropoietic porphyrias the porphyrins are elevated in the bone marrow, while in the hepatic porphyrias they are mainly in the liver.

1. Erythropoietic Porphyrias
   a. Congenital porphyria or Gunther's disease

   Congenital porphyria is a very rare disturbance which is characterized by a marked photosensitivity and an increased excretion of uroporphyrin and coproporphyrin, predominantly of the unphysiological series I type (Goldberg and Rimington, 1962). This disease can exist in utero and individuals possessing the genetic trait always develop symptoms before the age of six years. The biochemical abnormality appears to be lacking of uroporphyrinogen isomerase from red blood cells owing to a gene defect and because of this enzymic defect, series I porphyrins are released into the plasma and tissues. The photosensitivity associated with this disease is a result of the presence of these porphyrins in the epidermal layer of the skin.

   b. Erythropoietic protoporphyrria

   This condition was first reported by Magnus et al. in 1961 and is characterized by a mild photosensitivity and by an increased fecal excretion
of coproporphyrin and protoporphyrin IX which are present in excess in the circulating erythrocytes. In contrast to findings in erythropoietic congenital porphyria, there is no excess of uroporphyrin in blood or excreta.

2. Hepatic Porphyrias

a. Acute intermittent porphyria

This is the most important member of the group of porphyria diseases and is characterized clinically by abdominal and neurological symptoms which appear in acute attacks separated by latent phases (Goldberg and Rimington, 1962). In the acute and latent phases of this disease and often in states of remission, patients excrete in urine an excessive quantity of porphobilinogen and ALA (Haeger, 1958).

This disease occurs in members of the same family and Waldenstrom in 1937 established it as a hereditary disease. The biochemical trait is transmitted as an autosomal dominant characteristic (Goldberg and Rimington, 1962) and the clinical manifestations are more frequent in females. Goldberg and Rimington (1962) described fifty cases of acute intermittent porphyria and the most frequent age of onset was the third decade for females and the fourth for males. The youngest patient found was a girl eleven years of age.

The main symptoms of acute intermittent porphyria are abdominal pain, vomiting, constipation, peripheral paralysis, and psychological
disturbances and all these symptoms can be accounted for by the widespread neurological lesions (Goldberg, 1959) which can involve the central, peripheral, and autonomic nervous systems (Gibson and Goldberg, 1956). There is an absence of skin photosensitivity because in this condition the porphyrin precursors are the main excretory products.

A possible mechanism for the increased quantity of ALA and porphobilinogen eliminated in acute intermittent porphyria is that there is normally a control or inhibitor of ALA synthesis in the liver, but this control breaks down in the diseased subject. Shemin (1960) found evidence for a negative feed-back control of in vitro ALA synthesis by preparations derived from Rhodopseudomonas spheroides. In these preparations, ALA was found to be inhibitory to the system producing it. Granick (1965) stated that acute intermittent porphyria results in part from an increased synthesis of ALA synthetase due to a defective regulatory control mechanism governing the activity of the structural gene which does not sufficiently repress the formation of the enzyme. Tschudy et al. (1965) and Nakao et al. (1966) have recently reported that the livers from patients with acute intermittent porphyria have an increased ALA synthetase activity.

b. Hereditary coproporphyria

This rare disease is transmitted as an autosomal dominant characteristic and is characterized by an increased coproporphyrin III excretion
even during states of remission (Goldberg et al., 1967). The symptoms of acute attacks have been found to be very similar to those of acute intermittent porphyria.

c. Mixed porphyria or variegate porphyria

This porphyria is common among persons of European ancestry in South Africa (Dean, 1963) and is apparently inherited as a Mendelian dominant character. Acute attacks similar to those of acute intermittent porphyria occur most commonly in affected females, while photosensitivity is found in male members of the same family. Patients with South African porphyria have a high fecal porphyrin content in states of remission, with an increase in the urinary porphyrin, porphobilinogen, and ALA excretion during acute phases of the disease. The photosensitivity present in the variegate porphyria is due to the accumulation in the body of photodynamically active porphyrins.

Rimington (1967) has isolated hydrophilic porphyrins from the urine and feces of patients with mixed porphyria and has found that excretion of these porphyrins is characteristic of mixed porphyria. Normal persons and patients with porphyrias of other types excrete these hydrophilic porphyrins only in trace amounts if at all.

d. Symptomatic or acquired porphyria

This nonhereditary type of porphyria is characterized by photosensitivity and an absence of acute attacks. The excretion of porphyrins,
especially urinary uroporphyrin, is very high, while the excretion of porphobilinogen and ALA is usually normal. Several types of acquired porphyria have been described: (1) porphyria cutanea tarda symptomatica (Waldenstrom, 1957); (2) Bantu porphyria (Barnes, 1959); (3) Turkish porphyria (Cetingil and Ozen, 1960); and (4) porphyrin-producing hepatic adenoma (Tio et al., 1957). The porphyria which occurs in Bantus has been reported to be due to the hepatoxic effect of adulterated drinks brewed and sold in the urban areas, while the porphyria in Turkey was the result of the ingestion of bread made from hexachlorobenzene-treated bread.

i. The adverse effect of drugs on patients with hepatic porphyria

Certain drugs when administered to patients with either acute intermittent or mixed porphyria can worsen the course of the disease and also precipitate attacks in cases of latent porphyria. Patients with the genetic factor are very sensitive to these drugs and a very small amount has been found to cause a response. Waldenstrom (1939) and Goldberg (1959) reported that barbiturates not only precipitate attacks in persons with latent porphyria of the acute intermittent variety but they also seriously affect the prognosis of the disease. Dean (1963) also found that barbiturates may precipitate acute attacks in persons with mixed porphyria. Patients with South African mixed porphyria appear to be the most sensitive to barbiturates
those with acute intermittent porphyria less so, and those with hereditary coproporphyria even less (With, 1965).

Sulfonamides, nonbarbiturate sedatives and anticonvulsants, griseofulvin, and sex hormones have all been demonstrated to be capable of precipitating attacks of acute intermittent porphyria in patients possessing the genetic trait. Administration of chloroquine to patients with symptomatic porphyria results in an increased excretion of urinary uroporphyrin accompanied by transient fever, tachycardia, and general malaise (Embree, 1961; Cripps and Curtis, 1962). The increase in uroporphyrin excretion is not accompanied by an increased excretion of porphyrin precursors and it therefore appears that chloroquine causes a release of stored uroporphyrin from the liver (Sweeney et al., 1965). Felsher and Redeker (1966) have reported results which are in agreement with the suggestion that chloroquine causes a release of uroporphyrin from the liver rather than an increased synthesis of uroporphyrin by the liver.

ii. **Experimental hepatic porphyria**

Drugs such as 2-allyl-isopropylacetamide (AIA) and 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) have been found to induce experimental hepatic porphyria in both rats and rabbits (DeMatteis and Prior, 1962). Porphobilinogen and ALA were increased in the urine after treatment with these drugs and this is the same biochemical picture as seen in human acute intermittent porphyria. Ockner and Schmid (1961) have demonstrated that
rats fed 0.2% of hexachlorobenzene in their diet excrete large amounts of uroporphyrin and coproporphyrin and if the exposure is prolonged porphobilinogen also increases. This biochemical picture is similar to that seen in patients with cutaneous porphyria in southeastern Turkey with the exception that in the latter case porphobilinogen was not increased in the urine. Certain drugs, therefore, when administered to animals appear to be able to produce porphyric disturbances resembling those present in human porphyria.

iii. **Mechanism of drug induced porphyria**

Granick (1965) demonstrated that porphyrogenic drugs induce de novo synthesis of ALA synthetase in cultures of chicken embryo liver cells and this observation has now been confirmed in the whole animal (Narisawa and Kikuchi, 1965; Marver et al., 1966). A recent report by Granick (1966) stated that heme prevents the increased porphyrin production caused by AIA in cultured liver cells and he suggested that heme inhibits the increased synthesis of ALA synthetase by acting as a specific co-repressor in the regulation of this enzyme. Porphyrogenic drugs may interact with the portion of the apo-repressor molecule that normally binds heme and compete with heme for this site. Heme would then be prevented from combining with the apo-repressor and the operator gene would be kept open for continuous synthesis of the m-RNA necessary for synthesis of ALA synthetase.
H. METABOLIC FATE OF ALA

SUCCINATE-GLYCINE CYCLE

Shemin and Russell (1953) postulated a succinate-glycine cycle through which ALA can be oxidized back to succinic acid and this oxidation would thus provide a pathway for glycine oxidation. The first step of this cycle is the deamination of ALA to $\alpha$-ketoglutaraldehyde which then releases the ALA-carbon derived from glycine to form succinate. The ALA-carbon derived from glycine provides the $\alpha$-carbon atom to ureido groups of purines, the $\beta$-carbon atom of serine, the methyl group of methionine, and forms formic acid (Shemin et al., 1955).

I. PHARMACOLOGICAL EFFECTS OF ALA

The symptoms of lead intoxication and of acute intermittent porphyria are very similar. The clinical pictures are characterized by abdominal pain, constipation, vomiting, nonabdominal pain, and paralysis (Dagg et al., 1965). In both diseases there is also a similar type of segmental demyelination of the peripheral nerves. Secondary axonal degeneration found in experimental lead poisoning by de Villaverde (1926) has also been described by Gibson and Goldberg (1956) in cases of acute intermittent porphyria. Since ALA is increased in both the urine and blood of patients with either lead intoxication or acute intermittent porphyria, it is tempting to assume that ALA is responsible for the clinical pictures described above.

Berlin et al. (1954) and Scott (1955) administered ALA to humans per os and Granick and Vanden Schrick (1955) and Jarrett et al. (1956)
injected ALA into rats intraperitoneally or subcutaneously and the only pharmacological effect observed was a transient but marked photosensitivity. Jarrett et al. (1956) also showed that ALA did not possess any pharmacological activity when tested in the anesthetized cat or with isolated guinea pig ileum. In the cat, ALA at a dose of 100 µg/kg body weight did not alter the heart rate or blood pressure and neither potentiated nor inhibited the responses to adrenaline or to nor-adrenaline. ALA at a dose of 100 µg in a 20 ml bath neither caused a contraction of the isolated guinea pig ileum nor effected the contraction produced by histamine, by acetylcholine, or by 5-hydroxytryptamine. On isolated rat's colon, the same dose of ALA had no effect and did not alter the response produced by acetylcholine or by 5-hydroxytryptamine.

This pharmacological evidence was strengthened when AIA was found to produce experimental porphyria in rabbits, rats, and fowls (Goldberg and Rimington, 1954-55). Animals injected with this drug excreted large quantities of ALA and PBG, yet showed no features similar to acute intermittent porphyria in humans apart from constipation and loss of weight. In addition cases of latent acute intermittent porphyria are known to excrete large quantities of ALA and yet are symptomless.

Recently, Feldman et al. (1969) reported that ALA causes a reduction in twitch tension in vitro on phrenic nerve stimulation of rat hemidiaphragm.
preparations (ED50 of 7.5 mM). When the miniature end-plate potential frequency was augmented with 20 mM K⁺, 7.5 mM ALA reduced it by 26% and 15 mM ALA by 61%. Dual microelectrode studies showed that ALA caused a 3-fold increase in muscle membrane conductance and it was concluded that ALA inhibits the neuromuscular acetylcholine release.

J. PURPOSE OF THE DISSERTATION

Lead poisoning in children is a major public health problem in large cities. The possibility exists that this problem may be controlled by carrying out large-scale screening programs designed for the detection of early and potentially hazardous exposure to lead before the development of frank lead intoxication. Since numerous investigators have reported that urinary ALA levels are increased in cases of adult lead intoxication and in lead-exposed workers, a study of urinary ALA levels of children suspected of lead ingestion was undertaken. This type of study employing urinary ALA as a possible indicator for early lead poisoning has never been carried out in children.

The studies which were undertaken had three objectives. . . first, the modification and simplification of the method described by Mauzerall and Granick for the determination of urinary ALA levels, thus making the urinary ALA test a practical screening procedure for the early detection of increased lead absorption in children; second, making the urinary ALA test readily available for use throughout the country; and third, the initial correlation of the
urinary ALA test with the clinical incidence of childhood lead intoxication.
CHAPTER II

MATERIALS AND METHODS
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A. PREPARATION OF REAGENTS

1. Sodium acetate, 1.0M. 68 g of NaC$_2$H$_3$O$_2$·3H$_2$O (J. T. Baker Chemical Co., Phillipsburg, N. J., catalog no. 3460) are dissolved and diluted with water to 500 ml. 0.8% of boric acid is added as a preservative.

2. Acetylacetone. Obtained under the name of 2,4-pentanedione (Eastman Organic Chemicals, Rochester, New York, catalog no. 1088) and used undiluted.

3. Modified Ehrlich's reagent. 10 g of para-dimethylamino-benzaldehyde U.S. P. (Matheson Coleman and Bell, Norwood, Ohio, catalog no. 1606) is dissolved in 420 ml of glacial acetic acid (E.I. duPont de Nemours and Company, Wilmington, Delaware) and 80 ml of 70% perchloric acid (G. Frederick Smith Chemical Comp., Columbus, Ohio).

B. PREPARATION OF ALA STANDARD

1. Delta-aminolevulinic acid hydrochloride. Obtained from Calbiochem, Los Angeles, Calif., catalog no. 1584.

2. Acetate buffer, pH 4.6. 5.7 ml of glacial acetic acid and 13.6 g of NaC$_2$H$_3$O$_2$·3H$_2$O are diluted with water to 100 ml.

3. A stock solution of ALA is prepared by dissolving 12.5 mg of
ALA hydrochloride with acetate buffer, pH 4.6 to a 100 ml mark. Each ml of stock solution contains 125 µg of ALA·HCl which is equivalent to 100 µg of pure ALA.

C. PREPARATION OF AN ALA STANDARD CALIBRATION CURVE

(1). Six aliquots of the ALA hydrochloride stock solution (0.01 ml, 0.05 ml, 0.10 ml, 0.20 ml, 0.30 ml and 0.50 ml) are pipetted into test tubes and are used as working standards.

<table>
<thead>
<tr>
<th>Volume of standard stock solution</th>
<th>µg of ALA per total reaction volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 ml</td>
<td>1 µg</td>
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<tr>
<td>0.05 ml</td>
<td>5 µg</td>
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<tr>
<td>0.10 ml</td>
<td>10 µg</td>
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<td>0.20 ml</td>
<td>20 µg</td>
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<td>0.30 ml</td>
<td>30 µg</td>
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<tr>
<td>0.50 ml</td>
<td>50 µg</td>
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</table>

(2). Dilute each of the working standards to 7.0 ml with 1.0 M sodium acetate.

(3). Pipet 7.0 ml of 1.0 M sodium acetate into an empty test tube to be used as a reagent blank.

(4). Add 0.2 ml of acetylacetone to each test tube and mix.

(5). Place the test tubes in a boiling water bath for 10 minutes, remove, and cool to room temperature.

(6). Add 7.0 ml of modified Ehrlich's reagent to each test tube, mix and let stand for 15 minutes for complete color development.
(7). Read the optical density of the standards corrected for the blank at 553 mp in a spectrophotometer. The optical path of the cuvette is 1.0 cm.

(8). Prepare a standard curve by plotting the concentrations of the standards against their optical densities.

1. Effect of Varying the Amount of Acetylacetone

Ten sets of test tubes with each set consisting of a 0.05 ml, 0.10 ml, and 0.20 ml aliquot of the ALA hydrochloride stock solution were employed in this experiment and each tube was diluted to 7.0 ml with 1.0 M sodium acetate. Reagent blanks were prepared for each of the ten sets of test tubes and to each set of tubes a different amount of acetylacetone was added (0.01 ml, 0.05 ml, 0.10 ml, 0.15 ml, 0.20 ml, 0.25 ml, 0.30 ml, 0.40 ml, 0.50 ml, and 1.0 ml). After thoroughly mixing each tube, they were placed in a boiling water bath for 10 minutes, then removed and cooled to room temperature. Color development was attained with modified Ehrlich's reagent and the optical densities of the ALA standards in each set corrected for the appropriate blank were determined.

2. Effect of Changing the Boiling Time

Six sets of test tubes with each set consisting of a 0.05 ml, 0.10 ml, 0.20 ml, and 0.30 ml aliquot of the ALA hydrochloride stock solution were employed in this experiment and each test tube was diluted to 7.0 ml with 1.0 M sodium acetate. Reagent blanks were prepared for each of the six
sets of test tubes and 0.2 ml of acetylacetone was added to each test tube. The test tubes were then mixed and each set of tubes was placed in a boiling water bath for a different period of time (1 minute, 5 minutes, 10 minutes, 15 minutes, 20 minutes and 30 minutes). Color development of the pyrrole was attained with modified Ehrlich's reagent and the optical densities of the ALA standards in each set corrected for the blank determined.

3. Stability of the Color Developed with Modified Ehrlich's Reagent

Six aliquots of the ALA hydrochloride stock solution (0.01 ml, 0.05 ml, 0.10 ml, 0.20 ml, 0.30 ml, and 0.50 ml) were placed in test tubes and after the appropriate steps, color development was attained with modified Ehrlich's reagent. The optical density of each tube corrected for the blank was determined 15 minutes and also 2 hours after the addition of the modified Ehrlich's reagent.

