Effects of chronic lead exposure on pregnant sheep and their progeny

by

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INTRODUCTION

In humans there is evidence that a greater incidence of central nervous system aberrations occurs during the adolescent stage of children born to mothers who had elevated blood lead levels during pregnancy. There are also reports concerning intrauterine exposure of human fetuses to high levels of lead through the maternal circulation. Angle and McIntire (1964) reported that there is a definite fetal risk due to intrauterine exposure of high concentrations of lead in maternal blood, especially during the first trimester of pregnancy. Palmisano et al. (1969) reported evidence of neurologic defects, intrauterine growth retardation, and postnatal failure to thrive in a ten-week-old infant born to a mother who drank illegal lead-containing whiskey during pregnancy.

In 1969 the Toxicology Section of Veterinary Diagnostic Laboratory, College of Veterinary Medicine, obtained a contract from the National Air Pollution Control Administration to study the effects of prenatal sublethal exposure of lead in sheep and their progeny. This investigation was a part of that project.

OBJECTIVES

The specific objectives of this work unit were:

1. To produce sublethal lead poisoning in pregnant ewes by continual exposure to lead in their feed during the entire period of gestation (five months).
2. To observe and record any clinical manifestations of lead poisoning during this period of time and correlate such changes with:
   a. hematological changes
   b. levels of lead in blood and urine
   c. levels of delta-aminolevulinic acid in urine
3. To study and compare the above parameters in the progeny of dams exposed to lead during pregnancy to those born to dams not exposed to lead during pregnancy.

LITERATURE REVIEW

History

Lead was one of the first metals discovered by man and has been widely used for domestic, industrial, and medicinal purposes during the last two thousand years. Some of the clinical signs of lead poisoning were known to the ancients long before they were ascribed to the action of lead. Hippocrates (370 B.C.) was probably the first to recognize lead poisoning. He reported severe attacks of colic in a man who extracted metals. Nicander, in the second century (B.C.), reported that a relationship existed between constipation, abdominal pain, and pallor to the action of lead on the human body. Several reports about the development of this typical lead colic appeared in the literature in the seventeenth century. Citois (1616) reported that wine contaminated with lead (bad wine) was the cause of colic.

An experimental study of lead poisoning was conducted by Orfila (1814) who administered lead orally as well as intravenously and reported that lead was more toxic orally. Soldering, painting, and pottering industries constituted the largest industrial hazard during the nineteenth century leading to chronic lead poisoning in workers. Teleky (1909), Hamilton (1914), and Oliver (1914) did extensive work on the public and industrial hygiene aspects of lead poisoning in Germany, United States, and England, respectively. Haeger (1960) reported that while lead poisoning was formerly an extremely dangerous disease and often fatal, official statistics have shown a progressive decrease in the frequency of serious cases in Europe, as well as in America, during the last two-three decades. The

decline was likely a result of improved hygienic and medical supervision of workers in lead industries. However, Bloomfield (cited by Johnstone, 1957) attributed this decreased incidence of lead poisoning in workers to the development of modern techniques for the determination of lead in air, blood, and urine. Browning (1969) reported that between 1900 to 1958, the cases of industrial lead poisoning in Britain had fallen from 1,058 with 38 deaths in 1900 to 55 cases with no fatalities in 1958.

Haeger (1960) reported that lead poisoning is still very common in workers in ship and car building, storage battery, and pottery industries. She studied lead poisoning in 185 workers employed in nine different industries, namely, soldering, shipbuilding, shipbreaking, lead alloy melting, tin soldering, and storage battery industries. Using lead values in blood and urine and amino-levulinic acid values in urine, she reported that incidents of lead poisoning in these workers is still high.

The history of lead poisoning in animals is more recent. Morgan (1924) reported chronic lead poisoning in sheep and ponies and observed that animals might acquire a taste for lead. Gardner (1924) reported development of rickets in lambs when kept on lead mining areas of North Derbyshire, England. Since then many reports have appeared in the literature concerning incidents of lead poisoning in animals.

Lead Poisoning in Man

Incidence in man

Lead poisoning in human adults occurs largely because of industrial exposure or accidents (Aub et al., 1925; Ashe, 1943). However, in children lead poisoning results because of pica and concomitant lead ingestion

(Byers, 1959). Wiener (1970) observed that age in children is a critical factor and mouthing of foreign objects is frequent before 18 months of age. Chronic lead poisoning in children occurs most commonly as a result of ingestion of lead containing substances (Cohen and Ahrens, 1959). Paints containing high levels of lead from walls and woodworks are the primary source of lead poisoning in children (Millichap et al., 1952; Mellins and Jenkins, 1955; Chislom and Harrison, 1956).

Lead poisoning in children is still a very serious problem in slum areas of the major cities in the United States. Bradley et al. (1956) studied 664 children suspected of lead poisoning in Baltimore, Maryland, and reported that one-third of them had abnormally high blood lead levels. Mellins and Jenkins (1955) diagnosed 21 cases of lead poisoning in Chicago children during 1953. Griggs et al. (1964) studied 801 children between the ages of 12-60 months in Cleveland suspected of lead poisoning and reported that 27% had abnormally high blood lead levels. Lead poisoning can also occur in adults and children due to consumption of lead contaminated water. Bacon et al. (1967) reported three cases of lead poisoning caused by erosion of lead flakes from the inside of water pipes.

Clinical syndrome in man

In humans lead poisoning can result in three distinctly different clinical entities (Zavon, 1963): a) abdominal syndrome, b) neuromuscular syndrome, and c) central neurological syndrome (encephalopathy).

In the abdominal syndrome, the clinical signs are abdominal pain, recurrent vomiting, anorexia, constipation, and loss of weight. Muscular weakness and palsy are principal clinical signs in the neuromuscular syn-

drome. This kind of palsy is often unilateral, occasionally bilateral, and only rarely affects more than one or two extensor muscle groups. The central neurologic syndrome (encephalopathy) usually occurs in children who have consumed large quantities of flaked lead paint. It also develops in children and adults who have inhaled massive amounts of inorganic lead dust or fumes. The clinical signs in general are headache, tremors of the lips and hand, slight bilateral papilledema, loss of motor activity in the right arm, and unsteady gait. However, encephalopathy in children that have ingested large quantities of lead is more severe and frequently fatal. McKhann and Vogt (1933) reviewed 89 hospital cases in Boston and found 12 to be latent and 77 showing signs of lead intoxication. Of the 77, 11 died, 45 had encephalitis, 4 had neuritis, and 12 had permanent sequela with one-half being mentally retarded. Levison and Zeldes (1939) studied 26 cases of lead poisoning in children in Chicago and found that five of them died as a result of acute poisoning. Nine were studied for a period of three and one-half years. One was blind, mute, and helpless; two were epileptic and mentally subnormal; and the remainder were normal. Mellins and Jenkins (1955) also reported 21 cases of poisoning in Chicago during 1953. Five died because of encephalopathy, 14 out of 15 were retarded, and the rest had disturbed language ability.

Lead Poisoning in Animals

Accidental lead poisoning has been reported in dogs, cattle, sheep, horses, swine, cats, foxes, and waterfowl. Lead poisoning is very common in dogs and cattle and has been reported from England, New Zealand, Africa, United States, and many other countries.

Incidence in dogs

Six cases of lead poisoning in dogs were reported by Lieberman (1948), 60 by Zook et al. (1969) in the United States, 11 in New Zealand by Dodd and Staples (1956), and 28 in Rhodesia by Scott (1963). Zook et al. (1969) reported that common sources of lead for dogs are lead painted objects, linoleum, lead paints, and roofing material. Lead paints are the common sources of poisoning in dogs according to Molpus (1958).

Clinical syndrome in dogs

Lead poisoning in dogs occurs in two forms: 1) abdominal and 2) nervous (Dodd and Staples, 1956; Wilson and Lewis, 1963; Zook et al., 1969).

In the abdominal form, the clinical signs are abdominal pain, anorexia, vomiting, diarrhea, and dysentery. In the nervous form, hysterical barking, restlessness, muscular weakness and tremors, frothing at the mouth, and champing of the jaws are observed.

Anemia, stippling of red blood cells, and presence of many immature erythrocytes in the peripheral blood of the lead poisoned dogs has been reported by Bond and Kubin (1949). Similarly, Zook et al. (1969) reported numerous stippled and immature erythrocytes, anemia, leucocytosis, absolute neutrophilia with a shift to the left, eosinophilia, and monocytopenia in the blood of poisoned dogs.

Incidence in cattle

In ruminants, lead poisoning is most common in cattle, especially young calves. Allcroft and Blaxter (1950) reported 190 cases of lead poisoning in cattle, of which 91 were confirmed as positive. Orr (1952) mentioned that of 96 cases of lead poisoning in farm animals, 77 were cattle.

He further stated that the importance of lead poisoning on bovine toxicology could be estimated by the fact that out of 110 cattle poisoned, 77 were poisoned by lead. Todd (1962) reported that of deaths in young calves in Ireland, 4.5% were a result of lead poisoning. Acute lead poisoning has been and continues to be a significant cause of losses in calves and older cattle in the United States (Little and Sorenson, 1969). Buck (1970) reported 24 episodes of lead poisoning in cattle in Iowa. Hatch and Funnell (1969) in Canada reported 175 cases of lead poisoning in cattle during the last 15 years.

The usual sources for lead toxicosis in ruminants are paints, grease, used crankcase oil, roofing insulation, discarded lead acid batteries, and lead containing insecticides (lead arsenate) (Buck, 1970). Buck et al. (1971) reported that some of the old paint bases and machinery grease may contain upwards of 50% lead. He further stated that the grass growing near highways and roads may contain up to 500 ppm of lead, which comes from automobile exhaust. Chow (1970) reported that the concentration of lead in grass growing along U.S. Highway 1 and the Baltimore Washington Parkway ranged from 290 to 825 ppm lead on a dry weight basis.

Clinical syndrome in cattle

The clinical signs of lead poisoning are excessive salivation, abdominal discomfort, grinding of the teeth, twitching of the ears, anorexia, rumen atony, blindness, pushing the head against solid objects, circling, and convulsive seizures (Buck, 1970).

Incidence in wild birds

Lead poisoning has also been reported in wild birds. Wickware (1940) and Coburn et al. (1951) observed lead poisoning in wild ducks and other waterfowl, respectively. Rac and Crisp (1954) recorded a case of lead poisoning in domestic ducks which resulted in the death of five of 11 ducklings in England. Bellrose (1959) presented a thorough documentation of lead poisoning in waterfowl in the United States. The incidence of lead poisoning in the pen-raised mallard, black duck, and wild pheasant was discussed by Irby et al. (1967) and Hunter and Rosen (1965).

The usual cause of lead poisoning is the ingestion of lead shot from lakes and marshes which are retained by the gizzard (Bagley and Locke, 1967).

Metabolism of Lead

Absorption

Lead enters the body through the respiratory and alimentary tracts and through the skin. In children, absorption from the alimentary tract is important clinically because most cases of lead poisoning occur as a result of ingestion of paints containing high amounts of lead (Cohen and Ahrens, 1959; Bradley et al., 1956; Millichap et al., 1952; Jacobziner and Raybin, 1957; Berman, 1966).

Most industrial poisonings and a few cases of poisoning in children follow the inhalation of lead dust or fumes (Berman, 1966). Lead can be absorbed from all portions of the respiratory tract including the nasal passages (Minot, 1924). Aub et al. (1925) reported that absorption from lungs is more rapid and complete than from the gastro-intestinal tract.

In animals lead poisoning occurs most commonly as a result of ingestion of substances containing high concentration of lead (White and Cotchin, 1948; Blaxter, 1950; Allcroft, 1951; Little and Sorenson, 1969; Buck, 1970). However, slight absorption of lead from the respiratory tract takes place in industrial areas (Allcroft, 1951).

Allcroft (1950) demonstrated that comparable elevated blood lead levels were obtained when different compounds of lead (lead phosphate, oxide, and carbonate) were ingested in similar amounts by calves. It was concluded that the different forms of lead were absorbed at the same rate from the gastro-intestinal tract of animals.

Inorganic lead is very slightly, if at all, absorbed through the intact skin (Aub et al., 1925). However, organic lead (tetraethyl lead) is readily absorbed through skin (Kehoe et al., 1934).

Transportation

After absorption, whether from the respiratory or digestive tract, lead enters the circulation, and 90% is bound to red blood cells (Behrens and Pachur, 1927; Blumberg and Scott, 1935; Schubert and White, 1952). Recent in-vitro, radioactive studies have shown that 95% of the lead in the circulation enters the erythrocytes, and it is not adsorbed to the red blood cell membrane (Barltrop, 1968).

Deposition

Lead is deposited as diphosphates of lead in soft tissues, particularly brain, lung, liver, spleen, and marrow. From there it is transferred to bones and stored as insoluble lead triphosphates (Aub et al., 1925; Kehoe et al., 1933). This lead is quite inert and insoluble under conditions of

normal hydrogen ion concentration, but any significant change in the latter toward the acid or alkaline side will cause its excretion (Aub et al., 1925).

Excretion

Lead is eliminated in the urine, feces, and milk. In humans the fecal excretion of lead is usually greater than the urinary excretion (Aub et al., 1925). Kehoe et al. (1935) reported that in man, a daily excretion greater than 0.6 mg was abnormal. Kehoe and Thamann (1929) indicated that lead excretion proceeds gradually. Using rabbits, it was found that about one-fourth of the quantity ingested was excreted during the first week and the rest within 30 days.

After absorption has ceased, the rate of excretion of lead depends mainly on the rate of its mobilization from skeletal deposits. Excretion can be increased by factors which favor mobilization of lead from the bone such as acidosis, low calcium and phosphorus, and administration of parathyroid hormone (Aub et al., 1925). Lead is excreted in the milk of both man and animals. Normal values of lead in milk of man and cattle are 0.00-0.05 mg/l and 0.009 mg/l, respectively (Kehoe et al., 1940; Hammond and Aronson, 1964).

Effects of Lead on Body Systems

Reproductive

Lead has been considered a "race poison" because its effects are not confined to the individuals exposed but are passed on to their progeny (Hamilton, 1925). Lead crosses the placental barrier very easily (Flury, 1934; Kehoe et al., 1933). Baumann (1933) supported this view on the basis

of his observation that radioactive lead fed to pregnant mice appeared very quickly in the fetal tissues. Lead, from the time of Tanquerel (1839), has been known to act as an abortifacient, and it increases the frequency of miscarriages, stillbirths, and premature birth in lead workers. Paul (1860) and Flury (1934) reported disturbances of menstruation in women poisoned with lead and observed development of transitory sterility in women lead workers. Normal fertility returned after cessation of exposure to lead. Hamilton (1925) reported that if lead exposed animals conceived, they had miscarriages, intrauterine fetal deaths, premature births, and abortions. These observations were made in dogs, cats, rabbits, and guinea pigs. Paul (1860) reported that of 123 pregnancies in women lead workers, the pregnancy terminated in fetal death in 73 cases and that of the 50 live births, 35 died by three years of age. Pindborg (1945) stated that 60% of pregnancies in the first trimester ended in abortion when lead oxide was ingested as an abortifacient by Danish women. These observations, with or without control data, led to the widespread enactment by 1910 of labor codes forbidding the employment of women in industry involving lead hazard. This may be the reason that such cases are not reported in the more recent literature.

Allcroft (1950) reported two abortions out of four ewes when they were fed lead acetate at a rate of 50 mg/kg. James et al. (1966) reported that lead acetate, when given orally to two ewes at a rate of five mg/kg for 45 days of gestation, did not cause abortion, whereas two other ewes aborted and died after 59 and 106 days of gestation, when lead acetate was given at a rate of nine mg/kg.

Blood

A relationship between lead poisoning and the appearance of basophilic granules in red blood cells in peripheral blood was first reported by Behrend (1899). Grawitz (1900) reported that basophilic granules in lead poisoning differed from nuclear substances in staining and concluded that they are of different origin. Basophilic stippling of erythrocytes occurs as a result of action of lead on immature red blood cells. In lead poisoning, the percentage of stippled cells is higher in bone marrow than in the peripheral blood, indicating that lead attacks the red blood cells before they are released from the bone marrow (Henning and Keilhack, 1940; McFadzean and Davis, 1949; Pirrie, 1952). Sano (1955a, 1955b, 1958) reported that in lead poisoning, basophilic stippling in red blood cells results from inhibition of maturation, retention of mitochondria, and concentration of ribonucleic acid (RNA) in these cells.

Binnedjik (1880, cited by Stokvis, 1895) reported the occurrence of porphyrin in urine from a patient with lead poisoning. About two decades later, Gerrod (1900) suggested that porphyrinuria was a result of inhibitory effects of lead on heme synthesis. Watson (1936) also reported that lead interfered with the synthesis of hemoglobin. Dresel and Falk (1956) and Goldberg *et al*. (1956) were able to show an inhibition by lead of the *in-vitro* synthesis of porphobilinongen (PBG) from aminolevulinic acid (ALA) in hemolyzed chicken red blood cells. A corresponding inhibition was demonstrated *in vivo* in lead-poisoned rabbits (Sano, 1958; Tanabe, 1959). The increased urinary excretion of ALA by lead workers may be due to this inhibition (Haeger, 1957, 1958; Griggs and Harris, 1958; Tishkoff *et al*., 1958).

Rimington (1937) and Watson (1950) reported that lead inhibits various enzymes catalyzing different steps of heme biosynthesis. Studies by Licthman and Feldman (1963) made the enzymatic action of lead clearer when they reported evidence of decreased activity of the enzyme ALA dehydrase in erythrocytes obtained from patients with lead poisoning. Further studies on the effects of lead on heme biosynthesis revealed that increased urinary excretion of ALA resulted from the decreased activity of ALA dehydrase in erythrocytes. The amounts of urinary ALA closely correlated with the blood lead levels and the duration of exposure to lead (Nakao and Yano, 1968).

Brain

The first report of lead causing impairment of the normal processes of the growth and development of the cerebral cortex in children was given by Byers and Lord (1943). It was concluded that plumbism in children, whether manifested by encephalopathy or not, leads to failure or impairment of the normal processes of growth and development of the cerebral cortex even in those cases discharged from the hospital as completely recovered. Similar views were expressed by McKhann and Vogt (1933) and Levison and Zeldes (1939). Gibbs and Macmahon (1955) stated that prognosis of lead encephalopathy in children should be guarded at least until the age of 16 because it is not until then that the intellectual development normally approaches its peak. Byers and Lord (1943) recorded 20 nonencephalitic cases of lead poisoning in children and noted that 19 out of 20 were mentally retarded. Lead poisoning in children has been correlated with impaired intelligence quotient, speech, and fine motor coordination (Jenkins and Mellins, 1957).

MATERIALS AND METHODS

Experimental Design

On November 1, 1969, 21 aged Columbia-Rambouillet crossbred ewes were purchased and housed on a private farm. These animals were acclimated to the surroundings for a period of one month. During this period, they were fed chopped alfalfa and bromegrass hay plus one-fourth pound of pelleted grain mixture consisting of 600 parts corn, 300 parts soybean meal, and 50 parts binder material. This ration served as a basic concentration to which finely powdered lead was added at a calculated concentration of one percent (10,000 ppm). The concentrate was mixed by a commercial feed company, however, and the control pellets subsequently were found to be contaminated with 420 ppm lead. A fresh batch of "lead-free" control ration was prepared. The experimental concentrate was found to contain 7,050 ppm instead of 10,000 ppm. Trace minerals and salt were provided to each sheep ad libitum.

Samples of venous blood and urine were obtained from all sheep on three different days before starting the experiment (November 19 and 25 and December 2, 1969). These samples served as pre-exposure data. The following chemical and hematological determinations were made: 1) blood lead, 2) urine lead, 3) packed cell volume, 4) red blood cell count, 5) white blood cell count, 6) hemoglobin, and 7) urine delta-aminolevulinic acid.

On December 6, 1969, the animals were divided into exposed and control groups having 12 and nine sheep, respectively. Attempts were made to pair them on the basis of body weight and packed cell volume.

Beginning December 8, 1969, the exposed group of sheep were fed lead containing pellets at a rate of one-fourth pound/head/day. The control sheep were fed pellets without lead at the same rate. Sheep in both groups were given chopped alfalfa hay twice a day ad libitum.

Blood and urine samples were obtained from the exposed group every other day for the first week, twice weekly during the second week, and once weekly thereafter. Samples were collected from the control group once a week for the first two weeks and afterwards once a month.

Three days after the beginning of the experiment, a Dorset ram, leased from the Iowa State University sheep herd, was turned into both the experimental and control group every morning and evening, respectively. The dates that ewes were mated were noted.

