A COMPARATIVE STUDY OF LABORATORY METHODS FOR DETECTING LEAD TOXICOSIS

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ABSTRACT

A COMPARATIVE STUDY OF LABORATORY

METHODS FOR DETECTING LEAD TOXICOSIS

By

Norman J. Gatzemeyer

Blood and tissues from lead poisoned rabbits were utilized to compare various laboratory tests and procedures routinely used as aids in the diagnosis of lead toxicosis. The 2 most common methods to determine lead exposure in the living animal include chemical analysis of the blood for lead concentration and examination of stained blood smears for immature, abnormal erythrocytes.

Good statistical correlation was noted between blood lead levels and lead dosage. This correlation was determined by 2 dithizone lead methods. The trichloroacetic acid method, as suggested by Hammond *et al.* (1956) was the more accurate, simpler and faster test.

Basophilic stippling and polychromatophilic staining of erythrocytes in Wright's-stained smears increased significantly following ingestion of lead. Animals receiving lead had a wide range in the number of stippled cells counted. This would limit its use as a single diagnostic tool. There was a consistent increase in the number of polychromatophilic erythrocytes; therefore, this could be considered a more reliable test.

The gravimetric lead-chromate method for tissue lead levels had little correlation between its results and known amounts of lead ingested.

Examination of special stained tissue sections, sodium rhodizonate, Ziehl-Neelsen acid-fast stain for kidney inclusion bodies, and bone sections treated with hydrogen sulfide failed to aid in identifying lead poisoned animals.

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By

Norman J. Gatzemeyer

A THESIS

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Dedicated to

Mary

and the family

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INTRODUCTION

Lead toxicosis has been, and still is, considered a common and serious disease in both man and animals. Acute lead toxicosis usually does not cause great difficulty in diagnosis. However, the most persistent problem lies in the inability to determine poisoning early enough in the disease process to effectively initiate treatment.

Throughout the years many types of tests have been used or advocated for use in lead determinations. Differences in test animals (both species and individual), route of administration and dose levels are but 3 variables that make it difficult to evaluate laboratory findings effectively.

OBJECTIVES

The objectives of this research were to compare several types of laboratory procedures for lead determinations in animal tissues as reported in the literature. New Zealand White rabbits were chosen as the experimental animal because they could be easily dosed orally with known levels of lead and were sufficiently large for blood and tissue collections and subsequent determinations.

The specific aims of this research:

1. To evaluate several laboratory tests and procedures, described in the literature, for lead determinations.

2. To observe gross and microscopic lesions of lead toxicosis and to compare these with lead levels as revealed by selected clinical pathology tests.

3. To determine whether these laboratory tests are effective and accurate in the diagnosis of lead toxicosis.

LITERATURE REVIEW

A review of the literature reveals that much has been written on lead and its toxic effects. Reviews by Blansdorf (1922), Hutton (1923), Key (1924) and Aub *et al.* (1926) described some of the work on lead toxicosis. The symptoms of plumbism have been known since ancient times, but it was not until the early part of the 19th century that experimental investigations on lead and its toxic effects were begun. These investigators understood and described the correlation between lead and so-called "metallic colic" of lead miners and smelters. Other writers in historical books on medicine have written on palsy, colic and other symptoms following the ingestion of lead.

Historical

The history of lead and its toxic properties was well known and recognized as early as the 2nd century B.C. Nikander's description of the signs of poisoning, as seen in workers mining and refining lead, included constipation, abdominal pain, and pallor (Aub *et al.*, 1926; Gilfillan, 1965; Morgan *et al.*, 1966). Alderson (1852) quoted Dioscorides as saying,

> "the drinking of litharge (red lead) causes oppression to the stomach, belly and intestines, with intense wringing pains; sometimes it even wounds the intestines by its severe pressure, it suppresses the urine, while the body swells and acquires an unsightly leaden hue."

Hippocrates, 370 B.C., was the first to associate certain clinical signs with an exposure to lead (Waldron, 1966). The ease in refining lead

from natural ore, along with its malleability and resistance to corrosion, lent itself easily to a widespread use by man. The practice of lining cooking and drinking vessels with lead, the construction of plumbing systems in the homes of the wealthy and the sweetening of wine, as well as many other purposes, have recently been incriminated as one cause of the deterioration of the Roman civilization (Gilfillan, 1965). Water contaminated by lead in Amsterdam and cider contaminated by lead in Devon appeared to cause epidemics of poisoning during the 16th century (Morgan *et al.*, 1966).

In the 17th and 18th centuries, lead was used in medicine, especially for its action upon the blood. Lead was found to be hemostatic and, because of its power to coagulate albuminous tissue, was used in the treatment of ulcers. Lead was also used for the treatment of fevers and as a drug to combat malaria (Legge and Goodby, 1912). In 1839, the first detailed treatise describing clinical features of lead poisoning was published (Tanquerel des Planches, 1839). By the end of the 19th century there was an increase in use of lead as well as literature describing lead poisoning. At about this time pioneer investigators such as Legge, Teleky, Lehmann, Aub, Minot, Reznikoff and Fairhall initiated independent studies on the toxic effects and metabolism of lead.

Industrial Lead Toxicosis

One of the earliest indications that a health problem existed in the people working in the lead industry, in general, and the lead mines of England in particular can be determined from an 1842 subcommission report of the Children's Employment Commission. This commission not only was concerned about wages but also with miners' health. In a letter to the Weardale lead miners, they wrote,

> "by moving only twenty miles lower down into the coal country, a young man might nearly double his income and have the prospect of adding many years to his life." (Raistrick and Jennings, 1965)

This commission also commented on the state of poverty of the miners and concluded that the chief cause of pauperism was due to the large number of widows and orphans. The husbands of many of these widows had fallen victim to the "miner's complaint," which materially shortened the lives of the men and made the miners old men at the age of 50.

The harmful effects of lead in the painting and varnishing trades was the subject for a 38-page paper by Koelsch in 1921 given to the German Painters Guild. In this lecture, he detailed symptoms, modes of entrance into the body, and individual susceptibility. He also presented statistics in regard to the frequency of poisoning in various localities of Germany. Teleky (1922) described the dangers of using lead-based paints. This article was the result of world-wide attention given to the lead paint industry following World War I.

In 1912, cases of lead toxicosis in workers of smelting and refining plants in the United States numbered 1667 out of a total of 7400 men, or approximately 22%, while in England the rate of lead toxicosis for that year was 1.8%. For this same year, statistics from Germany revealed an incidence of 10.5% employee lead poisoning and one report from a large Austrian plant reported that 9% of their total work force were poisoned (U. S. Bureau of Labor Statistics Bull. 141, 1912).

Burnham (1923), in his study on industrial lead poisoning and its prevention in factories, presented statistics showing the reduction of reported cases of lead poisoning between 1900 and 1920 in England. A total of 1058 cases with 38 deaths was seen in 1900, while in 1920 only

243 cases were reported with 23 deaths. He further stated that in the United States during a 9-year period (1911 to 1920) about 2000 cases of chronic lead poisoning in humans was seen annually.

In a survey conducted by Pratt (1913) comparing European and American industries that were sources of lead poisoning to the workers, it was found that great variations of the problem existed. For example, companies that made dinnerware in Great Britain and Germany had a high incidence of lead toxicosis, while in America no lead poisoning was noted. According to Pratt, this was due to the use of lead in tin plating in Europe, while in America enamel dinnerware was used. He also concluded that workers hand-making files in Europe had more lead poisoning than workers using machines in America.

Edsall (cited by Hamilton, 1919), comparing plumbism in the dyeing and calico printing trades, found a high level of lead poisoning in employees of companies in Great Britain; however, he found no evidence of lead poisoning in the textile industry of Philadelphia.

Infants and Lead Toxicosis

Although industrial lead poisoning was recognized for many years in adults, few references are found concerning lead toxicosis in infants prior to 1945. Here the disease prevailed mainly in infants aged 1 to 4 and was reportedly the most common type of fatal poisoning in children (Perlstein and Attala, 1966). The source of poisoning usually was flaking paint or crumbling plaster, especially in slum areas where property upkeep was poor.

In infants, lead poisoning was usually quite severe. Abdominal pain, constipation, diarrhea, vomiting, and lethargy were commonly seen and, in more prolonged cases of lead ingestion, central nervous system

disturbances were noted. Even with modern forms of therapy the mortality rate was 22 to 65% (Coffin *et al.*, 1966). In a study in the Chicago area, Perlstein and Attala (1966) reported that 39% of 425 cases revealed persistent neurological after-effects of lead poisoning ranging from profound mental retardation to optic atrophy and grand mal.

Legge and Goodby (1912), Aub *et al.* (1926), Bradley *et al.* (1956), and Waldron (1966) reported basophilic stippling of RBCs, a mild hypochromic anemia with many immature red blood cells, proteinuria, glycosuria, and amino-aciduria, as some common laboratory findings in lead poisoning of children.