4. Stability of Refrigerated Modified Ehrlich's Reagent

A solution containing 10 g of para-dimethylaminobenzaldehyde (DMAB) in 420 ml of glacial acetic acid was prepared and a 100 ml aliquot of this solution was removed. The remaining 320 ml was placed in a refrigerator where it was frozen and stored for future experimentation. To the 100 ml aliquot 19 ml of 70% perchloric acid was added and the formed solution constitutes fresh modified Ehrlich's reagent. A standard calibration curve was prepared as described under the section "PREPARATION OF AN ALA STANDARD CALIBRATION CURVE" and this fresh modified Ehrlich's reagent was
used for color development. A 100 ml aliquot of the glacial acetic acid solution containing DMAB was removed 5 days, 10 days, and 15 days after initiation of freezing and modified Ehrlich's reagent was prepared from each of the aliquots. An ALA standard calibration curve was prepared using each of the modified Ehrlich's reagents and the optical densities of the ALA standards were compared to one another.

D. PREPARATION OF DISPOSABLE CHROMATOGRAPHY COLUMNS PREPACKED WITH AN ION-EXCHANGE RESIN

In order to isolate ALA from urine, the urine sample must first be passed through an anionic exchange resin which permits the ALA and urea to pass through while retaining porphobilinogen. The effluent passing through the anionic resin must then be passed through a cationic exchange resin which retains ALA while allowing the urea to pass through. The anionic resin employed in the following experiments was AG 1-X8, 100 to 200 mesh, acetate ionic form. The cationic exchange resin was AG 50W-X4, 100 to 200 mesh, hydrogen ionic form.

A slurry of either AG 1-X8 resin or AG 50W-X4 resin was made in water using 3 g of the respective resin and 7 ml of water for each column to be prepared. A 10 ml aliquot of the slurry was then pipetted into the appropriate disposable plastic chromatography column (Bio-Rad Laboratories) with the aid of an automatic syringe and the resin was held in the column by a glass wool plug placed in the bottom cap of the column. A second glass
wool plug was placed on top of the resin in order to prevent distortion of the resin bed. Using this method a column measuring 2 cm in height and 1.6 cm in diameter resulted.

E. PROCEDURE FOR THE DETERMINATION OF URINARY ALA

The procedure used for the determination of urinary ALA is illustrated in Figure 1 and is as follows:

1. Insert a dual disposable chromatographic column setup in the support rack with the column containing the anionic AG 1-X8 resin on top and the column containing the cationic AG 50W-X4 resin on the bottom.

2. Wash the top column with 10 ml of water and allow to drain through both columns into the drain tray.

3. Apply 0.5 ml of a random urine sample to the top column after having determined that the pH of the urine is 6 or below.

4. Wash the top column three times with 10 ml of water and allow the water to drain through both columns.

5. Discard the top disposable column.

6. Place the bottom column in a test tube (20x150 mm). Add 7.0 ml of 1.0 M sodium acetate to the column and collect the eluted ALA in the test tube.

7. Discard the bottom disposable column and add 0.2 ml of acetylacetone to the test tube and mix.

8. Place the test tube in a boiling water bath for 10 minutes.

9. Add 7.0 ml of modified Ehrlich's reagent to the cooled test tube.
(10). Read the optical density corrected for the blank after 15 minutes at 553 μ in a spectrophotometer and determine the concentration of ALA in the urine sample from the standard calibration curve. Normal values of urinary ALA produce a faint yellow color, while abnormally elevated values produce a distinct red color.

For the urinary ALA analysis, disposable pipettes, test tubes, and columns were used. An automatic pipetting machine was utilized for the addition of water and sodium acetate to the columns. Figure 2 shows the application of the urine samples to the columns and the utilization of a hand-operated continuous syringe type pipetter for the application of the water and sodium acetate. The procedure for urinary ALA analysis as described above has been found to permit one individual to perform 200 determinations of urinary ALA in approximately only 4 hours or as many as 1,000 analyses in a normal work week.

1. **Recovery of ALA from Columns**

Forty-three piggyback column units were employed in this experiment and 0.5 ml of an aqueous solution (distilled water) containing 20 μg of ALA per ml was applied to each of 25 columns, while 1.0 ml was added to each of the remaining 18. The procedure for the determination of the ALA content of the solution was the same as described above for the determination of ALA in urine. The concentration of ALA in the aqueous solution was
determined from the ALA standard calibration curve and this value was compared to the known amount of ALA in the solution. The per cent recovery of ALA from the columns was then calculated.

To determine the recovery of ALA from urine, ALA was added to freshly voided urine, pH 4.0-6.0, from a normal individual in such a manner so that a 0.55 ml aliquot of the urine sample would contain 5 μg of added ALA + the normal ALA content of the urine, a 0.60 ml aliquot would contain 10 μg of added ALA + the normal ALA content of the urine, and a 0.70 ml aliquot would contain 20 μg of added ALA + the normal ALA content of the urine. Four columns each received a 0.5 ml aliquot of the normal urine and 0.55 ml, 0.60 ml, and 0.70 ml aliquots of the urine samples containing the added ALA. The per cent recovery of the added ALA was then determined.

2. Variation between Duplicate Samples

A 0.5 ml aliquot of urine from a normal individual was added to each of five piggyback column units, while a 0.5 ml portion of urine from an individual with lead exposure was added to each of a second set of five columns. The ALA concentration of both the normal and high ALA urines was determined by each of the five columns to which they were added and the variation in ALA values between the columns receiving the same urine samples calculated.
3. **Effect of Varying the Mesh Size of the AG 50W-X4 Resin**

A 10 ml aliquot of water was passed through each of twelve columns prefilled with AG 1-X8 resin, 100 to 200 mesh, and the time required for the water to pass through each column was noted. The time required for 10 ml of water to pass through columns prefilled with AG 50W-X4 resin of either 20 to 50, 50 to 100, 100 to 200, or 200 to 400 mesh was also determined. The AG 1-X8 columns were then placed on top of the AG 50W-X4 columns forming piggyback column units. The piggyback units were then divided into three series each consisting of an AG 1-X8 column on top of an AG 50W-X4 column of either 20 to 50, 50 to 100, 100 to 200, or 200 to 400 mesh. The time required for 10 ml of water to pass through each of these piggyback column units was then determined. To each column in the first series, a 0.5 ml aliquot of an aqueous solution (distilled water) containing 20 μg of ALA per ml was added. To each of the columns in the second series, a 0.5 ml aliquot of urine from a normal individual was added, while each of the columns in the third series received a 0.5 ml aliquot of urine from a lead-exposed individual. The piggyback column units were washed three times with 10 ml of water and the time required for each wash to pass through the column units was determined. The top column containing AG 1-X8 resin was discarded and 7.0 ml of 1.0 M sodium acetate was added to each of the bottom AG 50W-X4 columns. The time required
for the 7.0 ml aliquot of sodium acetate to pass through each column was noted and the pH of the sodium acetate eluate was determined. Color development was attained as previously described and the recovery of ALA from the columns receiving the same sample was calculated.

4. Effect of Urine pH on the Recovery of ALA from Ion-Exchange Columns

A 0.2 ml aliquot of an aqueous solution containing 500 µg of ALA per ml was added to each of 10 test tubes. To each of these tubes 9.0 ml of normal urine, pH 5.1, was added and the pH of the urines was adjusted with hydrochloric acid and sodium hydroxide to either 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, or 10.0. The total volume of each tube was then adjusted to 10 ml with normal urine. A 0.5 ml aliquot of urine from each of the tubes was then added to a different piggyback column unit and the optical density of the ALA eluted from each column was determined as previously described.

Similar experiments using 1 M sodium carbonate and hydrochloric acid and ammonium hydroxide and hydrochloric acid to adjust the pH of urine were also performed.

F. COLLECTION OF URINE SAMPLES FOR THE CORRELATION OF URINARY ALA VALUES WITH CLINICAL IMPRESSION OF LEAD INTOXICATION

1. Study Number 1
The first investigation was performed during the months of February - April, 1967 and concerns 250 children living in high-incidence areas of lead poisoning in the city of Chicago who were found by the Chicago Board of Health Division of Laboratories to have an initial blood lead value greater than 40 µg/100 ml as measured by atomic absorption spectrometry. The percentage distribution of the initial screening blood lead values of the 250 children studied was 72% of the population between 40 and 59 µg/100 ml; 13% between 60 and 69 µg/100 ml; 8% between 70 and 79 µg/100 ml; 3% between 80 and 99 µg/100 ml; 3% between 100 and 149 µg/100 ml; and 1% between 150 and 200 µg/100 ml. Following the initial blood lead determination, each child was referred to the Chicago Board of Health Lead Clinic located at Municipal Contagious Disease Hospital for clinical examination of possible increased lead exposure. The ages of the children studied ranged from 9 months to 6 years with the majority of children (83%) being between the ages of 1 and 4 years. Of the children studied 11% were white and 89% nonwhite, 51% male and 49% female. A random urine sample for the analysis of delta-aminolevulinic acid (ALA) was obtained from each child during as many clinical visits as was possible. A total number of approximately 1,000 urine samples were analyzed for urinary ALA content. The random urine samples were collected using 15 ml capacity disposable plastic tubes and stored in the dark in a freezer.
prior to the analysis of urinary ALA. All determinations of urinary ALA were performed within 1 week after collection. Urinary ALA is extremely stable following the above storage procedure since it has been found that keeping frozen urines in a dark freezer for a period of 1 year results in only a 5% decrease in the original ALA content.

2. Study Number 2

Random urine samples were obtained during the months of May - August, 1967 from a second group of children who had initial screening blood lead values greater than 50 μg/100 ml and were examined for possible lead ingestion at Municipal Contagious Disease Hospital. This group consisted of 534 children and a random urine sample was obtained from each child during as many subsequent visits to MCDH as was possible. A special attempt was made to obtain successive daily random urine samples from all children receiving chelation therapy for lead exposure. A total number of 1,831 urine samples from the 534 children were analyzed for urinary ALA content.

G. Correlation of Urinary ALA Values with Clinical Impression of Lead Intoxication

Each child referred to the Chicago Board of Health Lead Clinic had a thorough history and physical examination as well as a number of the commonly employed laboratory tests for lead exposure. The clinical and
laboratory findings on each child's chart were summarized and compared with the corresponding urinary ALA level obtained. In addition, the presence or absence of a clinical diagnosis of lead ingestion in each child was compared with the urinary ALA level of the same child. The examining physicians at the Chicago Board of Health Lead Clinic were in no instance aware of the urinary ALA level of the patient when arriving at a decision as to possible therapy for lead exposure, thereby permitting a single-blind type of clinical study to establish a relationship between urinary ALA levels and possible lead exposure. The administration of either calcium disodium edetate or penicillamine by the examining physician on the basis of all clinical and laboratory data except the urinary ALA level was taken as a clinical impression of an increased lead exposure in the child.

H. EPIDEMIOLOGICAL FIELD STUDY OF URINARY ALA LEVELS

A total of 5,000 plastic disposable urine collection tubes were distributed to nine Urban Progress Centers in order to determine the frequency of elevated urinary ALA levels in children residing in high-incidence areas of lead poisoning in the city of Chicago. The location of the Urban Progress Centers are listed below.

<table>
<thead>
<tr>
<th>Urban Progress Center</th>
<th>Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Parkway UPC</td>
<td>4622 South Parkway</td>
</tr>
<tr>
<td>Woodlawn UPC</td>
<td>1030 E. 63rd Street</td>
</tr>
<tr>
<td>Englewood UPC</td>
<td>839 W. 64th Street</td>
</tr>
<tr>
<td>South Chicago Unit</td>
<td>9231 S. Houston Avenue</td>
</tr>
<tr>
<td>Division St. Unit</td>
<td>1940 W. Division Street</td>
</tr>
</tbody>
</table>
A blood sample and a fresh urine sample were obtained from every child examined at the Urban Progress Centers. The urine samples were refrigerated after collection and were sent with the blood samples to the Chicago Board of Health. As the urine samples arrived at the Chicago Board of Health they were frozen and were picked up twice a week by our laboratory in order to determine their ALA content. The analysis of blood lead was performed by the Chicago Board of Health Division of Laboratories. The procedure as outlined to the technicians is as follows:

1. Check the names on the tubes containing the blood samples, number each, and enter the names and numbers into the workbook.

*2. Measure the volume of the blood sample with a pipette and add this to 5 ml of 20% trichloroacetic acid (TCA). Mix well on a mixer and centrifuge 5 minutes at 2500 RPM. Decant the supernatant into a labeled 50 ml tube.

3. Add 2.5 ml of 20% TCA to the packed precipitate, resuspend by mixing and centrifuge 10 minutes at high speed.

4. Pour this supernatant into the tube containing the first supernatant. (AT THIS POINT THE SAMPLES MAY BE FROZEN).
If the samples are to be run in the same day continue to step #5.

Prepare standards having concentrations of 0.2, 0.4, 0.8 and 1.0 parts per million. Place a 5 ml aliquot of each standard into a 50 ml tube, add TCA and then proceed to step 5.

**5. Adjust pH to 2.2–2.8 with NaOH and TCA.

6. Add 1 ml of 1% (aqueous) ammonium pyrrolidine dithiocarbamate (APDC) made fresh each day. Mix well.

7. Add 2 ml methyl isobutyl ketone (MIBK) and mix well.

8. Centrifuge 3 minutes and then aspirate top layer. Read in atomic absorption spectrophotometer.

NOTES: The following are the settings for the atomic absorption spectrophotometer:

Slit width - variable from 0.05 to 0.3

Support - compressed air (17-18 lbs / sq in)

Triple pass

Hot mode with variac

Fuel - Acetylene (1 - 1.1/2 lbs / sq in)

Elevator Height - 2.35 to 2.4 (Adjust for greatest sensitivity).

Lamp - 11 ma to 15 ma. Wavelength - 283 mp

Scale expansion - 1X
All glassware and pipettes are put through a regular wash and then returned to the laboratory where they are washed with a 1:1 dilution of concentrated nitric acid. They are then rinsed several times with distilled water, rinsed with de-ionized water, and finally oven dried. All standards and solutions are stored in polyethylene bottles.

* 10 ml of blood are requested but usually only 5 ml are received. Samples as small as 2 ml whole blood can be run satisfactorily, but with 2 ml there is no concentration factor and the result is multiplied by 2 1/2.

** Use one drop of thymol blue (1% in methanol) which is deep rose in TCA; the end-point is pale salmon.

I. ANIMAL EXPERIMENTS

1. Storage of Urine Samples

   The pH of each urine sample was tested by indicator paper and occasionally, when necessary, adjusted to a pH range of 4-6 by the addition of a few drops of glacial acetic acid. The urine samples were then frozen and stored for future analysis of ALA. Delta-aminolevulinic acid levels were determined employing the dual disposable chromatographic columns as described previously.

2. Urinary ALA Excretion of Rats Injected with Lead Acetate

   Six male Sprague-Dawley rats 90 to 100 days of age and weighing 305 to 355 g were each placed in individual stainless steel metabolism
cages and urine samples were collected in continuous two hour portions with the aid of test tubes placed in a fraction collector. After collecting 24 hours of normal urine, three rats were injected intraperitoneally with 1 mg/kg of lead acetate and three with 15 mg/kg. The urinary ALA excretion of each rat was determined before and after the administration of lead acetate.

3. Urinary ALA Excretion of Rats Fed Lead Acetate in the Diet

Six male Sprague-Dawley rats 60 days of age and weighing 210 to 230 g were each placed in individual stainless steel metabolism cages and the 24 hour urinary outputs of the rats were collected. After two days of collecting normal urine samples, three rats were fed, ad libitum, diets containing 0.1% and three were fed diets containing 1% lead acetate. The lead acetate diets were maintained for seven days and then the rats were returned to a normal diet (powdered Rockland Rat Diet). The urinary ALA excretion per 24 hour urine sample was determined for each rat.

4. Effect of Calcium Disodium Edetate on the Urinary ALA Excretion of Rats Fed a 1% Lead Acetate Diet

Six male Sprague-Dawley rats 60 to 67 days of age and weighing 220 to 265 g were each placed in individual stainless steel metabolism cages and their 24 hour urinary outputs were collected. After a three day control period, three rats were injected intraperitoneally with 200 mg/kg of calcium disodium edetate for three consecutive days and then were fed a 1% lead
acetate diet for seven days. The three remaining rats were fed a 1% lead acetate diet for seven days and were injected intraperitoneally with 200 mg/kg of calcium disodium edetate during the fourth through seventh day of the lead acetate diet. The 24 hour urinary ALA excretion from each rat was determined.

5. Effect of Various Metals on the Urinary ALA Excretion of Rats

Male Sprague-Dawley rats 90 to 95 days of age and weighing 280 to 350 g were each placed in individual stainless steel metabolism cages and their 24 hour urinary outputs were collected. After three days of collecting normal urine samples, various metals were administered to the rats and each was administered as a single intraperitoneal injection which corresponded to approximately one-half its LD50 dosage. Three rats were injected with each of the metals studied. The metals and dosages that were administered are the following: lead acetate, 50 mg/kg; cadmium chloride, 4.5 mg/kg; barium chloride, 15 mg/kg; ferrous sulfate, 100 mg/kg; zinc chloride, 19 mg/kg; strontium bromide, 500 mg/kg; copper sulfate, 15 mg/kg; arsenic pentoxide, 2 mg/kg; titanium dioxide, 500 mg/kg; mercuric chloride, 2 mg/kg; and lithium chloride, 250 mg/kg. Tetraethyl lead was administered as a single oral dose of 10 mg/kg. The 24 hour urinary outputs of the rats were collected and the ALA levels were determined.
Most of the metals injected into the rats were bivalent (lead, cadmium, barium, iron, zinc, strontium, copper, and mercury) and were selected in order to determine the specificity of lead in causing an increased excretion of ALA in urine. Tetraethyl lead was administered to determine whether an organic form of lead is capable of increasing urinary ALA excretion.

