The Biochemistry of Lead:

Review of the Body Distribution and Methods of Lead Determination

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Lead is a cumulative poison. Though prevalent in man's environment, only a fraction of what is ingested is absorbed. Slow acting and subtle, lead is powerful and produces a variety of symptoms with many body tissues vulnerable.

LEAD is prevalent in modern man's environment, but its concentration varies from area to area. Soils of highly industrialized areas may contain up to several thousand parts per million, and soil in agricultural New Jersey shows a lead content of 13.9 to 95.7 ppm. Kehoe [1] found that an average adult's lead intake is from 0.1 mg. to 0.6 mg. daily.

Routes of Lead Absorption

The routes of lead absorption are governed by its chemical structure. Lead salts do not penetrate intact skin, but can be absorbed through abrasions. Significant amounts can also be absorbed from a bullet or shot wound, with lead shot considered particularly dangerous because of its larger surface area permitting greater absorption. There have been reports of lead poisoning occurring within a month after a bullet wound.

Organic lead compounds, such as tetraethyl lead in gasoline, rapidly penetrate the intact skin. Absorption into body tissues is more rapid with the organic than with the inorganic compounds, and also because of their higher lipid solubility, large amounts gain access to nerve tissue. Following absorption, lead is rapidly released in the body from organic combination. Vapors of organic lead compounds are also highly dangerous.

One of the two major routes of absorption of lead salts is the gastro-intestinal tract. Most cases of lead intoxication in children result from lead ingestion. Motor activity of the bowel is the only factor which significantly influences lead absorption by this route. As the speed of evacuation decreases, lead absorption increases. The reverse is also true.

The respiratory tract is the other major route of lead absorption. Most industrial cases and a few children's poisonings follow inhalation of lead dust or fumes. In 1925, Blumgart and Minot [2, 3] demonstrated that lead can be absorbed from all portions of the respiratory tract including the nasal passages. Absorption from these areas is actually much more rapid and complete than from the gastro-intestinal tract. Goodman and Gilman [4] state that the mechanism of lead absorption from the respiratory tract may be influenced by two factors: (1) the high carbon dioxide tension present creates an acid medium, causing solution of the lead particles; and (2) it is also possible that particles may be phagocytized and, thus, gain access to the circulation.

Tissue Distribution of Lead

Following absorption, lead is distributed to various tissues, with highest soft tissue concentration being in the liver and kidney. Lead quickly leaves soft tissue for deposition in bone as the relatively insoluble tri-lead phosphate. Though the site of deposition is in controversy, it is generally believed to be the cancellous and compact tissue. Long bones contain more lead than flat bones.

During the early period of lead deposition, especially in growing bone, concentration is highest in the epiphyseal portion. Skeletal lead is fairly inert, and the metal leaves the skeleton only very slowly. Under certain metabolic conditions, however, such as a slight acid-base disturbance or an upper respiratory infection, lead may suddenly be mobilized from bones and an acute lead intoxication may be precipitated.

E. M. Butt,[5] during his investigations of trace metal patterns in disease states, found that normal control tissues held the following contents

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TABLE 1. *Lead Content of Fresh Tissue from Two Children with Lead Intoxication*

Tissue	Case A (mg./100 Gm.)	Case B (mg./100 Gm.)
Bone (rib)	3.84	Not submitted
Diaphragm	0.133	Not submitted
Heart	0.095	Not submitted
Liver	3.05	Not submitted
Lung	0.024	Not submitted
Lymph node	0.126	Not submitted
Spleen	1.69	4.4
Thymus	0.371	Not submitted
Feces	2.27	Not submitted
Kidney	Not submitted	4.0
Brain	Not submitted	3.0

of lead per 100 Gm. of dry tissue: liver, 0.79 mg.; kidney, 0.42 mg.; heart, 0.21 mg.; brain, 0.31 mg.; lung, 0.46 mg.; spleen, 0.51 mg.; pancreas, 0.57 mg.; and adrenal, 0.30 mg.