Blood was drawn directly from either the right or left external jugular vein using 15 gauge stainless steel needles and was collected in heparinized tubes. Blood smears were made immediately to prevent morphological changes in the red blood cells.

Urine samples were collected by holding the nostrils of the sheep for 30 seconds to prevent breathing. This increased intra-abdominal pressure; the animal became nervous and passed urine. On some occasions, sheep did not pass a sufficient amount of urine, which resulted in incomplete data.

Blood and urine samples were analyzed, and the results were recorded.

Description of the Various Procedures

Determination of lead in blood

The method described by Hessel (1968) was used. The blood was hemolyzed by using 5% TX-100 solution[1] and was chelated with APDC[2] and extracted by methyl isobutyl ketone. The organic supernatant solution was analyzed by atomic absorption spectroscopy. Standards were made using the blood collected from sheep which were not exposed to lead.

The following reagents were prepared:

Triton TX-100 5% by volume was slowly dissolved in warm deionized water.

Ammonium pyrrolidine dithiocarbamate (1-pyrrolidine-carbodithioic acid ammonium salt, Eastman No. 9279) 2% W/V was dissolved in deionized water.

Standard stock solution containing 1,000 ug/ml of lead as $Pb(NO_3)_2$.

An intermediate stock solution of 100 ug/ml was made by dissolving 10 ml of standard stock solution in a 100 ml volumetric flask by adding deionized water.

Standards, having concentrations of 1, 2, 3, 4, and 5 ppm lead, respectively, were made by diluting 1, 2, 3, 4, and 5 ml, respectively, of intermediate stock solution. Thorough shaking of the standards was found to be a very important factor.

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[1] TX-100 is alkyl phenoxy polyethoxyethanol. Rohm and Hass, Philadelphia, Pa.

[2] APDC is an ammonium pyrrolidine dithiocarbamate.

Procedure -- Five ml of unclotted blood collected from sheep not exposed to lead was taken in each of six 20 mm x 150 mm test tubes. The unknown blood samples were added to other test tubes. One ml of respective standard solutions was added to tube nos. 2, 3, 4, 5, and 6, each containing sheep blood, to prepare standards containing the equivalent of 0.2, 0.4, 0.6, 0.8, and 1.0 ppm lead.

One ml of 5% TX solution was added to each test tube and mixed. One ml of 2% APDC solution was added to each test tube followed by additional mixing. Five ml of water-saturated methyl isobutyl ketone was added. The test tubes were sealed with screw caps and were shaken for at least one minute by hand, followed by centrifugation for ten minutes at 2,000 rpm. The organic supernatant was aspirated into an atomic absorption spectrophotometer[1] at wave length 2833A, range selector UV, and the burner was kept under normal flow condition of air and acetylene as described by Hessel (1968).

Determination of lead in urine

The following reagents were prepared:

2% W/V APDC solution made as previously described

5 N HCL

Glacial acetic acid

Lead standard solution

Procedure -- The pH of the urine samples collected from the unexposed sheep to be used as standards, as well as unknown urine samples, were

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[1]Perkin-Elmer Model 303, Perkin-Elmer Corp., Walthum, Massachusetts.

adjusted to pH 3 by adding a few drops of 5 N HCl and glacial acetic acid. No TX-100 solution was used. The rest of the extraction procedure was similar to that for blood lead.

Determination of delta-aminolevulinic acid in urine

The method described by Davis and Andelman (1967) was used. To separate ALA[1] from the urine, the sample was passed through an anionic ion exchange resin which retains porphobilinogen and releases the ALA and urea. The solution containing ALA and urea was passed through a cationic ion exchange resin which retains ALA while urea passes through both columns. The ALA retained on the cationic resin was then washed with one molar sodium acetate buffer solution.

ALA was analyzed after quantitative conversion to 3-acetyl-2-methyl-pyrrole 4-(3) propionic acid by heating with acetylacetone. With dimethyl-aminobenzaldehyde (DMAB) in acid solution, the pyrrole obtained on condensation of ALA and acetylacetone formed colored complexes which were spectrophotometrically determined.

The following reagents were prepared (full descriptions for each are given subsequently):

One molar sodium acetate solution buffered to pH 4.6

Erhlich's reagent

Standard ALA solution

Piggyback columns

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[1]ALA is an intermediate metabolite in the hemoglobin synthesis.

One molar sodium acetate solution was prepared by dissolving 82.04 gm of anhydrous sodium acetate powder in a small amount of distilled water and 57 ml of glacial acetic acid. More distilled water was added to bring the volume to one liter. The pH of this solution was adjusted with glacial acetic acid to 4.6 and refrigerated to prevent fungus growth.

Erhlich's reagent was prepared by dissolving ten grams of DMAB (reagent grade) into 420 ml of glacial acetic acid. The bottle was covered with foil after thorough mixing. This reagent was stable up to six months under refrigerated conditions. To prepare working Erhlich's reagent, 100 ml of this stock solution was mixed with 19 ml of concentrated (72%) perchloric acid. It was necessary to use this reagent within one hour.

The standard ALA solution (10 mg/ml) was prepared by dissolving 12.5 mg of 5-aminolevulinic acid hydrochloride (reagent grade) in one molar sodium acetate buffer solution and the volume brought to 100 ml in a volumetric flask.

Piggyback columns, manufactured by Bio-Rad[1], had a top and a bottom unit made of polyethylene. The top column was filled with 0.75 gm of AG1-X8 resin, 100 to 200 mesh, acetate form. The bottom column was filled with 0.75 gm AG50W-X4 resin, 100 to 200 mesh, hydrogen form.

Procedure -- The columns were placed in their respective positions, and the entire columns were washed with 10 ml of demineralized water. The frozen urine samples were thawed and their pH readjusted, if needed, to between 4 and 6 by adding a few drops of glacial acetic acid. An aliquot

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[1]Bio-Rad Laboratories, 32nd and Griffin Avenue, Richmond, California.

of 0.5 ml of urine was added to the top column and was allowed to drain through the bottom column. The columns were washed three times with 10 ml of demineralized water. The top columns were removed and test tubes were placed under each bottom column. ALA was eluted from the bottom column by adding 7 ml of one molar buffered sodium acetate solution and collected in a graduated test tube. Acetylacetone (0.2 ml) was added to each test tube, and the solution was mixed.

Six graduated test tubes were used to prepare a sequence of standards from which a standard curve was derived. Seven ml of one molar sodium acetate solution were added to the first test tube which served as a blank. Standard ALA solutions were prepared by adding 0.05, 0.10, 0.20, 0.30, and 0.50 ml to each of test tube numbers 2, 3, 4, 5, and 6, respectively. The volume in each tube was adjusted to 7 ml by adding one molar sodium acetate solution. To each tube, 0.2 ml acetylacetone was added.

The tubes were placed in a boiling water bath (above $90^{o}C$) for ten minutes. Seven ml of freshly prepared Erhlich's working reagent were added to each tube after cooling to room temperature. They were allowed to stand for 15 minutes for complete color development. The unknown samples and standards were read at 533 mu on a Coleman Junior II spectrophotometer[1]. A standard curve was plotted with optical density on the abscissa and concentration of ALA on the ordinate.

The following precautions were taken: the pH of the urine was adjusted to between 4 to 6 before freezing, fresh acetylacetone was always

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[1]Coleman Instruments, 42 Madison Street, Maryland, Illinois.

used, the Erhlich's working reagent was used within an hour of preparation, analytical or medium grade DMAB was used, and samples were kept in boiling water for not more than 10 minutes to prevent destruction of ALA.

Determination of various other blood constituents

Red blood cell and white blood cell counts were made using a Coulter-counter[1]. On some occasions, this instrument was not functioning and determinations were not made for those weeks. Packed cell volume determinations were done using the microhematocrit technique. Hemoglobin values were determined using the cyanmethemoglobin technique. Blood smears were stained with commercial Wright's stain and washed with phosphate buffer.

Statistical methods

Analysis of variance was made using the F test. Correlation coefficients were calculated to determine relationships between time, hemoglobin, packed cell volume, white blood cells, red blood cells, blood lead, urine lead, ALA, lead in feed, eosinophils, monocytes, neutrophils-band, neutrophils-segs, and lymphocytes.

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[1]Coulter Electronics, Inc., Hialeah, Florida.

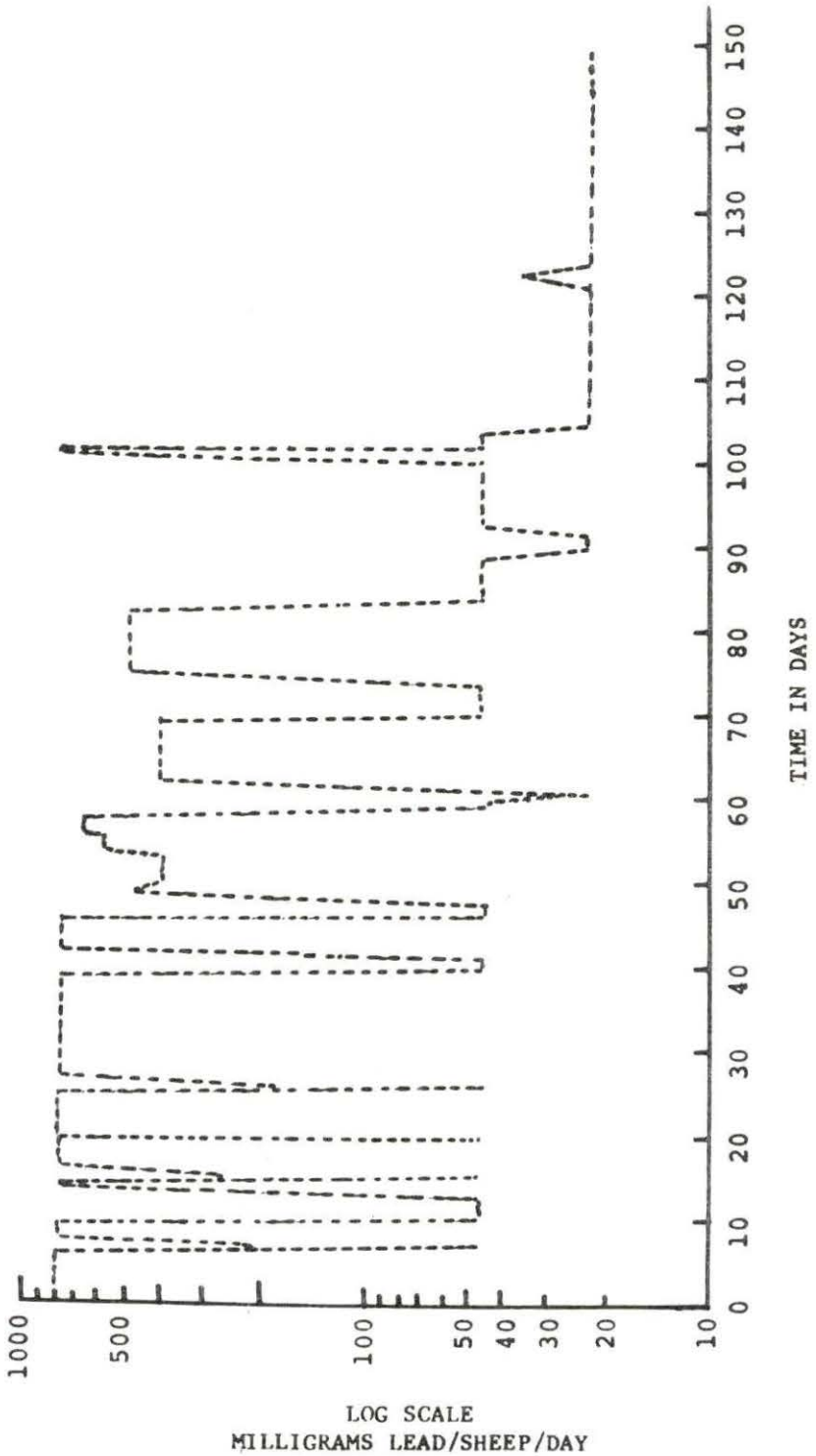
RESULTS AND DISCUSSION

Levels of lead fed to the 12 exposed ewes were varied between 24 mg and 800 mg/sheep/day for 164 days. Daily dosages were varied to maintain a blood lead level of at least 0.4 ppm without producing death. Assuming 50 kg as the average body weight of each exposed sheep, the daily dose of lead varied approximately from 0.5 mg/kg to 16 mg/kg (Figure 1).

The hematological, chemical, and clinical data for each animal are given in Appendix A (Tables 16-36). The relationship between blood lead and number of animals showing clinical signs is shown in Figure 2 and Table 1. The percentage of animals showing clinical signs (primarily anorexia and depression) fluctuated with blood lead levels. At an average blood lead level of 0.4 ppm, 50% of the animals were showing clinical signs and increased to 90% at a blood lead level of about 0.5 ppm. However, after 60 and 101 days of exposure, the percentage of animals showing clinical signs dropped to 45 percent, although blood lead levels remained high. None of the exposed sheep showed clinical signs after 115 days of exposure despite the fact that blood lead levels ranged between 0.3 - 0.4 ppm.

Three exposed sheep (343, 351, and 367) aborted. Sheep 343 and 351 aborted after 133 and 129 days of gestation, respectively. The period of gestation was not known for sheep 367. The rate of abortions in exposed and unexposed animals was 25% and 0%, respectively (Table 2). This is compatible with the findings of Allcroft (1951) and James et al. (1966). Abortion due to lead poisoning may be caused by: 1) spasms of the uterine muscles, 2) degenerative changes in the chorionic epithelium, and 3) placental injury and hemorrhages.

Figure 1. Average daily dosages of lead in milligrams per sheep per day for the exposed group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TIME IN DAYS

Figure 2. Percentage of exposed sheep showing clinical signs (anorexia and depression) and their average blood lead levels

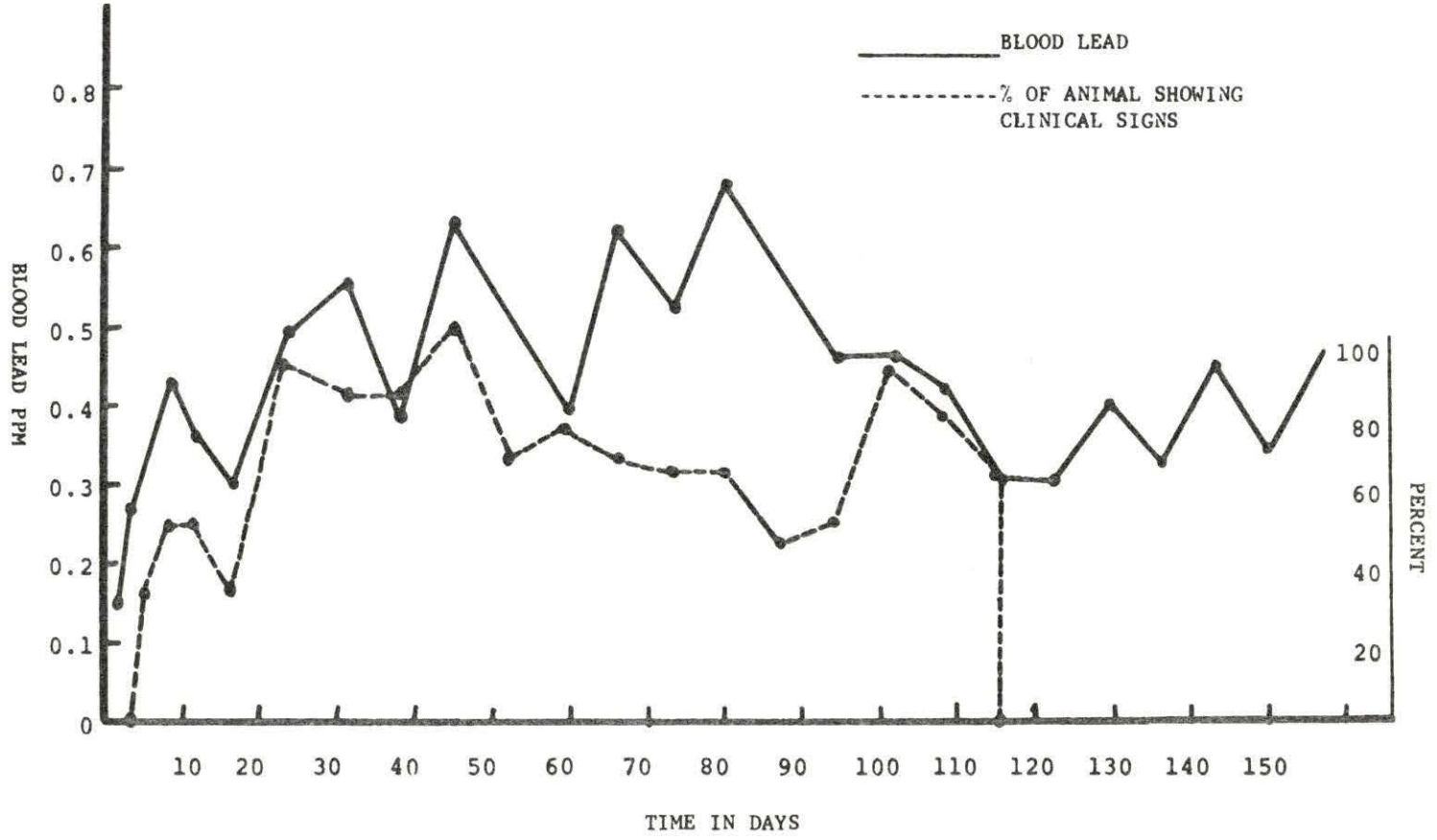

BLOOD LEAD
% OF ANIMAL SHOWING CLINICAL SIGNS
BLOOD LEAD PPM
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
PERCENT
100
80
60
40
20
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
TIME IN DAYS

Table 1. Clinical signs in exposed sheep

Date	Day	No. of animals showing clinical signs	Date	Day	No. of animals showing clinical signs
12-9-69	1	None	1-29-70	52	$\frac{8}{12}$
12-11-69	3	None	2-5-70	59	$\frac{9}{12}$
12-13-69	5	$\frac{4}{12}$	2-12-70	66	$\frac{8}{12}$
12-16-69	8	$\frac{6}{12}$	2-19-70	73	$\frac{7}{11}$
12-19-69	11	$\frac{6}{12}$	2-26-70	80	$\frac{7}{11}$
12-24-69	16	$\frac{4}{12}$	3-5-70	87	$\frac{5}{11}$
12-31-69	23	$\frac{11}{12}$	3-12-70	94	$\frac{5}{10}$
1-8-70	31	$\frac{10}{12}$	12-19-70	101	$\frac{8}{9}$
1-15-70	38	$\frac{10}{12}$	3-26-70	108	$\frac{7}{9}$
1-22-70	45	$\frac{12}{12}$	4-5-70	115[a]	$\frac{5}{8}$

[a]None of the exposed sheep showed clinical signs after 115 days of exposure.

Each of two exposed sheep (350 and 353) gave birth to a weak but physically normal lamb (Table 3). Seven of the unexposed animals (344, 346, 348, 359, 360, 362, and 366) gave birth to a total of eight normal lambs (Table 4). Sheep 359 had twins. Sheep 349 (unexposed) had dystocia, and the apparently normal fetus was born dead. Sheep 347 (unexposed) was euthanatized on the 92nd day of gestation for comparison of tissue lead

Table 2. Summary of the gestation records in the exposed and control groups

Group	No. animals	No. aborted	% abortion	No. lambed	% lambing
Exposed	12	3	25.0	2	25[4]
Control	9	None	0	9[1,2,3]	100.0

1. One control sheep had dystocia, lamb born dead.
2. One control sheep was enthanatized. Was eight weeks pregnant and had normal fetus.
3. One control sheep had twins.
4. One exposed sheep died of antibiotic anaphylactic shock; was eight weeks pregnant and had normal fetus.

levels and had an apparently normal fetus. Sheep 348 and 366 (unexposed) died about one week after normal parturition as a result of bacterial pneumonia. The rate of lambing in the exposed and unexposed sheep was 25 and 100%, respectively (Table 2). Five animals in the exposed group (354, 355, 357, 363, and 364) either did not conceive or their fetuses were resorbed. On postmortem examination, sheep 363 and 364 were nongravid. The remaining three sheep were not euthanatized and were neither observed to have been pregnant nor to have aborted. This could be attributed to the effects of lead. Sheep 365 (exposed) had a mummified fetus on postmortem.

Average blood and urine lead values were calculated for both groups for each week and are shown in Figures 3 and 4. Figure 3 indicates that blood lead levels fluctuated as the dosage of lead in the feed was increased or decreased.