Animals and Lead Toxicosis

Domesticated animals accidentally poisoned by lead included horses, dogs, cattle, sheep, swine, and cats (Zook *et al.*, 1969). Experimental lead poisoning has been carried out using all "common laboratory animals." The rabbit and the rat were the 2 most common small animals used, while in the large domestic animals cattle and sheep were the animals of choice (Blaxter, 1950; Allcroft, 1951).

The horse was the 1st animal reported to be subject to lead poisoning. These reports of poisoning were usually from lead mining and smelting areas where this animal was used as the major source of power. Haring (1915) reported the lead caused a paralysis of the laryngeal nerves causing failure of normal abduction of the laryngeal cartilages during inspiration, hence the term "roarers." In this case death was usually caused by foreign-body pneumonia due to failure of the epiglottis to close. The 2nd area where accidental lead poisoning was seen concerned fruit orchards where lead arsenate was commonly used as a pesticide. Today lead poisoning in horses is reported far less than in earlier years, due to the more

effective means of removing lead from smelter fumes and the use of the newer pesticides.

Although the horse was the first animal reported with lead toxicosis, in recent years lead poisoning has been reported as a disease of cattle almost exclusively. This was primarily due to the nature of cattle smelling, licking or chewing foreign objects. The source of the lead usually was found to be lead-based paints, plasters, greases or old storage batteries (Hammond *et al.*, 1956).

A wealth of data is available concerning experimental lead poisoning in the dog (Ophuls, 1907; Horwitt and Cowgill, 1937; Calvery *et al.*, 1938; Finner and Calvery, 1939; Fauts and Page, 1942); however, examination of available literature concerning accidental lead poisonings revealed few reported cases. Recently one investigator diagnosed and described lead poisoning in 60 dogs at the Angell Memorial Animal Hospital (Zook *et al.*, 1969). Other accidental lead poisonings in the United States include reports by Bond and Kubin (1949) and Berry (1966), while Scott (1963) reported on 28 cases of lead poisoning in dogs which occurred at Broken Hill, Northern Rhodesia, and Dodd and Staples (1956) reported on lead poisoning in New Zealand.

Lead Determinations

A search of the literature revealed that the chemical methods most frequently used for lead determinations were: (1) the colorimetric sulfide method, (2) gravimetric or volumetric sulfate, molybdate and chromate methods; and (3) the electrolytic method. Fourcroy and Hahnemann were the first investigators to propose the use of hydrochloric acid saturated with hydrogen sulfide as a test for lead (Friend, 1917). Pelouze (cited by Fairhall, 1922), first proposed a colorimetric method for the determination of small amounts of lead. He converted the lead into sulfide and compared the

intensity of the brownish coloration produced against standard lead solutions similarly treated.

A number of objections have been defined in the colorimetric methods of estimating lead as the sulfide. These included acid concentration, amount and character of the salts and the size of the lead particles. Any one of these variables could change the color intensity and would thereby give improper values (Fairhall, 1922).

The sulfate method was advocated for the analysis of larger amounts of lead. A number of substances such as ammonium chloride, tartrate, acetate, the alkaline chlorides and caustic alkalis increased the solubility of lead sulfate which produced low results. Potassium salts often gave high results due to the precipitation of a potassium and lead complex. This complex was practically impossible to remove. This method was not recommended for biological tissues because of the false high and low values (Fox, 1909).

Lead may be determined either gravimetrically or volumetrically as the molybdate since lead molybdate is extremely insoluble and lends itself readily to either process (Alexander, 1893; Weiser, 1916). Using ammonium molybdate as a precipitant, Eegriwe reported that the sensitivity was 1:800,000; however, because ammonium molybdate gave insoluble precipitates with both the calcium salts and phosphates in tissues, this method was not recommended for biological material (Fairhall, 1922).

The electrolytic process for lead determination has been subjected to much investigation (Frankel, 1893; Exner, 1903; Snoden, 1906; Patten, 1917; Denis and Minot, 1919). The difficulties of manipulation of the equipment plus the low results obtained when phosphates are present or when using sulfuric acid in the test solutions has resulted in its disuse for lead determination in biological materials (Patten, 1920).

The chromate method of lead determination proposed by Diehl has been modified by several investigators (Crookes, 1894; Cushman and Hayes-Campbell, 1895; Pope, 1896; Seeker and Clayton, 1915; Fairhall, 1922) in an attempt to prevent the lower results obtained in the presence of sodium acetate. Cox (cited by Fairhall, 1922) reported that by using acetic acid to convert the basic salt into the normal salt, one could precipitate all the lead present and thereby prevent the low, inaccurate analysis. Scott (1939) presented a simplified chromate method for lead determination that has since been used as a standard test in some laboratories.

Other tests for the estimation of lead, using urine, feces or tissues, included one by Finner and Calvery (1939). They described a method that consisted of placing bones in a 10% formaldehyde solution saturated with hydrogen sulfide. Pieces of the long bones were placed in the solution for up to 3 weeks and, if lead were present, it combined with the sulfide and black particles could be seen following treatment with formic acid.

In 1942, Bambach and Burkey described a micromethod of lead determination in tissues using the chemical, dithizone. This was an adaptation of the titrimetric method that was used by Horwith and Cowgill (1937). Hammond *et al.* (1956) further modified this test by replacing the ashing procedure with an extraction method using trichloroacetic acid.

Feigl and Suter (1924) described a chemical method for the simultaneous demonstration of barium, strontium and lead. Later this was adapted for use in tissue sections and was presented for use by Thompson (1966). This method used the chemical, sodium rhodizonate, to stain tissue sections. Four duplicate sections of the tissue to be examined were prepared and stained separately. Changing the pH of the staining solutions allowed a differentiation of the contaminated metals.

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The atomic absorption spectrophotometry method to determine small amounts of metals was suggested by Walsh (1955). Since that time investigators have improved on both the instrumentation and extraction methods. Simple procedures using the raw sample have been proposed for lead detection (Slavin *et al.*, 1964), while more elaborate methods required ashing or extraction (Willis, 1962; Berman, 1964; Kopito and Schwochman, 1967).

Garrod (1892) reported that lead inhibited heme synthesis from glycine. Because of this inhibition, 2 intermediate stages, delta amino levulinic acid and coproporphyrin III, were found in the urine. Recently the levels of the 2 intermediate stages in the urine were employed as a basis for determining lead toxicosis (Benson and Chisolm, 1960; Chisolm, 1964; Balbo *et al.*, 1965; Ellis, 1966; Bruin and Hoolboom, 1967).

Clinical pathology determinations for lead toxicosis include the examination of blood smears stained with Wright's stain for the presence of basophilic stippling of erythrocytes (Key, 1924; Finner and Calvery, 1939; Mitchell, 1940; Zook *et al.*, 1970). Slight anemia, as evidenced by a reduction in PCV and hemoglobin values, has been reported in hemograms from lead poisoning suspects (Zook *et al.*, 1969).

MATERIALS AND METHODS

Experimental Animals

Fifteen apparently healthy, 8- to 10-month-old female New Zealand White rabbits were used in this study. This experimental animal was chosen because it (1) was large enough to permit collection of blood and tissues needed for analytical work; (2) was easily obtained at a low cost; and (3) has known susceptibility to heavy metal poisoning. The experiment was designed to cause death in 21 days, and any rabbits still alive would be killed and necropsied at that time.

By random selection the rabbits were divided into 2 groups. Group 1 consisted of 10 animals identified as Nos. 1 to 10. Group 2 consisted of the remaining 5 rabbits, to serve as controls, and were identified as Nos. 11 to 15.

Animal Care

The rabbits were maintained in individual, wire-bottomed, stainless steel rabbit cages in the Center for Laboratory Animal Resources (CLAR) facilities at Michigan State University. They were fed free choice a standard rabbit pellet ration.* Water was provided *ad libitum*.

*John A. Van Den Bosch Co., Zeeland, Mich.

Source and Method of Toxicosis

Reagent grade lead carbonate (2 $PbCo_3Pb(OH)_2$)* M.W. 774.7 was used as the toxicologic agent and was supplied by the Michigan Department of Agriculture Regulatory Laboratory. A standard analytical balance was used to weigh 125 mg. of lead carbonate calculated to be 100 mg. pure lead, for each animal's daily dose and was placed into No. 00** gelatin capsules. One rabbit at a time was held on a table and, with the aid of a short piece of plastic tubing and plunger (Figure 1), the capsule containing the lead was placed as far back in the mouth as possible. The animal was kept on the table and observed until chewing and swallowing were completed and then was returned to its cage.

Sample Collection

To establish normal hemoglobin, packed cell volume and blood lead levels, blood samples were taken from each animal before the beginning of the experiment. A rabbit board was used to facilitate the holding of the animals. Blood was collected by cardiac puncture with the needle inserted caudolateral to the xiphoid cartilage toward the location of the heart. Heparin*** solution was used as the anticoagulant in the 10 cc. plastic syringes and 2-inch, 18 gauge needles. A minimum of 10 ml. of blood was collected every 48 hours from the treated animals during the experimental period and placed in plastic heparinized vials for individual analyses.

A second study was initiated using another group of 6 rabbits in an attempt to determine what effect the removal of 10 ml. of blood every 48

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*J. T. Baker Chemical Co., Phillipsburg, N.J.