J. **STATISTICS EMPLOYED FOR THE ANALYSIS OF DATA**

1. **Standard Deviation**

\[ S = \sqrt{\frac{S_X^2}{N-1}} \]

- \( S \) = standard deviation
- \( S_X^2 \) = sum of the deviations from the mean squared
- \( N \) = number of individual measurements

2. **Comparison of Paired Observations by the t-Test**

\[ t = \frac{\bar{d}}{S_d} \]

- \( t \) = t-value
- \( \bar{d} \) = mean difference
- \( S_d \) = standard error of mean difference

3. **Correlation Coefficient**

\[ r = \frac{SP_{XY}}{\sqrt{S_X^2S_Y^2}} \]

- \( r \) = correlation coefficient
- \( SP_{XY} \) = sum of the cross products \( XY \)
- \( S_X^2 \) = sum of the deviations from the mean squared for group X
- \( S_Y^2 \) = sum of the deviations from the mean squared for group Y

where \( X \) and \( Y \) are the deviations from their respective means.
4. Chi-Square Test

\[ X^2 = \frac{\left[ (ad - bc) - \frac{N}{2} \right]^2 N}{(a+b)(c+d)(a+c)(b+d)} \]

- \( X^2 \) = chi-square
- \( N \) = total number of subjects
- \( a, b, c, \) and \( d \) = components of a 2X2 contingency table

2X2 Contingency Table

<table>
<thead>
<tr>
<th></th>
<th>White Children</th>
<th>Negro Children</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Children</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Having Normal Urinary ALA Levels</td>
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<td>(b)</td>
<td>(a+b)</td>
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<td>857</td>
<td>2507</td>
<td>3364</td>
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<tr>
<td>Number of Children</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Having Elevated Urinary ALA Levels</td>
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<td>(d)</td>
<td>(c+d)</td>
</tr>
<tr>
<td></td>
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<td>190</td>
<td>212</td>
</tr>
<tr>
<td>Total</td>
<td>(a+c)</td>
<td>(b+d)</td>
<td>(N)</td>
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<tr>
<td></td>
<td>879</td>
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</table>
CHAPTER III

RESULTS
CHAPTER III

RESULTS

A. MODIFICATION OF MAUZERALL AND GRANICK'S METHOD FOR THE DETERMINATION OF URINARY ALA

The use of disposable plastic chromatography columns prefilled with ion-exchange resin has made the determination of urinary ALA levels a practical laboratory procedure. The average completion time for one individual to perform 40 measurements of urinary ALA has been found to be approximately one hour, while 200 measurements of urinary ALA requires only 4 hours. Only six urinary ALA determinations could be performed in one day using the method described by Mauzerall and Granick.

Chart 1 presents a typical ALA standard calibration curve obtained by following the procedure described in the section entitled "Preparation of an ALA Standard Calibration Curve." Urinary ALA concentrations up to 10 mg/100 ml (10 mg%) can be determined from this curve without dilution of the final red-colored reaction mixture.

Chart 2 presents the effect on final color development of changing the amount of acetylacetone added to the ALA standards. Color development of the formed pyrrole, 2-methyl-3-acetyl-4-(3-propionic acid) pyrrole, was attained with modified Ehrlich's reagent and maximal development resulted when 0.10 ml of acetylacetone was added to the ALA standards. A decreased
color intensity was not noted until 0.30 ml of acetylacetone was added. A volume of 0.20 ml of acetylacetone was therefore chosen as the quantity to be used for the condensation of ALA in both the preparation of the standard calibration curve and in the procedure for the determination of urinary ALA.

Chart 3 presents the effect on final color development of varying the boiling time of the sodium acetate solution containing ALA and acetylacetone. Color development of the formed pyrrole was attained with modified Ehrlich's reagent and maximal development was found to occur when the sodium acetate solution was boiled for 5 minutes. The color intensity remained maximal even when the sodium acetate solution was boiled for periods up to 30 minutes. A 10 minute boiling period was therefore chosen to be used in the preparation of the ALA standard calibration curve and in the procedure for the determination of urinary ALA.

Chart 4 presents the stability of the color developed with modified Ehrlich's reagent. The optical densities of the ALA standards when read 15 minutes and 2 hours after the addition of modified Ehrlich's reagent are similar and thus the color formed with this reagent is stable for at least 2 hours.

Table 1 presents the stability of refrigerated modified Ehrlich's reagent. When glacial acetic acid solution containing DMAB is frozen for
periods up to 15 days and is then used for the preparation of modified
Ehrlich's reagent, the color of the ALA standards developed with this
Ehrlich's reagent is exactly the same as that produced with fresh reagent.

Table 2 indicates the expected results using the modified method for
the determination of urinary ALA. The limit of detectability of ALA in
urine is 0.03 mg/100 ml; the sensitivity of the test is 0.15 microgram;
the variation between duplicate samples is ± 3%; the recovery of ALA from
aqueous solution is 97%; and the recovery of ALA from urine is 93%.

Table 2a presents the recovery of ALA from aqueous solution (distilled
water) using the piggyback column method. When aliquots containing either
10 or 20 μg of ALA were placed on piggyback column units, the recovery of
ALA was 94.9 ± 5.4% and 100.0 ± 3.4%, respectively. Table 2b presents
the recovery of ALA from urine using the piggyback column method. When
5, 10, and 20 μg of ALA were added to normal urine samples, the percent
recovery of the added ALA was 92.9 ± 6.8, 93.5 ± 9.5, and 93.9 ± 5.0,
respectively. Table 2c presents the variation between duplicate samples
using the piggyback column method. The concentration of ALA in the
urine of a normal individual was 0.31 ± 0.02 mg/100 ml and in the urine
of a lead-exposed individual was 1.63 ± 0.05 mg/100 ml. The variability
between duplicate samples was found to be ± 3%.

Table 3 presents the results of changing the mesh size of the AG
50W-X4 resin. A 25% recovery of ALA was obtained from columns pre-
filled with AG 50W-X4 resin, 20 to 50 mesh. When the mesh was 50 to
100, a 58% recovery of ALA was attained. Columns prefilled with 100 to
200 mesh AG 50W-X4 resin yielded a 97% ALA recovery, while columns
containing 200 to 400 mesh gave a 94% recovery of ALA. Since the time
required for 7.0 ml of 1.0 M sodium acetate to flow through the 20 to 50
mesh column was 54 seconds as compared to 4 minutes 33 seconds for the
100 to 200 mesh, it appears that the sodium acetate passes through the
larger mesh columns so rapidly that it can not quantitatively elute off the
retained ALA. It was of interest to note that the pH of the sodium acetate
eluate ranged from 4.57 to 4.71 depending on the mesh size of the AG
50W-X4 column. Mauzerall and Granick have stated that ALA should be
condensed with acetylacetone at a pH of 4.6 and using the presently
described method for the determination of urinary ALA, the pH of the sodium
acetate eluate is of such a magnitude that it does not have to be adjusted
prior to the addition of acetylacetone.

Table 4 presents the effect of urine pH on the recovery of ALA from
disposable ion-exchange columns. Urine samples having a pH of from
1.0 to 10.0 were applied to piggyback column units and there was found to
be an essentially identical recovery of ALA from those columns which
received urine samples with a pH of 1.0 to and including 6.0. However,
when the pH of the urine sample was 7.0 or above, a decrease in the
recovery of ALA was noted. It is therefore suggested that the pH of all urine samples applied to piggyback column units be 6.0 or below.

B. CORRELATION OF URINARY ALA LEVELS WITH THE CLINICAL IMPRESSION OF LEAD INTOXICATION

1. Study Number 1

Table 5 presents the percentage distribution of urinary ALA levels obtained from 250 children suspected of lead ingestion as a result of having an initial blood lead value greater than 40 μg/100 ml. A total of 187 children (75%) were found to have a urinary ALA level less than 1.00 mg/100 ml, while 63 children (25%) were found to have a urinary ALA level of 1.00 mg/100 ml or higher. Following clinical examination with the physician unaware of the urinary ALA level of the patient, only 4 to 5 per cent of children with a urinary ALA level less than 1.00 mg/100 ml were treated with chelating agents for lead exposure. However, 65 to 100% of the children with urinary ALA levels of 1.00 mg/100 ml or higher received chelation therapy for lead exposure. This data indicates that the practical upper limit of normal for urinary ALA values in children is 0.99 mg/100 ml. Since there was also an essentially linear increase in the incidence of chelation therapy with progressive elevation of the urinary ALA level from 1.00 mg/100 ml to 10.0 mg/100 ml, it seems feasible to classify urinary ALA levels in children according to the code indicated in
Table 5. Urinary ALA levels between 0.00 and 0.99 mg/100 ml may be considered normal. Urinary ALA levels above 1.00 mg/100 ml can be subdivided into five ranges with a 1+ code corresponding to 1.00 to 1.49 mg/100 ml, a 2+ code corresponding to 1.50 to 1.99 mg/100 ml, a 3+ code corresponding to 2.00 to 2.99 mg/100 ml, a 4+ code corresponding to 3.00 to 5.99 mg/100 ml, and a 5+ code corresponding to greater than 6.00 mg/100 ml.

Table 6 presents a comparison of urinary ALA levels with the incidence of chelation therapy in 250 children with suspected lead ingestion. The urinary ALA test was interpreted as being correctly negative when a child with a urinary ALA level of less than 1.00 mg/100 ml was not treated with chelating agents following clinical examination. The urinary ALA test was interpreted as being correctly positive when a child with a urinary ALA level of 1.00 mg/100 ml or higher was administered chelating agents. The minimum criteria used for the clinical diagnosis of increased lead exposure were two consecutive blood lead values greater than 60 μg/100 ml coupled with either a definite history of pica or X-ray evidence of lead ingestion. Based upon an overall comparison of urinary ALA levels with the incidence of chelation therapy in children with suspected lead ingestion it can be stated that urinary ALA levels are 91% accurate for the clinical diagnosis of increased lead exposure. The incidence of urinary
ALA being false negative is only 3%, while the incidence of urinary ALA being false positive is only 6%.

Table 7 presents a comparison of the various laboratory tests for lead exposure in 250 children with suspected lead ingestion in terms of frequency of abnormal test results. Employing the single-blind type of evaluation, 92% of all the children who were discharged without chelation therapy after clinical examination were found to have normal urinary ALA levels, whereas 86% of all the children who received chelation therapy after clinical examination had abnormally elevated urinary ALA values. None of the other laboratory tests studied had a comparable degree of accuracy for the detection of early lead exposure.

Table 8 presents a comparison of the various laboratory tests for lead exposure in 250 children with suspected lead ingestion in terms of frequency of associated chelation therapy. Employing the single-blind type of evaluation, 96% of all the children with a normal range of urinary ALA values were discharged without chelation therapy after clinical examination, whereas 76% of all the children with an abnormally elevated urinary ALA value did receive treatment for lead exposure following clinical examination. Again, none of the other tests studied could compare with the urinary ALA test in terms of accuracy of detection of early exposure in asymptomatic children. Following clinical examination, only 22% of the children who originally had initial blood lead values greater
than 40 µg/100 ml as determined by atomic absorption spectrometry were treated with chelating agents for lead exposure.

Table 9 presents a comparison of mean urinary ALA values with mean blood lead values in 250 children with suspected lead ingestion classified by range of urinary ALA levels. Each blood lead value was determined at the same time as the urinary ALA value. The mean blood lead value which corresponded to normal urinary ALA levels less than 1.00 mg/100 ml was 48 µg/100 ml. However, children with levels of urinary ALA ranging from 1.00 to 1.49 mg/100 ml had a mean blood lead value of 65 µg/100 ml. An increase in the urinary ALA level was accompanied by a statistically significant corresponding increase in blood lead levels. The significance for the correlation coefficients between mean urinary ALA and mean blood lead values was P < 0.001 for normal, 1+, 2+, and 3+ urinary ALA levels with P < 0.01 for 4+ and 5+ urinary ALA levels.

Table 10 presents the distribution of hemoglobin levels in children suspected of lead ingestion classified by urinary ALA levels. Of the 57 children with elevated urinary ALA levels, 22% had abnormal hemoglobin levels (less than 10 g/100 ml). Of the 166 children having normal urinary ALA levels, 16% had hemoglobin levels less than 10 g/100 ml.

Table 11 presents the distribution of hematocrit values in children suspected of lead ingestion classified by urinary ALA levels. Of the 180
children with normal urinary ALA levels, 18% had abnormal hematocrit values (less than 33%). Similarly 18% of the 61 children with elevated urinary ALA levels had hematocrit values less than 33%.

2. **Study Number 2**

Table 12 presents the percentage distribution of urinary ALA levels in children with suspected lead ingestion as a result of having an initial blood lead level greater than 50 μg/100 ml as determined by atomic absorption spectrometry. A total of 371 children (69.5%) had a urinary ALA level less than 1.00 mg/100 ml, while 163 (30.5%) had a urinary ALA level above 1.00 mg/100 ml.

Table 13 presents a comparison of elevated urinary ALA levels with the incidence of chelation therapy in children suspected of lead ingestion as a result of having an initial blood lead value greater than 50 μg/100 ml. As in the first study, in no instance was the examining physician aware of the urinary ALA values when prescribing chelation therapy. The minimum criteria used for clinical diagnosis of increased lead exposure was also the same as before. A total of 131 children were found to have urinary ALA levels of 1.00 mg/100 ml or higher and 68.4 to 100% of these children received chelation therapy for lead exposure. These results are in accord with those obtained in the first study in which 65 to 100% of the children with urinary ALA levels of 1.00 mg/100 ml or higher were treated.
Table 14 presents a comparison of elevated mean urinary ALA values with the corresponding mean blood lead values in children with suspected lead ingestion. Each blood lead value was determined at the same time as the urinary ALA value. The mean blood lead value which was found to correspond to urinary ALA levels ranging from 1.00 to 1.49 mg/100 ml was 60 μg/100 ml. The mean urinary ALA level which was found to correspond to this 60 μg/100 ml was 1.27 mg/100 ml. The mean blood lead levels which were found to correspond to mean urinary ALA levels of 1.67, 2.41, 4.10 and 7.97 mg/100 ml were 74, 80, 95, and 127 μg/100 ml respectively.

Chart 5 indicates the typical response obtained when either calcium disodium edetate, abbreviated as EDTA, or penicillamine, abbreviated as PNCM, were administered to children with elevated urinary ALA values. It was found that both calcium disodium edetate and penicillamine administration resulted in a rapid return to normal of previously elevated urinary ALA levels. A child with an initial urinary ALA level of 4.50 mg/100 ml after being injected intramuscularly with EDTA for five successive days at a dose of 50 mg/kg per day was found to have a normal urinary ALA level on the fifth day of treatment. A similar decrease was noted when a child with an initial urinary ALA of approximately 2.80 mg/100 ml was given penicillamine orally at a dose of 250 mg per day for 14 successive days.
C. EPIDEMIOLOGICAL STUDY OF URINARY ALA LEVELS

Table 15 presents the results of an epidemiological study to determine the frequency of abnormal urinary ALA values in apparently-well, asymptomatic children residing in high-incidence areas of lead poisoning in the city of Chicago. Random urine samples from a total of 4,189 children between the ages of 1 and 10 years were tested for urinary ALA content by the piggyback column method. A total of 5.90% of the children tested had elevated urinary ALA values. Assuming that these results are representative of the entire population of approximately 260,000 children residing in high-risk areas of the city, this data suggests a predicted number of 15,340 children in the entire city of Chicago who should be strongly suspected of an increased exposure to lead on the basis of an abnormally elevated urinary ALA screening test.

Table 16 presents the percentage distribution of the urinary ALA levels of the 4,189 children according to the Urban Progress Centers where the children were examined. A total of 7.6, 8.9, 5.6, 6.6, 2.3, 3.1, 4.2, 5.8, and 8.6% of the children examined at South Parkway UPC, Woodlawn UPC, Englewood UPC, South Chicago Unit, Division Street Unit, Montrose UPC, Halsted UPC, Lawndale UPC, and Midwest UPC, respectively, had urinary ALA levels of 1.00 mg/100 ml or higher. The Midwest UPC had the highest percentage of children with elevated urinary ALA levels and it was found that 98.3% of the children examined at this Urban
Progress Center were Negro. In contrast, the children examined at the Division Street Unit had the lowest incidence of elevated urinary ALA levels and only 5.9% of the children examined at this Urban Progress Center were Negro.

Table 17 presents the distribution of children with elevated urinary ALA levels by age. Of 227 children with urinary ALA levels of 1.00 mg/100 ml or higher, the highest percentage or 17.2% were 4 years of age. Only 4 children or 1.8% were in the 1 year age group.

Table 18 presents the distribution of the urinary ALA levels of 3,919 asymptomatic children classified by age. It was found that 3.9, 6.9, 5.3, 5.5, 4.0, 6.4, 6.3, 5.3, 7.6, and 7.9% of the children in the 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 year age group, respectively, had urinary ALA levels of 1.00 mg/100 ml or higher. The 10 year age group had the highest percentage of children with elevated urinary ALA levels.