Table 3. Summary of gestation records of the sheep exposed to lead

Animal number	Date bred	Date lambed or aborted	Sex	Comments
343	1/7/70	Aborted 5/20/70	Male	The fetus was physically normal and weighed 8.3 lbs.
350	12/12/69	5/11/70	Female	Lamb was weak but physically normal and weighed 5 lbs.
351	12/12/69	Aborted 4/23/70	Male	A macerated fetus was found in the bedding on 4/24/70.
353	Not known	5/28/70	Male	Lamb was weak but physically normal, weighed 5.5 lbs.
354	12/20/69	--	--	Apparently did not conceive or fetus was resorbed.
355	12/18/69	--	--	Apparently did not conceive or fetus was resorbed.
357	Not known	--	--	Apparently did not conceive, fetus was resorbed, or was not bred.
358	12/28/69	--	Male	Died of an antibiotic induced anaphylactic shock on 2/22/70, was pregnant with two-month term fetus, apparently normal.
363	1/1/70	--	--	Apparently did not conceive or fetus was resorbed.
364	Not known	--	--	Apparently did not conceive, fetus was resorbed, or was not bred.
365	12/20/69	--	--	Uterus contained a mummified fetus on postmortem on 3/17/70.
367	Not known	Aborted 5/11/70	Male	Fetus weighed 5 lbs. and had no apparent abnormality.

Table 4. Summary of gestation records of the sheep not exposed to lead

Animal number	Date bred	Date lambed	Sex	Comments
344	12/26/69	6/9/70	Female	Had a normal lamb weighing 7 lbs.
346	1/3/70	5/27/70	Female	Had a normal but weak lamb weighing 5 lbs.
347	12/21/70	3/23/70 (Dam euthanatized)	Female	Had a normal fetus on postmortem examination.
348	12/27/70	5/25/70	Male	Had a normal lamb weighing 5 1/2 lbs.
349	12/12/69	5/5/70 (Dystocia)	Male	Had a normal lamb which died as a result of dystocia.
359	12/24/69	5/20/70	Both female	Had normal twins both weighing 5 lbs.
360	12/24/69	5/20/70	Female	Had a normal lamb weighing 8 lbs.
362	12/17/69	5/13/70	Female	Had a normal lamb weighing 12 lbs.
366	12/27/69	5/19/70	Female	Had a normal lamb weighing 6 lbs.

Figure 3. Average blood lead levels in nine unexposed and in 12 sheep during exposure to lead throughout their gestation period. The presence of low levels of lead in the blood of unexposed sheep resulted from accidental contamination of control feed during pre-exposure period.

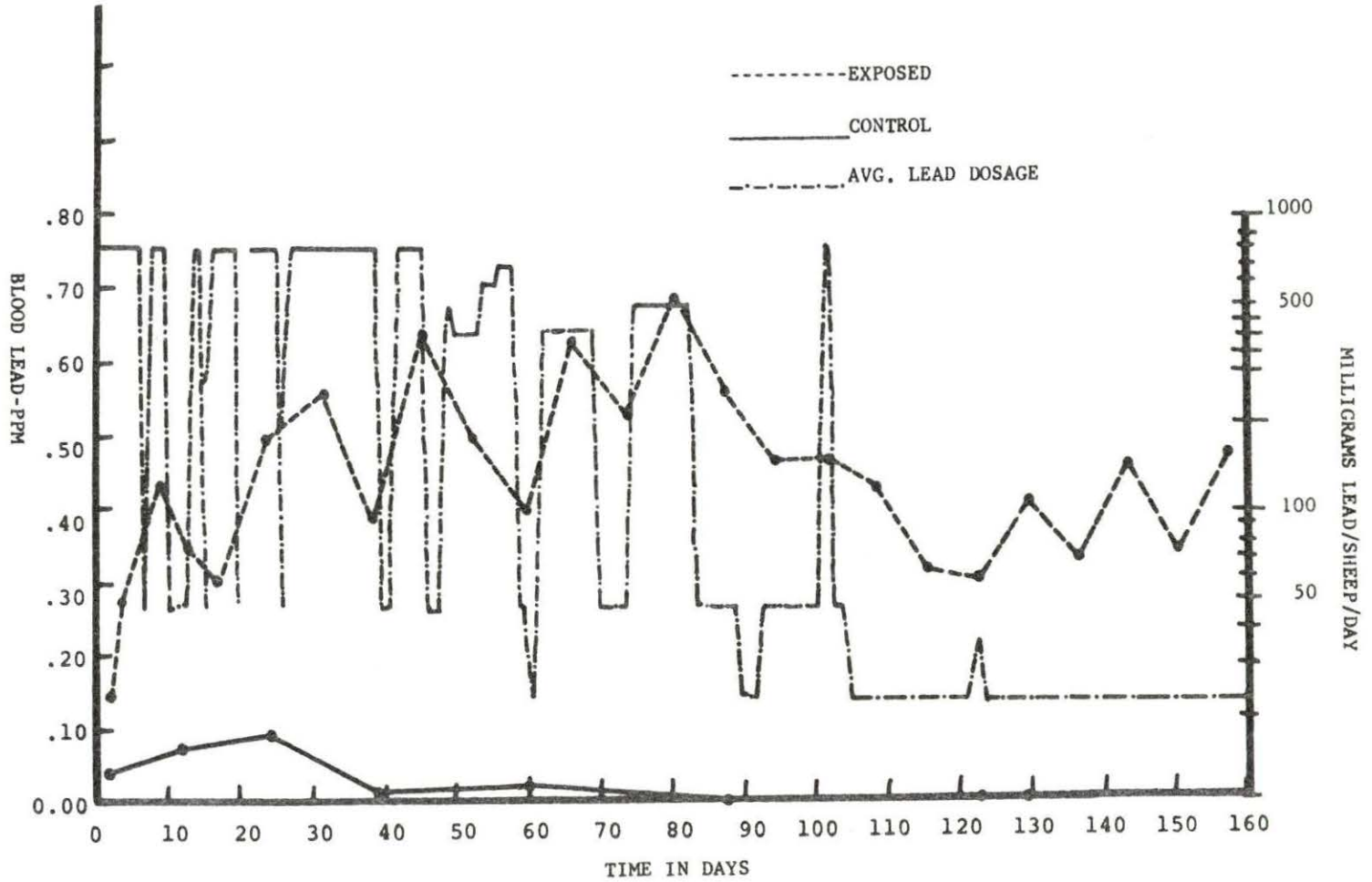
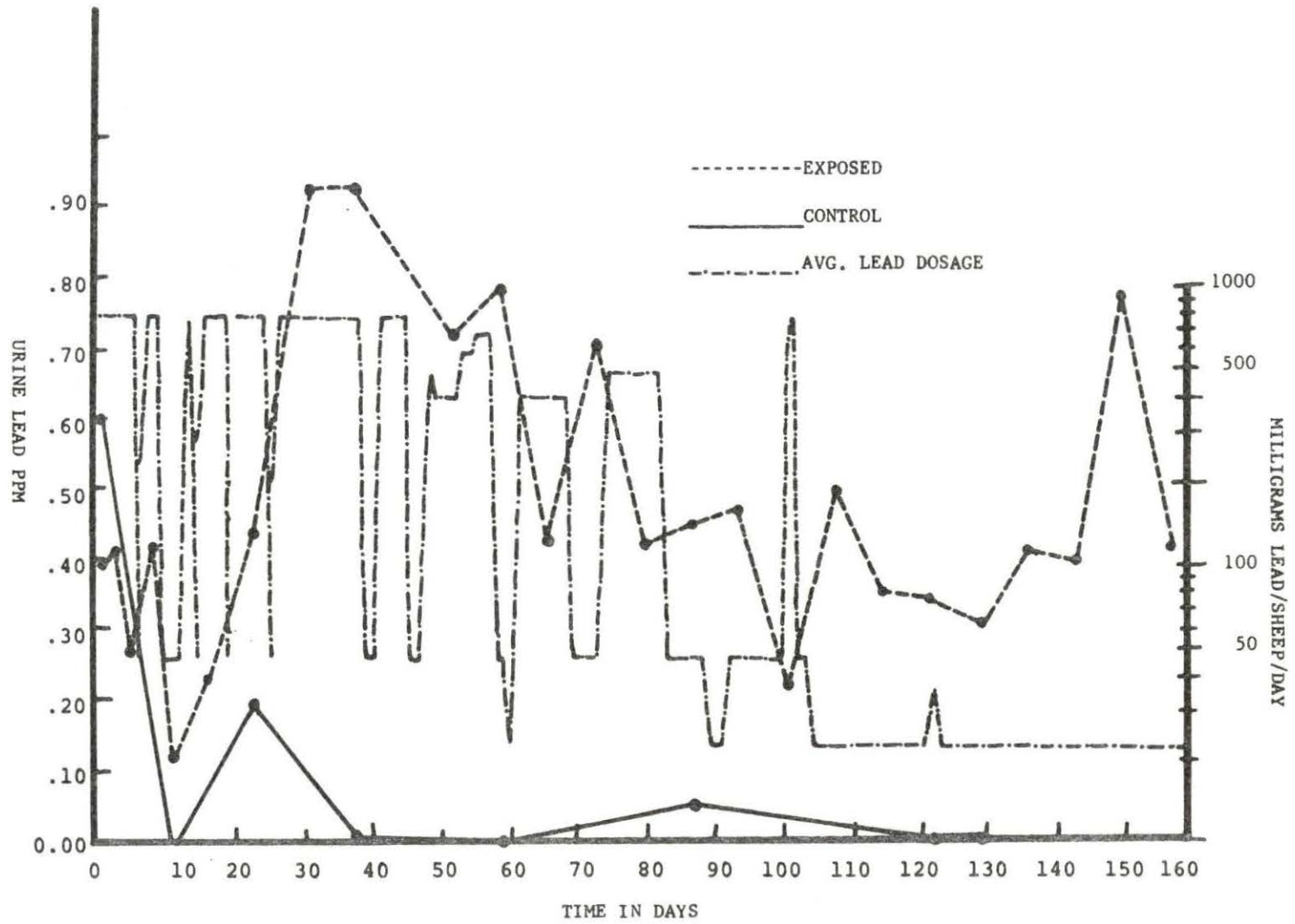

EXPOSED
CONTROL
AVG. LEAD DOSAGE
BLOOD LEAD-PPM
.80
.70
.60
.50
.40
.30
.20
.10
0.00
1000
500
100
50
MILLIGRAMS LEAD/SHEEP/DAY
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
TIME IN DAYS

Figure 4. Average urine lead levels in nine unexposed and in 12 sheep during exposure to lead throughout their gestation period. The presence of lead in the urine of unexposed sheep resulted from accidental contamination of control feed during pre-exposure period.


EXPOSED
CONTROL
AVG. LEAD DOSAGE
URINE LEAD PPM
.90
.80
.70
.60
.50
.40
.30
.20
.10
0.00
1000
500
100
50
MILLIGRAMS LEAD/SHEEP/DAY
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160
TIME IN DAYS

Excretion of lead in the urine varied with the amount of lead fed (Figure 4). However, increased excretion of lead in urine was observed during days 110 to 160 when low levels of lead (24 mg) were being fed. This is apparently a result of mobilization of lead from the tissues. Smaller quantities of lead were detected in the blood and urine of unexposed sheep for about five weeks after the beginning of the experiment. This was because these animals were given feed accidentally contaminated with lead before the start of the experiment.

Excretion of ALA in the urine of exposed sheep varied with the amount of lead fed and ranged from 0.1 to 1.4 mg/100 ml (Figure 5). In man, determination of ALA in the urine of lead-poisoned patients is frequently used as a preliminary diagnostic test. According to Haeger (1960), normal ALA values for man and the rabbit are 0.29 and 0.03 mg %, respectively. No data is available for normal ALA values in domestic animals. Urine ALA levels in humans exposed to lead are presented in Table 5. These studies indicate that sheep may not excrete as much ALA as does man following lead exposure, however, 24-hour collection determinations were not done.

Results of chemical analysis for lead in tissues collected from exposed and unexposed animals on postmortem examination are given in Table 6. A statistical analysis of these data is given in Table 7. Mean values of 10 and 39 ppm lead in the liver and kidney, respectively, are comparable with levels reported in cattle (Allcroft, 1951; Hatch and Funnell, 1969; Buck, 1970).

Lead levels in the rumen contents of exposed sheep varied between 2 and 87 ppm with a mean value of 31.2 ppm. A similar observation in cattle was made by Hatch and Funnell (1969). There are several reasons to account

Figure 5. Average urine aminolevulinic acid (ALA) in nine unexposed and in 12 sheep during exposure to lead throughout their gestation period

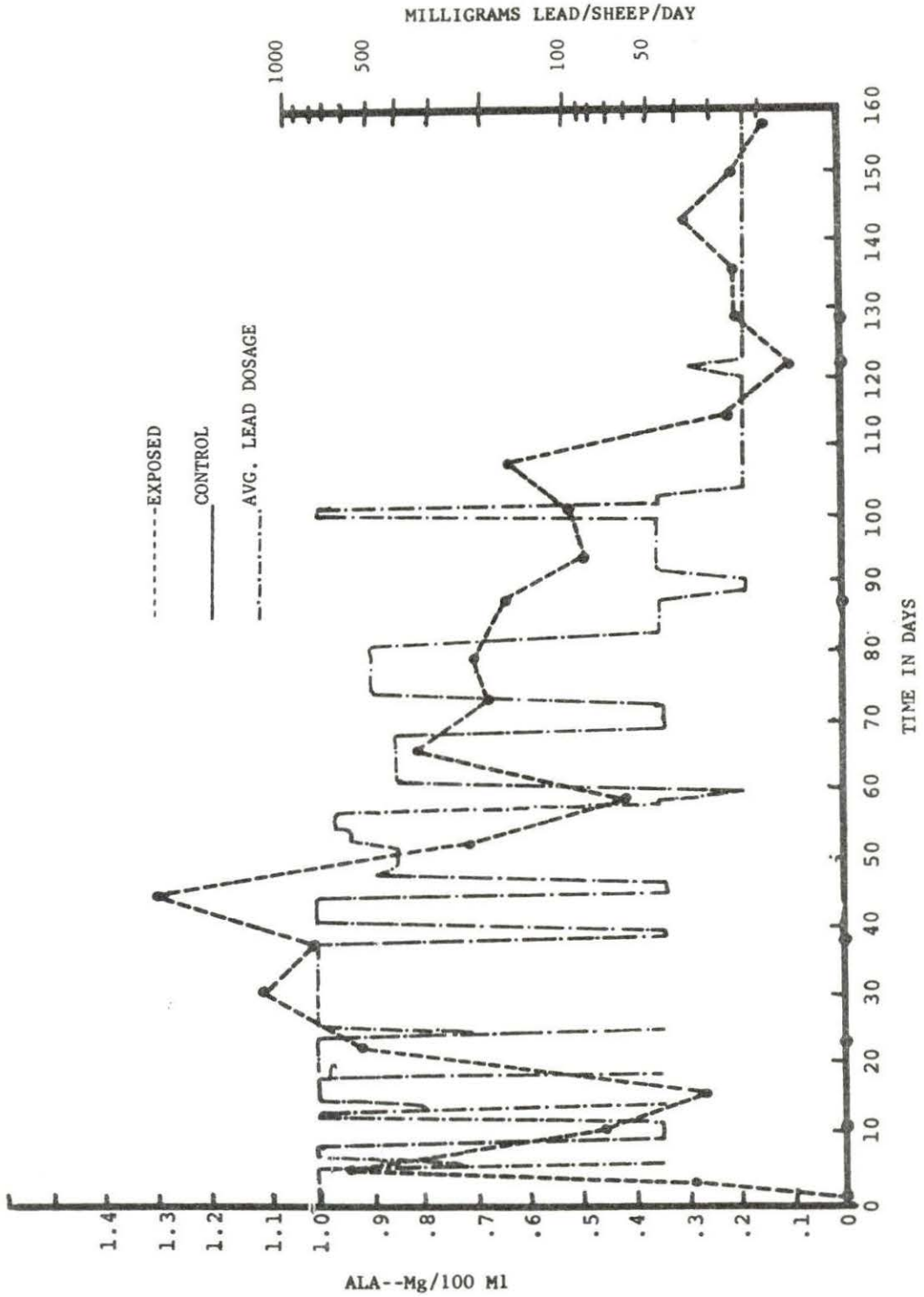

MILLIGRAMS LEAD/SHEEP/DAY
1000
500
100
50
EXPOSED
CONTROL
AVG. LEAD DOSAGE
TIME IN DAYS
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160
ALA--Mg/100 Ml
1.0 .9 .8 .7 .6 .5 .4 .3 .2 .1 0
1.1 1.2 1.3 1.4

Table 5. Urine aminolevulinic and levels in the human[a]

Urinary ALA range mg/100 ml	Urinary ALA codes	Relationship to lead exposure
.00-0.54	Normal	None
0.55-0.99	Trace	Slight
1.00-1.49	1 plus	Moderate
1.50-1.99	2 plus	Heavy
2.00-2.99	3 plus	Severe
3.00-5.99	4 plus	Critical
6.00-10.00	5 plus	Overwhelming

[a]Davis and Andelman (1967).

for variation in rumen lead levels, such as 1) source of the ingesta, 2) variation in the amount of lead eaten by the animals, and 3) period of time since ingestion of the lead.

Chemical analysis of the tissues collected from unexposed sheep revealed insignificant lead levels (Table 6).

Chemical analysis of the fetal tissues for lead are given in Table 8. Fetal livers from sheep 358 and 367 had 12 and 43 ppm lead, respectively. This is significant because it indicates placental transference of lead. Similar observations were made by Allcroft (1951), who reported 37 ppm of lead in the liver of a fetus from a sheep fed 50 mg of lead acetate daily during gestation. Chemical analysis of fetal tissues from unexposed sheep revealed insignificant lead levels (Table 8).

Results of analysis of blood from the two lambs born to exposed ewes and eight lambs born to controls are given in Table 9. Microscopic examination of blood smears from the exposed sheep revealed anisocytosis,

Table 6. Summary of the residual lead levels in the tissues of exposed and unexposed sheep

Sheep no.	Group	Lead (ppm) Liver	Kidney	Brain	Rumen contents	Spleen	Aorta	Heart muscle
351	Exposed	10.0	32.0	Less than 1	5.0	7.0	1.0	1.0
358	Exposed	11.0	51.0	4.0	2.0	3.0	N.A.[a]	1.0
363	Exposed	11.0	49.0	3.0	82.5	N.A.	3.0	2.0
364	Exposed	13.0	36.0	15.0	87.5	10.0	5.0	N.A.
365	Exposed	8.0	37.0	3.0	7.5	8.0	3.0	2.0
367	Exposed	9.0	31.0	6.0	2.5	8.0	N.A.	1.0
347	Unexposed	1.0	1.0	N.T.[b]	N.T.	N.T.	N.T.	N.T.
366	Unexposed	0.14	0.45	N.T.	N.T.	0.19	N.T.	N.T.

[a]Not analyzed.

[b]Negative to test.

poikilocytosis, hypochromasia, many immature red blood cells, and many basophilic stippled red cells (Figures 6, 7, and 8). The blood hemograph was normal in the unexposed sheep (Figure 9). The RBC morphology and differential WBC data for each sheep are given in Appendix B. A summary of changes in red blood cell morphology of exposed sheep is given in Table 10. Examination of this table indicates that anisocytosis, poikilocytosis, immature red blood cells, and basophilic stippling were highest between the sixth and seventh week of exposure, decreased as the dosage of lead was reduced, and disappeared during the later part of the experiment. This may be due either to reduced lead dosage or the development of resistance in the animals.

Histopathological examination of the kidneys collected from the exposed sheep revealed edematous changes in the glomerulus, hemosiderin-

Table 7. Average residual lead levels in the tissues of six exposed and two control sheep

Specimen	Exposed Group		Control Group	
	Lead (ppm), mean, S.D. and range[a]	No. of samples	Lead (ppm), mean, S.D. and range[a]	No. of samples
Kidney	39.3 ± 8.8 (31 - 51)	6	0.7 ± 0.7 0.5 - 1.0	2
Liver	10.3 ± 1.7 (8 - 11)	6	Negative	2
Spleen	7.2 ± 2.6 (3 - 10)	5	0.1 ± 0.9 0.0 - 0.2	2
Brain	5.3 ± 7.8 (1 - 15)	6	Negative	2
Rumen contents	31.2 ± 41.8 (2 - 87)	6	Negative	2
Heart muscle	1.5 ± 0.56 (1 - 2)	4	Negative	2
Aorta	3.0 ± 1.4 (1 - 5)	4	Negative	2

[a]S.D. = Standard Deviation; values in parentheses are ranges.

like pigment in the cytoplasm of the tubular epithelium, and disruption of the cytoplasmic continuity. Hemotoxylin-eosin stained sections showed large intranuclear inclusions in the tubular epithelium (Figure 10).