**Parke, Davis & Company, Detroit, Mich.

***American Hospital Supply Corp., Evanston, Ill.

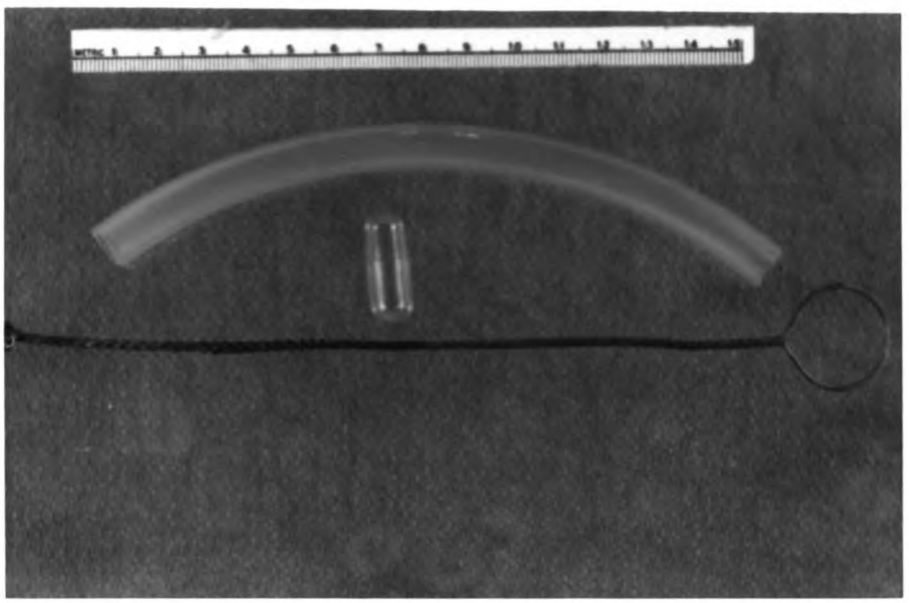

Figure 1. Plastic tube and plunger used as balling gun.

hours would have on the hemoglobin, packed cell volume, and morphology of the Wright's-stained blood smears.

Tissue collections were taken at the time of death or as soon as possible following death. Tissues collected from all animals included the following: (1) a portion of the right and left lateral lobe of the liver; (2) right and left kidney; (3) duodenum; (4) jejunum; (5) ileum; (6) brain; (7) heart; and (8) right and left femur. Following necropsy, collected tissues were divided into 2 groups. One portion was fixed in a 10% buffered formalin solution for sectioning and appropriately stained for histopathologic examination. The 2nd portion was refrigerated until chemical analyses were performed for lead.

Experiments

During the 21 days of the experiment, the 5 nontreated controls and the 10 rabbits given the daily dose of lead were maintained in the same room under identical conditions. In each trial a comparison was made between controls and poisoned animals. The trials are summarized as follows:

1. Two blood smears were made at the time of bleeding of each animal and stained by Wright's method (Benjamin, 1961) for stippled cell determinations.

2. Hemoglobin values were determined by the cyanmethemoglobin method (Benjamin, 1961). The microhematocrit method (Benjamin, 1961) was used for the packed cell volume (PCV) values.

3. Blood lead levels were determined by 2 different dithizone (diphenylthiocarbazone) methods. Method I (Hammond *et al.*, 1956) described a trichloroacetic acid solution to precipitate the blood proteins. This step replaced ashing of the sample. The lead was extracted using dithizone

reagent and the lead level was determined by visual comparison to a group of color blocks. Method II involved the ashing of the sample in a muffle furnace, extracting the lead using dithizone reagent and titrating against a standard lead solution (Hurwitt and Cowgill, 1937).

4. Lead levels in the tissues were determined by the lead chromate method as described by Scott (1939).

5. Bone lead levels were determined by a method used by Finner and Calvery (1939). One femur from each animal was fixed for 3 weeks in a solution of formaldehyde diluted 1:10 with distilled water and saturated with hydrogen sulfide. Following fixation, bone was treated with formic acid. The other femur was fixed in buffered 10% formalin. Both femurs were decalcified using Cal-Ex decalcifying solution,* sectioned and stained with hematoxylin and eosin. A histopathologic comparison was made between the 2 fixation methods and the control animals.

6. Special stains. Kidney sections were stained using the Ziehl-Neelsen acid-fast method for inclusion body determinations. Kidney and liver sections were stained using the sodium rhodizonate** method for lead (Thompson, 1966).

7. A histopathologic comparison was made between hematoxylin and eosin-stained sections of major organs from poisoned and control animals.

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*No. S-391, Fisher Scientific Co., Fairlawn, N.J.

**Fisher Scientific Co., Fairlawn, N.J.

RESULTS

The rabbits were weighed prior to and again at the end of the experimental period, or following death, to determine weight changes. The 10 rabbits that were given lead had an average weight loss of 0.015 kg. (Table 1), while there was an average weight gain of 0.254 kg. in the 5 control animals (Table 2).

Signs attributable to lead toxicosis were observed in only 1 rabbit. In this animal there were signs of ataxia, incoordination and mental derangement. This rabbit repeatedly charged the walls of the cage and had high-pitched crying sounds with open-mouthed breathing. The above signs lasted only a short period of time and were followed by death.

All rabbits given lead had roughened hair coats, paleness of the mucous membranes, and signs of dehydration. Terminal diarrhea was evidenced by the soiled hair coat of the hindquarters in all but 2 animals. In contrast, control rabbits appeared normal in all respects.

Upon death, an autopsy was performed on every animal. The first to die, rabbit No. 1, was pregnant. The 4 approximately 25-day-old fetuses were removed from the uterus for tissue lead determination. Subsequent chemical examination of the composite sample (all tissue of the 4) revealed a tissue lead level of 44.8 ppm. Pathologic changes in rabbit No. 1 included a pale yellowish liver, a thickened edematous pericardium, and a few small, circumscribed white spots on the surface of the kidney. No other lesions were seen.

Table 1. Weight changes of rabbits given lead

Animal No.	Pretrial Wt. (kg.)	Final Wt. (kg.)	Net Change	Total Lead Given (mg.)
1	4.20	4.12	-0.08	800
2	4.10	4.01	-0.09	1500
3	3.50	3.50	---	1700
4	3.54	3.50	-0.04	1700
5	2.62	2.74	+0.12	1900
6	3.12	2.98	-0.14	1700
7	4.00	4.14	+0.14	1900
8	3.30	3.24	-0.06	900
9	2.80	2.80	---	1900
10	3.94	3.94	---	1700
Totals	35.12	34.97		
	35.12 - 10	34.97	= 0.015 kg. average loss	

Table 2. Weight changes of control rabbits

Animal No.	Pretrial Wt. (kg.)	Final Wt. (kg.)	Net Change
11	3.50	3.80	+0.30
12	3.40	3.51	+0.11
13	4.22	4.55	+0.33
14	4.12	4.20	+0.08
15	3.54	3.98	+0.44
Totals	18.78	20.04	
	20.04 - 5	18.78	= 0.254 kg. average gain

Necropsy revealed Animal No. 8 had an acute pneumonia. All areas of the lungs were solidified and full of a yellowish, tenacious material. Microbiologic examination of portions of the lung identified the causative organism as *Pasteurella multocida*. No other gross lesions were noted.

Examination of the remaining 8 poisoned animals and the 5 controls did not reveal any gross lesions, except in the area of the heart. Some pericardial thickening with an increase in fibrous connective tissue and edema around the heart was noted in these animals.

Trial 1

Examination of the Wright's-stained blood smears revealed anisocytosis, poikilocytosis, and hypochromia of the red blood cells in lead-poisoned animals. Approximately 10,000 red blood cells were examined on each of 53 blood smears. Forty-four of the smears had an average of 39 basophilic stippled "reticular" and/or "punctate" red blood cells (Table 3, Figure 2). Nine of the smears did not have red blood cells with basophilic stippling. A sharp increase in erythrocytes with basophilic stippling was seen when the total cumulative dose of lead exceeded 1400 mg. per animal (Figure 3).

Blood smears were made and examined from all animals, prior to the experiment, and from the 5 control animals before they were killed at the termination of the experiment, to establish normal values. Only 1 stippled cell was observed in these slides.

Examination of the blood smears revealed the number of polychromatophilic erythrocytes were noticeably increased with each additional level of lead (Table 4, Figure 4), with an average of 232 polychromatophilic erythrocytes in the 53 smears.

Table 3. Total number of basophilic stippled cells per 10,000 erythrocytes counted in lead treated animals

Animal No.	Pretrial Examination	Final Examination	Cumulative Lead Intake (mg.) 200	700	900	1100	1400	1600	1800
1	0		1	118**					
2	0		23	20	17	50	64**		
3	0		18	14	0	20	10**		
4	0		0	2	18	12	10	44**	
5	0		2	2	22	28	---	292	469***
6	0		0	0	5	7	14	154**	
7	0		4	10	4	2	4	3	7***
8	0		2	2	2**				
9	0		0	0	2	0	5	16	64***
10	0		0	0	14	10	20	134**	
11*	0	0							
12*	0	0							
13*	0	0							
14*	0	1							
15*	0	0							
Mean	0.05		5	17	8	14	18	107	180
± S.E.			2.74	11.46	2.82	6.28	7.91	49.1	178
P <			0.05	.01	.01	.01	.01	.01	.01

*Control animals.