In Cumings'[5] compilation of reported lead levels in tissues of normal adult subjects determined since 1940, brain was found to contain up to 0.11 mg./100 Gm. of fresh tissue; liver up to 0.5 mg.; kidney up to 2.2 mg. and rib up to 2.8 mg. Values for tissues from children with no history of lead exposure were not presented. It is expected that these would be much lower. In his compilation of reported lead levels in tissues in encephalopathies due to exposure to inorganic lead, brain was reported to contain up to 1.7 mg. per 100 Gm.; liver up to 26.1 mg.; kidney, up to 9.5 mg.

Kehoe[7] found normal liver to contain from 0.04 to 0.28 mg. of lead per 100 Gm. of fresh tissue. Levels in bone from apparent normals varied from 0.67 to 3.59 mg. per 100 Gm. On the other hand, bone from those with lead intoxications held concentrations 20 to 30 times greater. Brain tissue taken from cases of encephalopathy contained levels varying from 0.2 to 0.6 mg./100 Gm.

TABLE 2. *Comparison of Blood Lead Levels in Children*

Lead Level mcg.%	Cases Not Considered as Lead Intoxication Clinically	Cases Diagnosed as Lead Intoxication
0–20	45	0
21–40	12	14
41–50	8	9
51–60	2	1
61–70	1	5
71–80	0	12
81–100	0	11
101–150	0	8
151–200	0	5
201–300	0	5

We had occasion to determine the lead content in tissues obtained at autopsy from two cases of lead intoxication. The findings are outlined in Table 1. Case B was of special interest. This child was a two-year-old female admitted with signs of encephalopathy. She was afebrile. Previous history was noncontributory. Laboratory findings —blood, urine, spinal fluid—were not remarkable. The child expired in less than 24 hours following admission. Autopsy findings were also non-illuminating. To determine tissue lead contents was an after-thought.

Lead Excretion

The excretion of lead is mediated in part by the gastro-intestinal and urinary tracts. Lead is also excreted in the sweat, the concentration here being similar to that in the urine. Balance studies show that all but 5 to 10 per cent of ingested lead is absorbed, but a portion of this is excreted back into the gastro-intestinal tract and evacuated.

An average adult excretes approximately 0.3 mg. lead into the feces and 0.03 mg. in the urine daily. Fecal lead mostly represents lead that was ingested, but not absorbed. Urinary lead indicates the degree of lead absorption, and concentrations above 15 mg./liter in adults and 0.08 mg./liter in children are considered dangerous.

Blood Lead Levels

Blood levels also indicate the degree of lead absorption, but these are of value only in acute cases since the blood is rapidly cleared of lead. Blood levels in chronic cases are not remarkable.

What constitutes a diagnostic blood lead level is a point of dispute. The older literature considered a blood lead level of 0.07 mg. per cent (70 mcg.%) as still within normal limits for an adult. We found that 100 adults admitted to Illinois Masonic Hospital for a variety of problems, but presenting neither history nor clinical evidence of lead exposure, had lead levels below 20 mcg. per cent.

Forty-five of 68 children seen at Cook County Hospital and considered not to be cases of clinical lead intoxication had levels of 20 mcg. per cent or below. Twelve others in this group showed lead contents as high as 40 mcg. per cent. Ten had levels between 40 and 60 mcg. per cent. Only one had a level as high as 70 mcg. per cent. Not one case of another group of 70 children diagnosed as clinical lead intoxication had a level below 20 mcg. per cent. Fourteen of these showed levels between 21 and 40 mcg. per cent. Fifteen had lead levels between 41 and 70 mcg. per cent and 41 of the group had higher levels. A com-

parison of the distribution of lead levels in clinical lead intoxications and other ill children at Cook County Hospital is illustrated in Table 2. On the basis of these figures we feel that perhaps blood lead levels should now be interpreted as follows:

0–21 mcg. per cent—negative
21–60 mcg. per cent—evidence of increased lead exposure
above 60 mcg. per cent—lead intoxication indicated

Since lead clears the blood stream fairly rapidly, a blood lead level alone may not be sufficient to substantiate the diagnosis of lead intoxication. Urine lead content is also of value. Table 3 lists the comparison of lead contents found in urine from a series of children. Care was taken to eliminate the possibility of contamination during the collection process.