Table 19 presents the distribution of children with elevated urinary ALA levels according to race. In the epidemiological study, 24.4% of the children tested were white, 74.8% were Negro, and 0.8% were classified as other. Of 214 children with urinary ALA levels of 1.00 mg/100 ml or higher, 10.3% were white, 88.8% were Negro, and 0.9% were in the other category.
Table 20 presents the distribution of the urinary ALA levels of 3,606 asymptomatic children classified by race. A total of 2,697 Negro children were tested and 7.0% were found to have elevated urinary ALA levels. Only 2.5% of the 879 white children demonstrated an increased ALA excretion. The difference between the number of Negro children having elevated urinary ALA levels and the number of white children is highly significant ($P < 0.001$).

Table 21 presents the distribution of the children with elevated urinary ALA levels according to sex. Of 4,019 children tested, 52.9% were males and 47.1% females. A total of 233 children had elevated urinary ALA levels and of this total 62.7% were males and 37.3% were females.

Table 22 presents the distribution of the urinary ALA levels of 4,019 asymptomatic children classified by sex. Of 2,126 male children, 6.9% had urinary ALA levels of 1.00 mg/100 ml or higher. Only 4.6% of the 1,893 female children had elevated urinary ALA levels. The difference in the incidence of elevated urinary ALA levels between the two sexes is statistically significant ($P < 0.005$).

Table 23 presents the distribution of blood lead values in 3,068 children with normal levels of urinary ALA. Each blood lead value was determined at the same time as the urinary ALA value. Using a blood lead value of 60 μg/100 ml as the upper limit of normal, 3,031 out of 3,068 children with normal urinary ALA levels also had normal blood lead levels. Only 37
children with normal urinary ALA levels had blood lead levels greater than
60 μg/100 ml.

Table 24 presents the distribution of urinary ALA values in 3,148
children with normal levels of blood lead. Only 2,982 out of 3,148 children
with normal blood lead levels also had normal urinary ALA levels. A
total of 166 children with normal blood lead levels were found to have
elevated urinary ALA levels.

D. ANIMAL EXPERIMENTS

Chart 6 shows the typical response obtained when rats were injected
intraperitoneally with a single dose of either 1 mg/kg or 15 mg/kg of lead
acetate. It was found that 1 mg/kg of lead acetate, shown by the open circles,
did not cause an increased amount of urinary ALA. On the other hand,
15 mg/kg shown by the dark circles and equivalent to only 1/10 of the LD50
of lead acetate was capable of causing an increase in urinary ALA within
6 hours after injection, which reached a level of almost 5 mg/100 ml 24
hours after injection.

Chart 7 shows the effect on urinary ALA excretion of different concen-
trations of lead acetate in ad libitum diets fed to adult rats. Within 1 day,
a 0.1% lead acetate diet resulted in an elevation of the urinary ALA excretion
from a control level of 50 μg/24 hours to 96 μg/24 hours. A 1% diet of lead
acetate was found to cause an increase in urinary ALA excretion from
50 to 400 µg/24 hours. The urinary ALA excretion remained elevated for the duration of the seven day feeding period of the lead-containing diet and was found to return to normal following the removal of lead from the diet.

Chart 8 shows the effect of calcium disodium edetate on the urinary excretion of ALA in rats fed a 1% lead acetate diet. The top graph shows the elevation of urinary ALA caused by a 1% lead acetate diet as previously described. The middle graph shows the urinary ALA excretion obtained when rats pretreated with calcium disodium edetate were then fed a 1% lead acetate diet. The rats were injected intraperitoneally with 200 mg/kg of calcium disodium edetate for three consecutive days prior to the onset of the 1% lead acetate diet. It was found that the calcium disodium edetate itself had no effect on the urinary ALA excretion of normal rats. In addition, the pretreatment of rats with calcium disodium edetate had no effect on the increase in urinary ALA excretion resulting from a 1% lead acetate diet. The bottom graph shows the urinary ALA excretion in rats fed a 1% lead acetate diet for seven days and which were also injected intraperitoneally with 200 mg/kg of calcium disodium edetate during the fourth thru seventh day of the lead acetate diet. The urinary ALA level was observed to decrease from 424 µg/24 hours just prior to the injection of calcium disodium edetate to 197 µg/24 hours following it. The urinary ALA excretion then remained depressed for the remainder of the seven day
period of lead acetate feeding.

Table 25 indicates the effect of various metals on the urinary ALA excretion in adult rats. Each metal was administered as a single intra-peritoneal injection which corresponded to approximately one-half its LD50 dosage. Tetraethyl lead, on the other hand, was administered as a single oral dose of 10 mg/kg. On Day 0 which was the day preceding administration of the various metals, the mean urinary ALA excretion per 24 hour urine sample in 36 adult rats was 37 µg with an upper limit of normal of 69 µg. Mean values of urinary ALA on Day 1 following injection of lead acetate rose to 291 µg, remaining elevated throughout the time periods studied. The difference between the urinary ALA levels obtained the day before and the day after the injection of lead acetate is statistically significant (P < 0.05). Tetraethyl lead, which is an organic form of lead, did not cause any increase in the urinary ALA excretion of treated rats. In addition, none of the other metals studied, including cadmium, barium, iron, zinc, strontium, copper, arsenic, titanium, mercury, and lithium were found to produce a statistically significant increase in urinary ALA excretion.
CHAPTER IV

DISCUSSION
A. CHILDHOOD LEAD POISONING AS A PUBLIC HEALTH PROBLEM

Lead poisoning in children is one of the major public health problems in large cities. In a three year period, 1959-1961, lead poisoning accounted for 4.7% of 9,853 cases of accidental poisoning in children reported to the Chicago Board of Health, but was responsible for nearly 80% of all deaths due to accidental poisoning (Christian et al., 1964). Of 61,167 poisonings reported to the New York City Poison Control Center between 1955 and 1963, 3% or 1,704 cases were due to lead (Jacobziner, 1966). In Baltimore from 1956 through 1964 there were 1,337 known cases of lead poisoning in children (Schucker et al., 1965).

New York City reported a 6.3% mortality rate for childhood lead poisoning from 1954-1964 (Jacobziner, 1966). In Cleveland, Griggs et al. (1964) reported that from 1952 through 1958 lead poisoning had a mortality rate of 30%. Between 1959 and 1963, a mortality rate of 25% was reported for a group of 182 children treated for acute lead encephalopathy at Cook County Children's Hospital in Chicago and this fatality rate occurred despite the use of chelating agents and other techniques for reducing the intracranial pressure. Perlstein and Attala (1966) have found that in childhood lead encephalopathy, the mortality rate is between 28 and 45%, and four out of 84
five who survive have sequelae. This sequelae includes mental retardation, recurrent seizures, cerebral palsy, and optic atrophy. Recurrent seizures in their study was the most common sequela and occurred in 54% of the surviving patients, while optic atrophy was the least common having occurred in only 6%.

Lead has become a major cause of poisoning among children, particularly those residing in the slum areas of large cities. High risk areas for lead poisoning exist in all large cities and these areas usually contain old, dilapidated substandard homes. In such homes children have a ready access to flaking paint and broken plaster and due to a lack of parental supervision these homes provide an excellent environment for lead poisoning. The problem of lead poisoning is not, however, confined to the children in the lower socio-economic groups since it has also been reported in children from advantaged homes (Moncrieff et al., 1964).

Children between the ages of 1 and 6 are the main victims of lead poisoning with those between 1 and 3 years comprising nearly 85% of the cases (Jacobziner, 1966). The two year age group contains nearly 54% of all deaths due to lead intoxication. Jacobziner (1966) in his epidemiological study of childhood lead poisoning found no significant difference in the mortality incidence based on sex, except among whites. Here, deaths of females outnumbered males two to one, however, the total number of deaths were too few to warrant any conclusions as to whether the female is more
Lead poisoning and lead encephalopathy in children are most frequently encountered during the summer months. About 45% of the total number of childhood lead poisoning cases reported in New York City between 1954 and 1963 were found to occur between the months of June and September (Jacobziner, 1966). Christian et al. (1964) have reported that 80 to 85% of the cases of childhood lead poisoning occur during these months. Lead poisoning, however, should not be considered strictly a summertime disease since a number of cases have also been reported during the winter months. Epidemiological studies indicate that even though lead encephalopathy occurs more frequently during the summer, asymptomatic lead poisoning is a year round disease. The reasons postulated for the seasonal variation in the number of lead poisoning cases reported include: 1) a metabolic disturbance caused by the actinic rays of the summer sun which results in an increased lead absorption; and 2) a greater opportunity during the summer months to ingest lead containing paint used on the exterior surfaces of the home (Jacobziner, 1966).

B. USE OF SCREENING TESTS FOR THE DETECTION OF EARLY LEAD EXPOSURE

Lead poisoning will only be wiped out when old housing is eliminated and new homes employing lead free paint constructed. Since at present this task is not possible, the best approach to the control of lead poisoning in children would be the carrying out of large-scale screening programs.
designed for the detection of early and potentially hazardous exposure to lead prior to the development of the symptoms of frank lead intoxication. The Chicago Board of Health has been able to obtain an average of 2,000 venous blood samples per month from children residing in high-incidence areas of lead poisoning in the city of Chicago as part of their blood lead screening program. This rate of collection would only be able to screen a total of 24,000 children per year and since there are at least 260,000 children between the ages of 1 and 7 residing in these high-risk areas, an annual screening program of only 24,000 children does not seem adequate. Another disadvantage to the implementation of a blood lead screening program is that the drawing of blood and the determination of the lead content in the blood both require highly skilled personnel.

In 1956 Mauzerall and Granick developed a colorimetric method for the determination of the ALA content in urine. Using this method Haeger-Aronsen (1960) found that the concentration of urinary ALA is increased in adult workers exposed to lead. Several other investigators have also demonstrated that urinary ALA levels are increased in cases of lead poisoning and in lead-exposed workers (deKretzer and Waldron, 1963; Kleinstein et al., 1963; Efe, 1964; Cramer et al., 1965; Cramer et al, 1966; deBruin and Hoolboom, 1967).
Urine contains glucosamine and aminoketones and these compounds represent potential interfering substances in the determination of urinary ALA. Shuster (1956) reported that both glucosamine and aminoketones resembling ALA when heated with ethyl acetoacetate give condensation products which react with para-dimethylaminobenzaldehyde to form colored complexes. However, the relative molar color yield of glucosamine and most of the aminoketones was much less than that of ALA.

Mauzerall and Granick (1956) separated by paper chromatography the Ehrlich positive products associated with the determination of ALA in normal urine and found that only 10 to 20% of these products was the pyrrole corresponding to free ALA. Another 20 to 40% behaved as the pyrrole corresponding to an aminoketone without a free carboxyl group e.g. aminoacetone and the remainder was an organic solvent-insoluble material such as might be formed from glucosamine. Shuster (1956) has shown that using the ethyl acetoacetate method both aminoacetone and glucosamine give only a 10% relative molar color yield as compared to ALA. Mauzerall and Granick's method for the determination of urinary ALA uses condensation with acetylacetone at a pH of 4.6 and "this method is less sensitive than the ethyl acetoacetate method to color interference with high concentrations of amino acids, ammonia, or glucosamine." Thus, even though only 10 to 20% of the total Ehrlich positive products in normal urine are due to the pyrrole
corresponding to free ALA, the color intensity of this 10 to 20% would be far greater than that of the sum of the other products. Haeger-Aronsen (1960) has found that essentially all the ALA-like substances occurring in increased amounts in urine from lead workers is ALA.

C. URINARY ALA LEVELS OF CHILDREN SUSPECTED OF AN INCREASED LEAD EXPOSURE

The choice of urine as the biological sample to be used in a screening program for the detection of early lead exposure in children has the advantage that a large number of samples could easily be obtained. Therefore, after reading the above reports it was deemed of interest to investigate the urinary ALA levels of children suspected of an increased lead exposure. The method described by Mauzerall and Granick was utilized, however, it was found that only six urinary ALA determinations could be performed in one working day. The method of Mauzerall and Granick was modified and the present study indicates that the determination of urinary ALA levels is now possible as a practical laboratory procedure. As shown in Figure 3, a complete kit for performing the analysis of urinary ALA is now commercially available and utilizing this kit one individual can perform either 40 analyses of urinary ALA in one hour or approximately 1,000 analyses in a normal work week. The collection, handling, and storage of large numbers of urine specimens has been found to be feasible because only 0.5 ml of a random urine sample is adequate for the quantitation of urinary
Cramer and Selander (1967) have reported that collection of 24-hour urine specimens is not necessary for the accurate quantitation of urinary ALA since the ALA values expressed in mg/100 ml from freshly voided urine specimens closely agree with those from 24-hour specimens. The ALA output expressed in mg/g creatinine in freshly voided urine was also found to closely correlate with the values obtained from 24-hour specimens. When the urinary ALA output expressed in mg/100 ml was compared to ALA output expressed in mg/g creatinine a strong positive correlation was obtained. This thus indicates that it is not necessary to relate the urinary output of ALA to the output of creatinine or to the specific gravity of urine.

Haeger-Aronsen (1960) collected freshly voided urine samples from 100 apparently healthy individuals (50 males, 50 females), 20 to 65 years of age, and reported a mean urinary ALA concentration of 0.29 mg/100 ml with a standard deviation of 0.14. The 95% confidence limit was found to be 0.01-0.57 mg/100 ml. Davis and Andelman (1967) reported that the normal urinary ALA level in children ranging in age from 9 months to 5 years averaged 0.22 mg/100 ml with a standard deviation of 0.16 mg/100 ml and a 95% confidence limit of 0.54 mg/100 ml.

In the present clinical study only 4 to 5% of the children with urinary ALA levels less than 1.00 mg/100 ml were treated with chelating agents for lead
exposure. This data indicates that the practical upper limit of normal for urinary ALA values in children is 0.99 mg/100 ml. Since the incidence of chelation therapy was found to increase as the urinary ALA levels increased, it was possible to subdivide the ALA levels of 1.00 mg/100 ml and above into five ranges. A 1+ ALA code corresponded to an ALA level of from 1.00 to 1.49 mg/100 ml, a 2+ code corresponded to 1.50 to 1.99 mg/100 ml, a 3+ code corresponded to 2.00 to 2.99 mg/100 ml, a 4+ code corresponded to 3.00 to 5.99 mg/100 ml, and a 5+ code corresponded to 6.00 mg/100 ml and above.

A 91% agreement was found to exist between urinary ALA levels and the administration of chelation therapy to asymptomatic children suspected of lead ingestion and this offers evidence that urinary ALA levels are useful as a screening technique for the detection of childhood lead exposure. The incidence of urinary ALA being false positive was only 6% and this might have been due to the fact that urinary ALA levels have been reported to be the earliest indication of lead exposure (Efe, 1964; Djuric et al., 1966). Since the examining physician was unaware of the urinary ALA levels of the patients in this clinical study, he would not be expected to administer chelating agents in the absence of other signs of an increased lead exposure.

When comparing the various laboratory tests available for determining increased lead exposure, it was found that none of these could compare with the urinary ALA test in terms of accuracy of detection of early lead exposure.
in children. Employing the single-blind type of evaluation, 96% of all the children with a normal range of urinary ALA values were discharged without treatment after clinical examination, whereas 76% of all the children with abnormally elevated urinary ALA values did receive chelation therapy for lead exposure after clinical examination. Only 22% of the children who originally had initial screening blood lead values greater than 40 μg/100 ml as determined by atomic absorption spectrometry were treated with chelating agents.

When lead-poisoned children with elevated urinary ALA levels were treated with chelating agents, the ALA levels returned to normal limits within a few days. Haeger-Aronsen (1960) treated lead-poisoned adults and rabbits with chelating agents and also found that the excretion of ALA in the urine decreased after initiation of treatment. A return of elevated urinary ALA levels to normal after treatment with chelating agents has been reported by numerous investigators (Griggs, 1964; Koziolowa-Lipska and Gutniakowa, 1964; Selander et al., 1966; Selander, 1967). Haeger-Aronsen (1960) and Selander et al. (1966) have also reported that the excretion of lead in urine increases soon after the administration of chelating agents. The fact that the recommended chelation therapy for lead poisoning causes an increased excretion of ALA to return to normal limits provides additional evidence that urinary ALA determinations correctly indicate abnormal lead exposure.
in children and adults. The use of urinary ALA determinations as a clinical index for following the therapeutic management of patients receiving chelating agents for lead poisoning, however, is questionable since urinary ALA levels have been found to increase once chelation therapy has ended. Griggs (1964) has reported that the reduction in the excretion of ALA from a lead-poisoned patient treated with versenate was maintained for only a few days; within two weeks excretion had returned to pretreatment levels. Perhaps the urinary ALA test is so sensitive that the small amount of lead left in the body after chelation therapy is capable once again of increasing the urinary excretion of ALA.

It is generally recognized that the upper limit of normal values for blood lead in children approximates 60 µg/100 ml, with a level of 80 µg/100 ml or higher being indicative of frank lead intoxication (Smith, 1964; Greengard, 1966). The data from this study indicates that urinary ALA values below 1.00 mg/100 ml which are considered normal values were associated with blood lead levels which averaged below the accepted upper limit of normal of 60 µg/100 ml. Urinary ALA levels above 1.00 mg/100 ml which are considered abnormally elevated values were associated with blood lead values above 60 µg/100 ml. In the present study, children with a mean urinary ALA level of 1.22 mg/100 ml had a mean blood lead level of 65 µg/100 ml. Selander et al. (1966) have found that in lead-poisoned adults a lead level of 60 µg/100 ml blood corresponded to an ALA value in urine of 2 mg/100 ml.
It should be noted that the highest mean elevated value of urinary ALA represents almost an eighteenfold increase over the mean normal value of urinary ALA, while the highest mean elevated value of blood lead represents only a threefold increase over the mean normal value of blood lead.