**Last sample taken prior to death.

***Last sample taken prior to euthanasia.

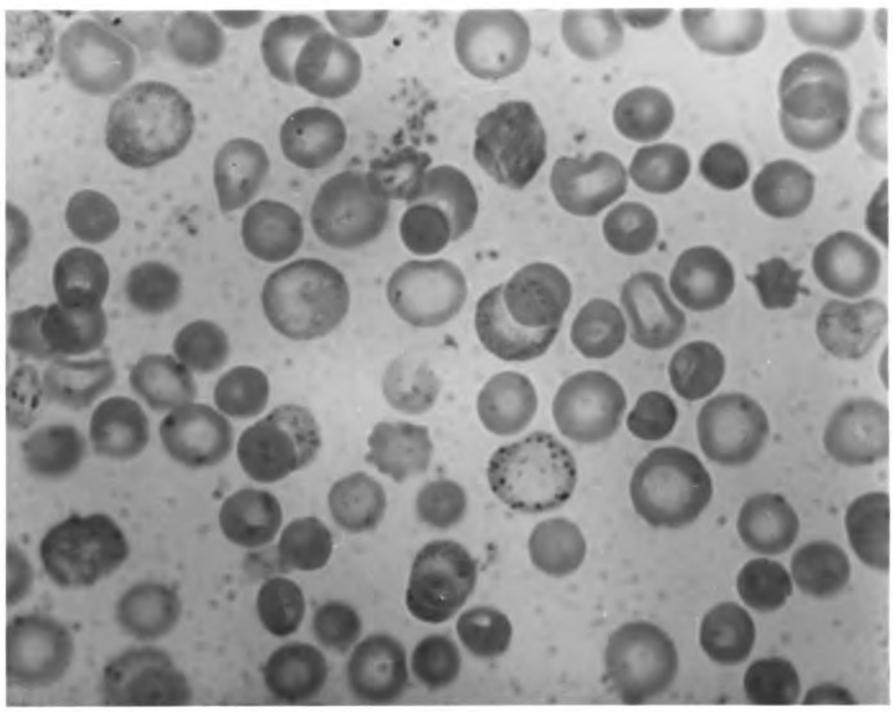

Figure 2. Photomicrograph of Wright's-stained blood smear with basophilic stippling. Notice both "punctate" and "reticular" forms of stippling. x 1500.

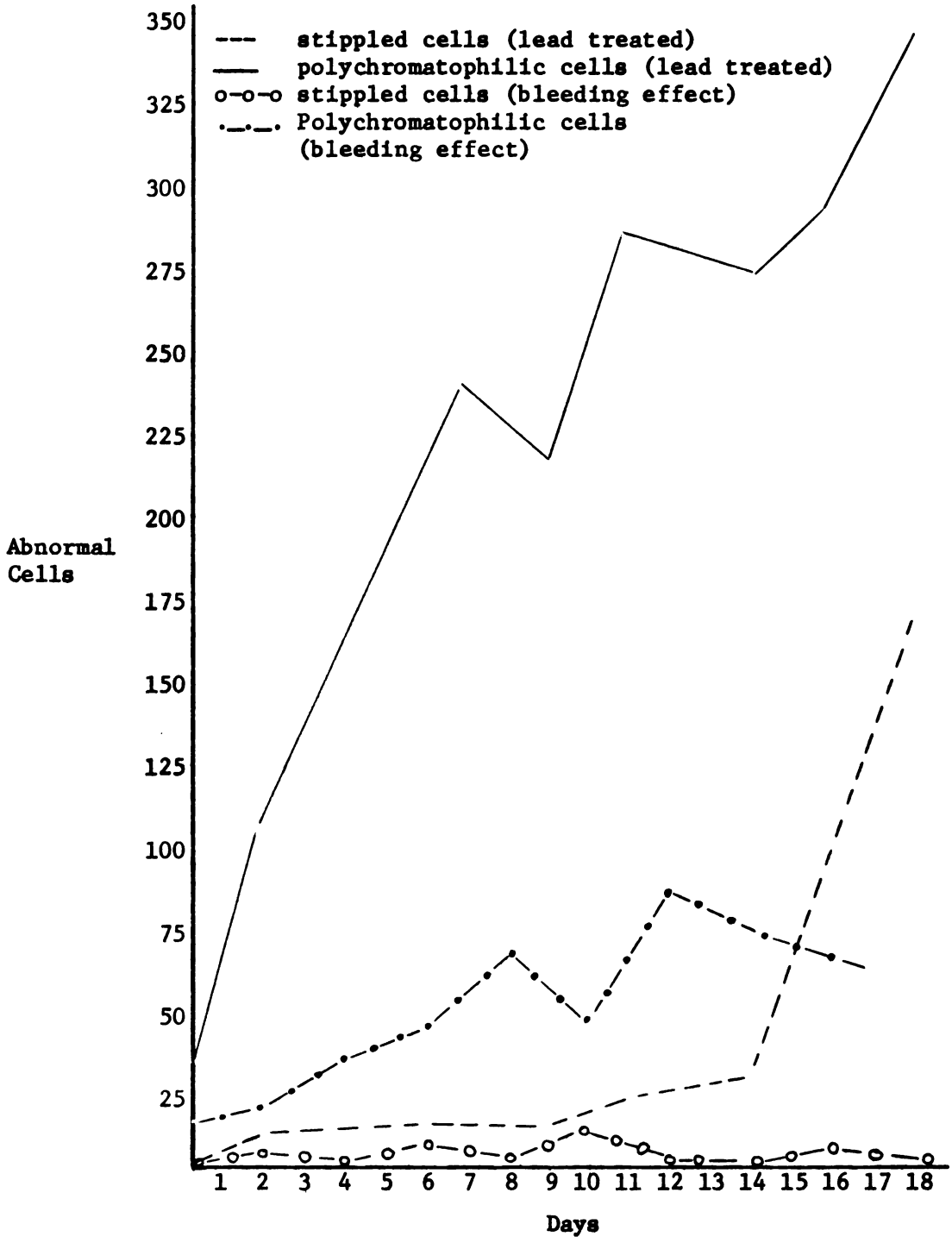

Figure 3. Average number of stippled and polychromatophilic erythrocytes per 10,000 erythrocytes counted. Lead treated animals received 100 mg. lead per day.

Table 4. Total number of polychromatophilic erythrocytes per 10,000 erythrocytes counted in lead treated animals

Animal No.	Pretrial Examination	Final Examination	Cumulative Lead Intake (mg.) 200	700	900	1100	1400	1600	1800
1	21		168	258*					
2	70		234	156	150	152	156**		
3	54		238	344	290	278	146**		
4	28		136	272	252	254	222	206**	
5	16		41	160	274	456	---	446	802***
6	22		30	195	146	236	398	326**	
7	40		47	166	141	234	206	200	121***
8	17		32	148	142**				
9	19		87	84	123	208	270	234	84***
10	44		200	464	417	470	514	292**	
11*	29	78							
12*	34	58							
13*	16	24							
14*	47	77							
15*	36	49							
Mean	39		121	225	215	286	273	284	336
± S. E.	4.51		26.7	11.3	33.2	39.5	51.2	38.1	233.5
P <			.001	.001	.001	.001	.001	.001	.001

*Control animals.

**Last sample taken prior to death.

***Last sample taken prior to euthanasia.

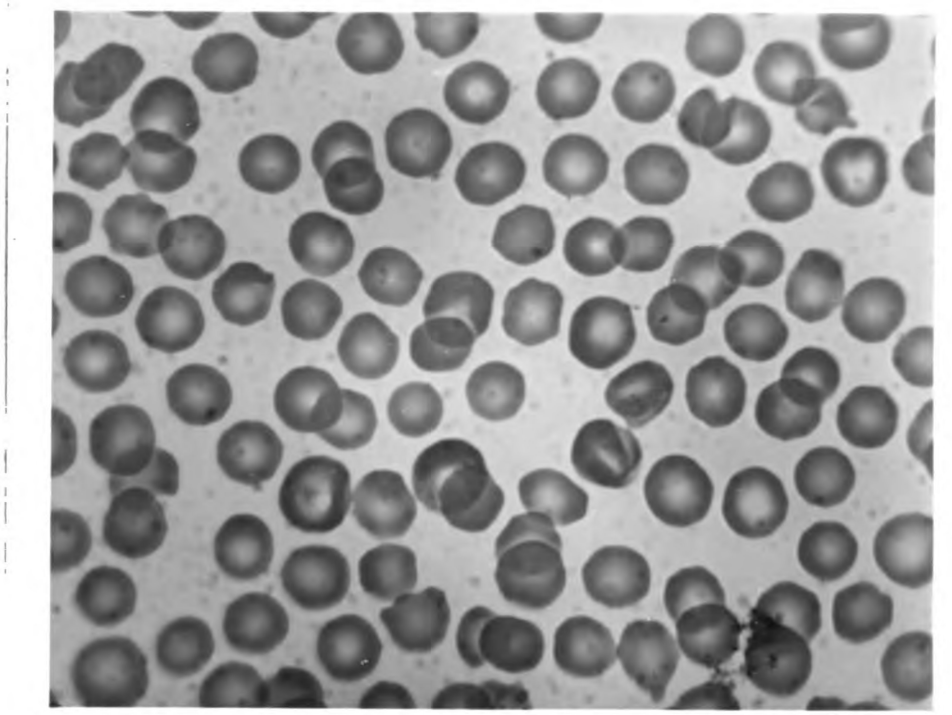

Figure 4. Photomicrograph of Wright's-stained blood smear with polychromatophilic erythrocytes. x 1500.