Average values for hemoglobin (Figure 11), packed cell volume (Figure 12), WBC's (Figure 13), and RBC's (Figure 14) were plotted for exposed and unexposed sheep.

An analysis of variance was made for hemoglobin, packed cell volume, white blood cells, red blood cells, blood lead, urine lead, and urine delta-aminolevulinic acid (Table 11).

Table 8. Summary of the residual lead levels in the tissues of fetuses from the exposed and unexposed sheep

Sheep no.	Term of fetus in days	Weight of fetus	Lead (ppm)
343 (Exposed)	133	8.3 lbs.	Liver--0.93 ppm Kidney--0.29 ppm Brain--Negative Stomach contents--Negative Aorta--Negative
358 (Exposed)	The sheep died of anaphylactic shock after being given penicillin injection at 75 days of exposure.	6.0 lbs.	Liver--12.0 ppm Kidney--2.0 ppm Brain--1.0 ppm Aorta--Negative
365 (Exposed)	A mummified fetus found on postmortem on 3/17/70.	———	Liver--1.0 ppm Kidney--1.0 ppm Brain--Negative Aorta--Negative
367 (Exposed)	Date of breeding not known. Sheep aborted on 5/11/70.	4 lbs. 15 oz.	Liver--43.00 ppm Kidney--1.20 ppm Brain--0.65 ppm Stomach contents--0.78 ppm Aorta--Negative
349 (Unexposed)	Dystocia after 144 days.	9 lbs. 11 oz.	Liver--0.70 ppm Kidney--1.40 ppm Brain--0.43 ppm Stomach contents--0.34 ppm Aorta--Negative
347 (Unexposed)	Dam euthanatized on 3/23/70.	Not weighed	Liver--Less than 1.0 ppm Kidney--Less than 1.0 ppm Brain--Less than 1.0 ppm Aorta--Negative

Table 9. Comparative blood lead levels of lambs born to exposed and unexposed ewes

Lamb number	Dam number	Dam exposed to lead	Days after birth					
			1	2	3	4	5	6
1	350	Yes	No test	No test	0.14	0.16	N.T.[a]	N.T.
15	353	Yes	0.17	0.15	N.T.	N.T.	N.A.	N.A.
3	362	No	N.A.[b]	N.A.	N.T.	N.T.	N.T.	N.T.
5	366	No	N.A.	N.T.	N.A.	N.A.	N.A.	N.A.
6	359	No	N.A.	N.T.	N.T.	N.T.	N.T.	N.T.
7	359	No	N.A.	N.T.	N.A.	N.A.	N.A.	N.A.
10	360	No	N.A.	N.T.	N.A.	N.A.	N.A.	N.A.
11	348	No	N.A.	N.T.	N.A.	N.A.	N.A.	N.A.
12	346	No	N.A.	N.T.	N.A.	N.T.	N.A.	N.A.

[a]Negative to test.

[b]Not analyzed.

The data used for analysis of variance were from the monthly determinations when both the control and exposed populations were sampled. The remaining weekly determinations in which the exposed sheep only were sampled were not used. From Table 11, it can be seen that there are no significant differences between the exposed and unexposed sheep for hemoglobin, packed cell volume, white blood cells, and red blood cells. However, significant differences ($P < .05$) for urine lead and highly significant ($P < .01$) differences for the blood lead and urine ALA were observed between the exposed and unexposed sheep. Table 12 shows the mean values for the above mentioned parameters for the exposed and control sheep along with published normal values. Analysis of variance for differential WBC revealed that the increased number of monocytes and neutrophils (bands)

Figure 6. Photomicrograph of blood smear from sheep 357 (exposed) showing poikilocytosis. Wright's stain. Oil immersion. (Magnification 2000X)

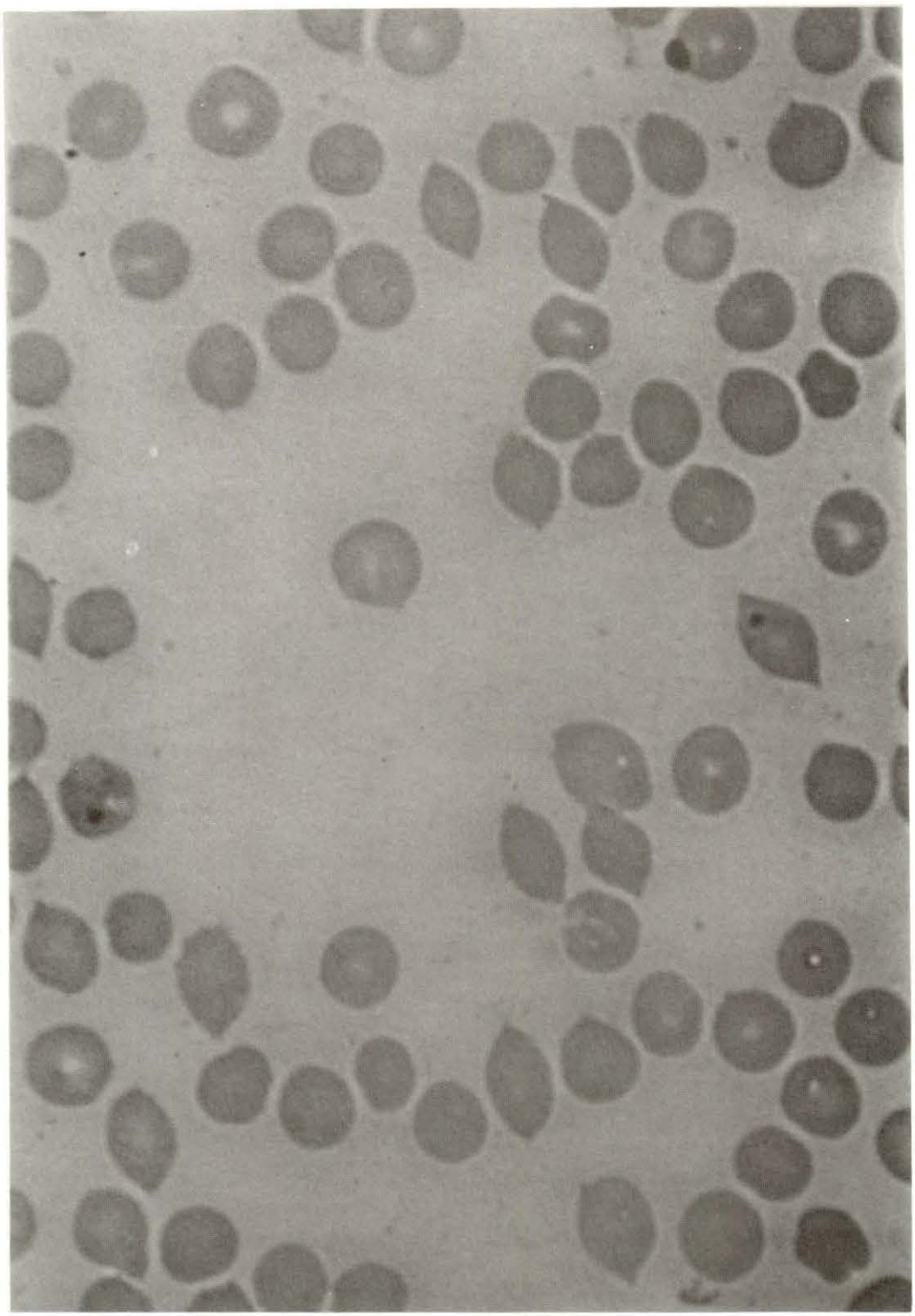


Figure 7. Photomicrograph of blood smear from sheep 353 (exposed) showing an immature red blood cell with basophilic stippling. Note hypochromasia of the cells. Wright's stain. Oil immersion. (Magnification 2000X)

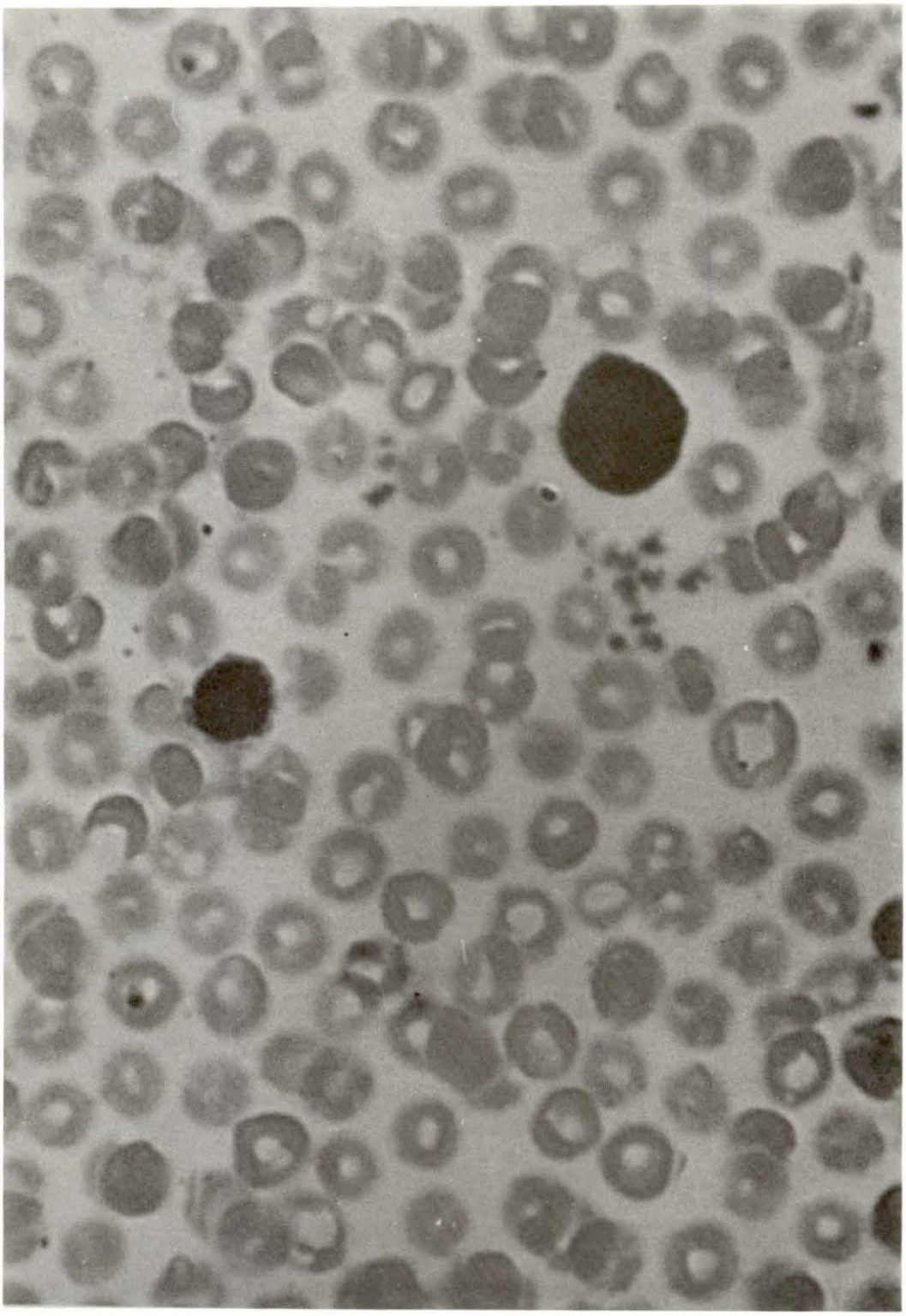


Figure 8. Photomicrograph of blood smear from sheep 357 (exposed) showing anisocytosis and poikilocytosis. Wright's stain. Oil immersion. (Magnification 2000X)

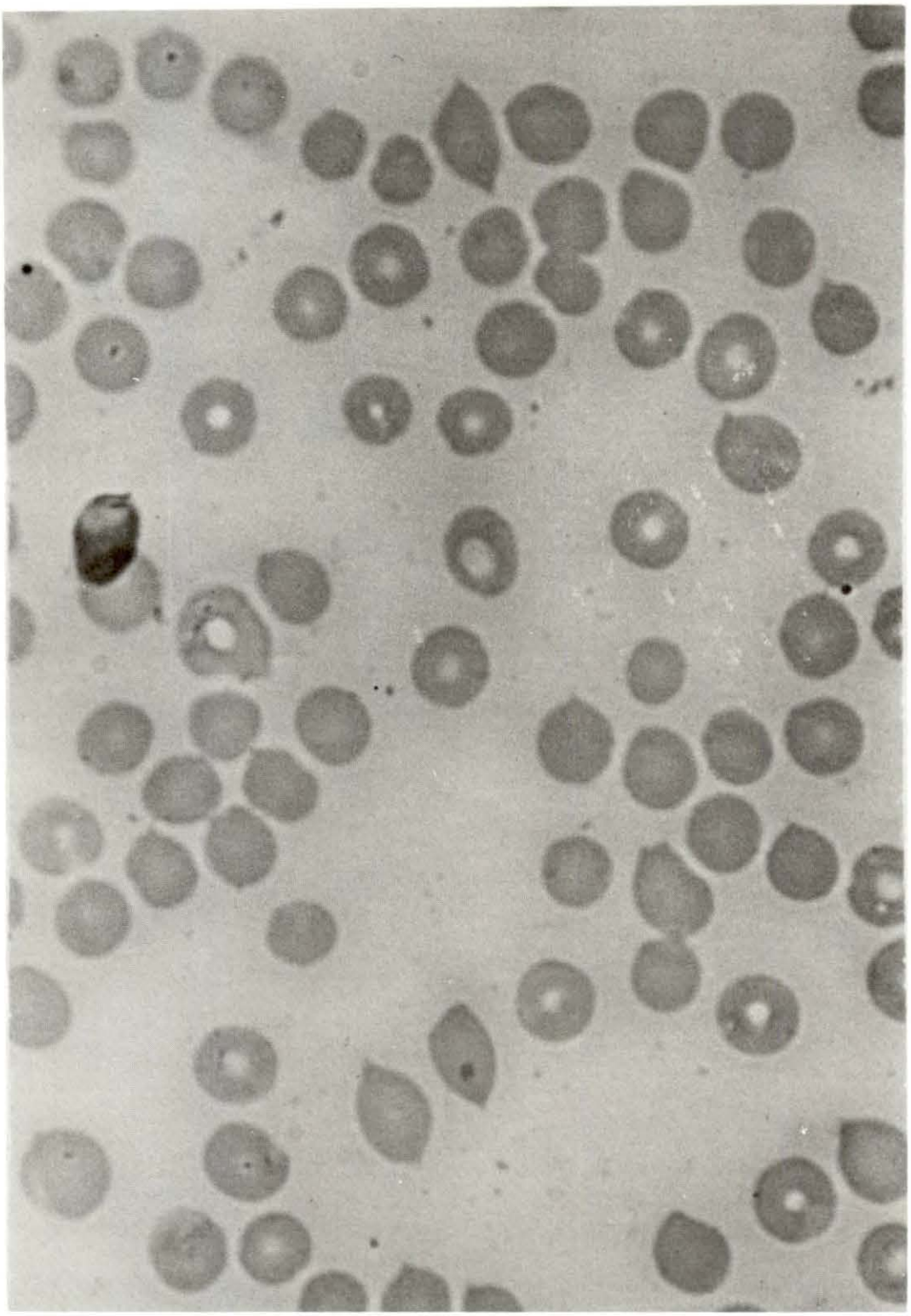


Figure 9. Photomicrograph of blood smear from sheep 346 (unexposed) showing normal blood. Wright's stain. Oil immersion. (Magnification 2000X)

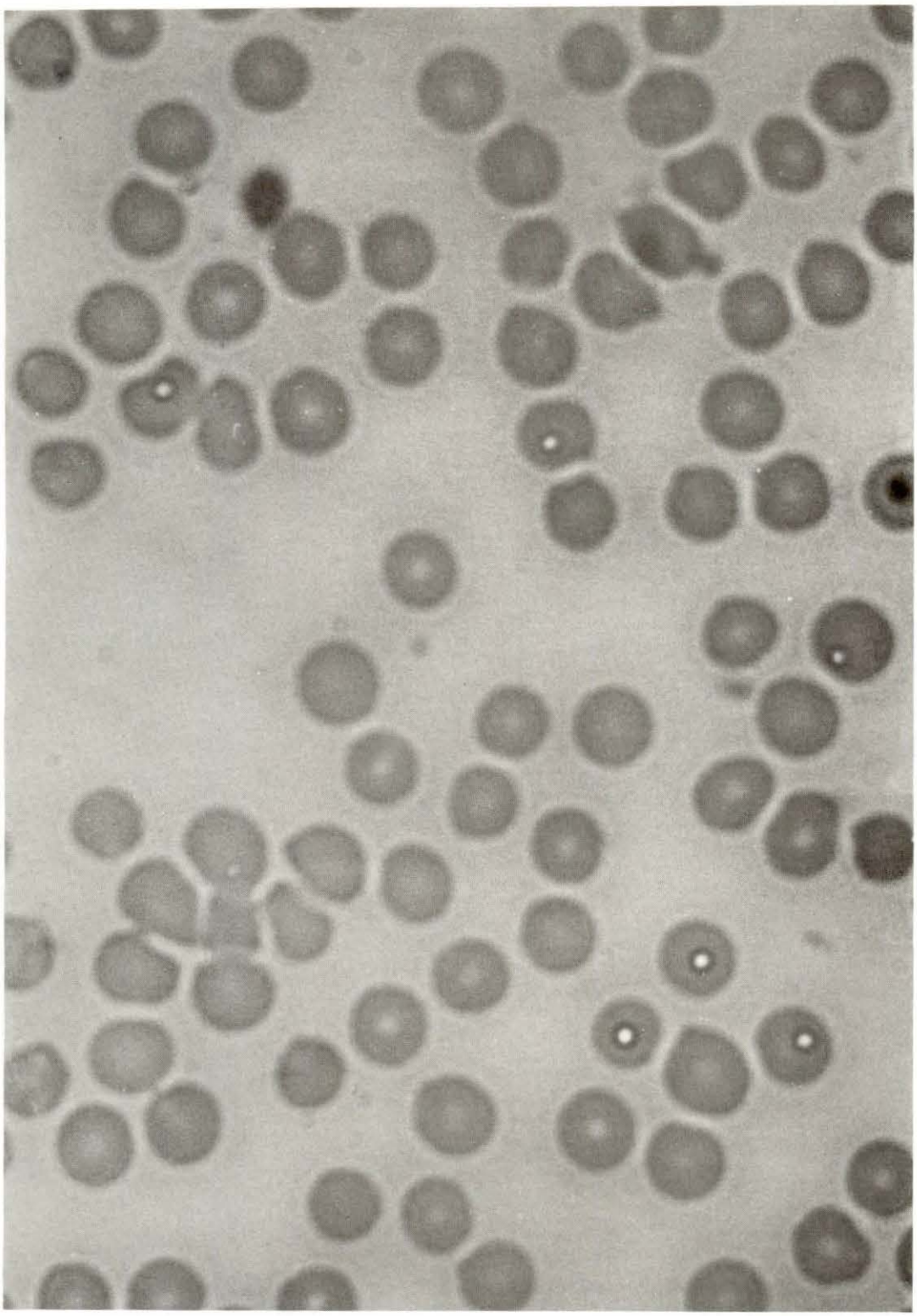


Table 10. Summary of changes in red blood cells of sheep exposed to lead

Days of exposure	Number of sheep with RBC changes			
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling
1	$\frac{0}{12}$	$\frac{0}{12}$	$\frac{0}{12}$	$\frac{0}{12}$
3	$\frac{1}{12}$	$\frac{0}{12}$	$\frac{0}{12}$	$\frac{0}{12}$
5	$\frac{1}{12}$	$\frac{0}{12}$	$\frac{0}{12}$	$\frac{0}{12}$
8	$\frac{2}{12}$	$\frac{0}{12}$	$\frac{0}{12}$	$\frac{0}{12}$
11	$\frac{4}{12}$	$\frac{0}{12}$	$\frac{3}{12}$	$\frac{0}{12}$
16	$\frac{6}{12}$	$\frac{4}{12}$	$\frac{3}{12}$	$\frac{1}{12}$
23	$\frac{7}{12}$	$\frac{5}{12}$	$\frac{4}{12}$	$\frac{4}{12}$
31	$\frac{11}{12}$	$\frac{8}{12}$	$\frac{6}{12}$	$\frac{6}{12}$
38	$\frac{11}{12}$	$\frac{8}{12}$	$\frac{6}{12}$	$\frac{6}{12}$
45	$\frac{10}{12}$	$\frac{9}{12}$	$\frac{7}{12}$	$\frac{6}{12}$
52	$\frac{9}{12}$	$\frac{6}{12}$	$\frac{6}{12}$	$\frac{6}{12}$
59	$\frac{10}{12}$	$\frac{8}{12}$	$\frac{4}{12}$	$\frac{7}{12}$
66	$\frac{9}{11}$	$\frac{9}{12}$	$\frac{6}{12}$	$\frac{4}{12}$
73	$\frac{9}{11}$	$\frac{8}{11}$	$\frac{6}{11}$	$\frac{6}{12}$
80	$\frac{9}{10}$	$\frac{8}{11}$	$\frac{5}{11}$	$\frac{6}{11}$
87	$\frac{7}{10}$	$\frac{7}{11}$	$\frac{6}{11}$	$\frac{6}{11}$
94	$\frac{5}{9}$	$\frac{5}{10}$	$\frac{3}{11}$	$\frac{7}{11}$