Lead poisoning in adults usually results from the accidental exposure to lead in an industrial atmosphere, while in children is usually the result of oral ingestion of chips of lead-containing paint primarily in the older undercoats of paint. The chronic nature of lead poisoning enables the employment of a suitable screening technique carried out at periodic intervals which would detect an abnormal exposure to lead prior to the development of clinical symptoms. Since lead is rapidly stored in the skeletal system shortly after exposure of the child, initially high blood lead levels may quickly decrease to normal values. This can occur even if the exposed child has an accumulation of potentially dangerous quantities of stored lead which can be released at any time to cause the severe manifestations characteristic of lead intoxication. Therefore, unless blood lead values are determined within a short period of time following exposure to lead, they may be of little value in any annual screening program designed for detecting early lead exposure in asymptomatic individuals. Saita and Moreo (1964) have shown that ALA levels remain elevated for at least three years following removal of the
individual from the source of lead exposure. This prolonged increase makes it possible for the use of urinary ALA measurements in annual screening programs for the detection of early lead exposure in asymptomatic individuals. An additional advantage for the use of urinary ALA levels as a screening technique is its low incidence of false-positive results. In contrast, the frequency of false-positive results obtained for abdominal x-ray films, hematocrit determination, hemoglobin determination, wrist x-ray films, and blood lead values greater than 40 μg/100 ml were 49, 64, 58, 68 and 78%, respectively. Coproporphyrin determination of urines obtained from asymptomatic children living in high-incidence areas of lead poisoning has been reported to be 85% false positive (Davis and Andelman, 1967).

Children with lead poisoning have been reported to demonstrate a pronounced microcytic, hypochromic anemia. In this study, however, children having elevated urinary ALA levels had similar hemoglobin and hematocrit values as children having normal ALA levels. It therefore appears that an elevated urinary ALA excretion occurs prior to the onset of anemia.

D. URINARY ALA LEVELS OF ASYMPTOMATIC CHILDREN

The results of the epidemiological study indicate that 5.90% of the asymptomatic children residing in high-incidence areas of lead poisoning in the city of Chicago have urinary ALA levels of 1.00 mg/100 ml or greater.
Since 260,000 children reside in high-risk areas of the city, this data suggests that 15,340 children in the city of Chicago should be suspected of an increased lead exposure on the basis of an abnormally elevated urinary ALA screening test. Of 227 children with urinary ALA levels of 1.00 mg/100 ml or greater, 17.2% were 4 years of age and only 1.8% were in the 1 year age group. Even though lead poisoning has been reported to occur most frequently in children between 1 and 6 years of age, this study demonstrated that the highest percentage of children having elevated urinary ALA levels is in the 9 and 10 year age groups. This latter result can be explained by the finding of Saita and Moree that urinary ALA levels may remain increased for up to three years after removal of the individual from the source of lead exposure. If children had ingested lead when they were 6 or 7 years of age, the ALA levels in their urine would remain elevated until the age of 10.

The epidemiological study showed that 7.0% of the Negro children tested had elevated urinary ALA levels, while only 2.5% of the white children demonstrated an increased ALA excretion. This disparity is probably a reflection of the housing in which the children reside. Since homes in Negro ghettos are more dilapidated than those in white neighborhoods, it is entirely reasonable to see such a high incidence of lead poisoning in Negro children. Dilapidated homes provide a ready access to broken, painted plaster from walls and paint peelings from window sills and ceilings.
Jacobziner (1966) has reported that in 90% of the cases of childhood lead poisoning, the affected child ingested chips of paint or plaster for several months prior to the diagnosis of lead intoxication. Since lead poisoning associated with pica is a chronic process, it becomes apparent that there is not only a necessity for new housing in slum areas but also a crying need for closer parental supervision of children.

The epidemiological study also demonstrated that 6.9% of the male children tested had urinary ALA levels of 1.00 mg/100 ml or higher, while only 4.6% of the female children had elevated urinary ALA levels. Jacobziner (1966) also found that more cases of lead poisoning occurred in males. In the present study, however, the difference in the incidence between the sexes is statistically significant.

E. EFFECT OF VARIOUS METALS ON THE URINARY ALA EXCRETION OF RATS

In the animal studies, rats were injected intraperitoneally with a single dose of 15 mg/kg of lead acetate and an increased urinary ALA excretion was demonstrated within 6 hours. This dose represents only 1/10 the LD50 of lead acetate and illustrates the rapid rise in the urinary ALA excretion after exposure to a low dose of lead acetate. Haeger-Aronsen (1960) found that rabbits injected subcutaneously with lead acetate at a dose of 125 mg/kg reached a maximum urinary ALA level on the thirty-fifth day.
after injection when it was 1.96 mg/100 ml. Rats injected with 15 mg/kg had a value of almost 5 mg/100 ml 24 hours after injection. The rat, therefore, appears to be an excellent animal to study the acute effect of chemicals and drugs on urinary ALA excretion.

When each of several metals were administered to rats as a single intraperitoneal injection which corresponded to one-half its LD50 dosage, inorganic lead was the only one found to cause an elevation of urinary ALA excretion. Tetraethyl lead, which was given orally, was found incapable of causing an increased urinary ALA excretion. Haeger-Aronsen (1960) injected rabbits with either cobalt, copper, zinc, arsenic, silver, lead, tin, mercury, thallium, or bismuth and also found that lead was the only metal capable of increasing the ALA content of the urine. It was of particular interest to note that the administration of copper, mercury, and zinc did not increase the excretion of ALA by the intoxicated animals since Gibson et al. (1955) have demonstrated that in vitro these metals have a much stronger inhibitory effect on ALA dehydrase than lead. If ALA dehydrase inhibition was the sole cause for increasing the ALA excretion, these other metals should also elicit an increased ALA content in the urine. A recent report by Heilmeyer (1967) shows that the normal metabolism of ALA to \( \delta \) dioxovaleric acid (DVA) is inhibited in patients with lead intoxication. Shemin et al. (1953) were the first to show that ALA was metabolized to DVA and
perhaps the inhibition of this pathway is important. If lead causes a
build-up of ALA by inhibiting the ALA dehydrase enzyme and then also
blocks the normal metabolism of ALA to DVA, there is no recourse but for
ALA to be excreted as such in the urine.

Goldwater and Joselow (1967) have reported that mercury-poisoned
individuals have a normal content of ALA in the urine and this agrees with
the rat data. Tetraethyl lead exposed individuals have also been shown to
have normal urinary ALA levels (Koziolowa-Lipska and Gutniakowa, 1964;
Gherardi and Vidoni, 1965). It appears that only the inorganic form of lead
is capable of eliciting an increased urinary excretion of ALA.

F. DISADVANTAGES OF USING A SINGLE CATIONIC COLUMN RATHER
THAN A PIGGYBACK COLUMN UNIT FOR THE ISOLATION AND DETER-
MINATION OF URINARY ALA

The presently used method for urinary ALA determinations employs
two chromatography columns held in tandem position so that both the urine
sample and the water washes can pass through both columns consecutively.
The top column retains the porphobilinogen (PBG) and removes it as an
interfering substance in the colorimetric determination of urinary ALA with
Ehrlich's reagent. In two recent reports (Williams and Few, 1967; Sun et al.,
1969) it has been suggested that acceptable estimates of urinary ALA might
be obtained without prior removal of PBG and this is based on the assumption
that PBG is little affected by lead absorption. The available literature tends to indicate that PBG levels in the urine become increased with the onset of symptoms of lead poisoning. Griggs and Harris (1958), Haeger-Aronsen (1960), and deKretzer and Waldron (1963) have found that lead-exposed workers have normal levels of PBG in urine. However, Bashour (1954), Tanabe (1959), Schlenker et al. (1964), and Gibson et al. (1968) have reported that PBG is increased in the urine of lead-poisoned individuals. In addition to cases of lead intoxication, urinary PBG excretion has been reported to increase in diseases of the liver, including cirrhosis, as well as a number of anemias other than those due to iron-deficiency (Saita et al., 1966). Williams and Few (1967) state that their single column method might result in falsely high values in pathological conditions such as porphyria. The possibility therefore exists that measurement of urinary ALA levels without the prior removal of PBG will result in a high incidence of false positives for lead exposure due to urinary increases of PBG. Since no additional time is required for the urine sample to pass through both a top anionic column which removes the PBG and a bottom cationic column which retains ALA for subsequent elution, a dual disposable column unit measuring urinary ALA levels alone is the method of choice. However, if urinary ALA levels are used as a screening technique for the detection of early lead exposure in asymptomatic children, the cost of using the dual column units
may be prohibitive. Since children do not demonstrate acute intermittent porphyria and liver diseases in childhood are rare, it may be possible to use a single disposable chromatography column containing AG 50W-X4 resin to obtain an acceptable estimate of urinary ALA for the early detection of childhood lead exposure. In industry where cost is not that important and the number of workers to be tested not that large, the use of the dual piggyback column units is recommended.

G. USE OF URINARY ALA LEVELS AS A SCREENING TECHNIQUE FOR THE DETECTION OF EARLY LEAD EXPOSURE

In order to perform an annual urinary ALA screening program to detect early lead exposure, large numbers of urine samples would have to be collected. If 100,000 random urine samples were to be collected annually, it would be necessary to obtain random urine samples from approximately 2000 children per week. This can be accomplished by utilizing the following sources for collecting urine samples from children: 1) community representatives as part of their normal assigned duties in visiting the homes in slum areas; 2) high-risk infant welfare stations; and 3) neighborhood health centers. The major responsibility in collecting urine samples should lie with the community representatives representing the Urban Progress Centers. With the large number of community representatives available at the Urban Progress Centers, each one would have to collect only a few urine samples per day in order to attain the desired 2,000 urine samples per week.
The utilization of the community representatives in obtaining the urine samples would not involve any additional expense since they would perform the collection of urine samples during the course of their normal visits. The only instruction necessary would be the proper labeling of the data-tab attached to the urine collection tube. Each community representative would fill out the data-tab and then slip it around the urine collection tube which is then filled with as little as 1 to 5 ml of urine and stoppered. At the end of the day, the representatives would return their collected urine samples to their respective Urban Progress Center where they would be placed in a freezer. The following day these urine samples would be brought to the laboratory where the determination of urinary ALA would be performed.

The urine can be stored in a freezer prior to the determination of its ALA content.

The following is the presently recommended procedure for the collection and storage of random urine samples whose ALA levels are subsequently to be determined:

1. Place 0.1 ml of glacial acetic acid in an empty screw-capped vial in order to insure that the stored urine will have a pH below 6.

2. Collect a random urine sample. It is advisable not to use the first voided morning specimen, late evening specimens after 8:00 P.M., or specimens obtained following excessive fluid intake. This eliminates any concentration or dilution of the urinary ALA content.
3. Add approximately 10 ml of the random urine sample to the vial containing the glacial acetic acid, replace the cap, and gently mix.

4. Place the vial in a refrigerator or freezer as soon as possible. Excessive light exposure of the urine sample should be avoided prior to storage since ALA has been found to be light-sensitive. This can easily be accomplished by placing the vials in either a dark brown plastic bag or a light-tight box. The urinary ALA content of samples stored in a dark refrigerator will remain constant up to 4 months, while the urinary ALA content of samples stored in a dark freezer have been found to remain constant up to 1 year.

5. After removal from storage, the urine in each vial should be gently shaken to provide a homogeneous sample from which to pipet the 0.5 ml aliquot required for the urinary ALA determination.

In order to test 100,000 children for urinary ALA levels per year, it is necessary that a total of 400 urinary ALA determinations be performed daily. This can easily be accomplished by using the previously described method. A major advantage of the colorimetric determination of urinary ALA levels in the visible spectrum is that normal values of urinary ALA produce a colorless to faint yellow color, while abnormally elevated values of urinary ALA produce a definite and distinct red color. It is therefore possible in a large-scale screening program to immediately classify all non-red colors
as being normal ALA values and determine only the optical densities of the few red colored samples. The optical densities of the samples can be quickly converted to mg/100 ml (mg%) of urinary ALA by using a conversion chart as shown in Table 26.

Table 27 classifies urinary ALA levels in terms of their relationship to lead exposure in children. Values of urinary ALA below 1.00 mg/100 ml are considered to be normal, indicating an insignificant exposure to lead. Values of urinary ALA 1.00 mg/100 ml or higher can be considered to be abnormally elevated with five subdivisions of increasing severity. A 1+ ALA code indicates a moderate exposure to lead; a 2+ indicates a heavy exposure to lead; a 3+ indicates a severe exposure to lead; a 4+ indicates a critical exposure to lead; and a 5+ indicates an overwhelming exposure to lead. The greatest priority should thus be given the children with highest urinary ALA levels. Children with urinary ALA levels corresponding to a code of 5+, 4+, 3+, and 2+ should be immediately referred to a hospital for clinical examination. Such a clinical visit might include not only a history and physical examination, but also a blood lead determination, X-ray examination of both the abdomen and wrists, and hemoglobin and hematocrit determinations. If desired, the determination of urinary coproporphyrin and basophilic stippling can also be performed. Children with a urinary ALA code of 1+ should be scheduled for the earliest possible clinical examination.
after all the children in the range of 2+ to 5+ have been examined and treated with deleading agents.

In summary, the following are the major advantages of using the urinary ALA levels of asymptomatic children as a screening program for the detection of early lead exposure:

1. The collection of random urine samples is the only practical method of obtaining biological specimens from thousands of small children.

2. The determination of urinary ALA requires only 0.5 ml of a urine sample.

3. ALA is stable in frozen urine for at least one year and therefore urine samples can be collected and stored without any decrease in their ALA content.

4. On the basis of performing 400 urinary ALA determinations per day, 100,000 urine samples can be analyzed for their ALA content per year.

5. Urinary ALA levels are 91% accurate for the clinical diagnosis of increased lead exposure in children based on a single-blind type of evaluation.

6. Urinary ALA levels have a statistically significant relationship to blood lead levels in children ($p < 0.001$).

7. Urinary ALA levels remain elevated up to 3 years following
exposure to lead, thereby permitting the implementation of an annual ALA screening program.

8. Elevated urinary ALA levels return to normal within 3 to 5 days after calcium disodium edetate therapy and this indicates that urinary ALA determinations correctly reflect an abnormal lead exposure in both adults and children.

9. The 5.90% incidence of elevated urinary ALA levels found in the 4,189 children residing in high-risk areas of lead poisoning agrees with the predicted 6.0% incidence of childhood lead poisoning based on the Department of Health, Education, and Welfare's figures for urban areas.

10. Acute intermittent porphyria and occasionally certain liver diseases are the only other pathological conditions besides lead poisoning which cause an increased excretion of ALA in the urine. Acute intermittent porphyria does not occur in children and liver disease in childhood is rare and therefore lead intoxication is the only known condition to cause an increased ALA content in the urine of children.

11. Finally, the cost of determining the ALA content of urine is much less than the cost of performing most of the other laboratory tests commonly used for the detection of an increased lead absorbance, e.g. blood lead determination.
CHAPTER V

SUMMARY
CHAPTER V

SUMMARY

1. The method for the isolation of urinary ALA from random urine samples has been modified utilizing a dual disposable chromatographic column unit prefilled with resin that can be conveniently held in a support rack over a drain tray. The method for the colorimetric determination of urinary ALA has been simplified by the elimination of a number of transfer and dilution steps. By using the presently described procedure, one individual is able to perform without difficulty as many as 1,000 analyses of urinary ALA in a normal working week.

2. A single-blind type of evaluation was made of the laboratory findings and clinical impression of increased lead exposure versus the urinary ALA levels obtained in each of 250 children suspected of lead ingestion. In no instance was the examining physician aware of the urinary ALA level of the patient when deciding on possible therapy for lead exposure. It was found that urinary ALA levels are 91% accurate for the clinical diagnosis of increased lead exposure in children. None of the other laboratory tests studied had a comparable degree of accuracy for the detection of early lead exposure.
3. It was found that a practical upper limit of normal for urinary ALA levels in children is 0.99 mg/100 ml. Elevated urinary ALA values have been subdivided into 5 ranges of increasing severity with a 1+ code corresponding to 1.00-1.49 mg/100 ml; a 2+ code corresponding to 1.50-1.99 mg/100 ml; a 3+ code corresponding to 2.00-2.99 mg/100 ml; a 4+ code corresponding to 3.00-5.99 mg/100 ml; and a 5+ code corresponding to greater than 6.00 mg/100 ml.

4. A significant correlation was found to occur between levels of urinary ALA and levels of blood lead as determined by atomic absorption spectrometry in children suspected of lead ingestion. The mean blood lead values found to correspond to normal urinary ALA levels was 48 µg/100 ml. The mean blood lead values found to correspond to abnormally elevated urinary ALA levels ranged from 65 to 147 µg/100 ml.

5. The frequency of abnormal urinary ALA values in apparently-well, asymptomatic children residing in high-incidence areas of lead poisoning in the city of Chicago has been determined. Of 4,189 children tested, a total of 5.90% had elevated urinary ALA values. Based upon this data, it has been predicted that nearly 15,000 children in the city of Chicago may suffer from an increased exposure to lead.