The greatest number of polychromatophilic erythrocytes seen in blood from untreated animals was 78. The average number of polychromatophilic cells counted per smear is illustrated (Figure 3).

Six additional animals were bled to determine the effect of blood loss on the number of abnormal red blood cells present in Wright's-stained smears and on hemoglobin and hematocrit (Tables 5, 6, 7, and 8, Figures 3 and 5). The total number of cells with basophilic stippling in this study ranged from no stippled cells to a high of 8 cells with stippling. While the average number of polychromatophilic erythrocytes gradually increased with repeated bleeding from a low of 13 to a high of 84, the total number counted did not approach the numbers noted in the animals given lead.

No appreciable change in hemoglobin or hematocrit values due to bleeding could be seen when compared to initial readings from the same animals.

Trial 2

Hemoglobin and hematocrit values from lead poisoned animals indicated mild anemia (Tables 9 and 10, Figure 5).

An average of the last PCV and Hb values taken before death on the poisoned animals compared to the average pretrial reading of the same animals revealed a reduction of 21.2% hematocrit and a 20% reduction in hemoglobin (Table 11).

Trial 3

A comparison of 2 methods to determine blood lead levels using dithizone are presented in Table 12. In general, good correlation was noted between the 2 methods. Initial levels prior to poisoning indicated normal lead values in the rabbit blood ranged from 0.16 to 0.23 ppm with an average of 0.204 ppm. Examination of the average blood lead levels

Table 5. Basophilic stippling as affected by repeated bleeding

Animal No.	Bleedings (48-hour interval) 1	2	3	4	5	6	7	8	9
16	0	0	4	1	4	3	2	0	0
17	0	-	0	1	-	0	1	0	4
18	1	0	0	0	2	1	0	2	0
19	0	0	8	5	0	0	1	0	0
20	0	0	0	0	0	0	0	0	0
21	0	-	0	0	7	0	4	5	0
Mean	0.2	0.0	2.0	1.2	2.3	0.2	1.2	1.2	0.7
± S. E.	.20	.0	1.36	1.28	1.33	.54	.62	.83	.66
P <		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Table 6. Polychromatophilic erythrocyte count as affected by repeated bleeding

Animal No.	Bleedings (48-hour interval) 1	2	3	4	5	6	7	8	9
16	8	10	51	48	55	54	71	48	62
17	16	-	38	31	-	41	78	116	111
18	24	23	44	56	87	51	62	56	27
19	13	30	88	72	67	79	84	44	58
20	12	5	15	23	44	36	80	90	103
21	4	-	9	68	91	89	130	78	50
Mean	13	17	41	48	68	58	84	72	68
± S. E.	2.81	5.75	11.6	8.06	9.06	8.67	9.69	11.5	13.2
P <		N.S.	.025	.001	.001	.001	.001	.001	.001

Table 7. Hemoglobin values as affected by repeated bleeding (gm/100 ml.)

Animal No.	Bleedings (48-hour interval)								
	1	2	3	4	5	6	7	8	9
16	11.7	10.8	12.0	10.8	9.7	10.8	10.3	10.0	12.5
17	12.0	12.0	---	11.7	---	11.7	11.7	10.2	10.4
18	11.2	10.0	9.8	10.5	9.7	11.1	10.8	10.5	11.7
19	11.6	9.5	10.0	12.3	13.0	11.1	10.8	11.4	12.5
20	11.4	11.5	10.5	10.8	11.0	---	10.3	10.0	10.3
21	13.5	12.0	12.3	11.5	10.5	10.8	11.7	11.2	10.4
Mean	11.9	11.3	10.9	11.3	10.8	11.1	10.9	10.5	11.3
± S. E.	.31	.35	.52	.28	.61	.20	.22	.20	.43
P <		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Table 8. Hematocrit values as affected by repeated bleeding (percent)

Animal No.	Bleedings (48-hour interval)								
	1	2	3	4	5	6	7	8	9
16	38	34	--	33	31.5	33	34	33	37
17	39	36	--	35	--	38	37	32	37
18	35	32	--	33	33	34	36	33	37
19	36	32	--	38	38	37	37	37	42
20	36	37	--	34	34.5	--	34	34	36
21	40	37	--	37	37	36	37	36	38
Mean	37	35	--	34	36	36	36	34	38
± S. E.	.82	.97	--	.97	1.41	.95	.61	.79	.87
P <		N.S.	--	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

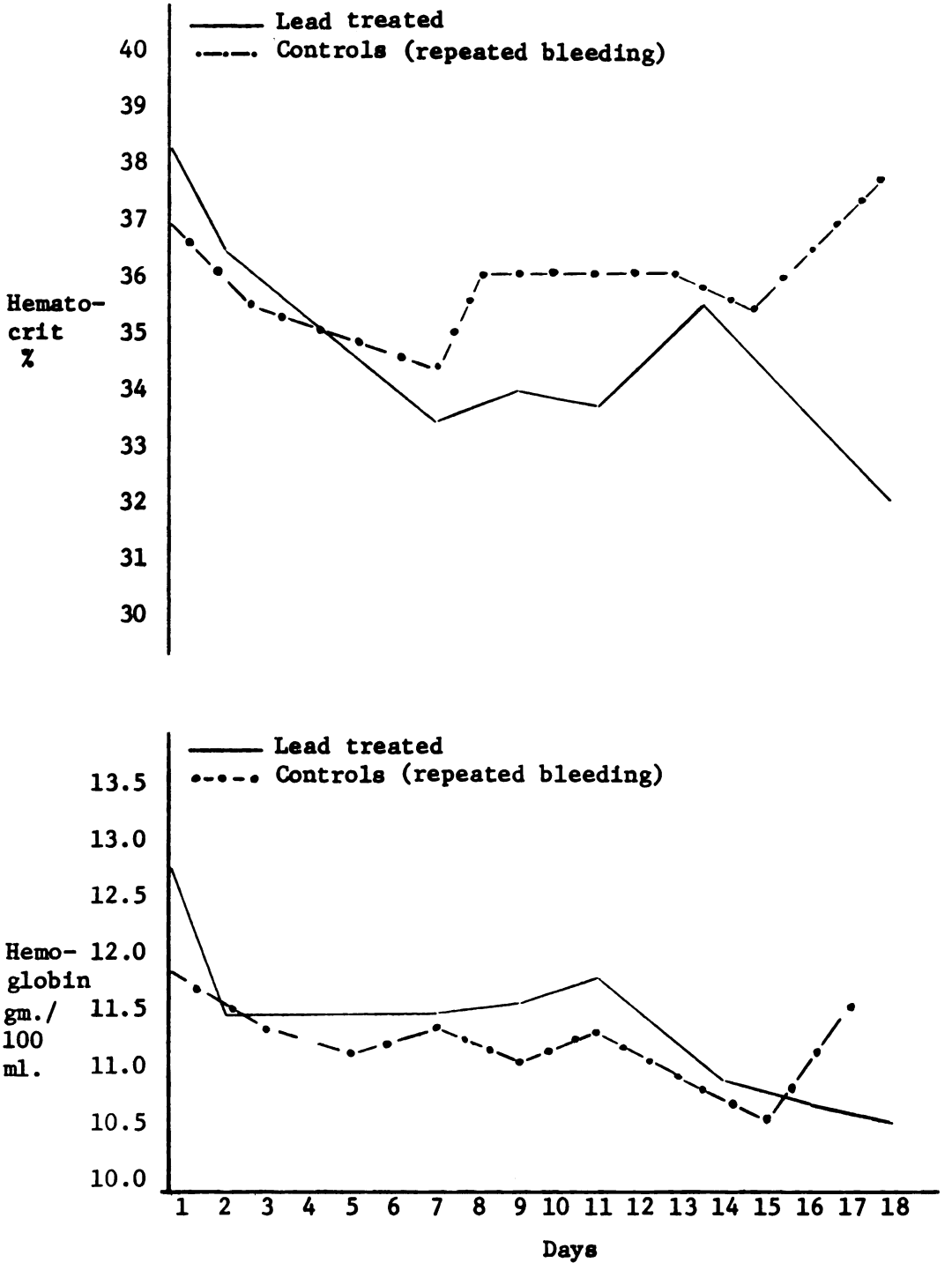

Figure 5. Average hemoglobin and hematocrit change. Lead treated animals received 100 mg. lead per day.