Table 10. (Continued)

Days of exposure	Number of sheep with RBC changes			
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling
101	$\frac{5}{9}$	$\frac{3}{9}$	$\frac{0}{9}$	$\frac{4}{9}$
108	$\frac{3}{8}$	$\frac{3}{8}$	$\frac{0}{8}$	$\frac{2}{8}$
115	$\frac{3}{8}$	$\frac{2}{8}$	$\frac{0}{8}$	$\frac{0}{8}$
122	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$
129	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$
136	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$
143	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$
150	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$
157	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$
164	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$	$\frac{0}{8}$

and decreased number of eosinophiles were highly significant in the exposed sheep (Table 13). However, no significant difference was observed in the number of neutrophiles (segs) and lymphocytes. The mean percentage of eosinophiles in the exposed group was significantly less than the control group ($P < .01$), while the mean percentage of monocytes and neutrophiles (bands) was significantly higher than in the control group (Table 14). The importance of the small but statistically significant decrease in eosino-

Figure 10. Section of renal tubular epithelium from sheep 364 (exposed) showing large intra-nuclear inclusion bodies. H and E. (Magnification 2000X)

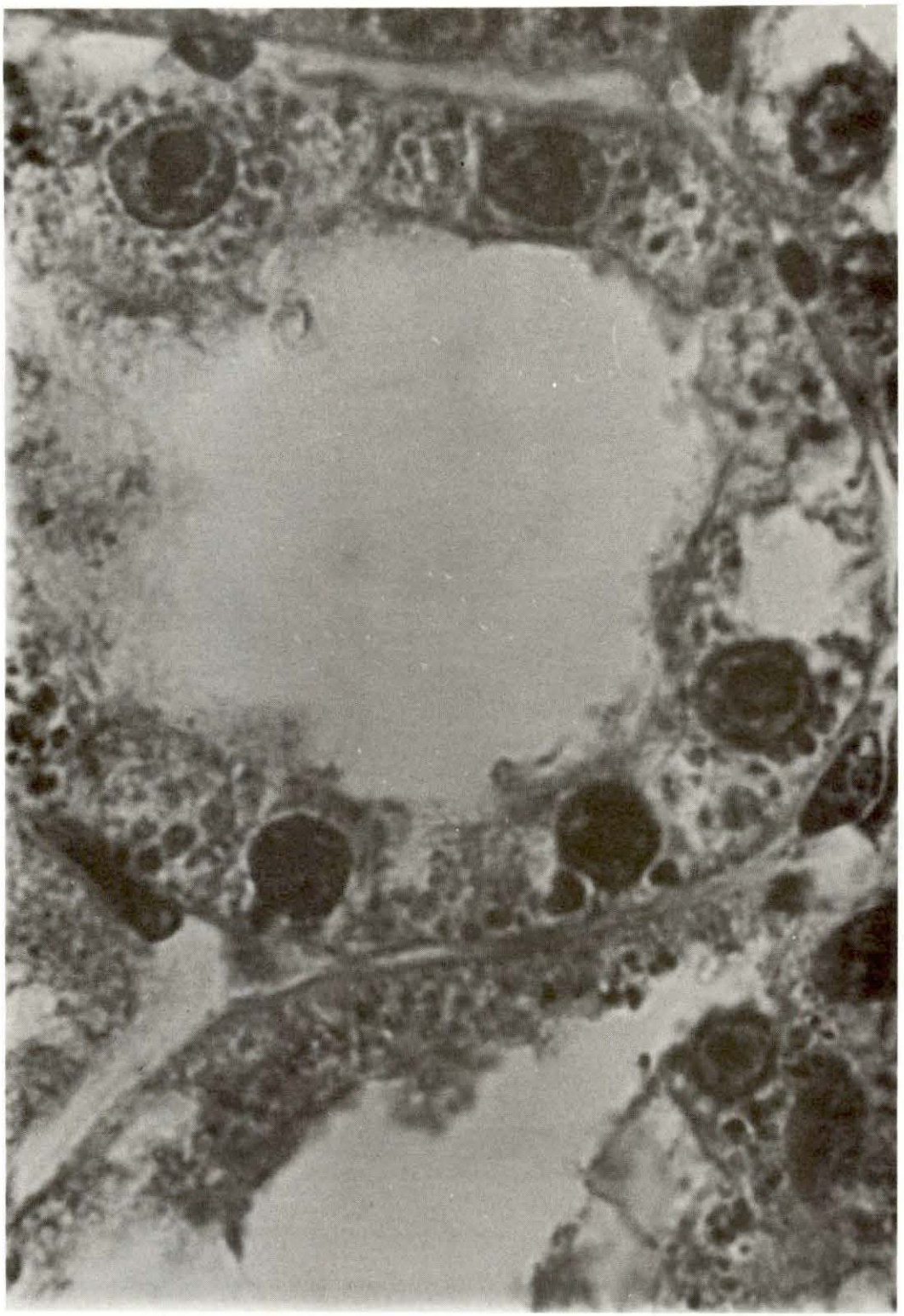


Figure 11. Average hemoglobin values in 12 sheep during exposure to lead throughout their gestation period and nine unexposed controls. Daily dosages of lead fed are given in Figure 1

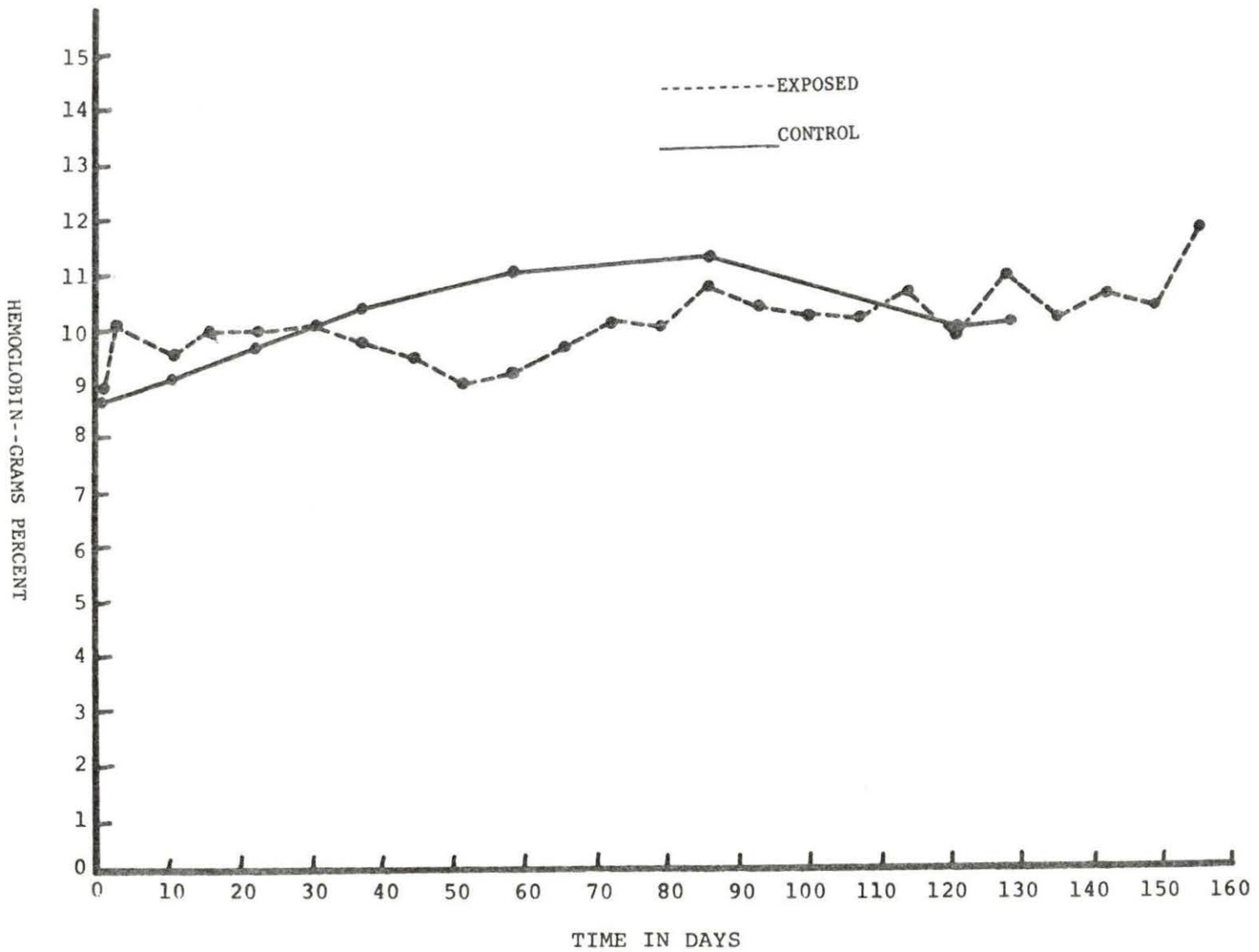

EXPOSED
CONTROL
HEMOGLOBIN--GRAMS PERCENT
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
TIME IN DAYS

Figure 12. Average packed cell volume values in 12 sheep exposed to lead throughout their gestation period and in nine sheep unexposed to lead. Daily dosages of lead fed are given in Figure 1

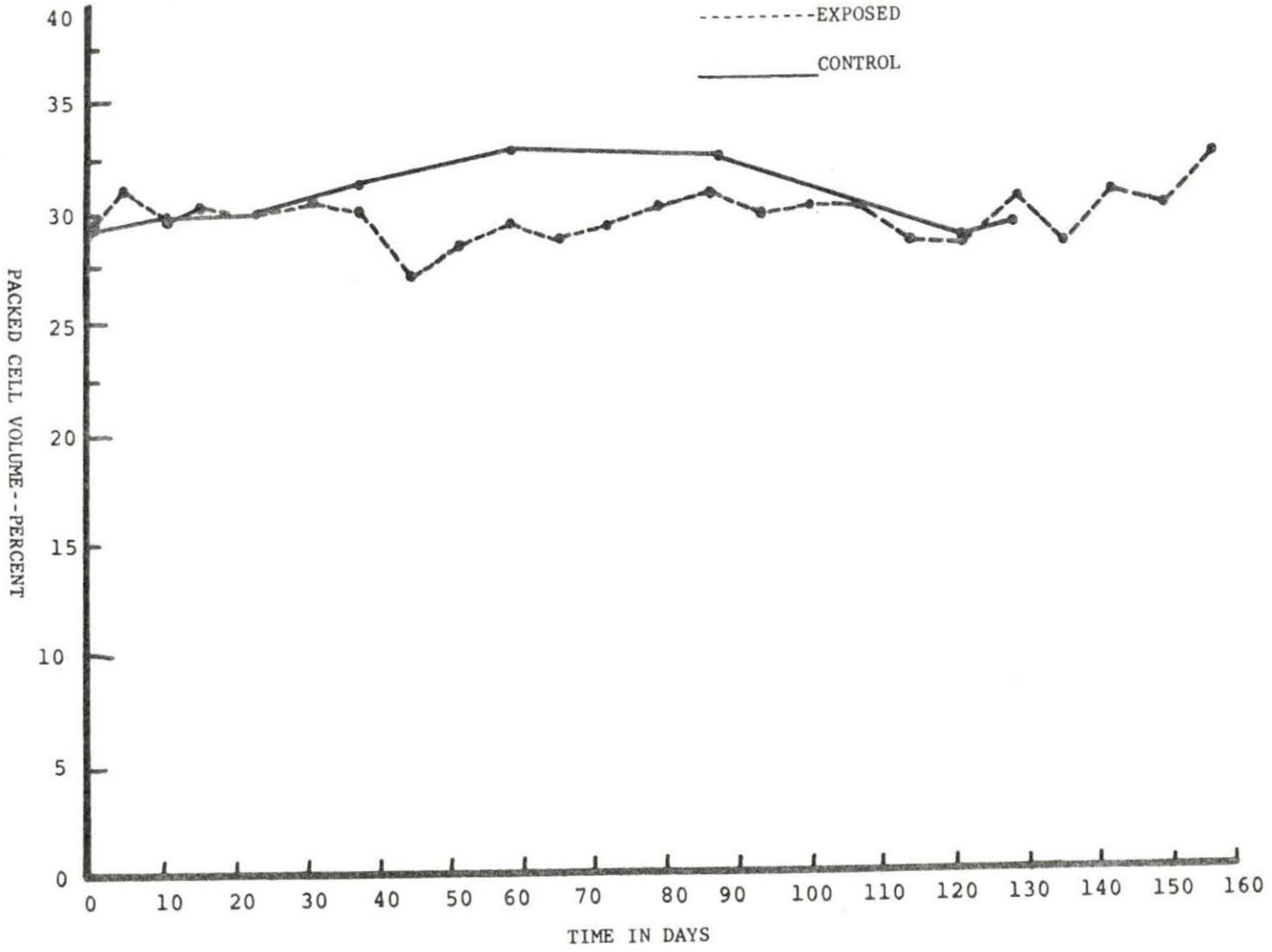

----------EXPOSED
CONTROL
PACKED CELL VOLUME--PERCENT
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
TIME IN DAYS

Figure 13. Average white blood cell counts in 12 sheep during exposure to lead throughout their gestation period and nine unexposed controls. Daily dosages of lead fed are given in Figure 1

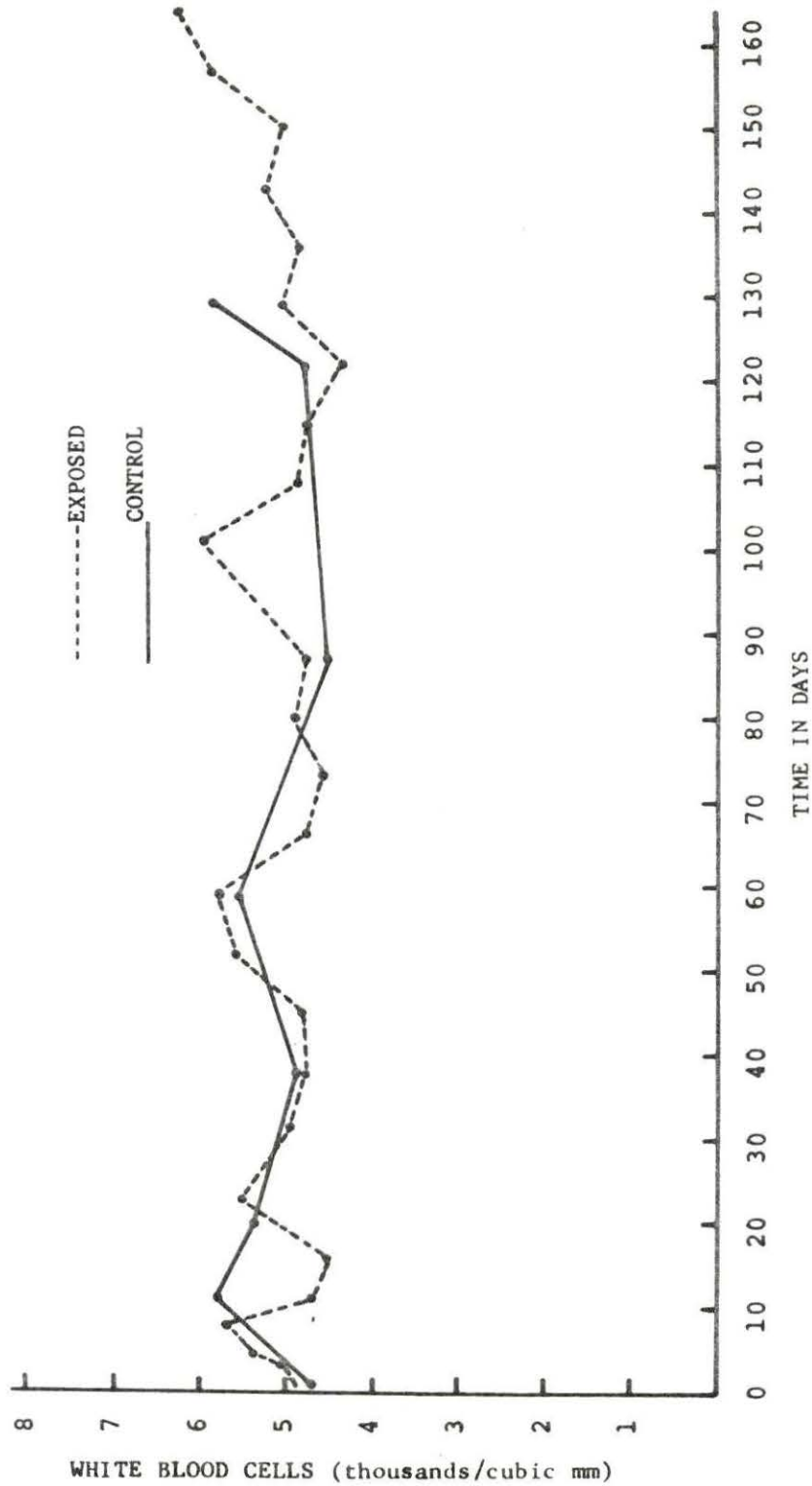

EXPOSED
CONTROL
WHITE BLOOD CELLS (thousands/cubic mm)
8
7
6
5
4
3
2
1
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
TIME IN DAYS

Figure 14. Average red blood cell counts in 12 sheep exposed and nine sheep unexposed to lead during their gestation period. Daily dosages of lead fed are given in Figure 1

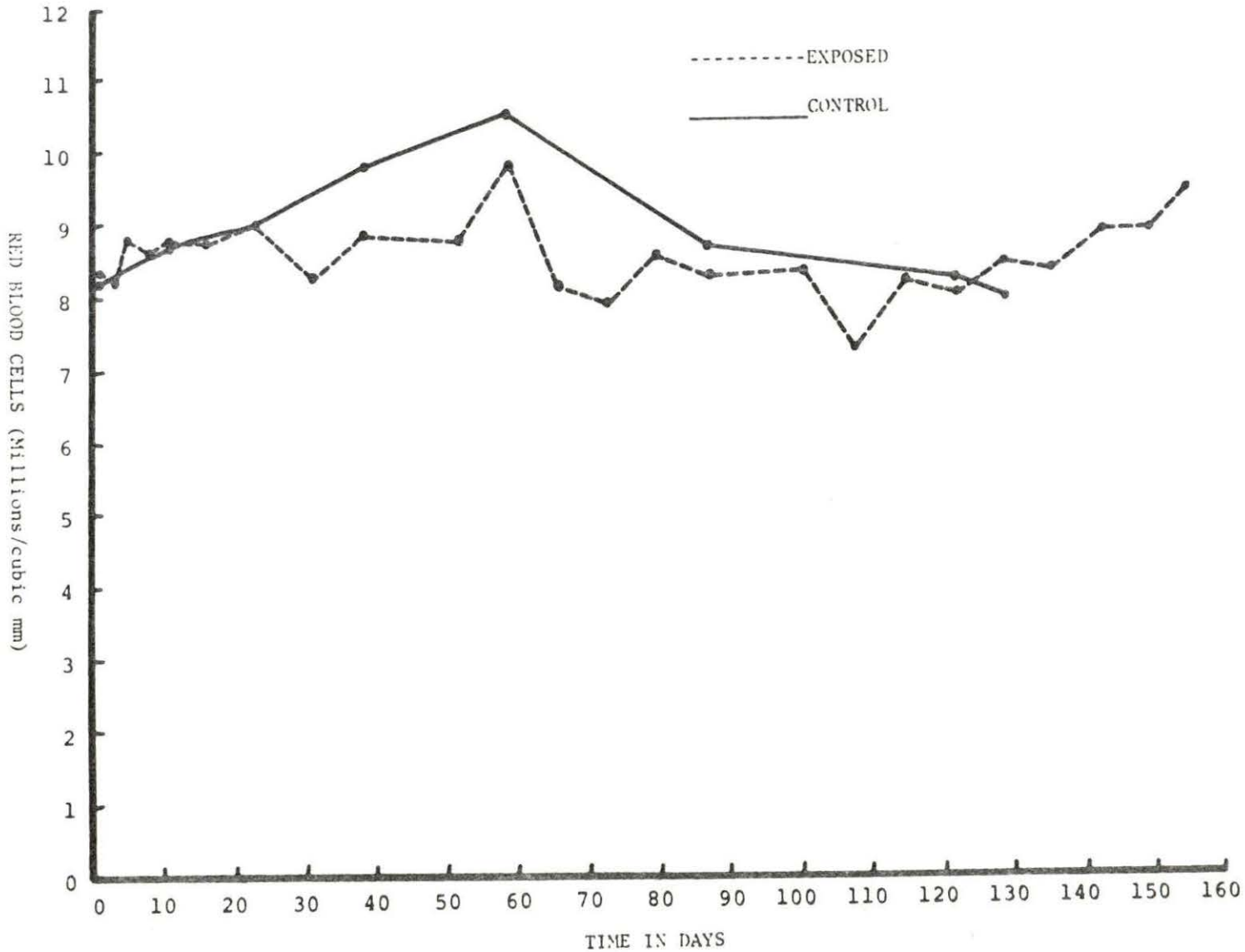

EXPOSED
CONTROL
RED BLOOD CELLS (Millions/cubic mm)
TIME IN DAYS
0
1
2
3
4
5
6
7
8
9
10
11
12
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160

Table 11. Analysis of variance for the studied parameters

	d.f.	MSB[a]	MSW[b]	$F = \frac{MSB}{MSW}$[c]
Hemoglobin	1	0.0054	0.5040	0.0107
Packed cell volume	1	0.0430	2.7801	0.0155
White blood cells	1	512	295,974	0.0017
Red blood cells	1	0.1453	0.3873	0.3750
Blood lead	1	0.3929	0.0222	17.7042**
Urine lead	1	0.3823	0.0598	6.3906*
ALA	1	0.6703	0.0768	8.7251**

[a]MSB = Mean square between groups.