All with clinical symptoms of lead intoxication excreted more than 80 mcg./L. of urine. Kehoe considers a urinary lead concentration above that level as dangerous in children. Blood values in many of the urinary 80–250 mcg. group ranged between 20 and 40 mcg. per cent.

The protean expressions of lead poisoning develop as increasing concentrations gain access to the circulation. It was formerly believed that lead circulated as a salt (diphosphate, diphosphoglycerate, citrate, or lead and calcium phosphate). It is now known, however, that over 90 per cent of the circulating lead is associated with the erythrocyte. Lead added to blood rapidly combines with cells and is removed from plasma. Lead also injures the red cell surface and causes mild hemolysis.

Lead also interrupts porphyrin metabolism and, no doubt, interferes with other metabolic pathways as well. Heavy metals are known to inactivate many enzyme systems and Watrach[9] reports degeneration of cellular mitochondria in lead poisoning. Enzyme systems are now known to be concentrated in the mitochondria.

Methods for Lead Analysis

The literature is rich with descriptions of procedures for lead analysis. Various analytical technics applying spectrography, polarography, or colorimetry have been employed in industry for the determination of lead in blood, urine, and other materials.[4] Each of these procedures leaves something to be desired under clinical pediatric conditions. A fairly large sized sample is usually required for analysis, and the time consumed in the analytical procedure renders results so obtained of academic interest only.

TABLE 3. *Lead Contents in Urine of Children*

Content mcg./l.	Cases Not Considered Lead Intoxicated	Cases Diagnosed as Lead Intoxicated
0–60	23	5
61–80	10	3
81–250	0	16
251–350	0	5
351–500	0	8
500–1,000	0	6
Above 1,000	0	15

Some modification of a colorimetric technic involving the formation of a colored complex of lead and dithizone is the most frequently employed technic in lead determinations in clinical laboratories. Though this method is sensitive, it lacks specificity. Approximately 17 heavy metals including thallium, cadmium, and mercury form colored complexes with dithizone. With the exception of thallium, all possible interfering elements are eliminated in the procedure prior to the formation of the colored complex. Even an experienced and careful chemist may upon occasion mistake thallium for lead and vice versa, unless prewarned by an adequate description of physical findings.

Polarography, though fairly suitable in industrial screening programs, lacks the sensitivity necessary to make it a useful tool in a clinical laboratory. An emission spectrograph is a sensitive but expensive instrument. Emission flame spectrometers which are used for routine sodium and potassium analyses do not possess the sensitivity necessary for lead analyses in a clinical laboratory.

In 1962,[10] Willis in Australia reported on the application of atomic absorption flame spectrometry to the determination of lead and other trace metals in urine. This instrumentation, previously used in analyses of copper in soils and calcium and magnesium among others in serum, has proved to have the sensitivity and specificity desired for clinical procedures.

Adapting a previously established colorimetric method to the Perkin-Elmer Atomic Absorption Spectrometer model 214 enabled us to determine the lead content in blood. An analysis requires but one and one-half hours. For routine screening purposes a 5 ml. sample is adequate. During the acute phase when blood lead levels are markedly elevated, the determination can be done on a lesser quantity, at times even as little as 1 ml.