6. In the epidemiological field study, the four year age group contributed
the greatest number of children having elevated urinary ALA levels. Negro children with elevated urinary ALA levels outnumbered white almost nine to one and males outnumbered females almost two to one.

7. Animal studies using adult rats indicate that a single intraperitoneal injection of 1/10 the LD50 of lead acetate is capable of causing an increase in urinary ALA excretion within six hours.

8. Tetraethyl lead was not capable of increasing the urinary ALA excretion of adult rats.

9. Pretreatment with calcium disodium EDTA did not protect against an increase in urinary ALA excretion from adult rats subsequently fed lead acetate. However, calcium disodium EDTA when administered during the period of elevated urinary ALA excretion, caused a rapid fall in urinary ALA levels.

10. Inorganic lead has been shown to be specific in causing an increased excretion of urinary ALA from adult rats with respect to 10 other metals tested (cadmium, barium, iron, zinc, strontium, copper, arsenic, titanium, mercury, and lithium).

11. It is suggested that urinary ALA levels are the earliest and most sensitive indicator of childhood lead exposure and that the present development of a practical laboratory method for the analysis of urinary ALA can make possible both large-scale screening for early
lead exposure in asymptomatic individuals as well as the routine determination of urinary ALA levels in all individuals suspected of lead intoxication. The fact that about 15,000 children in the city of Chicago alone might suffer from an increased exposure to lead emphasizes the need to carry out such large-scale urinary ALA screening in apparently-well, asymptomatic children before the possible development of frank lead intoxication.
CHAPTER VI

FIGURES, CHARTS, AND TABLES
FIGURE 1

INDIVIDUAL STEPS OF THE TEST PROCEDURE FOR THE RAPID ANALYSIS OF URINARY ALA EMPLOYING PREFILLED DISPOSABLE PLASTIC CHROMATOGRAPHY COLUMNS ARRANGED IN PIGGY-BACK FASHION
FIGURE 2

Simplified system for the isolation of ALA from 40 samples of urine employing two disposable plastic chromatographic columns in tandem position held by a support rack measuring 10 inches in length, 5 inches in width and 10 inches in height. **Left,** The top column contains an anionic exchange resin consisting of AG 1-X8, 100 to 200 mesh, in the acetate ionic form. The bottom column contains a cationic exchange resin consisting of AG 50W-X4, 100 to 200 mesh, in the hydrogen ionic form. The columns are situated in the support rack above a suitable drain tray. The urine sample is applied to the top column by inserting a pipette through the numbered hole in the top removable plastic sample guide and, after passage through both columns, allowed to drain into the drain tray. **Center,** Following removal of the plastic sample guide, the top column is washed with water allowing the water to drain through both columns into the drain tray. **Right,** Following removal of the top column, the bottom columns are placed in numbered test tubes held in a standard test tube support rack. Elution of ALA from the bottom column into the test tube is then carried out with sodium acetate solution.
FIGURE 3

COMPLETE KIT FOR PERFORMING THE ANALYSIS OF URINARY ALA FROM 40 URINE SAMPLES, INCLUDING DISPOSABLE PLASTIC CHROMATOGRAPHY COLUMNS PREFILLED WITH AG 1-X8 AND AG 50W-X4 RESINS, SUPPORT RACK AND NUMBERED PIPET GUIDE, DRAIN TRAY AND ALL NECESSARY REAGENTS.
CHART 1

ALA STANDARD CALIBRATION CURVE
Effect on final color development of changing the amount of acetylacetone added to ALA. Graph A represents the optical density of 0.05 ml (5 μg) of the ALA hydrochloride stock solution. Graph B represents the optical density of 0.10 ml (10 μg) of the ALA stock solution. Graph C represents the optical density of 0.20 ml (20 μg) of the ALA stock solution.
Effect on final color development of varying the boiling time of the sodium acetate solution containing ALA and acetylacetone. **Graph A** represents the optical density of 0.05 ml (5 μg) of the ALA hydrochloride stock solution. **Graph B** represents the optical density of 0.10 (10 μg) of the ALA hydrochloride stock solution. **Graph C** represents the optical density of 0.20 ml (20 μg) of the ALA hydrochloride stock solution. **Graph D** represents the optical density of 0.30 ml (30 μg) of the ALA hydrochloride stock solution.
CHART 4

STABILITY OF THE COLOR DEVELOPED WITH

MODIFIED EHRlich'S REAGENT
Chart 5

Typical response obtained when either calcium disodium edetate (EDTA) or penicillamine (PNCM) are administered to children with elevated urinary ALA values.
CHART 6

EFFECT OF AN INTRAPERITONEAL INJECTION OF LEAD ACETATE ON THE URINARY ALA EXCRETION OF RATS
CHART 7

EFFECT OF LEAD ACETATE IN THE DIET ON THE URINARY ALA EXCRETION OF RATS
CHART 8

EFFECT OF CALCIUM DISODIUM EDETATE ON THE URINARY ALA EXCRETION OF RATS FED A 1% LEAD ACETATE DIET
TABLE 1

STABILITY OF REFRIGERATED MODIFIED EHRLICH'S REAGENT

<table>
<thead>
<tr>
<th>Quantity of ALA per 14.2 ml Final Reaction Volume</th>
<th>Day 0</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 µg</td>
<td>0.070</td>
<td>0.070</td>
<td>0.065</td>
<td>0.073</td>
</tr>
<tr>
<td>5 µg</td>
<td>0.168</td>
<td>0.163</td>
<td>0.158</td>
<td>0.170</td>
</tr>
<tr>
<td>10 µg</td>
<td>0.345</td>
<td>0.352</td>
<td>0.335</td>
<td>0.355</td>
</tr>
<tr>
<td>20 µg</td>
<td>0.670</td>
<td>0.648</td>
<td>0.640</td>
<td>0.645</td>
</tr>
<tr>
<td>30 µg</td>
<td>0.940</td>
<td>0.900</td>
<td>0.925</td>
<td>0.900</td>
</tr>
<tr>
<td>50 µg</td>
<td>1.45</td>
<td>1.45</td>
<td>1.45</td>
<td>1.40</td>
</tr>
</tbody>
</table>
TABLE 2
EXPECTED RESULTS USING THE MODIFIED METHOD FOR THE DETERMINATION OF URINARY ALA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of detectability of ALA in urine</td>
<td>0.03 mg/100 ml</td>
</tr>
<tr>
<td>Sensitivity of test</td>
<td>0.15 µg ALA</td>
</tr>
<tr>
<td>Variation between duplicate samples</td>
<td>± 3%</td>
</tr>
<tr>
<td>Recovery of ALA from aqueous solution (distilled water)</td>
<td>97%</td>
</tr>
<tr>
<td>Recovery of ALA from urine</td>
<td>93%</td>
</tr>
</tbody>
</table>
TABLE 2a

RECOVERY OF ADDED ALA FROM AQUEOUS SOLUTION (DISTILLED WATER) USING THE PIGGYBACK COLUMN METHOD

<table>
<thead>
<tr>
<th>ALA Recovered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µg ALA Applied to Column</td>
</tr>
<tr>
<td>87.5</td>
</tr>
<tr>
<td>98.4</td>
</tr>
<tr>
<td>98.4</td>
</tr>
<tr>
<td>103.2</td>
</tr>
<tr>
<td>94.7</td>
</tr>
<tr>
<td>86.8</td>
</tr>
<tr>
<td>92.7</td>
</tr>
<tr>
<td>101.4</td>
</tr>
<tr>
<td>103.8</td>
</tr>
<tr>
<td>103.8</td>
</tr>
<tr>
<td>85.0</td>
</tr>
<tr>
<td>92.1</td>
</tr>
<tr>
<td>98.4</td>
</tr>
<tr>
<td>93.9</td>
</tr>
<tr>
<td>100.0</td>
</tr>
<tr>
<td>92.9</td>
</tr>
<tr>
<td>96.4</td>
</tr>
<tr>
<td>88.7</td>
</tr>
<tr>
<td>87.2</td>
</tr>
<tr>
<td>94.1</td>
</tr>
<tr>
<td>95.5</td>
</tr>
<tr>
<td>95.8</td>
</tr>
<tr>
<td>94.1</td>
</tr>
<tr>
<td>97.1</td>
</tr>
<tr>
<td>91.2</td>
</tr>
</tbody>
</table>

\[
\bar{X} = 94.9 \quad S.D. = 5.4
\]

100.0 \quad +3.4
TABLE 2b

RECOVERY OF ADDED ALA FROM URINE USING THE PIGGYBACK COLUMN METHOD

<table>
<thead>
<tr>
<th>ALA Recovered (%)</th>
<th>5 µg ALA Applied to Column</th>
<th>10 µg ALA Applied to Column</th>
<th>20 µg ALA Applied to Column</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>92.6</td>
<td>89.2</td>
<td>95.3</td>
</tr>
<tr>
<td></td>
<td>87.9</td>
<td>91.6</td>
<td>95.3</td>
</tr>
<tr>
<td></td>
<td>102.5</td>
<td>107.4</td>
<td>86.7</td>
</tr>
<tr>
<td></td>
<td>88.5</td>
<td>85.9</td>
<td>98.4</td>
</tr>
<tr>
<td>( \bar{X} )</td>
<td>92.9</td>
<td>93.5</td>
<td>93.9</td>
</tr>
<tr>
<td>S. D.</td>
<td>±6.8</td>
<td>±9.5</td>
<td>±5.0</td>
</tr>
<tr>
<td>ALA Recovered from Urine of Normal Individual (mg/100 ml)</td>
<td>Variation from Mean (+%)</td>
<td>ALA Recovered from Urine of Individual With Lead Exposure (mg/100 ml)</td>
<td>Variation from Mean (+%)</td>
</tr>
<tr>
<td>----------------------------------------------------------</td>
<td>--------------------------</td>
<td>-------------------------------------------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>0.31</td>
<td>0.0</td>
<td>1.70</td>
<td>4.3</td>
</tr>
<tr>
<td>0.32</td>
<td>3.2</td>
<td>1.60</td>
<td>1.8</td>
</tr>
<tr>
<td>0.27</td>
<td>12.9</td>
<td>1.61</td>
<td>1.2</td>
</tr>
<tr>
<td>0.32</td>
<td>3.2</td>
<td>1.66</td>
<td>1.8</td>
</tr>
<tr>
<td>0.31</td>
<td>0.0</td>
<td>1.56</td>
<td>4.3</td>
</tr>
<tr>
<td>( \bar{X} ) 0.31</td>
<td>3.9</td>
<td>( \bar{X} ) 1.63</td>
<td>2.7</td>
</tr>
<tr>
<td>S. D. ( \pm 0.03 )</td>
<td></td>
<td>( \pm 0.05 )</td>
<td></td>
</tr>
<tr>
<td>Mesh Size of AG 50W-X4 Resin</td>
<td>Flow Rate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Piggyback Flow Rate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Flow Rate of 1st Wash&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>----------------------</td>
<td>-------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>20-50</td>
<td>1 min 56 sec</td>
<td>6 min 33 sec</td>
<td>6 min 30 sec</td>
</tr>
<tr>
<td>50-100</td>
<td>2 min 42 sec</td>
<td>6 min 9 sec</td>
<td>6 min 27 sec</td>
</tr>
<tr>
<td>100-200</td>
<td>6 min 30 sec</td>
<td>7 min 49 sec</td>
<td>7 min 46 sec</td>
</tr>
<tr>
<td>200-400</td>
<td>10 min 27 sec</td>
<td>11 min 43 sec</td>
<td>11 min 40 sec</td>
</tr>
</tbody>
</table>

* Each Value represents the mean of two experiments.

a  Time required for 10 ml of water to pass through an AG 50W-X4 column.

b  Time required for 10 ml of water to pass through a piggyback column unit composed of an AG 1-X8 column on top of an AG 50W-X4 column. The time required for 10 ml of water to pass through an AG 1-X8 column alone was 5 min 47 sec.

c  Time required for 10 ml of water to pass through the piggyback column unit after addition of 0.5 ml of an ALA sample to the AG 1-X8 column.
### TABLE 3 (Continued)

**EFFECT OF VARYING THE MESH SIZE OF THE AG50W-X4 RESIN**

<table>
<thead>
<tr>
<th>Flow Rate of 1M Sodium Acetate&lt;sup&gt;d&lt;/sup&gt;</th>
<th>pH of Sodium Acetate Eluate</th>
<th>% Recovery of 10 µg ALA</th>
<th>% Recovery of ALA from Normal Urine&lt;sup&gt;e&lt;/sup&gt;</th>
<th>% Recovery of ALA from the Urine of a Lead-Exposed Individual</th>
</tr>
</thead>
<tbody>
<tr>
<td>54 sec</td>
<td>4.69</td>
<td>25</td>
<td>35</td>
<td>31</td>
</tr>
<tr>
<td>1 min 30 sec</td>
<td>4.57</td>
<td>58</td>
<td>58</td>
<td>61</td>
</tr>
<tr>
<td>4 min 33 sec</td>
<td>4.57</td>
<td>97</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>7 min 28 sec</td>
<td>4.71</td>
<td>94</td>
<td>97</td>
<td>98</td>
</tr>
</tbody>
</table>

<sup>d</sup> Time required for 7.0 ml of 1M sodium acetate to pass through the AG 50W-X4 column.

<sup>e</sup> 100% recovery was arbitrarily set for the amount of ALA eluted from the 100-200 mesh AG 50W-X4 column. The ALA eluted from the columns containing 20-50, 50-100, and 200-400 mesh resin were then compared to this value.
TABLE 4

EFFECT OF URINE pH ON RECOVERY OF ALA FROM COLUMNS

<table>
<thead>
<tr>
<th>pH</th>
<th>HCl + NaOH</th>
<th>HCl + NH₄OH</th>
<th>HCl + Na₂CO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.250</td>
<td>0.271</td>
<td>0.241</td>
</tr>
<tr>
<td>2.0</td>
<td>0.245</td>
<td>0.289</td>
<td>0.252</td>
</tr>
<tr>
<td>3.0</td>
<td>0.232</td>
<td>0.260</td>
<td>0.256</td>
</tr>
<tr>
<td>4.0</td>
<td>0.249</td>
<td>0.273</td>
<td>0.230</td>
</tr>
<tr>
<td>5.0</td>
<td>0.225</td>
<td>0.260</td>
<td>0.230</td>
</tr>
<tr>
<td>6.0</td>
<td>0.232</td>
<td>0.240</td>
<td>0.228</td>
</tr>
<tr>
<td>7.0</td>
<td>0.210</td>
<td>0.225</td>
<td>0.212</td>
</tr>
<tr>
<td>8.0</td>
<td>0.188</td>
<td>0.161</td>
<td>0.200</td>
</tr>
<tr>
<td>9.0</td>
<td>0.127</td>
<td>0.022</td>
<td>0.095</td>
</tr>
<tr>
<td>10.0</td>
<td>0.085</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>
### TABLE 5
PERCENTAGE DISTRIBUTION OF URINARY ALA LEVELS IN 250 CHILDREN WITH SUSPECTED LEAD INGESTION

<table>
<thead>
<tr>
<th>Urinary ALA (mg/100 ml)</th>
<th>Urinary ALA Code</th>
<th>Number Children in Each Range</th>
<th>% Total Children in Each Range</th>
<th>% Each Range Treated With Chelating Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00-0.54</td>
<td>Normal</td>
<td>123</td>
<td>49.2</td>
<td>4</td>
</tr>
<tr>
<td>0.55-0.99</td>
<td>Trace</td>
<td>64</td>
<td>25.8</td>
<td>5</td>
</tr>
<tr>
<td>Abnormal range</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00-1.49</td>
<td>1+</td>
<td>27</td>
<td>10.8</td>
<td>65</td>
</tr>
<tr>
<td>1.50-1.99</td>
<td>2+</td>
<td>14</td>
<td>5.6</td>
<td>70</td>
</tr>
<tr>
<td>2.00-2.99</td>
<td>3+</td>
<td>12</td>
<td>4.8</td>
<td>84</td>
</tr>
<tr>
<td>3.00-5.99</td>
<td>4+</td>
<td>3</td>
<td>3.2</td>
<td>100</td>
</tr>
<tr>
<td>6.00-10.00</td>
<td>5+</td>
<td>2</td>
<td>0.6</td>
<td>100</td>
</tr>
</tbody>
</table>

*All children had an initial blood lead level greater than 40 μg/100 ml as determined by atomic absorption spectrometry.*
<table>
<thead>
<tr>
<th>Urinary ALA Level</th>
<th>Chelation Therapy</th>
<th>Interpretation of ALA Test</th>
<th>Number of Children</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal **</td>
<td>Not treated</td>
<td>Correct negative</td>
<td>179</td>
<td>72</td>
</tr>
<tr>
<td>High ***</td>
<td>Treated</td>
<td>Correct positive</td>
<td>48</td>
<td>19</td>
</tr>
<tr>
<td>Normal **</td>
<td>Treated</td>
<td>False negative</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>High ***</td>
<td>Not treated</td>
<td>False positive</td>
<td>15</td>
<td>6</td>
</tr>
</tbody>
</table>

*All children had an initial blood lead level greater than 40 \( \mu \text{g}/100 \text{ ml}\) as determined by atomic absorption spectrometry.