[b]MSW = Mean square within, error term.

[c]Degrees of freedom for F are 1, 20.
Table values for F are 4.35 at 5% and 8.10 at 1%

**Significant at $P < 0.01$.

*Significant at $P < 0.05$.

phils and small but statistically significant increase in monocytes and neutrophils (bands) in lead poisoning is not presently known; and further, it cannot be explained on the basis of the experiment because of the large standard deviations and overlap between the two groups. Further studies are needed to clarify this finding.

A correlation analysis was made among the different variables, and the entire correlation matrix is given in Appendix C (Table 37). Table 15 contains the correlation coefficients related to the toxic effects of lead.

Table 12. Values of mean, standard deviations, and variance for studied parameters in the exposed and control groups

	Mean	Standard deviations	Variance	Published normal values for sheep
Hemoglobin				
Control	10.02	0.71	0.50	Schalm (1958)
Exposed	9.99	0.64	0.42	12(8-16)
PCV				
Control	30.60	1.58	2.49	38(24-50)
Exposed	30.51	1.60	2.56	
WBC				
Control	4.969×10^3	621	386,504	9(4-12)
Exposed	4.977×10^3	389	151,757	
RBC				
Control	8.73×10^6	0.76	0.57	12(8-16)
Exposed	8.57×10^6	0.36	0.13	
Blood Lead				
Control	0.035	0.044	0.002	.05-.25 ppm Hammond and Aronson (1964)
Exposed	0.302	0.196	0.038	
Urine Lead				
Control	0.105	0.184	0.034	0.07 ppm Blaxter (1950)
Exposed	0.369	0.274	0.075	
ALA				
Control	0.003	0.008	0.000	Not known
Exposed	0.352	0.374	0.140	

Table 13. Analysis of variance table for differential white blood cell counts of control and exposed groups

Variable	d.f.	MSB[a]	MSW[b]	$F = \frac{MSB}{MSW}$
Eosinophils	1	526.20	17.4	30.24**
Monocytes	1	259.67	28.87	8.99**
Neutrophils-Band	1	261.92	18.66	14.04**
Neutrophils-Segs	1	183.88	213.01	0.86
Lymphocytes	1	67.94	183.48	0.37

[a]MSB = Mean square between groups.

[b]MSW = Mean square within, error term.

**Significant at $P < .01$.

Table 14. Means, standard deviations, and variance of white blood cells of control and exposed groups

Variable	Number of observations	Mean %	Standard deviations	Variance	Published normal values (Schalm 1958)
Eosinophils					
Control	56	7.98	5.22	27.19	4.5%(1-10)
Exposed	189	4.49	3.78	14.31	
Monocytes					
Control	56	1.13	1.58	2.50	2.5%(1-6)
Exposed	189	3.58	6.03	36.37	
Neutrophils-Bands					
Control	56	0.13	0.47	0.22	0.5%(0-2)
Exposed	189	2.59	4.90	23.92	
Neutrophils-Segs					
Control	56	35.42	13.88	192.53	30%(10-50)
Exposed	189	37.49	14.72	216.82	
Lymphocytes					
Control	56	52.86	14.83	220.05	62%(40-75)
Exposed	189	51.60	13.07	170.07	

Table 15. Correlation coefficients among the parameters of interest

	Variable x Variable	Correlation coefficient	Significance
1.	Blood Lead x Urine Lead Says that as blood lead increased, urinary output of lead also increased. This is an expected relationship.	0.220	$P < .01$
2.	Blood Lead x Urine ALA At the doses used in this experiment, the amount of ALA excreted was correlated with blood lead levels.	0.473	$P < .01$
3.	Urine Lead x ALA This indicates that urine lead and urine ALA are directly related.	0.387	$P < .01$
4.	ALA x Feed Lead This indicates that the amount of ALA excreted is related to the amount of lead fed. Again, it is possible that a higher correlation coefficient would be obtained if lead levels were not varied so much from day to day.	0.298	$P < .01$
5.	Feed Lead x Eosinophils Eosinophils were the only blood cells correlated with lead exposure. This indicates that the number of eosinophils is directly related to exposure to lead.	0.260	$P < .01$
6.	Feed Lead x Urine Lead This indicates that the amount of lead excreted is proportional to the amount of lead fed. Lack of a higher degree of correlation reflects the fact that the amount of lead fed was varied quite often between high and low levels as the experiment progressed.	0.176	$P < .05$
7.	Feed Lead x Hemoglobin The magnitude of change in hemoglobin level over the exposure period was approximately 1 gm/100 ml. The negative correlation coefficient indicates that when lead exposure was high, hemoglobin tended to be depressed, and as lead exposure decreased during the latter part of the experiment, hemoglobin concentration increased (see Figure 11).	-0.198	$P < .05$
8.	ALA x Hemoglobin The excretion of ALA in the urine is inversely related to hemoglobin. As hemoglobin levels declined initially, ALA excretion increased, and later as hemoglobin levels increased, ALA excretion decreased (see Figures 5 and 11).	-0.158	$P < .05$

SUMMARY AND CONCLUSIONS

Twelve sheep were exposed to sublethal daily doses of powdered lead in their feed during the entire period of gestation, and nine were treated as unexposed controls. Most of the exposed sheep showed anorexia and depression characteristic of lead poisoning one week after beginning the experiment. The percentage of animals showing clinical signs was related to the blood lead levels. However, none of the exposed sheep showed clinical signs after 115 days of the experiment, even though blood lead levels were only slightly lower than during the first week of the experiment. The rate of lambing was 25 and 100% in the exposed and unexposed sheep, respectively. Abortions occurred at a rate of 25% in the exposed group but did not occur in the control group. Five exposed sheep either did not conceive or their fetuses were resorbed.

Levels of lead in blood and urine of exposed sheep were related to the amount of lead in the feed. Urinary excretion of delta-aminolevulinic acid was also related to the amount of lead fed.

Analysis of the tissues for lead from six exposed sheep revealed mean values of 10 ppm in the liver, 39 ppm in the kidney, 7 ppm in the spleen, 5 ppm in the brain, 31 ppm in the rumen contents, 1.5 ppm in the heart muscle, and 3 ppm in the aorta. Analysis of comparable tissues from control sheep revealed less than 1 ppm lead, which is considered to be background and of no clinical significance. Levels of 12 and 43 ppm lead were found in the livers of two nonterm fetuses from exposed dams. Levels from 0.14 to 0.17 ppm lead were found in the blood of two lambs born to exposed dams. Blood from eight lambs from control dams contained no lead.

Microscopic examination of the blood smears from exposed sheep revealed anisocytosis, poikilocytosis, many immature red blood cells, and many basophilic stippled cells. These changes were related to the dosage of lead. The blood picture was normal in the unexposed sheep.

Histopathological examination of the kidneys from the exposed sheep revealed large intranuclear inclusions in the tubular epithelium.

Statistical analysis indicated that lead exposure did not produce significant changes in hemoglobin, PCV, WBC, and RBC values between the control and experimental groups. However, significant differences were observed in blood lead, urine lead, and urine ALA values between the exposed and unexposed sheep. Statistical analysis of the differential white blood cell counts indicated that the increased numbers of monocytes and neutrophils (bands) and decreased numbers of eosinophils were highly significant in the exposed sheep, even when the total number of WBC's was less than in the unexposed sheep. There was no significant increase in lymphocytes and neutrophils (segs).

On the basis of correlation analysis, the following conclusions were made:

A. Highly significant positive correlations were found between the following:
    1. Blood lead and urine lead
    2. Blood lead and urine ALA
    3. Urine lead and urine ALA
    4. Feed lead and urine ALA
    5. Feed lead and eosinophils

B. A significant correlation was found between lead and urine lead.

C. Significant negative correlations were found between:

1. Feed lead and hemoglobin
2. ALA and hemoglobin

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APPENDIX A: CHEMICAL AND HEMATOLOGICAL DATA IN THE EXPOSED AND UNEXPOSED SHEEP

Table 16. Chemical and hematological data from sheep 343 in the lead exposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	10.7	34.0	4,840	---[a]	0.20	--	--	Pre-exposure period
11-25-69	11.5	36.4	6,400	---	N.T.**	--	--	
12-2-69	10.0	29.0	4,650	---	0.03	--	--	
12-9-69	9.0	30.0	7,780	8.77	0.09	0.48	N.T.	Post-exposure period
12-11-69	11.0	33.0	6,350	8.85	0.17	0.31	0.20	
12-13-69	9.4	30.0	6,530	10.05	0.13	0.27	0.30	Reduced appetite: 12/15/69, 12/18/69, 12/21/69, 12/22/69,
12-16-69	10.5	32.0	6,320	9.69	0.68	0.25	1.40	12/24/69, 12/31/69, 2/2/70,
12-19-69	9.3	32.5	5,100	---	0.45	0.35	0.80	2/11/70, 3/25/70, 3/27/70.
12-24-69	10.5	31.5	5,500	---	0.21	0.25	0.30	
12-31-69	9.0	26.5	7,640	8.51	0.30	0.65	0.50	Reduced appetite and depres-
1-8-70	10.5	29.8	6,000	8.44	0.47	--	1.30	sion: 12/16/69, 1/16/70,
1-15-70	10.3	30.0	6,000	---	0.44	0.49	0.80	1/24/70, 2/1/70, 2/3/70, 2/7/70, 2/10/70, 3/20/70,
1-22-70	10.5	23.0	5,200	---	0.75	--	1.30	3/21/70.
1-29-70	10.0	31.5	7,500	---	0.33	0.16	0.30	
2-5-70	10.5	32.8	8,600	10.00	0.55	1.20	1.00	Depression observed: 1/11/70,
2-12-70	9.3	28.5	5,500	8.09	0.67	0.39	1.10	1/20/70, 1/26/70, 1/27/70,
2-19-70	11.5	30.5	5,600	8.74	0.58	--	1.00	2/5/70, 2/9/70.
2-26-70	9.8	30.0	4,010	8.75	0.74	0.71	2.40	

[a]Data not available.

**Negative to test.

Table 16. (Continued)

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
3-5-70	11.8	30.0	4,300	8.67	0.69	0.93	1.70	No clinical signs were
3-12-70	9.8	29.0	5,290	7.87	0.60	1.10	1.70	observed after 3/27/70.
3-19-70	9.3	29.0	9,800	8.74	0.60	--	1.80	
3-26-70	9.3	28.9	4,800	7.04	0.50	1.20	1.40	Aborted on 5/20/70 (fetus was
4-2-70	9.5	27.8	6,000	8.03	0.39	0.62	0.60	normal male weighing 8.3 lbs.).
4-9-70	10.3	31.0	10,700	7.96	0.40	0.32	0.20	
4-16-70	11.0	31.5	5,000	8.75	0.41	0.60	0.40	Chemical analyses of the fetal specimens for lead revealed
4-23-70	10.8	31.0	5,160	9.32	0.37	0.61	0.50	the following:
4-30-70	11.8	34.0	5,800	9.80	0.45	--	--	Fetus
5-7-70	11.3	33.5	5,125	9.40	0.31	--	--	Liver--0.93 ppm
5-14-70	13.5	40.0	8,370	10.20	0.47	--	0.20	Kidney--0.29 ppm
5-21-70	14.5	41.0	4,720	9.41	0.21	0.49	0.20	Brain--Negative
								Stomach Contents--Negative

Table 17. Chemical and hematological data from sheep 350 in the lead exposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	11.1	35.0	3,960	---[a]	0.18	--	--	Pre-exposure period
11-25-69	10.0	33.2	4,200	---	N.T.**	N.T.	--	
12-2-69	9.3	27.0	4,500	---	N.T.	--	--	
12-9-69	8.3	26.0	5,000	7.92	0.28	0.23	N.T.	Post-exposure period
12-11-69	9.5	27.5	4,500	7.70	0.25	0.36	0.55	
12-13-69	9.4	31.0	5,000	9.23	0.20	0.34	0.60	Bred 12/11/69. Rebred 12/13/69.
12-16-69	10.0	31.0	5,350	8.99	0.50	--	1.60	
12-19-69	9.5	28.8	5,650	---	0.34	0.15	0.50	Reduced appetite: 12/15/69,
12-24-69	9.3	28.5	2,050	---	0.18	0.08	0.20	12/16/69, 12/18/69, 12/21/69,
12-31-69	8.3	24.5	4,900	8.03	0.25	--	0.20	12/22/69, 1/2/70, 1/18/70,
1-8-70	10.0	29.5	4,000	8.00	0.38	--	--	2/3/70, 3/1/70, 3/16/70,
1-15-70	8.3	26.5	4,250	---	0.37	0.51	0.95	3/19/70, 3/28/70.
1-22-70	7.5	22.0	6,000	---	0.47	0.91	1.20	No clinical signs were
1-29-70	7.5	24.5	6,100	---	0.46	0.82	0.80	observed after 3/28/70.
2-5-70	8.3	26.5	9,500	9.33	0.35	0.87	0.14	
2-12-70	9.5	29.5	5,400	8.45	0.40	0.34	1.00	Depression observed: 1/10/70,
2-19-70	8.3	25.3	4,100	7.51	0.36	--[a]	--	1/11/70, 1/30/70, 2/16/70,
2-26-70	8.8	28.0	5,070	8.10	0.42	--	0.80	2/17/70.

[a]Data not available.

**Negative to test.

Table 17. (Continued)

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
3-5-70	9.0	26.5	5,200	7.64	0.32	--	0.90	Reduced appetite and depression: 12/17/69, 12/31/69, 1/14/70, 1/15/70, 1/22/70, 1/22/70, 1/23/70, 2/4/70.
3-12-70	9.8	25.5	4,900	8.42	0.28	0.58	0.20	
3-19-70	10.3	25.8	6,600	7.95	0.50	--	--	
3-26-70	9.5	27.5	5,050	7.04	0.34	0.68	0.70	
4-2-70	9.3	27.0	4,700	7.81	0.20	0.36	0.10	
4-9-70	9.5	27.0	3,800	7.77	0.15	0.32	0.30	Gave birth to weak but normal female lamb weighing 5 lbs.
4-16-70	9.5	27.0	4,700	7.77	0.28	--	0.20	
4-23-70	10.3	27.8	5,430	8.42	0.22	0.43	0.20	
4-30-70	10.3	30.0	4,600	9.03	0.36	--	0.50	
5-7-70	9.3	27.0	4,950	8.58	0.28	0.77	--	
5-14-70	11.0	33.0	5,710	8.91	0.32	0.66	0.18	
5-21-70	12.3	35.0	8,725	10.90	0.18	--	--	

Table 18. Chemical and hematological data from sheep 351 in the lead exposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	13.6	39.0	4,400	---[a]	0.20	--	--	Pre-exposure period
11-25-69	11.0	35.0	6,750	---	N.T.**	N.T.	--	
12-2-69	12.5	37.0	5,950	---	0.04	0.16	--	
12-9-69	9.3	29.0	5,400	8.44	0.054	--	N.T.	Post-exposure period
12-11-69	10.3	30.0	4,300	8.37	0.23	0.75	0.60	
12-13-69	9.8	30.0	3,840	8.37	0.21	0.34	1.30	Bred 12/12/69. Rebred 12/13/69.
12-16-69	9.3	27.5	4,630	8.16	0.35	0.08	1.60	
12-19-69	10.0	29.0	4,050	---	0.31	0.15	1.20	Reduced appetite: 1/1/70,
12-24-69	10.0	30.0	2,550	---	0.28	0.34	0.80	1/3/70, 1/5/70, 1/6/70,
12-31-69	10.5	31.5	4,760	10.61	0.56	N.T.	2.80	1/18/70, 1/19/70, 2/3/70,
1-8-70	11.5	33.5	4,100	8.37	0.49	1.1	1.48	2/7/70, 2/9/70, 2/22/70,
1-15-70	11.5	36.0	3,700	---	0.35	0.66	0.80	3/2/70, 3/7/70, 3/8/70.
1-22-70	10.8	33.0	3,300	---	0.65	0.41	0.60	Depression observed: 12/17/69,
1-29-70	10.5	31.0	4,950	---	0.35	--	0.32	1/30/70, 1/31/70, 2/16/70,
2-5-70	11.3	33.0	3,800	9.60	0.64	0.56	0.99	2/17/70.
2-12-70	11.5	35.0	3,700	8.92	0.72	0.38	0.80	
2-19-70	11.5	36.0	3,200	9.12	0.48	--[a]	0.50	No clinical signs were
2-26-70	11.3	34.0	4,700	9.07	0.70	--	--	observed after 3/20/70.

[a]Data not available.