Atomic absorption spectrometry possesses the sensitivity to detect less than 0.2 ppm. (20 mcg.%-200 mcg./L.) lead in an aqueous solution. This

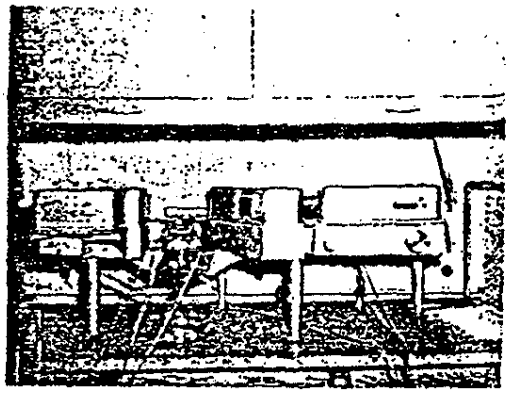

FIG. 1. Perkins-Elmer Atomic Absorption Spectrometer, model 214 used in determining lead content in the blood.

implies that urine specimens with an abnormal content of lead can be detected by screening with a minimum of sample preparation. Since the other urinary constituents do not interfere with this determination, a centrifuged urine specimen is merely aspirated into the instrument. Lead analyses can thus be completed in less than a minute.

When excretory levels are less than 0.2 ppm. the lead present can be concentrated by first chelating the metal in the urine sample with ammonium pyrollidine dithiocarbamate and then extracting a 30 ml. aliquot into a 5 ml. volume of organic solvent. This takes less than 30 minutes.[1] The instrument used in these analyses is shown in Figure 1.

We observed that the lead versenate complex excreted during versenate therapy is not removed from the aqueous phase by this procedure. Thus, by applying atomic absorption spectrometry, we hoped to learn more about the nature of lead excreted during the phases of versenate therapy. Findings in specimens kindly sent to use from four cases treated at Michael Reese Hospital are shown in Table 4. Total lead was determined by direct aspiration. "Unbound lead" is that quantity which was extractable into the organic phase. In some instances coproporphyrin levels were also determined.

All patients described in Table 4 presented historical and clinical evidence of lead intoxication. Blood levels were in an abnormal range of 41–82 mcg. per cent. Initial urinary lead concentrations, however, except in one case, were not remarkable. Following the initiation of BAL-versenate therapy, total lead excretion increased three to ten fold and then gradually fell. Less than 2 per cent of the lead excreted during treatment was in the unbound state. The lead-versenate complex appeared to be excreted slowly. Seven days after cessation of therapy, 75 per cent of the lead excreted was still in the bound state.

TABLE 4. *Lead and Coproporphyrin Excretion During Versenate Therapy*

Patient	Date	Blood Lead μg.%	Total Urine Lead μg./l.	Unbound Lead μg./l.	% Unbound	% Bound	Coproporphyrin μg./24 hr.
R. M.	11/16	58 μg.%	60 μg./l.				313 μg.
	11/23		Initiate therapy to 11/28				
	11/24		5,030	0	0	100	5.2
	11/25		1,100	20	1.8	98.2	10.8
	11/26		2,600	40	1.5	98.5	9.7
	12/4		100	25	25	75	95
	12/21	7					
E. Q.	11/16	41 μg.%	350				Not done
	11/22		Initiate therapy to 11/27				
	11/23		1,050	0	0	100	2.3
	11/25		1,400	23	1.6	98.4	11.7
	11/27		300	33	11	89	4.8
	11/28		200	50	25	75	—
	12/3	25	100	69	69	31	24
K. T.	11/11	58	33				329.3
	11/15		Initiate therapy to 11/20				
	11/27		198	43	22	78	
J. R.	11/16	82	56				556
	11/20		Initiate therapy to 11/25				
	11/20		2,300	30	1.3		12.4
	12/4	30	250	60	24	76	150

Urinary coproporphyrin levels also decrease during therapy. Before treatment, levels excreted by three subjects were 313, 329, and 556 mcg. per 24 hr. with the upper limit of normal being approximately 120 μg. per 24 hr. During therapy, coproporphyrin levels dropped to 12 μg. or less and then increased. In one instance the level rose to 150 μg. per 24 hr. ten days after cessation of the versenate.

Preliminary data such as these, though admittedly incomplete, suggest strongly that here is a means of more adequately studying lead metabolism in treated and untreated patients.