Table 9. Hemoglobin values for each animal at various total dosage levels (gm./100 ml.)

Animal No.	Pretrial Examination	Final Examination	Cumulative Lead Intake (mg.) 200	700	900	1100	1400	1600	1800
1	12.3		12.1	9.2**					
2	13.8		13.5	---	13.6	13.6	10.0**		
3	10.7		9.3	---	10.2	10.2	10.7	8.0**	
4	13.0		11.0	12.0	12.2	12.2	12.1	13.2**	
5	13.1		---	13.0	11.0	11.0	11.6	10.1	8.6***
6	12.8		11.4	11.8	11.6	10.8	10.4	9.4**	
7	13.8		11.9	---	12.2	12.2	11.8	13.2	12.4***
8	13.1		11.8	---	10.6**				
9	13.6		12.6	12.8	12.0	12.2	12.0	13.4	11.2***
10	10.0		8.7	8.8	8.6	7.8	9.1	8.8**	
11*	---	13.4							
12*	12.9	12.2							
13*	13.5	14.0							
14*	13.0	13.0							
15*	11.0	11.4							
Mean	12.7		11.4	11.3	11.3	11.3	10.9	10.8	10.7
± S. E.	.26		.51	.74	.48	.62	.38	.88	1.12
P <			.01	.025	.01	.01	.001	.005	.001

*Control animals.

**Last sample taken prior to death.

***Last sample taken prior to euthanasia.

Table 10. Hematocrit values for each animal at various dosage levels (percent)

Animal No.	Pretrial Examination	Final Examination	Cumulative Lead Intake (mg.) 200	700	900	1100	1400	1600	1800
1	38		38	27**					
2	42		41	---	39	40	30**		
3	33		29	---	29	29	33	22**	
4	39		36	37	37	37	39	42**	
5	39		---	37	35	33	35	25	25***
6	41		38	36	36	32	33	26**	
7	40		37	---	35	36	37	40	38***
8	40		39	---	33**				
9	40		40	38	35	36	38	40	35***
10	30		27	25	24	22	29	22**	
11*	---	40							
12*	38	36							
13*	40	42							
14*	39	40							
15*	34	34							
Mean	38		36	33	34	33	34	31.0	33.0
± S. E.	.75		1.59	2.35	1.52	1.98	1.29	3.46	3.93
P <			N.S.	.01	.01	.01	.01	.01	.05

*Control animals.

**Last sample taken prior to death.

***Last sample taken prior to euthanasia.

Table 11. A comparison of hemoglobin (gm./100 ml.) and hematocrit values (percent)

	Pretrial values	Final values	% Change
Hematocrit average (poisoned)	38.2	30.0	-21.2
Hemoglobin average (poisoned)	12.6	10.1	-20.0
Hematocrit average (control)	37.7	38.4	+ 1.8
Hemoglobin average (control)	12.6	12.8	+ 1.0

Table 12. Comparison of 2 dithizone methods for blood lead level determinations (ppm)

Animal No.	Method*	Pretrial Examination	Final Examination	Cumulative Lead Intake (mg.) 200	700	900	1100	1400	1600	1800
1	I	0.2		0.3	0.8					
	II	0.17		0.27	0.74					
2	I	0.2		0.3	---	0.5	0.7	1.1		
	II	0.22		0.29	0.46	0.46	0.65	1.40		
3	I	0.2		0.2	---	0.3	0.5	0.6	1.2	
	II	0.19		0.21	0.36	0.39	0.51	0.55	1.31	
4	I	0.2		0.4	0.5	0.4	0.4	0.6	0.5	
	II	0.23		0.38	0.43	0.44	0.40	0.61	0.68	
5	I	0.2		0.3	0.4	0.3	0.5	0.5	1.2	1.0
	II	0.20		0.30	0.40	0.40	0.46	0.54	1.00	1.00
6	I	0.2		0.4	0.5	0.4	0.6	0.5	0.9	
	II	0.21		0.38	0.41	0.40	0.49	0.56	1.10	
7	I	0.2		0.3	0.5	---	0.5	0.6	0.6	1.2
	II	0.18		0.26	0.39	0.51	0.48	0.55	0.60	1.00
8	I	0.2		0.5	---	0.5				
	II	0.20		0.43	0.50	0.54				
9	I	0.2		0.4	0.6	0.5	---	0.5	0.5	1.2
	II	0.23		0.37	0.51	0.48	---	0.50	0.56	1.24
10	I	0.2		0.3	0.5	0.5	0.5	0.6	1.3	
	II	0.21		0.30	0.49	0.50	0.48	0.55	1.12	

Table 12 (cont'd.)

Animal No.	Method*	Pretrial Examination	Final Examination	Cumulative Lead Intake (mg.) 200	700	900	1100	1400	1600	1800
11	I	0.2	0.2							
	II	0.20	0.19							
12	I	0.2	0.2							
	II	0.19	0.21							
13	I	---	0.2							
	II	---	0.20							
14	I	0.2	0.2							
	II	0.20	0.18							
15	I	0.2	0.2							
	II	0.19	0.20							
Mean	I	0.2		0.34	0.54	0.41	0.53	0.62	0.89	1.10
	II	0.2		0.32	0.47	0.45	0.50	0.66	0.91	1.08
± S. E.	I	0.0		.026	.048	.032	.04	.07	.13	.09
	II	.004		.022	.040	.018	.031	.11	.11	.08
P <	I			.001	.001	.001	.001	.001	.001	.001
	II			.001	.001	.001	.001	.001	.001	.001

*Method I - Trichloroacetic acid method (Hammond *et al.*, 1956).

Method II - Ashing method (Hurwitt and Cowgill, 1937).

when figured by days prior to death indicated a rise in ppm the closer the animals approached death (Figure 6).

Trial 4

Following death, composite liver and kidney samples from each poisoned animal were analyzed to determine tissue lead levels (Table 13). Two animals had tissue lead levels over 45 ppm, while lead was not detected in 1 animal. Lead was not detected in the tissues from the 5 control animals.

Trial 5

In the comparison between bone sections fixed in 10% buffered formalin and bone sections fixed in formalin saturated with hydrogen sulfide, significant histopathologic differences were not noted. Positive identification of lead could not be made by either method.

Trial 6

Kidney sections stained by the Ziehl-Neelsen acid-fast stain did not reveal intranuclear inclusion bodies. Liver and kidney tissue sections stained by the method presented by Thompson (1966) using sodium rhodizonate also failed to identify the presence of lead in any of the poisoned animals.

Trial 7

No obvious significant differences were noted between the H & E-stained tissue sections from poisoned animals as compared to control animals that could be considered as an aid in the diagnosis of suspected lead poisonings.