**Negative to test.

Table 18. (Continued)

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
3-5-70	12.0	36.0	3,800	8.77	0.71	0.30	0.50	Reduced appetite and depression: 12/31/69, 1/14/70, 1/24/70, 1/25/70, 2/15/70, 3/1/70, 3/6/70, 3/10/70, 3/11/70, 3/12/70, 3/13/70, 3/19/70, 3/20/70.
3-12-70	13.0	36.0	5,000	9.12	0.60	0.58	0.20	
3-19-70	11.5	33.0	6,700	8.39	0.50	0.34	0.40	
3-26-70	10.8	31.0	3,650	7.10	0.51	0.32	0.72	
4-2-70	14.0	25.0	4,900	7.58	0.35	--	--	
4-9-70	9.5	27.5	5,300	6.79	0.34	--	0.10	
4-16-70	12.3	31.0	4,000	8.39	0.51	0.14	0.70	On 4/23/70, fetal membranes were passed by sheep. Abortion was suspected. Next day a premature macerated male aborted fetus was found in barn. Postmortem exam of dam revealed a large number of small abscesses in lungs. Bacteriological studies did not result in isolation of pathogens.
4-23-70	9.5	26.0	4,460	7.23	0.31	--	--	

Chemical analyses of maternal specimens for lead revealed:

Liver--10.0 ppm	Spleen--7.0 ppm
Kidney--32.0 ppm	Aorta--1.0 ppm
Brain--less than 1.0 ppm	Heart muscle--1.0 ppm
Rumen contents--5.0 ppm	Muscle--1.0 ppm

Chemical analyses of the fetus for lead revealed:

Lumbar region--0.45 ppm
Thoracic region--0.21 ppm
Head region--0.12 ppm

Table 19. Chemical and hematological data from sheep 353 in the lead exposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	12.5	36.5	3,650	---[a]	0.10	--	--	Pre-exposure period
11-25-69	12.0	36.2	1.900	---	N.T.**	0.18	--	
12-2-69	10.5	33.0	3,750	---	N.T.	0.40	--	
12-9-69	10.0	34.0	2,470	9.48	0.03	0.20	N.T.	Post-exposure period
12-11-69	10.5	32.0	5,150	9.04	0.20	--	0.15	
12-13-69	11.1	34.0	5,380	9.70	0.31	0.35	1.20	Reduced appetite: 12/15/69, 12/25/69, 12/31/69, 1/19/70,
12-16-69	10.0	31.5	4,410	8.85	0.22	0.07	0.01	1/24/70, 2/5/70, 2/6/70,
12-19-69	10.3	31.8	2,500	---	0.25	N.T.	N.T.	2/19/70, 2/21/70, 2/22/70,
12-24-69	11.0	32.9	3,950	---	0.27	N.T.	N.T.	2/28/70, 3/16/70, 3/17/70,
12-31-69	12.0	37.3	3,100	10.60	0.53	0.38	0.15	3/19/70, 3/20/70, 3/21/70.
1-8-70	8.0	35.8	3,700	9.19	0.49	0.81	1.30	
1-15-70	10.5	32.0	3,550	---	0.48	2.00	0.01	Depression observed: 1/20/70, 2/14/70, 2/17/70.
1-22-70	10.0	29.5	3,000	---	0.63	--	0.58	
1-29-70	8.8	28.5	3,000	---	0.44	0.16	N.T.	No clinical signs were observed
2-5-70	10.3	30.0	3,750	9.53	0.28	0.76	0.01	after 3/21/70.
2-12-70	9.8	29.5	3,570	8.36	0.50	N.T.	0.20	
2-19-70	9.8	30.0	2,400	7.92	0.45	--	1.00	Gave birth to normal male lamb
2-26-70	9.0	27.0	3,700	7.58	0.49	N.T.	0.05	weighing 5 lbs.

[a] Data not available.

**Negative to test.

Table 19. (Continued)

Date	Hematology				Lead (ppm)		Urine ALA	Comments
	Hb gm/100 ml	PCV	WBC	RBC (10^6)	Blood	Urine	mg/100 ml	
3-5-70	10.8	33.0	3,700	8.37	0.46	N.T.	0.001	
3-12-70	10.8	31.0	4,100	7.67	0.38	0.09	0.10	
3-19-70	11.5	34.0	6,700	8.39	0.50	0.14	0.38	
3-26-70	11.8	36.0	3,950	8.20	0.31	0.40	0.35	
4-2-70	13.0	38.0	3,450	9.73	0.24	0.28	0.05	
4-9-70	12.0	34.0	4,600	9.64	0.23	--	N.T.	
4-16-70	13.5	37.0	5,750	9.55	0.32	0.42	N.T.	
4-23-70	11.8	33.0	3,650	9.49	0.23	0.09	N.T.	
4-30-70	12.3	35.3	4,900	9.90	0.46	0.28	0.05	
5-7-70	12.8	36.5	3,425	10.36	0.22	--	0.20	
5-14-70	15.0	39.5	4,390	10.76	0.40	0.61	0.40	
5-21-70	14.5	40.3	7,795	9.83	0.13	0.58	0.30	

Table 20. Chemical and hematological data from sheep 354 in the lead exposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	9.8	29.0	2,640	---[a]	0.30	--	--	Pre-exposure period
11-25-69	9.0	29.0	3,300	---	N.T.**	0.12	--	
12-2-69	9.3	27.0	2,900	---	N.T.	0.40	--	
12-9-69	8.0	28.0	3,120	6.95	0.10	0.15	N.T.	Post-exposure period
12-11-69	8.5	24.5	5,980	6.68	0.24	0.25	0.13	
12-13-69	8.0	26.0	5,100	6.83	0.53	0.22	0.80	Bred on 12/21/69 (apparently did not conceive).
12-16-69	8.0	22.8	8,100	6.26	0.43	0.45	1.00	
12-19-69	6.0	18.0	3,200	---	0.36	N.T.	0.30	Reduced appetite: 12/28/69,
12-24-69	6.5	20.5	5,400	---	0.31	N.T.	0.01	12/31/69, 1/5/70, 1/7/70,
12-31-69	7.0	21.5	5,600	5.94	0.53	--	1.40	1/8/70, 1/13/70, 1/14/70,
1-8-70	8.0	26.3	5,100	5.85	0.59	0.38	1.20	1/16/70, 1/19/70, 1/24/70,
1-15-70	8.0	25.0	3,900	---	0.34	0.51	1.00	2/20/70, 2/22/70, 3/15/70,
1-22-70	8.3	26.8	4,900	---	0.63	0.63	1.20	3/16/70, 3/17/70, 3/20/70, 3/21/70, 3/26/70, 3/30/70,
1-29-70	8.5	28.3	3,900	---	0.34	0.80	0.50	3/31/70.
2-5-70	8.5	27.5	6,250	8.21	0.37	0.62	0.60	
2-12-70	8.5	28.3	4,500	6.96	0.50	0.33	0.50	No clinical signs were observed
2-19-70	8.3	26.5	3,800	6.59	0.44	--	0.85	after 3/31/70.
2-26-70	8.3	26.5	3,750	6.85	0.45	--	0.50	

[a]Data not available.

**Negative to test.

Table 20. (Continued)

Date	Hematology				Lead (ppm)		Urine ALA	Comments
	Hb gm/100 ml	PCV	WBC	RBC (10^6)	Blood	Urine	mg/100 ml	
3-5-70	9.8	28.0	3,800	6.77	0.44	--	0.50	Depression observed: 1/20/71,
3-12-70	8.5	25.0	3,200	7.05	0.33	0.29	0.40	2/19/70.
3-19-70	8.0	25.5	3,600	6.51	0.39	0.26	0.60	
3-26-70	8.0	26.0	5,000	5.69	0.34	0.54	0.60	Reduced appetite and depression:
4-2-70	9.5	28.0	3,650	7.49	0.24	0.32	0.22	1/3/70, 1/21/70, 1/22/70.
4-9-70	9.5	28.5	3,650	7.62	0.18	0.31	N.T.	
4-16-70	9.8	29.3	3,860	8.10	0.30	0.39	N.T.	
4-23-70	10.3	31.0	4,200	8.63	0.28	--	0.10	
4-30-70	10.5	32.5	5,000	8.86	0.40	--	0.10	
5-7-70	11.3	33.0	4,960	9.50	0.27	--	--	
5-14-70	11.5	34.3	5,170	9.77	0.34	0.25	0.05	
5-21-70	11.0	34.0	4,980	8.77	0.16	0.23	0.10	

Table 21. Chemical and hematological data from sheep 355 in the lead exposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	11.0	30.0	6,380	---[a]	0.09	--	--	Pre-exposure period
11-25-69	10.0	36.2	7,400	---	N.T.**	N.T.	--	
12-2-69	11.0	37.0	4,950	---	N.T.	N.T.	--	
12-9-69	9.5	33.0	5,250	9.47	0.55	--	N.T.	Post-exposure period
12-11-69	11.5	37.0	5,200	9.87	0.24	0.82	0.20	
12-13-69	11.1	33.0	5,450	9.04	0.28	0.17	0.50	Bred 12/18/69 (apparently did not conceive).
12-16-69	10.5	32.3	3,700	9.18	0.63	0.18	0.20	
12-19-69	10.3	31.5	5,750	---	0.45	0.11	0.52	Reduced appetite: 12/15/69,
12-24-69	11.5	32.0	6,860	---	0.27	0.11	0.01	12/17/69, 12/24/69, 12/27/69,
12-31-69	11.5	33.3	7,150	10.10	0.50	0.49	0.65	1/3/70, 1/4/70, 1/5/70,
1-8-70	11.5	34.0	6,900	10.05	0.49	0.44	0.45	1/7/70, 1/8/70, 1/14/70,
1-15-70	11.3	34.0	6,050	---	0.35	0.63	0.80	1/15/70, 1/16/70, 1/17/70, 1/22/70, 1/29/70, 2/2/70,
1-22-70	12.0	33.0	5,800	---	0.61	0.63	0.80	2/9/70, 2/22/70, 3/2/70,
1-29-70	10.5	32.0	6,900	---	0.39	0.40	0.30	3/6/70, 3/7/70, 3/8/70, 3/9/70,
2-5-70	10.8	35.5	6,290	10.71	0.35	0.10	N.T.	3/11/70, 3/19/70, 3/21/70,
2-12-70	10.8	33.8	6,400	9.99	0.67	0.43	0.30	3/22/70, 3/23/70, 3/25/70,
2-19-70	11.0	37.5	6,200	9.47	0.60	--	0.40	3/26/70, 3/28/70.
2-26-70	9.8	25.3	5,840	8.37	0.74	0.48	0.80	

[a]Data not available.

**Negative to test.

Table 21. (Continued)

Date	Hematology				Lead (ppm)			Comments
	Hb gm/100 ml	PCV	WBC	RBC (10^6)	Blood	Urine		
3-5-70	10.0	28.5	5,900	8.41	0.66	--	0.80	No clinical signs were observed
3-12-70	9.5	27.0	7,010	8.34	0.56	0.44	0.60	after 3/28/70.
3-19-70	9.3	27.5	5,500	8.32	0.50	0.24	0.38	
3-26-70	10.3	28.5	6,300	7.18	0.60	0.36	0.50	Reduced appetite and depres-
4-2-70	10.3	27.0	5,500	8.12	0.40	--	0.20	sion: 1/1/70, 2/14/70.
4-9-70	9.8	28.5	5,200	8.49	0.46	--	0.10	
								Depression observed: 2/15/70.
4-16-70	11.0	32.5	5,150	9.03	0.56	0.46	0.30	
4-23-70	10.0	27.5	5,350	7.96	0.46	0.70	0.70	
4-30-70	10.0	29.0	5,550	8.62	0.61	0.62	0.80	
5-7-70	10.0	28.0	5,880	8.59	0.49	--	--	
5-14-70	11.5	33.8	5,680	9.51	0.59	0.42	N.T.	
5-21-70	10.8	29.6	6,200	10.37	0.39	--	0.20	

Table 22. Chemical and hematological data from sheep 357 in the lead exposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	11.4	34.5	4,290	---[a]	0.15	--	--	Pre-exposure period
11-25-69	11.0	36.2	4,600	---	N.T.**	N.T.	--	
12-2-69	9.5	29.0	4,350	---	0.04	0.24	--	
12-9-69	8.5	30.0	5,300	8.23	0.12	0.75	N.T.	Post-exposure period
12-11-69	11.0	31.0	4,560	8.39	0.28	--	0.40	
12-13-69	10.4	34.0	6,000	9.00	0.38	0.30	1.00	Reduced appetite: 12/13/69, 12/26/69, 12/30/69, 12/31/69,
12-16-69	9.5	31.0	6,250	8.30	0.28	0.02	0.10	1/7/70, 1/8/70.
12-19-69	10.0	30.0	6,200	---	--	N.T.	0.25	
12-24-69	9.5	30.5	5,100	--	0.31	N.T.	0.20	Bred 1/8/70 (apparently did not
12-31-70	10.3	31.8	4,700	8.93	0.51	0.38	0.85	conceive).
1-8-70	9.3	28.5	4,200	7.91	0.49	--	2.20	
1-15-70	10.0	32.0	6,400	---	0.45	0.52	1.80	Reduced appetite and depression on 1/13/70.
1-22-70	9.0	29.0	6,000	---	0.73	0.19	1.50	
1-29-70	9.5	30.5	5,900	---	0.79	1.00	1.40	Reduced appetite: 1/14/70,
2-5-70	9.3	30.8	5,020	10.37	0.45	0.30	0.80	1/15/70, 1/16/70, 1/19/70,
2-12-70	13.3	28.0	5,300	9.25	0.76	N.T.	0.50	1/24/70, 2/19/70, 2/22/70,
2-19-70	10.5	31.0	3,900	8.84	0.60	--	0.60	3/7/70, 3/8/70, 3/11/70,
2-26-70	11.0	35.0	4,760	10.22	1.5	0.34	0.40	3/15/70, 3/16/70, 3/17/70,

[a]Data not available.

**Negative to test.

Table 22 (Continued).

Date	Hematology				Lead (ppm)		Urine ALA	Comments
	Hb gm/100 ml	PCV	WBC	RBC (10^6)	Blood	Urine	mg/100 ml	
3-5-70	10.0	30.5	5,000	8.65	0.66	0.87	1.6	3/19/70, 3/20/70, 3/21/70,
3-12-70	10.5	30.5	5,000	9.39	0.62	0.49	--	3/26/70, 3/27/70, 3/28/70.
3-19-70	11.8	36.3	4,300	10.14	0.52	--	0.20	
3-26-70	10.8	31.5	5,600	8.70	0.50	--	0.20	No clinical signs were observed
4-2-70	11.5	33.0	5,700	10.04	0.40	0.48	0.04	after 3/28/70.
4-9-70	11.0	33.0	6,000	9.60	0.38	0.40	N.T.	
4-16-70	12.0	35.0	6,750	9.70	0.48	--	N.T.	
4-23-70	11.0	31.5	5,450	9.82	0.38	0.40	0.10	
4-30-70	12.0	35.5	6,600	10.19	0.44	--	--	
5-7-70	11.3	34.0	4,900	10.16	0.35	--	--	
5-14-70	13.5	41.0	5,690	10.38	0.52	0.33	0.15	
5-21-70	13.8	41.5	5,950	9.97	0.25	--	0.15	

Table 23. Chemical and hematological data from sheep 358 in the lead exposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	12.5	35.0	3,410	---[a]	0.14	--	--	Pre-exposure period
11-25-69	--	Blood clotted		---	--	N.T.**	--	
12-2-69	10.5	31.0	7,000	---	N.T.	--	--	
12-9-69	10.0	32.0	4,200	8.96	0.10	--	N.T.	Post-exposure period
12-11-69	12.0	37.0	4,800	9.26	0.20	0.10	N.T.	
12-13-69	12.5	37.0	5,900	10.69	0.37	0.30	1.40	Bred 12/28/69
12-16-69	11.5	33.8	6,370	9.04	0.36	0.15	0.75	Reduced appetite: 1/6/70,
12-19-69	11.0	33.5	4,300	---	0.38	N.T.	0.25	1/7/70, 1/8/70, 1/15/70,
12-24-69	12.0	35.5	4,800	---	0.39	--	0.70	1/16/70, 1/22/70, 1/24/70,
12-31-69	11.5	34.3	5,740	9.42	0.55	0.72	1.20	1/29/70, 2/11/70, 2/15/70,
1-8-70	13.0	38.0	4,600	0.63	0.79	1.10	2.85	2/16/70, 2/17/70, 2/19/70,
1-15-70	12.0	36.0	7,000	---	0.26	--	2.40	2/21/70, 2/22/70.
1-22-70	11.5	28.3	5,800	---	0.56	--	3.00	Reduced appetite and depres-
1-29-70	12.0	36.0	3,350	---	0.57	--	1.40	sion: 1/13/70, 1/21/70,
2-5-70	11.0	35.0	4,910	10.63	0.34	1.08	0.35	2/10/70.
2-12-70	10.5	29.5	4,100	8.92	0.68	--	0.20	
2-19-70	16.0	30.5	4,500	0.32	0.56	--	--	Died of anaphylectic shock from
2-26-70	12.3	36.0	4,430	10.19	0.56	--	0.80	I/m injection of penicillin-

[a]Data not available.

**Negative to test.

Table 23. (Continued)

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
3-5-70	12.0	34.0	7,700	9.23	0.48	--	0.70	streptomycin. A postmortem examination revealed enlarged and abscessed mediastinal lymph nodes. Was pregnant and fetus was about 2 months term. Chemical analyses of the maternal and fetal specimens revealed the following for lead: Liver--11.0 ppm Kidney--51.0 ppm Brain--4.0 ppm Rumen contents--2.0 ppm Spleen--3.0 ppm Aorta--3.0 ppm Heart muscle--1.0 ppm Placenta--Negative to test Fetal specimens: Liver--12.0 ppm Kidney--2.0 ppm Brain--1.0 ppm Bacteriological studies of mediastinal lymph nodes from sheep resulted in the isolation of Corynebacterium ovis.
3-12-70	11.5	34.0	6,630	9.22	0.46	--	--	
3-19-70	11.8	33.0	4,700	9.97	0.40	--	--	
3-26-70	13.3	35.0	4,700	8.25	0.37	--	1.00	

Table 24. Chemical and hematological data from sheep 363 in the lead exposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	11.1	35.5	7,000	---[a]	0.13	--	--	Pre-exposure period
11-25-69	11.5	35.7	5,500	---	N.T.**	N.T.	--	
12-2-69	10.3	32.0	5,950	---	N.T.	0.42	--	
12-9-69	7.0	23.0	6,800	7.36	0.14	0.20	N.T.	Post-exposure period
12-11-69	9.3	28.0	6,700	7.81	0.45	0.065	0.20	
12-13-69	9.8	32.0	7,000	9.06	0.43	0.35	1.35	Bred 1/1/70
12-16-69	10.0	33.8	7,310	9.54	0.52	0.15	1.00	Reduced appetite: 12/13/69,
12-19-69	9.3	29.0	6,250	---	0.43	0.04	N.T.	12/16/69, 12/17/69, 12/18/69,
12-24-69	10.0	30.5	5,700	---	0.33	0.39	0.50	12/25/69, 12/26/69, 12/29/69,
12-31-69	10.0	30.5	7,200	10.37	0.52	N.T.	1.10	1/4/70, 1/14/70, 1/19/70,
1-8-70	9.0	28.5	5,900	8.44	0.68	0.60	1.00	2/1/70, 2/22/70, 2/26/70,
1-15-70	8.3	28.5	3,900	---	0.34	2.00	0.78	2/28/70.
1-22-70	7.5	22.0	5,000	---	0.67	2.00	1.80	Reduced appetite and depres-
1-29-70	7.0	18.5	9,860	---	0.68	0.70	0.70	sion: 1/13/70, 1/21/70,
2-5-70	8.0	25.0	5,400	8.05	0.39	1.30	0.58	1/24/70, 2/7/70, 3/5/70.
2-12-70	8.0	25.5	5,500	8.29	0.77	0.80	0.90	
2-19-70	9.5	28.0	6,900	8.34	0.76	0.71	0.60	Depression observed: 1/10/70,
2-26-70	9.3	29.0	7,700	8.32	0.87	0.62	0.30	1/20/70.
3-5-70	10.5	31.0	2,500	8.25	0.78	--	--	

[a] Data not available.

**Negative to test.

Table 24. (Continued)

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
								On 3/5/70 was extremely weak. Could not get up. Was separated from experimental group and was given control pellets. Died 3/6/70. Postmortem examination revealed pleuritis and pneumonia. Was not pregnant. Chemical analyses of maternal specimens for lead revealed: Brain--3.0 ppm Kidney--49.0 ppm Aorta--3.0 ppm Heart muscle--2.0 ppm Liver--11.0 ppm Rumen contents--82.5 ppm Bacteriological examination of lungs did not result in the isolation of pathogenic bacteria.

Table 25. Chemical and hematological data from sheep 364 in the lead exposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	9.4	28.5	6,400	---[a]	0.30	--	--	Pre-exposure period
11-25-69	9.5	29.0	1,500	---	N.T.**	N.T.	--	
12-2-69	8.5	35.5	2,600	---	0.02	0.55	--	
12-9-69	8.3	28.0	1,200	8.52	0.11	--	N.T.	Post-exposure period
12-11-69	8.0	25.0	3,400	6.64	0.33	--	0.50	
12-13-69	8.2	26.0	2,550	7.14	0.34	0.16	1.10	Reduced appetite: 12/15/69, 12/16/69, 12/17/69, 12/27/69,
12-16-69	8.5	27.8	2,890	8.56	0.39	0.85	0.70	12/29/69, 12/30/69, 12/31/69,
12-19-69	8.5	26.5	3,250	---	0.32	0.08	0.52	1/1/70, 1/8/70, 1/15/70,
12-24-69	8.5	28.3	2,400	---	0.25	0.24	0.20	1/17/70, 1/19/70, 2/5/70,
12-31-69	8.3	25.5	3,500	8.69	0.41	0.51	0.95	2/9/70.
1-8-70	8.0	25.5	4,750	6.53	0.47	1.23	N.T.	
1-15-70	6.3	21.8	2,800	---	0.30	--	--	Reduced appetite and depression: 1/6/70, 1/14/70, 1/16/70,
1-22-70	6.3	21.0	2,100	---	0.58	1.10	1.80	1/22/70, 2/3/70, 2/4/70,
1-29-70	5.5	17.0	4,100	---	0.42	1.80	1.70	2/6/70, 2/7/70, 2/10/70.
2-5-70	5.3	16.5	3,800	5.64	0.30	1.30	0.32	
2-12-70	5.3	16.3	3,800	4.48	0.41	1.24	--	Observed depression: 1/10/70,
2-19-70	4.2	13.5	2,640	3.66	0.34	--	--	1/11/70, 1/26/70, 1/28/70, 1/30/70, 1/31/70, 2/11/70, 2/13/70, 2/14/70, 2/15/70.