References

1. Berman, E.: Atomic Absorption Newsletter 3: 111, 1964.
2. Blumgart, H. L.: J. Indust. Hyg. 5: 153, 1923.
3. Butt, E. M. *et al.*: Metal Binding in Medicine. Philadelphia, J. B. Lippincott Co., 1960, pp. 43 ff.
4. Cholak, J.: Arch. Environ. Health 8: 222, 1964.
5. Cumings, J. N.: Heavy Metals and the Brain. Springfield, Ill., Charles C Thomas, 1959, pp. 95 ff.
6. Goodman, L. S. and Gilman, A.: The Pharmacological Basis of Therapeutics. New York, The Macmillan Co., 1955.
7. Kehoe, R.: Arch. Environ. Health 8: 232, 1964.
8. Minot, A. S.: J. Indust. Hyg. 6: 125, 1924.
9. Watrach, A. M.: J. Ultrastruct. Res. 10: 177, 1964.
10. Willis, J. B.: Anal. Chem. 34: 614, 1962.

READER'S CHECK LIST

(Continued from page 276)

land and Sweden. Pertinent to the newborn period there are discussions dealing with physiology, respiratory and circulatory adaptations, hypoglycemia, respiratory distress, idiopathic hypoglycemia, and the late effects of obstetric hazards. There are excellent surveys also of contemporary knowledge of chromosomal abnormalities, obesity, kwashiorkor, renal biopsy, disorders of growth, cancer, steroid treatment of asthma, hydrocephalus, and spina bifida cystica.

In short, here is a refresher course for the physician in practice, within the framework of the subjects covered.

Hematology

Atlas of Haematology. G. A. McDonald, T. C. Dodds, and B. Cruickshank. Baltimore, Williams and Wilkins, 1965. 163 pp. $16.50.

An extensive and carefully selected collection of magnificent blood and bone marrow photographs. Though presented as being primarily for "trainees" in hematology, this can serve as a useful reference for all who work in clinical laboratories. Many photographs of organ biopsies and of cell appearances under phase contrast microscopy are included. Most of the smears are stained with either Leishman's or the May-Grunwald-Giemsa method, but the appearances with these are not significantly different from what one sees with Wright's stain. Along with the text comes a separable poster-like colored chart showing the morphologic changes of the major cell series during maturation.

Referral Book

American Pediatric Directory, 1965–1966. 12th ed. Joe T. Smith, M.D., F.A.A.P., Editor and Publisher, 1800 Magnolia Ave., N.E., Knoxville, Tenn. $12.00.

"A Roster of Pediatricians of the United States and Canada, with Complete Professional Biography" (from the Preface).

Therapy

New Drugs, Evaluated by the A.M.A. Council on Drugs, 1965. Chicago, Ill., American Medical Association. 516 pp.

"The drugs that are discussed in detail are individual agents, generally available in the United States, that have been introduced within the past ten years. The statements on each of these drugs are based on an evaluation of the available laboratory and clinical evidence by the Council and its consultants." Nearly 300 such drugs are arranged and discussed according to therapeutic purpose. Without question this is the most authoritative and all-inclusive reference of its type for the practicing clinician, inasmuch as all statements made have been approved by at least six independent consultants.

Neuromuscular

The Chemistry and Therapy of Disorders of Voluntary Muscles. E. G. Murphy, M.B., D.C.H., Toronto. Springfield, Ill., Charles C Thomas, 1964. 123 pp. $6.50.

An outline-like review of "the main features" of the two facets mentioned in the title.

Therapeutics

Diets for Sick Children. D. J. W. Dixon, Former Senior Dietitian, Hospital for Sick Children, Great Ormond Street, London. Oxford, Blackwood; Philadelphia, F. A. Davis Co., 1965. 111 pp. $2.50.

A valuable paper-back compendium of diet lists and ancillary instructions for use in a wide variety of childhood illnesses.