**Normal range of urinary ALA = 0.00 to 0.99 mg/100 ml.

***Abnormal range of urinary ALA = 1.00 to 10.00 mg/100 ml.
**TABLE 7**

COMPARISON OF VARIOUS LABORATORY TESTS FOR LEAD EXPOSURE IN 250 CHILDREN WITH SUSPECTED LEAD INGESTION* IN TERMS OF FREQUENCY OF ABNORMAL RESULTS

<table>
<thead>
<tr>
<th>Test</th>
<th>Children discharged without Chelation Therapy</th>
<th>Children Treated with Chelation Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% with Normal Test</td>
<td>% with Abnormal Test</td>
</tr>
<tr>
<td>Urinary ALA</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>Wrist X-ray</td>
<td>46</td>
<td>54</td>
</tr>
<tr>
<td>Abdominal X-ray</td>
<td>86</td>
<td>14</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>87</td>
<td>13</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>85</td>
<td>15</td>
</tr>
</tbody>
</table>

*All children had an initial blood lead level greater than 40 µg/100 ml as determined by atomic absorption spectrometry.

Abnormal urinary ALA = 1.00 mg/100 ml or higher
Abnormal wrist X-ray = increased density at metaphysis
Abnormal abdominal X-ray = radiopaque material in intestine
Abnormal hemoglobin = less than 10.0 g/100 ml
Abnormal hematocrit = less than 33%
TABLE 8

COMPARISON OF VARIOUS LABORATORY TESTS FOR LEAD EXPOSURE IN 250 CHILDREN WITH SUSPECTED LEAD INGESTION* IN TERMS OF FREQUENCY OF CHELATION THERAPY.

<table>
<thead>
<tr>
<th>Test</th>
<th>% of Children with Normal Test Values Who Were Discharged without Treatment</th>
<th>% of Children with Abnormal Test Values Who Received Chelation Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary ALA</td>
<td>96</td>
<td>76</td>
</tr>
<tr>
<td>Abdominal X-ray</td>
<td>82</td>
<td>51</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>81</td>
<td>42</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>80</td>
<td>36</td>
</tr>
<tr>
<td>Wrist X-ray</td>
<td>85</td>
<td>32</td>
</tr>
<tr>
<td>Blood Lead</td>
<td>--</td>
<td>22</td>
</tr>
</tbody>
</table>

* All children had an initial blood lead level greater than 40 &mu;g/100 ml as determined by atomic absorption spectrometry.

Abnormal urinary ALA = 1.00 mg/100 ml or higher
Abnormal wrist X-ray = increased density at metaphysis
Abnormal abdominal X-ray = radiopaque material in intestine
Abnormal hemoglobin = less than 10.0 g/100 ml
Abnormal hematocrit = less than 33%
Abnormal blood lead = greater than 40 &mu;g/100 ml
TABLE 9
COMPARISON OF MEAN URINARY ALA VALUES WITH MEAN BLOOD LEAD VALUES IN 250 CHILDREN WITH SUSPECTED LEAD INGESTION* CLASSIFIED BY VARIOUS RANGES OF URINARY ALA LEVELS

<table>
<thead>
<tr>
<th>Urinary ALA Code</th>
<th>Number of Children</th>
<th>Mean Urinary ALA + S. D. (mg/100 ml)</th>
<th>Urinary ALA Range (mg/100 ml)</th>
<th>Mean Blood Lead + S. D. ** (µg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ALA Values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>123</td>
<td>0.32 ± 0.13</td>
<td>0.00-0.54</td>
<td>48 ± 11</td>
</tr>
<tr>
<td>Trace</td>
<td>64</td>
<td>0.72 ± 0.12</td>
<td>0.55-0.99</td>
<td>49 ± 11</td>
</tr>
<tr>
<td>Abnormal ALA Values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+</td>
<td>27</td>
<td>1.22 ± 0.14</td>
<td>1.00-1.49</td>
<td>65 ± 17</td>
</tr>
<tr>
<td>2+</td>
<td>14</td>
<td>1.72 ± 0.16</td>
<td>1.50-1.99</td>
<td>68 ± 16</td>
</tr>
<tr>
<td>3+</td>
<td>12</td>
<td>2.45 ± 0.35</td>
<td>2.00-2.99</td>
<td>83 ± 23</td>
</tr>
<tr>
<td>4+</td>
<td>8</td>
<td>4.66 ± 0.79</td>
<td>3.00-4.99</td>
<td>107 ± 46</td>
</tr>
<tr>
<td>5+</td>
<td>2</td>
<td>8.69 ± 1.37</td>
<td>6.00-10.00</td>
<td>147 ± 65</td>
</tr>
</tbody>
</table>

* All children had an initial blood lead level greater than 40 µg/100 ml as determined by atomic absorption spectrometry.

** Represents corresponding blood lead value at time of urinary ALA determination.
<table>
<thead>
<tr>
<th>Urinary ALA Level</th>
<th>Total Samples</th>
<th>6.0-</th>
<th>7.0-</th>
<th>8.0-</th>
<th>9.0-</th>
<th>10.0-</th>
<th>11.0-</th>
<th>12.0-</th>
<th>13.0-</th>
<th>14.0-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>166</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>18</td>
<td>29</td>
<td>23</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>High</td>
<td>57</td>
<td>4</td>
<td>0</td>
<td>7</td>
<td>11</td>
<td>23</td>
<td>26</td>
<td>25</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

* Expressed as percent of total samples.

** All children had an initial blood lead level greater than 40 μg/100 ml as determined by atomic absorption spectrometry.

Normal range of urinary ALA = 0.00-0.99 mg/100 ml

High range of urinary ALA = 1.00-10.00 mg/100 ml
TABLE 11
DISTRIBUTION OF HEMATOCRIT VALUES* IN CHILDREN SUSPECTED OF LEAD INGESTION** CLASSIFIED BY URINARY ALA LEVELS

<table>
<thead>
<tr>
<th>Urinary ALA Level</th>
<th>Total Samples</th>
<th>Hematocrit Values (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21- 24- 27- 30- 33- 36- 39- 42-</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>23 26 29 32 35 38 41 44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 1 2 15 31 32 18 1</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 3 5 10 39 30 11 2</td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as percent of total samples.

** All children had an initial blood lead level greater than 40 μg/100 ml as determined by atomic absorption spectrometry.

Normal range of urinary ALA = 0.00-0.99 mg/100 ml

High range of urinary ALA = 1.00-10.00 mg/100 ml
**TABLE 12**

PERCENTAGE DISTRIBUTION OF URINARY ALA LEVELS IN 534 CHILDREN WITH SUSPECTED LEAD INGESTION*

<table>
<thead>
<tr>
<th>Urinary ALA (mg/100 ml)</th>
<th>Urinary ALA Code</th>
<th>Number of Children in Each Range</th>
<th>% of Total Children in Each Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal Range</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00-0.54</td>
<td>Normal</td>
<td>251</td>
<td>47.0</td>
</tr>
<tr>
<td>0.55-0.99</td>
<td>Trace</td>
<td>120</td>
<td>22.5</td>
</tr>
<tr>
<td><strong>Abnormal Range</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00-1.49</td>
<td>1+</td>
<td>64</td>
<td>12.0</td>
</tr>
<tr>
<td>1.50-1.99</td>
<td>2+</td>
<td>26</td>
<td>4.9</td>
</tr>
<tr>
<td>2.00-2.99</td>
<td>3+</td>
<td>36</td>
<td>6.7  30.5</td>
</tr>
<tr>
<td>3.00-5.99</td>
<td>4+</td>
<td>27</td>
<td>5.0</td>
</tr>
<tr>
<td>6.00-10.00</td>
<td>5+</td>
<td>10</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* All children had an initial blood lead level greater than 50 µg/100 ml as determined by atomic absorption spectrometry.
### TABLE 13

**COMPARISON OF ELEVATED URINARY ALA LEVELS WITH THE INCIDENCE OF CHELATION THERAPY IN CHILDREN WITH SUSPECTED LEAD INGESTION**

<table>
<thead>
<tr>
<th>Urinary ALA (mg/100 ml)</th>
<th>Urinary ALA Code</th>
<th>Number of Children in Each Range</th>
<th>No. in Each Range Treated with Chelating Agents</th>
<th>% of Each Range Treated with Chelating Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00-1.49</td>
<td>1+</td>
<td>38</td>
<td>26</td>
<td>68.4</td>
</tr>
<tr>
<td>1.50-1.99</td>
<td>2+</td>
<td>23</td>
<td>18</td>
<td>78.3</td>
</tr>
<tr>
<td>2.00-2.99</td>
<td>3+</td>
<td>32</td>
<td>29</td>
<td>90.6</td>
</tr>
<tr>
<td>3.00-5.99</td>
<td>4+</td>
<td>28</td>
<td>28</td>
<td>100.0</td>
</tr>
<tr>
<td>6.00-10.00</td>
<td>5+</td>
<td>10</td>
<td>10</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>131</td>
<td>111</td>
<td>84.7</td>
</tr>
</tbody>
</table>

*All children had an initial blood lead level greater than 50 μg/100 ml as determined by atomic absorption spectrometry.*
### TABLE 14

**COMPARISON OF ELEVATED MEAN URINARY ALA VALUES WITH CORRESPONDING MEAN BLOOD LEAD VALUES IN 107 CHILDREN WITH SUSPECTED LEAD INGESTION**

<table>
<thead>
<tr>
<th>Urinary ALA Code</th>
<th>Urinary ALA Range (mg/100 ml)</th>
<th>Number of Children</th>
<th>Mean Urinary ALA (mg/100 ml)</th>
<th>Mean Blood Lead (µg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+</td>
<td>1.00-1.49</td>
<td>31</td>
<td>1.27</td>
<td>60</td>
</tr>
<tr>
<td>2+</td>
<td>1.50-1.99</td>
<td>20</td>
<td>1.67</td>
<td>74</td>
</tr>
<tr>
<td>3+</td>
<td>2.00-2.99</td>
<td>30</td>
<td>2.41</td>
<td>80</td>
</tr>
<tr>
<td>4+</td>
<td>3.00-5.99</td>
<td>21</td>
<td>4.10</td>
<td>95</td>
</tr>
<tr>
<td>5+</td>
<td>6.00-10.00</td>
<td>5</td>
<td>7.97</td>
<td>127</td>
</tr>
</tbody>
</table>

* All children had an initial blood lead level greater than 50 µg/100 ml as determined by atomic absorption spectrometry.
TABLE 15

PERCENTAGE DISTRIBUTION OF URINARY ALA LEVELS IN 4,189 APARENTLY-WELL, ASYMPTOMATIC CHILDREN RESIDING IN HIGH-INCIDENCE AREAS OF LEAD POISONING IN THE CITY OF CHICAGO

<table>
<thead>
<tr>
<th>Urinary ALA (mg/100 ml)</th>
<th>Urinary ALA Code</th>
<th>Number of Children in Each Category</th>
<th>% of Total in each Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00-0.54</td>
<td>Normal</td>
<td>3251</td>
<td>77.61</td>
</tr>
<tr>
<td>0.55-0.99</td>
<td>Trace</td>
<td>691</td>
<td>16.50</td>
</tr>
<tr>
<td>Abnormal range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00-1.49</td>
<td>1+</td>
<td>212</td>
<td>5.06</td>
</tr>
<tr>
<td>1.50-1.99</td>
<td>2+</td>
<td>20</td>
<td>0.48</td>
</tr>
<tr>
<td>2.00-2.99</td>
<td>3+</td>
<td>13</td>
<td>0.31</td>
</tr>
<tr>
<td>3.00-5.99</td>
<td>4+</td>
<td>2</td>
<td>0.05</td>
</tr>
</tbody>
</table>


**TABLE 16**

**DISTRIBUTION OF THE URINARY ALA LEVELS* OF 4,189 ASYMPTOMATIC CHILDREN CLASSIFIED BY THE LOCATION OF THE URBAN PROGRESS CENTERS WHERE THEY WERE EXAMINED**

<table>
<thead>
<tr>
<th>Number of Children Examined at Each UPC</th>
<th>0.00-0.54</th>
<th>0.55-0.99</th>
<th>1.00-1.49</th>
<th>1.50-1.99</th>
<th>2.00-2.99</th>
<th>3.00-5.99</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Parkway UPC</td>
<td>872</td>
<td>74.4</td>
<td>18.0</td>
<td>6.7</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Woodlawn UPC</td>
<td>427</td>
<td>71.4</td>
<td>19.7</td>
<td>7.5</td>
<td>0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Englewood UPC</td>
<td>588</td>
<td>79.1</td>
<td>15.3</td>
<td>4.4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>South Chicago Unit</td>
<td>121</td>
<td>76.0</td>
<td>17.4</td>
<td>6.6</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Division St. Unit</td>
<td>258</td>
<td>82.2</td>
<td>15.5</td>
<td>2.3</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Montrose UPC</td>
<td>259</td>
<td>78.8</td>
<td>18.1</td>
<td>2.3</td>
<td>0.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Halsted UPC</td>
<td>857</td>
<td>82.0</td>
<td>13.8</td>
<td>3.7</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Lawndale UPC</td>
<td>595</td>
<td>78.5</td>
<td>15.8</td>
<td>4.9</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Midwest UPC</td>
<td>212</td>
<td>72.6</td>
<td>18.9</td>
<td>7.1</td>
<td>0.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Expressed as percent of children examined at each UPC.
**TABLE 17**

**DISTRIBUTION OF CHILDREN HAVING ELEVATED URINARY ALA LEVELS* BY AGE**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number of Children in Each Age Group Having Elevated Urinary ALA Levels</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>14.1</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>15.4</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>17.2</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>13.7</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>14.1</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>9.7</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>4.8</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>4.8</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>4.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>227</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

* Elevated Urinary ALA Levels = 1.00 mg/100 ml or higher.

** In this table the number of children reported to have had elevated urinary ALA levels is less than the actual number found during the course of the epidemiological field study. This difference occurs because in several instances the age of the child having an elevated urinary ALA level was not indicated on the physician's report form and therefore these children could not be included in the table.
TABLE 18

DISTRIBUTION OF THE URINARY ALA LEVELS* OF 3,919** ASYMPTOMATIC CHILDREN CLASSIFIED BY AGE

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number of Children in Each Age Group Tested for Urinary ALA Excretion</th>
<th>URINARY ALA LEVELS (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.00-0.99 1.00-1.49 1.50-1.99 2.00-2.99 3.00-5.99</td>
</tr>
<tr>
<td>1</td>
<td>103</td>
<td>96.1 3.9 0.0 0.0 0.0</td>
</tr>
<tr>
<td>2</td>
<td>459</td>
<td>93.1 5.4 0.9 0.4 0.2</td>
</tr>
<tr>
<td>3</td>
<td>666</td>
<td>94.7 4.8 0.5 0.0 0.0</td>
</tr>
<tr>
<td>4</td>
<td>704</td>
<td>94.5 4.4 0.7 0.3 0.1</td>
</tr>
<tr>
<td>5</td>
<td>655</td>
<td>95.2 4.1 0.5 0.2 0.0</td>
</tr>
<tr>
<td>6</td>
<td>502</td>
<td>93.6 6.0 0.2 0.2 0.0</td>
</tr>
<tr>
<td>7</td>
<td>353</td>
<td>93.7 5.4 0.6 0.3 0.0</td>
</tr>
<tr>
<td>8</td>
<td>206</td>
<td>94.7 5.3 0.0 0.0 0.0</td>
</tr>
<tr>
<td>9</td>
<td>145</td>
<td>92.4 6.2 0.0 1.4 0.0</td>
</tr>
<tr>
<td>10</td>
<td>126</td>
<td>92.1 5.6 0.8 1.5 0.0</td>
</tr>
</tbody>
</table>

* Expressed as % of children in each age group tested for urinary ALA excretion.

** In this table the number of asymptomatic children reported to have been tested for urinary ALA excretion is less than the actual number tested during the course of the epidemiological field study. This difference occurs because in several instances the age of the child tested was not indicated on the physician's report form and therefore these children could not be included in the table.
TABLE 19

DISTRIBUTION OF CHILDREN HAVING ELEVATED URINARY ALA LEVELS* ACCORDING TO RACE

<table>
<thead>
<tr>
<th>Race</th>
<th>Number of Children of Each Race Having Elevated Urinary ALA Levels</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>22</td>
<td>10.3</td>
</tr>
<tr>
<td>Negro</td>
<td>190</td>
<td>88.8</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>Total</td>
<td>214**</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* Elevated Urinary ALA Levels = 1.00 mg/100 ml or higher.

** In this table the number of children reported to have had elevated urinary ALA levels is less than the actual number found during the course of the epidemiological field study. This difference occurs because in several instances the race of the child having an elevated urinary ALA level was not indicated on the physician's report form and therefore these children could not be included in the table.
**TABLE 20**

**DISTRIBUTION OF THE URINARY ALA LEVELS* OF 3,606** ASYMPTOMATIC CHILDREN CLASSIFIED BY RACE

<table>
<thead>
<tr>
<th>Race</th>
<th>Number of Children of Each Race Tested for Urinary ALA Excretion</th>
<th>URINARY ALA LEVELS (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.00-0.99</td>
</tr>
<tr>
<td>White</td>
<td>879</td>
<td>97.5</td>
</tr>
<tr>
<td>Negro</td>
<td>2,697</td>
<td>93.0</td>
</tr>
<tr>
<td>Other</td>
<td>30</td>
<td>93.3</td>
</tr>
</tbody>
</table>

* Expressed as % of children of each race tested for urinary ALA excretion.