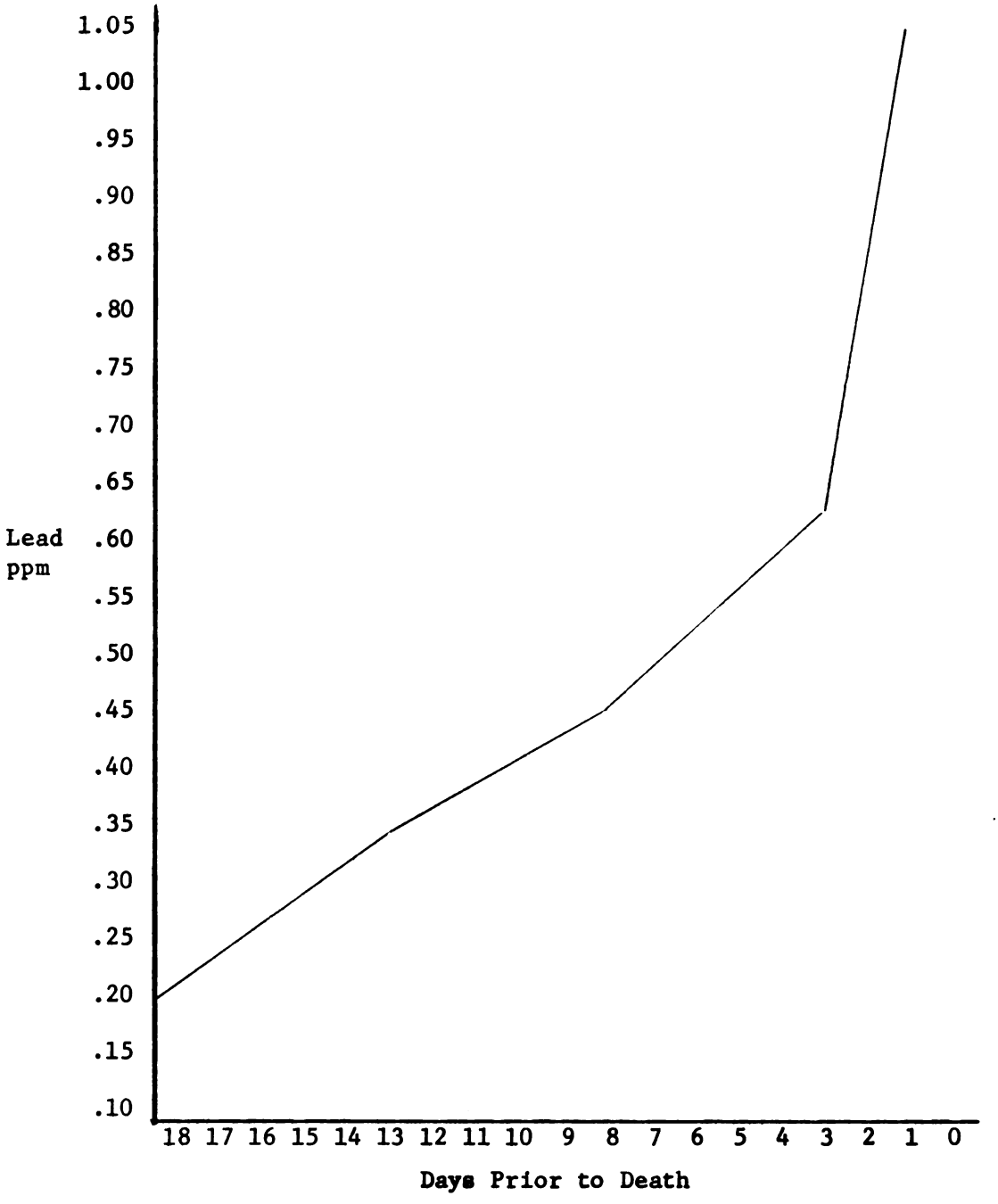

Figure 6. **Average blood lead levels figured in days prior to death (lead treated animals).**

Table 13. Lead levels in liver and kidney composite samples (gravimetric lead-chromate method)

Animal No.	Tissue Lead Levels (ppm)
1	41.0 (fetus-44.8)
2	45.5
3	45.1
4	8.0
5	34.2
6	10.0
7	7.7
8	none detected
9	7.3
10	23.8
11	none detected
12	none detected
13	none detected
14	none detected
15	none detected

DISCUSSION

This study indicated that accurate laboratory tests are available to aid in diagnosis of lead poisoning in rabbits. Common variables, such as age, sex, species, breed, care, chemical dose and route of administration were carefully controlled. However, factors unrelated to experimental design or laboratory procedures were encountered that made interpretation difficult. Individual animal variation and physical factors, unknown at the beginning of the trials, caused the greatest alteration in results.

There were 2 unexpected deaths of animals given lead orally, rabbit No. 1 and 8. The pregnancy of rabbit No. 1 and the pneumonia (*Pasteurella multocida*) of rabbit No. 8 placed added stress on the animals and possibly were contributory to early death.

Hammond *et al.* (1956) considered bovine blood containing lead between 0.3 and 0.5 ppm to be suspicious for lead exposure and any blood containing over 0.5 ppm to be diagnostic. Even on rabbits No. 1 and No. 8, using this criterion, a positive diagnosis of lead toxicosis would have been made. Examination of the blood smears stained by Wright's stain in rabbits No. 1 and No. 8 for basophilic stippling of erythrocytes would have resulted in only 1 positive lead diagnosis using standards for the dog suggested by Zook *et al.* (1970). He considered the finding of 15 BSE/10,000 RBC to be suggestive of lead poisoning and 40 or more to be pathognomonic. In this study, if one were to base a diagnosis of lead exposure on the above criteria, 3 of the 10 animals would have been overlooked.

Along with an increase in the number of basophilic stippled erythrocytes, an increase in the number of polychromatophilic erythrocytes was noted. This rise was seen in all 10 rabbits with a steady significant rise in numbers beginning at the 200 mg. lead dosage level. This rise in number would suggest the possibility of using this procedure as a screening test for lead exposure. Much lower counts were noted in the rabbits used in the bleeding effect study.

The present studies emphasize the individual differences of test animals when exposed to lead. Rabbits No. 5, 7 and 9 were alive at the end of the experiment, having received 1800 mg. of oral lead. However, while 1 rabbit had a basophilic stippled erythrocyte count of 7/10,000 RBC, another had over 65 times as many. In general, as the total dosage of lead was increased, an increase in the number of stippled cells was seen.

The 2 chemical tests for blood lead using dithizone had a very good correlation. They also compared favorably to the known lead dosage. At the 200 mg. dosage level, 9 out of 10 of the rabbits would be considered suspicious using previously established criteria. At the 700 mg. dosage level, all rabbits would be considered as having levels diagnostic for lead exposure. Except in the "added stressed" animals, all rabbits had blood lead levels in the area of 1.0 ppm just prior to death or at the end of the experimental period. While no significant difference in final results was noted between these 2 dithizone tests, a comparison of the equipment and skill needed to perform both tests revealed that the trichloroacetic acid method suggested by Hammond was much better than the ashing method. The necessity of a muffle furnace with accurate temperature controls to prevent excess lead vaporization and the extreme skill needed in the back titrating procedure are 2 reasons to preclude the use of the ashing method. Another advantage of the trichloroacetic

acid method was in the shorter total time needed to perform the tests. The average time for completion of an individual determination was approximately 2-1/2 hours for method I, while 5 hours were required to run the ashing method. Neither method should be considered sensitive enough to be used in situations requiring the quantitative determinations of lead, such as in a research laboratory. Where this type of need exists, newer, more sensitive methods, including the atomic absorption spectrophotometer, should be considered. However, these dithizone methods, when used as diagnostic aids, can be useful in determining possible lead exposure.

In this study routine H & E-stained sections as well as special staining methods (Trials 5, 6 and 7) failed to aid in identifying lead or animals poisoned by lead. While various reports have indicated the presence of lead produced inclusion bodies in kidney sections of man and animals (Calvery *et al.*, 1938; Fauts and Page, 1942; Morgan *et al.*, 1966; Locke *et al.*, 1969), acid-fast intranuclear inclusion bodies are not always present and are not specific (Jubb and Kennedy, 1970). In this experiment and in this species, inclusion bodies were not observed. Further work needs to be done on this aspect of lead toxicosis to determine whether this test could be improved to aid in lead poison identification.

SUMMARY

Blood and tissues from lead poisoned rabbits were utilized to compare various laboratory tests and procedures routinely used as aids in the diagnosis of lead toxicosis. The 2 most common methods to determine lead exposure in the living animal include chemical analysis of the blood for lead concentration and examination of stained blood smears for immature, abnormal erythrocytes.

Good correlation was noted between blood lead levels and lead dosage. This correlation was determined by 2 dithizone lead methods. The trichloroacetic acid method, as suggested by Hammond *et al.* (1956) was the more accurate, simpler and faster test.

Basophilic stippling and polychromatophilic staining of erythrocytes in Wright's-stained smears increased significantly following ingestion of lead. Animals receiving lead had a wide range in the number of stippled cells counted. This would limit its use as a single diagnostic tool. There was a consistent increase in the number of polychromatophilic erythrocytes; therefore, this could be considered a more reliable test.

Results of the gravimetric lead-chromate method for tissue lead levels had little correlation with the known amounts of lead ingested.

Tissue sections stained with sodium rhodizonate, acid fast stain, and bone sections treated with hydrogen sulfide failed to aid in identifying lead poisoned animals.

REFERENCES

REFERENCES

Alderson, J.: Effects of lead on the organism. Lancet, ii, (1852): 73-75, 95-98.

Alexander, H.: Volumetric determination of lead. Engineering and Mining J., 55, (1893): 298.

Allcroft, R.: Lead poisoning in cattle and sheep. Vet. Rec., 63, (1951): 5183.

Aub, J. C., Fairhall, L. T., Minot, A. S., and Reznikoff, P.: *Lead Poisoning.* Medicine Monographs, Williams and Wilkins Co., Baltimore, Md., (1926).

Balbo, W., Gualdi, G., and Marucci, V.: Latest contribution in the study of behavior of delta aminolevulinic acid in blood and urine in subjects exposed to the prolonged toxic action of lead. Folia Medica, 48, (1965): 161-167.

Bambach, K., and Burkey, R. L.: Microdetermination of lead by dithizone. Ind. Eng. Chem. Ann. Ed., 14, (1942): 904.

Benjamin, M. M.: *Outline of Veterinary Clinical Pathology*, 2nd ed. The Iowa State Univ. Press, Ames, Iowa, (1961): 56-58.

Benson, P. F., and Chisolm, J. J.: A reliable qualitative urine coproporphyrin test for lead intoxication in young children. J. Ped., 56, (1960): 759-767.

Berman, E.: The determination of lead in blood and urine by atomic absorption spectrophotometry. Atomic Absorption Newsletter, 3, (1964): 111-114.

Berry, A. P.: Lead poisoning in a litter of 5-week-old puppies. Vet. Rec., 79, (1966): 248.

Blansdorf, E.: Bleiliteratur Schriften ans dem Gesamtgebiet der Gewerbehygiene, Vii, part ii, (1922): 201-240.

Blaxter, K. L.: Lead as a nutritional hazard to farm livestock. II. The absorption and excretion of lead by sheep and rabbits. J. Comp. Path., 60, (1950): 140-159.