** In this table the number of asymptomatic children reported to have been tested for urinary ALA excretion is less than the actual number tested during the course of the epidemiological field study. This difference occurs because in several instances the race of the child tested was not indicated on the physician's report form and therefore these children could not be included in the table.
**TABLE 21**

DISTRIBUTION OF CHILDREN HAVING ELEVATED URINARY ALA LEVELS* ACCORDING TO SEX

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of Children of Each Sex Having Elevated Urinary ALA Levels</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>146</td>
<td>62.7</td>
</tr>
<tr>
<td>Female</td>
<td>87</td>
<td>37.3</td>
</tr>
<tr>
<td>Total</td>
<td>233**</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* Elevated Urinary ALA levels = 1.00 mg/100 ml or higher.

** In this table the number of children reported to have had elevated urinary ALA levels is less than the actual number found during the course of the epidemiological field study. This difference occurs because in several instances the sex of the child having an elevated urinary ALA level was not indicated on the physician's report form and therefore these children could not be included in the table.
### TABLE 22

DISTRIBUTION OF THE URINARY ALA LEVELS* OF 4,019** ASYMPTOMATIC CHILDREN CLASSIFIED BY SEX

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of Children of Each Sex Tested for Urinary ALA Excretion</th>
<th>URINARY ALA LEVELS (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.00-0.99</td>
</tr>
<tr>
<td>Male</td>
<td>2,126</td>
<td>93.1</td>
</tr>
<tr>
<td>Female</td>
<td>1,893</td>
<td>95.4</td>
</tr>
</tbody>
</table>

* Expressed as % of children of each sex tested for urinary ALA excretion.

** In this table the number of asymptomatic children reported to have been tested for urinary ALA excretion is less than the actual number tested during the course of the epidemiological field study. This difference occurs because in several instances the sex of the child tested was not indicated on the physician's report form and therefore these children could not be included in the table.
### TABLE 23

**DISTRIBUTION OF BLOOD LEAD VALUES IN 3,068 CHILDREN WITH NORMAL LEVELS OF URINARY ALA (0.00-0.99 mg/100 ml)**

<table>
<thead>
<tr>
<th>Blood Lead Levels (μg/100 ml)</th>
<th>0-9</th>
<th>10-19</th>
<th>20-29</th>
<th>30-39</th>
<th>40-49</th>
<th>50-59</th>
<th>60-64</th>
<th>65-69</th>
<th>70-79</th>
<th>80-89</th>
<th>90-99</th>
<th>100-129</th>
<th>130-300</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of children in each blood lead range</td>
<td>7</td>
<td>481</td>
<td>1246</td>
<td>868</td>
<td>334</td>
<td>95</td>
<td>15</td>
<td>11</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>% of children in each blood lead range</td>
<td>0.2</td>
<td>15.7</td>
<td>40.6</td>
<td>28.3</td>
<td>10.9</td>
<td>3.1</td>
<td>0.5</td>
<td>0.4</td>
<td>0.2</td>
<td>0.1</td>
<td>0.03</td>
<td>0.03</td>
<td>0.0</td>
</tr>
<tr>
<td>URINARY ALA LEVELS (mg/100 ml)</td>
<td>Normal</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
<td>4+</td>
<td>5+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------</td>
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<td>----</td>
<td>----</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.00-0.99)</td>
<td>2,982</td>
<td>152</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1.00-1.49)</td>
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<td></td>
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</tr>
<tr>
<td>(1.50-1.99)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2.00-2.99)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3.00-5.99)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6.00-10.00)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of children in each urinary ALA range:

- Normal: 2,982
- 1+: 152
- 2+: 12
- 3+: 1
- 4+: 1
- 5+: 0

% of children in each urinary ALA range:

- Normal: 947 (4.8%)
- 1+: 4.8%
- 2+: 0.4%
- 3+: 0.03%
- 4+: 0.03%
- 5+: 0.0%
### TABLE 25

**EFFECT OF VARIOUS METALS ON URINARY ALA EXCRETION IN RATS**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlb</td>
<td></td>
<td>33+25</td>
<td>37+9</td>
<td>39+20</td>
<td>39+17</td>
<td>34+17</td>
<td>35+18</td>
<td>24+7</td>
</tr>
<tr>
<td>Lead acetate</td>
<td>50</td>
<td>27+11</td>
<td>29+13</td>
<td>36+61</td>
<td>34+109</td>
<td>18+52</td>
<td>201+152</td>
<td>246+266</td>
</tr>
<tr>
<td>Tetraethyl lead</td>
<td>10</td>
<td>37+5</td>
<td>32+1</td>
<td>39+3</td>
<td>22+2</td>
<td>16+6</td>
<td>27+16</td>
<td>44+24</td>
</tr>
<tr>
<td>Cadmium chloride</td>
<td>4.5</td>
<td>37+4</td>
<td>23+8</td>
<td>32+8</td>
<td>20+6</td>
<td>24+4</td>
<td>22+3</td>
<td>27+4</td>
</tr>
<tr>
<td>Barium chloride</td>
<td>15</td>
<td>34+11</td>
<td>35+4</td>
<td>30+27</td>
<td>28+11</td>
<td>36+22</td>
<td>31+17</td>
<td>37+15</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>100</td>
<td>47+21</td>
<td>35+6</td>
<td>32+15</td>
<td>75+33</td>
<td>47+16</td>
<td>51+12</td>
<td>35+9</td>
</tr>
<tr>
<td>Zinc chloride</td>
<td>19</td>
<td>33+19</td>
<td>33+4</td>
<td>36+20</td>
<td>26+21</td>
<td>33+11</td>
<td>33+18</td>
<td>38+15</td>
</tr>
</tbody>
</table>

**URINARY ALA (µg/24 HOURS)a**

---

**a** Data are expressed as mean of 3 animals ± standard deviation.

**b** Each metal with the exception of tetraethyl lead was dissolved in distilled water. A single intraperitoneal injection of 1 ml was used to administer the desired quantity of each metal and a 1 ml intraperitoneal injection of distilled water was used as control. Tetraethyl lead was diluted to the desired strength with peanut oil and 1 ml of this solution was administered orally.
TABLE 25 (Continued)

EFFECT OF VARIOUS METALS ON URINARY ALA EXCRETION IN RATS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strontium bromide</td>
<td>500</td>
<td>48+26</td>
<td>42+12</td>
<td>61+10</td>
<td>47+34</td>
<td>46+32</td>
<td>50+27</td>
<td>54+30</td>
</tr>
<tr>
<td>Copper sulfate</td>
<td>15</td>
<td>33+14</td>
<td>29+21</td>
<td>25+18</td>
<td>28+19</td>
<td>25+13</td>
<td>24+14</td>
<td>34+16</td>
</tr>
<tr>
<td>Arsenic pentoxide</td>
<td>2</td>
<td>40+13</td>
<td>52+22</td>
<td>38+12</td>
<td>49+38</td>
<td>40+20</td>
<td>30+11</td>
<td>25+8</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>500</td>
<td>40+26</td>
<td>34+4</td>
<td>31+16</td>
<td>31+18</td>
<td>50+6</td>
<td>35+19</td>
<td>35+20</td>
</tr>
<tr>
<td>Mercuric chloride</td>
<td>2</td>
<td>37+6</td>
<td>13+8</td>
<td>16c</td>
<td>4+4</td>
<td>1+0.4</td>
<td>3d</td>
<td>e</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>250</td>
<td>37+19</td>
<td>20+9</td>
<td>35+2</td>
<td>33+10</td>
<td>22+5</td>
<td>54+17</td>
<td>35+22</td>
</tr>
</tbody>
</table>

- c On Day 2 anuria occurred in 2 animals.
- d On Day 5 two animals died.
- e By Day 14 all animals had died.
## Table 26

### Urinary ALA Conversion Chart for a 0.5ml Aliquot of Urine

<table>
<thead>
<tr>
<th>µg ALA per 14.2ml Final</th>
<th>µg ALA per 14.2ml Final</th>
<th>µg ALA per 14.2ml Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. D. Reaction Vol.</td>
<td>mg%</td>
<td>O. D. Reaction Vol.</td>
</tr>
<tr>
<td>0.010 = 0.15 = 0.03</td>
<td>0.360 = 9.25 = 1.85</td>
<td>0.710 = 18.60 = 3.72</td>
</tr>
<tr>
<td>0.020 = 0.40 = 0.08</td>
<td>0.370 = 9.50 = 1.90</td>
<td>0.720 = 18.85 = 3.77</td>
</tr>
<tr>
<td>0.030 = 0.65 = 0.13</td>
<td>0.380 = 9.75 = 1.95</td>
<td>0.730 = 19.10 = 3.82</td>
</tr>
<tr>
<td>0.040 = 1.00 = 0.20</td>
<td>0.390 = 10.00 = 2.00</td>
<td>0.740 = 19.50 = 3.90</td>
</tr>
<tr>
<td>0.050 = 1.25 = 0.25</td>
<td>0.400 = 10.25 = 2.05</td>
<td>0.750 = 19.75 = 3.95</td>
</tr>
<tr>
<td>0.060 = 1.50 = 0.30</td>
<td>0.410 = 10.50 = 2.10</td>
<td>0.760 = 20.00 = 4.00</td>
</tr>
<tr>
<td>0.070 = 1.75 = 0.35</td>
<td>0.420 = 10.75 = 2.15</td>
<td>0.770 = 20.25 = 4.05</td>
</tr>
<tr>
<td>0.080 = 2.00 = 0.40</td>
<td>0.430 = 11.10 = 2.22</td>
<td>0.780 = 20.60 = 4.12</td>
</tr>
<tr>
<td>0.090 = 2.25 = 0.45</td>
<td>0.440 = 11.35 = 2.27</td>
<td>0.790 = 21.00 = 4.20</td>
</tr>
<tr>
<td>0.100 = 2.50 = 0.50</td>
<td>0.450 = 11.60 = 2.32</td>
<td>0.800 = 21.25 = 4.25</td>
</tr>
<tr>
<td>0.110 = 2.75 = 0.55</td>
<td>0.460 = 11.85 = 2.37</td>
<td>0.810 = 21.50 = 4.30</td>
</tr>
<tr>
<td>0.120 = 3.00 = 0.60</td>
<td>0.470 = 12.10 = 2.42</td>
<td>0.820 = 21.85 = 4.37</td>
</tr>
<tr>
<td>0.130 = 3.25 = 0.65</td>
<td>0.480 = 12.35 = 2.47</td>
<td>0.830 = 22.25 = 4.45</td>
</tr>
<tr>
<td>0.140 = 3.50 = 0.70</td>
<td>0.490 = 12.60 = 2.52</td>
<td>0.840 = 22.60 = 4.52</td>
</tr>
<tr>
<td>0.150 = 3.75 = 0.75</td>
<td>0.500 = 13.00 = 2.60</td>
<td>0.850 = 23.00 = 4.60</td>
</tr>
<tr>
<td>0.160 = 4.00 = 0.80</td>
<td>0.510 = 13.25 = 2.65</td>
<td>0.860 = 23.35 = 4.67</td>
</tr>
<tr>
<td>0.170 = 4.25 = 0.85</td>
<td>0.520 = 13.50 = 2.70</td>
<td>0.870 = 23.60 = 4.72</td>
</tr>
<tr>
<td>0.180 = 4.50 = 0.90</td>
<td>0.530 = 13.75 = 2.75</td>
<td>0.880 = 24.00 = 4.80</td>
</tr>
<tr>
<td>0.190 = 4.75 = 0.95</td>
<td>0.540 = 14.10 = 2.82</td>
<td>0.890 = 24.35 = 4.87</td>
</tr>
<tr>
<td>0.200 = 5.00 = 1.00</td>
<td>0.550 = 14.35 = 2.87</td>
<td>0.900 = 24.75 = 4.95</td>
</tr>
<tr>
<td>0.210 = 5.35 = 1.07</td>
<td>0.560 = 14.60 = 2.92</td>
<td>0.910 = 25.10 = 5.02</td>
</tr>
<tr>
<td>0.220 = 5.60 = 1.12</td>
<td>0.570 = 14.85 = 2.97</td>
<td>0.920 = 25.35 = 5.07</td>
</tr>
<tr>
<td>0.230 = 5.85 = 1.17</td>
<td>0.580 = 15.10 = 3.02</td>
<td>0.930 = 25.75 = 5.15</td>
</tr>
<tr>
<td>0.240 = 6.10 = 1.22</td>
<td>0.590 = 15.35 = 3.07</td>
<td>0.940 = 26.25 = 5.25</td>
</tr>
<tr>
<td>0.250 = 6.35 = 1.27</td>
<td>0.600 = 15.60 = 3.12</td>
<td>0.950 = 26.60 = 5.32</td>
</tr>
<tr>
<td>0.260 = 6.60 = 1.32</td>
<td>0.610 = 15.85 = 3.17</td>
<td>0.960 = 27.00 = 5.40</td>
</tr>
<tr>
<td>0.270 = 6.85 = 1.37</td>
<td>0.620 = 16.25 = 3.25</td>
<td>0.970 = 27.35 = 5.47</td>
</tr>
<tr>
<td>0.280 = 7.10 = 1.42</td>
<td>0.630 = 16.50 = 3.30</td>
<td>0.980 = 27.75 = 5.55</td>
</tr>
<tr>
<td>0.290 = 7.35 = 1.47</td>
<td>0.640 = 16.75 = 3.35</td>
<td>0.990 = 28.10 = 5.62</td>
</tr>
<tr>
<td>0.300 = 7.60 = 1.52</td>
<td>0.650 = 17.00 = 3.40</td>
<td>1.000 = 28.50 = 5.70</td>
</tr>
<tr>
<td>0.310 = 7.85 = 1.57</td>
<td>0.660 = 17.25 = 3.45</td>
<td>1.100 = 32.50 = 6.50</td>
</tr>
<tr>
<td>0.320 = 8.10 = 1.62</td>
<td>0.670 = 17.50 = 3.50</td>
<td>1.200 = 37.00 = 7.40</td>
</tr>
<tr>
<td>0.330 = 8.35 = 1.70</td>
<td>0.680 = 17.75 = 3.55</td>
<td>1.300 = 41.60 = 8.32</td>
</tr>
<tr>
<td>0.340 = 8.75 = 1.75</td>
<td>0.690 = 18.10 = 3.62</td>
<td>1.400 = 46.25 = 9.25</td>
</tr>
<tr>
<td>0.350 = 9.00 = 1.80</td>
<td>0.700 = 18.35 = 3.67</td>
<td>1.500 = 51.00 = 10.17</td>
</tr>
</tbody>
</table>
TABLE 27

CLASSIFICATION OF URINARY ALA LEVELS IN TERMS OF THEIR RELATIONSHIP TO LEAD EXPOSURE IN CHILDREN

<table>
<thead>
<tr>
<th>Urinary ALA Range (mg/100 ml)</th>
<th>Urinary ALA Code</th>
<th>Relationship to Lead Exposure*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 - 0.99</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>1.00 - 1.49</td>
<td>1+</td>
<td>Moderate</td>
</tr>
<tr>
<td>1.50 - 1.99</td>
<td>2+</td>
<td>Heavy</td>
</tr>
<tr>
<td>2.00 - 2.99</td>
<td>3+</td>
<td>Severe</td>
</tr>
<tr>
<td>3.00 - 5.99</td>
<td>4+</td>
<td>Critical</td>
</tr>
<tr>
<td>6.00 - 10.00</td>
<td>5+</td>
<td>Overwhelming</td>
</tr>
</tbody>
</table>

* Terms employed were arbitrarily selected.
CHAPTER VII

BIBLIÖGRAPHY


Bell, W. B. 1924 Influence of Lead on Normal and Abnormal Cell-Growth. Lancet 1, 267-276.


Chiesura, P. and Brugnone, F. 1963 δ-Aminolevulinic Acid in the Serum and Relation to Its Urinary Elimination in Subjects with Lead Poisoning Med. Lavora 54, 88-94.


de Kretzer, A.J. and Waldron, H.A. 1963 Urinary Delta-Aminolevulinic


Granick, S. and Mauzerall, D. 1958 Porphyrin Biosynthesis in Erythrocytes II. Enzymes Converting 5-Aminolevulinic Acid to Coproporphyrin-


Marver, H.S., Collins, A., Tschudy, D.P., and Recheigl, M., Jr. 1966 
\(-\text{Aminolevulinic Acid Synthetase. II. Induction in Rat Liver. J. Biol. Chem. 241, 4323-4329.}

Mauzerall, D. and Granick, S. 1956 The Occurrence and Determination of
Delta-Aminolevulinic Acid and Porphobilinogen in Urine. J. Biol. Chem. 219, 435-446.


Narisawa, K. and Kikuchi, G. 1965 Effect of Inhibitors of DNA Synthesis
on Allylisopropylacetamide-Induced Increases of $\delta$-Aminolevulinic Acid Synthetase and Other Enzymes in Rat Liver. Biochim. Biophys. Acta 99, 580-583.


Oliver, T. 1914 Diseases of Occupation. London.


Pirrie, R. 1952 The Effect of Splenectomy and Reticuloendothelial Blockade Upon the Anaemia of Lead Poisoning in Guinea-Pigs. J. Path.


Shuster, L. 1956 The Determination of \( \delta \)-Aminolevulinic Acid. Biochem. J. 64, 101-106.


Smith, H. D., King, L. R., and Margolin, E. G. 1965 Treatment of


APPROVAL SHEET

The dissertation submitted by Ronald H. Abrahams has been read and approved by five members of the faculty of Loyola University Stritch School of Medicine.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form, and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

Jan. 10, 1970
Date

Signature of Advisor