Bond, E., and Kubin, R.: Lead poisoning in dogs. Vet. Med., 44, (1949): 118-123.

Bradley, J. E., Powell, A. E., Niermann, W., McGrady, K. R., and Kaplan, E.: The incidence of abnormal blood levels of lead in a metropolitan pediatric clinic. J. Pediat., 49, (1956): 1-6.

Bruin, A., and Hoolboom, H.: Early signs of lead exposure. Brit. J. Indust. Med., 24, (1967): 203-212.

Burnham, A. C.: The prevention of industrial lead poisoning in English factories. J. Indust. Hygiene, 5, (1923): 57-61.

Calvery, H. O., Laug, E. P., and Morris, H. J.: The chronic effects on dogs of feeding diets containing lead acetate, lead arsenate and lead trioxide in varying concentrations. J. Pharmacol. & Exptl. Therap., 64, (1938): 364-387.

Chisolm, J. J.: Disturbances in the biosynthesis of heme in lead intoxication. J. Pediat., 64, (1964): 174-187.

Christian, R. G., and Tryphonas, L.: Lead poisoning in cattle: Brain lesions and hematologic changes. Am. J. Vet. Res., 32, (1971): 203-216.

Coffin, R., Phillips, J. L., Staples, W. I., and Spector, S.: Treatment of lead encephalopathy in children. J. Pediat., 69, (1966): 198-206.

Crookes, W.: *Select Methods of Chemical Analysis*. Longmans, Green and Co., Ltd., London, (1894): 318.

Cushman, A. S., and Hayes-Campbell, J.: On the volumetric determination of lead. J. Am. Chem. Soc., 17, (1895): 901.

Denis, W., and Minot, A. S.: A method for determination of minute amounts of lead in urine, feces and tissues. J. Biol. Chem., 38, (1919): 449.

Dodd, D. C., and Staples, E. L. J.: Clinical lead poisoning in the dog. New Zealand Vet., 4, (1956): 1-7.

Ellis, R. W.: Urinary screening tests to detect excessive lead absorption. Brit. J. Ind. Med., 23(4), (1966): 263-270.

Exner, F. F.: The rapid precipitation of metals in the electrolytic way. J. Am. Chem. Soc., 25, (1903): 896.

Fairhall, L. T.: Lead studies. I. The estimation of minute amounts of lead in biological material. J. Indust. Hygiene, 4, (1922): 9-20.

Fauts, P., and Page, J.: The effect of chronic lead poisoning and arterial blood pressure in dogs. Am. Heart J., 24, (1942): 329.

Feigl, F., and Suter, H. A.: Analytical use of sodium rhodizonate. Indust. & Engr. Chem., 14, (1924): 840-842.

Finner, L. L., and Calvery, H. O.: Pathologic changes in rats and in dogs fed diets containing lead and arsenic compounds. Arch. Path., 27, (1939): 433-446.

Fox, J. J.: Solubility of lead sulfate in concentrated solutions of sodium and potassium acetates. J. Am. Chem. Soc., 95, (1909): 880.

Frankel, L. K.: The detection of lead in urine. Chem. News, 68, (1893): 5.

Friend, J. N.: *Inorganic Chemistry*. Charles Griffin & Co., Ltd., London, 5, (1917): 441.

Garrod, A. E.: The occurrence and detection of haematoporphyrin in the urine. J. Physiol., 13, (1892): 598-620.

Gilfillan, S. C.: Lead poisoning and the fall of Rome. J. Occup. Med., 7, (1965): 53-60.

Hamilton, A.: Lead poisoning in American industry. J. Indust. Hygiene, 1, (1919): 8-21.

Hammond, P. B., Wright, H. N., and Roepke, M. H.: A method for the detection of lead in bovine blood and liver. Univ. of Minnesota Agricultural Expt. Sta. Bull. No. 221, (1956).

Haring, C. M.: Investigations of livestock condition and losses in the Selby Smoke zone. U. S. Bureau of Mines Bull., 98, (1915).

Horwitt, M. K., and Cowgill, G. R.: A titrimetric method for the quantitative estimation of lead in biological materials. J. Biol. Chem., 3, (1937): 553-564.

Hutton, R. M.: Lead poisoning: A compilation of present knowledge. Provincial Board of Health of Ontario, Toronto, (1923).

Jubb, K. V. F., and Kennedy, P. C.: *Pathology of Domestic Animals*, Vol. 2. Academic Press, New York, (1970): 388-391.

Key, J. A.: Lead studies. IV. Blood changes in acute lead poisoning in rabbits. Am. J. Physiol., 70, (1924): 86-101.

Kopito, L., and Schwochman, H.: Determination of lead in urine by atomic absorption spectroscopy using coprecipitation with bismuth. J. Lab. Clin. Med., 70(2), (1967): 326-332.

Lane, R. E.: The role of punctate basophilia in the control of industrial plumbism. J. Indust. Hygiene, 13, (1931): 276.

Legge, T. M., and Goodby, K. W.: *Lead Poisoning and Lead Absorption.* Edward Arnold, London, (1912).

Locke, L. N., Bagley, G. E., Frickie, D. N., and Young, L. T.: Lead poisoning and aspergillosis in an Andean condor. J.A.V.M.A., 155, (1969): 1052-1056.

McCord, C. P., Holden, F. R., and Johnson, J.: Basophilic aggregation test in the lead poisoning epidemic of 1934-1935. Am. J. Public Health, 25, (1935): 1089.

Mitchell, G. A.: Lead poisoning of dogs. Canad. J. Comp. Med. and Vet. Sci., 4, (1940): 170-173.

Morgan, J. M., Hartley, M. W., and Miller, R. E.: Nephropathy in chronic lead poisoning. Arch. Int. Med., 118, (1966): 17-29.

Ophuls, W.: Experimental chronic nephritis. J.A.M.A., 48, (1907): 483-490.

Patten, N. E.: Report on baking powders. J. Assoc. Official Agric. Chem., 2, (1917): Part 2, 214.

Patten, H. E.: Report on baking powders. J. Assoc. Official Agric. Chem., 3, (1920): 429.

Perlstein, M. A., and Attala, R.: Neurological sequelae of plumbism in children. Clin. Pediat., 5, (1966): 292-298.

Pope, F. J.: Volumetric estimation of lead. J. Am. Chem. Soc., 18, (1896): 737.

Pratt, E. E.: Report of the New York State Factory Investigating Commission, (1913): 2.

Raistrick, A., and Jennings, G.: *A History of Lead Mining in the Pennines.* Longmans, Green & Co., Ltd., London, (1965): 296.

Scott, H. M.: Lead poisoning in small animals. Vet. Rec., 75, (1963): 830-833.

Scott, W. W.: *Standard Methods of Chemical Analysis*, 5th ed. D. Van Nostrand Co., New York, (1939): 523-524.

Seeker, A. F., and Clayton, H. D.: The determination of lead in phosphate and alum baking powders. J. Assoc. Official Agric. Chem., 1, (1915): 264.

Slavin, W., Rieders, R., and Cordova, V.: The determination of certain toxicological trace metals by atomic absorption spectrophotometry. Atomic Absorption Newsletter, 3(17), (1964): 7-10.

Slavin, W., and Sprague, S.: The determination of trace metals in blood and urine by atomic absorption spectrophotometry. Atomic Absorption Newsletter, 3, (1964): 1-6.

Snowden, R. C.: The electrolytic precipitation of lead from acetate solution. J. Phys. Chem., 10, (1906): 500.

Tanquerel des Planches, L.: Traite des Maladies de Plomb ou Saturnines. Ferra, Paris, (1839).

Teleky, L.: German literature on the white lead question. J. Indust. Hygiene, 4, (1922): 100-123.

Thompson, S. W.: *Selected Histochemical and Histopathological Methods.* Charles C. Thomas & Co., Springfield, Ill., (1966): 587-588.

U. S. Bureau of Labor Statistics Bulletin 141: Lead poisoning in the smelting and refining of lead, 1912.

Waldron, H. A.: The anaemia of lead poisoning: A review. Brit. J. Indust. Med., 23, (1966): 83-100.

Walsh, A.: The application of atomic absorption spectra to chemical analysis. Spectrochim. Acta., 7, (1955): 108-117.

Weiser, H. B.: The physical character of precipitated lead molybdate and its importance in the estimation of molybdenums and lead. J. Phys. Chem., 20, (1916): 640.

Williams, M. K.: Blood lead and haemoglobin in lead absorption. Brit. J. of Indust. Med., 23, (1966): 105.

Willis, J. B.: Determination of lead and other heavy metals in urine by atomic absorption spectroscopy. Anal. Chem., 34, (1962): 614-617.

Zook, B. C., Carpenter, J. L., and Leeds, E. B.: Lead poisoning in dogs. J.A.V.M.A., 155, (1969): 1329-1342.

Zook, B. C., McConnell, G., and Gilmore, C. E.: Basophilic stippling of erythrocytes in dogs with special reference to lead poisoning. J.A.V.M.A., 157, (1970): 2092-2099.

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