[a]Data not available.

**Negative to test.

Table 25. (Continued)

Date	Hematology: Hb gm/100 ml	PCV	WBC	RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
								On 2/16/70 she was very weak. Was lying down. Clinical signs were grinding of teeth, emaciation, weakness. Died. Postmortem examination revealed gelatinous atropy of fat around heart, aorta, and spinal cord. Was not pregnant. Chemical analyses of maternal specimens for lead revealed: Liver--13.0 ppm Kidney--13.0 ppm Brain--15.0 ppm Rumen contents--87.5 ppm Spleen--10.0 ppm Aorta--5.0 ppm Muscle--Negative to test

Table 26. Chemical and hematological data from sheep 365 in the lead exposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	12.5	34.5	4,950	---[a]	0.04	--	--	Pre-exposure period
11-25-69	12.5	35.2	4,300	---	N.T.**	N.T.	--	
12-2-69	8.8	26.5	4,900	---	0.03	0.18	--	
12-9-69	10.3	33.0	5,300	8.34	0.03	0.77	N.T.	Post-exposure period
12-11-69	11.0	33.0	3,750	8.44	0.34	0.61	0.30	
12-13-69	11.8	35.0	5,600	9.26	0.48	0.12	1.25	Reduced appetite: 12/13/69, 12/25/69, 12/26/69, 12/31/69,
12-16-69	10.5	33.0	5,780	9.02	0.44	1.00	0.20	1/4/70, 1/5/70, 1/6/70,
12-19-69	11.5	35.0	5,700	---	0.46	0.52	1.00	1/7/70, 1/8/70, 1/16/70,
12-24-69	11.5	33.0	3,200	---	0.48	0.40	0.30	1/18/70, 1/25/70, 1/31/70,
12-31-69	11.5	34.5	6,500	9.12	0.67	1.00	1.00	2/5/70, 2/6/70, 2/7/70,
1-8-70	11.0	32.0	5,000	8.41	0.44	0.68	0.26	2/9/70, 2/10/70, 2/11/70,
1-15-70	10.5	31.0	6,000	---	0.52	1.13	1.20	2/19/70, 2/20/70, 2/21/70, 2/28/70, 3/12/70, 3/14/70,
1-22-70	10.8	33.0	5,400	---	0.65	0.61	0.80	3/15/70, 3/16/70, 3/17/70.
1-29-70	10.3	31.0	5,150	---	0.58	1.20	1.20	
2-5-70	9.5	29.0	5,650	9.76	0.37	0.77	0.35	Bred 12/20/69
2-12-70	11.0	33.0	4,600	8.62	0.67	--	1.10	
2-19-70	12.3	33.8	4,570	8.13	0.58	--	0.85	Separated from experimental
2-26-70	12.3	37.0	3,900	9.63	0.51	--	0.95	group 3/3/70.
3-5-70	12.5	35.0	7,400	8.86	0.33	0.13	0.01	Reduced appetite and depres-
3-12-70	11.0	31.5	8,400	8.92	0.38	0.64	0.30	sion: 1/17/70, 1/19/70,
3-17-70	10.0	32.0	8,000	7.92	0.37	--	--	2/26/70, 3/1/70, 3/4/70,

[a]Data not available.

**Negative to test.

Table 26. (Continued)

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
								3/5/70, 3/6/70, 3/7/70, 3/10/70, 3/17/70. Depression observed: 1/11/70, 3/2/70. Was weak and anemic. Placenta was protruding from the vulva. Postmortem examination revealed a mummified fetus in one horn of uterus. Bacteriological examination of uterus did not result in the isolation of pathogenic organisms. Chemical analyses of maternal tissues for lead revealed: Liver--8.0 ppm Kidney--37.0 ppm Brain--3.0 ppm Rumen contents--7.5 ppm Spleen--8.0 ppm Aorta--3.0 ppm Heart muscle--2.0 ppm Amniotic fluid--1.8 ppm Chemical analyses of fetal tissues for lead revealed: Liver--1.0 ppm Kidney--1.0 ppm Brain--Negative

Table 27. Chemical and hematological data from sheep 367 in the lead exposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	9.4	30.0	6,500	---[a]	0.086	--	--	Pre-exposure period
11-25-69	8.5	29.0	4,650	---	N.T.**	N.T.	--	
12-2-69	8.0	25.0	7,000	---	0.06	0.30	--	
12-9-69	9.0	27.0	7,100	7.43	0.11	--	N.T.	Post-exposure period
12-11-69	9.3	29.0	6,500	7.43	0.30	0.53	0.40	
12-13-69	8.3	29.0	6,900	7.51	0.34	--	0.60	Reduced appetite: 12/13/69, 12/21/69, 12/26/69, 12/28/69,
12-16-69	9.0	29.0	7,500	7.83	0.37	--	0.25	12/30/69, 12/31/69, 1/1/70,
12-19-69	8.8	27.0	4,800	---	--	N.T.	0.20	1/3/70, 1/4/70, 1/5/70, 1/8/70,
12-24-69	9.5	30.3	6,550	---	0.37	N.T.	0.15	1/15/70, 1/19/70, 1/21/70,
12-31-69	9.3	27.5	6,200	7.83	0.59	0.24	0.40	1/24/70, 1/26/70, 1/28/70,
1-8-70	10.5	28.5	6,300	8.58	0.80	2.00	0.82	1/29/70, 1/31/70, 2/1/70,
1-15-70	9.5	32.5	4,600	---	0.34	0.85	0.60	2/9/70, 2/10/70, 2/19/70,
1-22-70	8.8	27.5	6,000	---	0.62	0.78	0.90	2/22/70, 3/7/70, 3/8/70, 3/9/70, 3/12/70, 3/13/70,
1-29-70	8.3	27.5	7,150	---	0.55	0.20	0.30	3/14/70, 3/16/70, 3/17/70,
2-5-70	8.3	28.0	6,810	10.26	0.34	0.58	0.01	3/18/70, 3/19/70, 3/20/70,
2-12-70	9.0	29.0	6,310	7.46	0.75	0.27	0.40	3/21/70, 3/22/70.
2-19-70	9.3	27.0	8,070	7.67	0.55	--	0.40	
2-26-70	8.5	27.0	7,150	7.24	0.54	0.37	0.30	Observed depression: 1/10/70, 1/27/70, 2/16/70.
								Reduced appetite and depression on 1/13/70 and 1/14/70.

[a] Data not available.

**Negative to test.

Table 27. (Continued)

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
3-5-70	9.5	30.5	4,150	7.79	0.50	--	N.T.	Aborted 5/11/70. Fetus was
3-12-70	9.3	28.3	5,443	7.83	0.38	0.01	0.01	male and weighed 4 lbs. 15 oz.
3-19-70	8.8	27.0	4,700	7.51	0.33	0.05	0.34	
3-26-70	8.5	27.0	5,900	6.40	0.34	0.05	0.16	No congenital abnormality was
4-2-70	8.5	23.5	5,000	6.71	0.23	0.04	0.06	observed.
4-9-70	8.0	19.5	5,650	6.49	0.28	0.70	0.20	
4-16-70	7.3	21.0	5,950	6.00	0.38	0.11	N.T.	A postmortem examination of fetus did not reveal any
4-23-70	7.5	22.0	6,140	6.15	0.34	0.26	0.40	abnormality.
4-30-70	6.5	20.8	4,500	6.00	0.43	0.28	0.10	
5-7-70	6.3	21.0	6,860	5.91	0.37	--	--	Chemical analyses of the
5-14-70	7.0	21.5	6,900	6.84	0.59	0.17	0.18	fetal specimens for lead
5-21-70	7.5	22.0	6,225	5.56	0.35	--	N.T.	revealed:
						Kidney--1.20 ppm		Abomasal contents--0.78 ppm
						Liver--43.00 ppm		Amniotic fluid--0.06 ppm
						Brain--0.65 ppm		

Sheep was observed sick 6/7/70. Was emaciated, weak, and anemic. Euthanatized 6/13/70. Postmortem examination revealed an abscess in spleen and many small abscesses in lungs. Bacteriological examination of the lungs and spleen resulted in the isolation of Corynebacterium ovis.

Chemical analyses of the maternal specimens for lead revealed:

Liver--9.0 ppm	Rumen contents--2.5 ppm
Kidney--31.0 ppm	Spleen--8.0 ppm
Brain--6.0 ppm	Muscle--1.0 ppm

Table 28. Chemical and hematological data from sheep 344 in the unexposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	10.9	33.5	4,510	---[a]	0.08	--	--	Pre-exposure period
11-25-69	10.5	32.0	3,600	---	N.T.**	N.T.	--	
12-2-69	9.3	27.0	4,500	---	N.T.	0.16	--	
12-9-69	7.8	27.0	3,570	7.75	0.043	0.71	N.T.	Post-exposure period
12-19-69	8.3	28.0	5,450	---	N.T.	N.T.	N.T.	
12-31-69	9.0	27.0	4,700	8.44	N.T.	N.T.	N.T.	Bred 12/24/69
1-15-70	10.8	30.0	5,250	---	N.T.	N.T.	N.T.	Reduced appetite: 2/8/70,
2-5-70	10.3	30.0	4,400	10.30	N.T.	N.T.	N.T.	2/9/70, 2/10/70.
3-5-70	9.5	26.5	3,900	8.45	N.T.	0.21	N.T.	
4-9-70	10.3	31.0	5,100	8.23	N.T.	N.T.	N.T.	Was observed sick 2/10/70.
5-7-70	9.8	27.0	4,540	8.23	N.T.	N.T.	N.T.	Temperature 104.2°F. Gave 4 cc of penicillin-streptomycin I/m plus 1 cc Vitamin B_{12}. Began to eat.
								Gave birth to normal female lamb weighing 7 lbs. 6/9/70.

[a]Data not available.

**Negative to test.

Table 29. Chemical and hematological data from sheep 346 in the unexposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	9.4	31.5	3,100	---[a]	0.09	--	--	Reduced appetite on 2/8/70.
11-25-69	8.5	30.0	3,750	---	N.T.**	N.T.	--	
12-2-69	8.0	26.0	3,300	---	N.T.	--	--	Observed sick 2/10/70.
12-9-69	8.3	26.0	4,700	7.90	0.08	--	N.T.	
12-19-69	8.3	27.3	3,000	---	N.T.	N.T.	N.T.	Gave 4 cc penicillin-strepto-
12-31-69	8.5	30.5	3,100	9.25	N.T.	N.T.	N.T.	mycin I/m and 1 cc Vitamin B_{12}.
1-15-70	10.3	33.0	3,500	---	N.T.	N.T.	N.T.	Recovered and eating the next
2-5-70	10.5	30.0	4,100	9.53	N.T.	N.T.	N.T.	day.
3-5-70	10.0	30.5	5,100	8.45	N.T.	N.T.	N.T.	
4-9-70	8.5	25.0	4,100	7.33	N.T.	N.T.	N.T.	Gave birth to normal but weak
5-7-70	9.8	30.0	4,430	8.23	N.T.	N.T.	N.T.	female lamb weighing 5 lbs.

[a]Data not available.

**Negative to test.

Table 30. Chemical and hematological data from sheep 347 in the unexposed group

Date	Hematology: Hb gm/100 ml	PCV	WBC	RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	9.0	30.0	5,700	---[a]	0.21	--	--	Bred 12/21/69.
11-25-69	10.0	33.0	4,350	---	N.T.**	N.T.	--	
12-2-69	9.3	28.0	5,350	---	N.T.	N.T.	--	To compare residual lead levels
12-9-69	9.0	30.0	4,960	7.92	0.032	1.50	N.T.	in tissues with the exposed
12-19-69	8.8	29.3	6,700	---	--	N.T.	N.T.	group, it was decided to sacri-
12-31-69	8.5	28.0	6,500	8.11	0.05	0.40	N.T.	fice this sheep. A postmortem
1-15-70	10.3	32.0	5,800	---	N.T.	N.T.	N.T.	examination revealed pleuritis,
2-5-70	11.3	32.5	5,420	10.32	N.T.	N.T.	N.T.	pneumonia along with multifocal
3-5-70	11.8	35.0	4,900	9.17	N.T.	N.T.	N.T.	abscesses. Was pregnant. The fetus was of two months term.

Bacteriological exam of lung resulted in the isolation of Pasteurella multocida.

Chemical analyses of the maternal specimens for lead revealed:

Liver--Less than 0.1 ppm	Spleen--Negative to test
Kidney--Less than 1.0 ppm	Aorta--Negative to test
Brain--Negative to test	Heart muscle--Negative to test
Rumen contents--negative to test	Placenta--Less than 0.1 ppm

Fetal Tissue:

Liver--Less than 1.0 ppm	Amniotic fluid--Negative to test
Kidney--Less than 1.0 ppm	Placenta--Less than 1.0 ppm
Brain--Less than 1.0 ppm	

[a] Data not available.

**Negative to test.

Table 31. Chemical and hematological data from sheep 348 in the unexposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	9.0	28.0	4,600	---[a]	0.16	--	--	Bred 12/27/69
11-25-69	10.5	32.5	4,750	---	N.T.**	N.T.	--	
12-2-69	10.3	30.5	7,050	---	N.T.	0.53	--	Gave birth to normal lamb
12-9-69	9.0	28.5	6,300	10.96	0.035	0.50	N.T.	weighing 5-1/2 lbs. 5/25/70.
12-19-69	9.3	31.0	6,700	---	N.T.	N.T.	N.T.	
12-31-69	9.5	29.0	8,600	8.91	0.11	0.75	N.T.	Sheep died 5/26/70
1-15-70	10.5	31.0	6,600	---	N.T.	N.T.	N.T.	Postmortem examination revealed
2-5-70	11.5	34.0	8,600	10.90	N.T.	N.T.	N.T.	serous pleuritis, fibrinous
3-5-70	11.0	32.0	3,600	8.65	N.T.	N.T.	N.T.	peritonitis, and abscesses in
4-9-70	8.8	25.0	5,700	7.70	N.T.	N.T.	N.T.	the lung including the
5-7-70	9.3	25.0	7,200	6.77	N.T.	N.T.	N.T.	mediastinal lymph nodes.
								Bacteriological studies resulted in the isolation of Corynebacterium pyogenes from the abscesses.

[a] Data not available.

**Negative to test.

Table 32. Chemical and hematological data from sheep 349 in the unexposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	10.4	35.0	3,630	---[a]	0.15	--	--	Bred 12/12/69.
11-25-69	10.5	36.2	4,200	---	N.T.**	N.T.	--	
12-2-69	9.8	30.0	2,950	---	N.T.	--	--	Gave birth to dead male lamb
12-9-69	10.0	31.5	3,070	8.85	0.01	0.07	N.T.	weighing 9 lbs. 11 ozs. 5/5/70
12-19-69	9.5	31.0	4,100	---	N.T.	N.T.	N.T.	which died as a result of
12-31-69	10.3	33.0	4,500	9.69	0.10	0.39	N.T.	dystocia.
1-15-70	10.3	32.0	2,700	---	N.T.	N.T.	N.T.	Postmortem examination of fetus
2-5-70	11.0	34.5	3,900	10.47	N.T.	--	--	did not reveal any abnormality.
3-5-70	10.5	33.0	3,600	8.65	N.T.	--	--	
4-9-70	9.8	30.0	3,000	7.83	N.T.	N.T.	N.T.	Chemical analyses of fetal
5-7-70	10.5	33.0	5,450	8.34	N.T.	N.T.	N.T.	specimens for lead revealed: Liver--0.70 ppm; Kidney--1.40 ppm; Brain--0.43 ppm; Aorta--0.66 ppm; Heart muscle--0.75 ppm
								Bacteriological examination of specimens did not result in isolation of pathogenic bacteria.

[a]Data not available.

**Negative to test.

Table 33. Chemical and hematological data from sheep 359 in the unexposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	10.3	30.5	2,500	---[a]	0.14	--	--	Bred 12/24/69
11-25-69	10.3	31.2	3,200	---	N.T.**	N.T.	--	
12-2-69	9.8	20.5	3,400	---	0.032	0.35	--	Reduced appetite: 2/8/70,
12-9-69	8.0	26.0	3,400	8.04	0.018	--	N.T.	2/9/70.
12-19-69	9.5	29.0	2,650	---	--	N.T.	N.T.	
12-31-69	10.0	30.5	3,100	8.95	0.14	--	N.T.	Recovered and began eating 2/11/70.
1-15-70	10.3	31.0	3,300	---	N.T.	--	N.T.	
2-5-70	11.0	33.3	3,800	10.50	N.T.	N.T.	--	Gave birth to normal female
3-5-70	11.0	31.5	3,800	7.83	N.T.	0.20	N.T.	twins weighing 5 lbs. each
4-9-70	10.0	22.5	3,800	7.83	N.T.	N.T.	N.T.	5/20/70.
5-7-70	6.3	18.5	5,400	5.03	N.T.	--	--	

[a]Data not available.

**Negative to test.

Table 34. Chemical and hematological data from sheep 360 in the unexposed group

Date	Hematology				Lead (ppm)		Urine ALA	Comments
	Hb gm/100 ml	PCV	WBC	RBC (10^6)	Blood	Urine	mg/100 ml	
11-19-69	10.1	29.5	5,100	---[a]	0.23	--	--	Bred 12/24/69
11-25-69	12.0	30.5	4,400	---	N.T.**	N.T.	--	
12-2-69	10.5	30.5	4,500	---	N.T.	0.30	--	Gave birth to normal female
12-9-69	8.5	30.0	4,700	7.73	N.T.	0.42	N.T.	lamb weighing 8 lbs. 5/20/70.
12-19-69	9.5	28.8	6,400	---	--	N.T.	N.T.	
12-31-69	10.3	30.0	5,100	8.12	0.09	N.T.	N.T.	
1-15-70	11.5	34.5	6,000	---	N.T.	--	N.T.	
2-5-70	11.3	32.0	8,090	10.30	N.T.	N.T.	N.T.	
3-5-70	12.8	35.0	6,300	8.63	N.T.	N.T.	N.T.	
4-9-70	12.8	36.0	6,150	10.42	N.T.	N.T.	N.T.	
5-7-70	12.8	34.5	7.225	10.09	N.T.	N.T.	N.T.	

[a]Data not available.

**Negative to test.

Table 35. Chemical and hematological data from sheep 362 in the unexposed group

Date	Hematology: Hb gm/100 ml	Hematology: PCV	Hematology: WBC	Hematology: RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	10.2	31.5	5,390	---[a]	0.10	--	--	Bred 12/17/69.
11-25-69	11.0	34.5	4,600	---	0.05	0.10	--	
12-2-69	10.5	33.0	6,200	---	0.01	0.14	--	Sheep jumped over fence and ate
12-9-69	9.6	30.0	2,100	6.42	0.03	0.44	N.T.**	some pellets contaminated with
12-19-69	10.3	31.0	6,250	---	--	N.T.	N.T.	lead on 2/1/70.
12-31-69	11.0	32.5	6,300	9.21	0.15	N.T.	N.T.	
1-15-70	10.0	31.0	5,000	---	N.T.	0.09	N.T.	Reduced appetite: 2/5/70, 2/8/70, 2/9/70.
2-5-70	11.5	37.0	6,470	10.36	0.15	N.T.	0.20	
3-5-70	12.5	35.0	6,300	8.99	--	N.T.	N.T.	Gave birth to normal female
4-9-70	10.8	32.0	5,600	8.36	N.T.	N.T.	N.T.	lamb weighing 12 lbs. 5/13/70.
5-7-70	11.8	34.0	6,625	8.77	N.T.	N.T.	N.T.	

[a]Data not available.

**Negative to test.

Table 36. Chemical and hematological data from sheep 366 in the unexposed group

Date	Hematology: Hb gm/100 ml	PCV	WBC	RBC (10^6)	Lead (ppm): Blood	Lead (ppm): Urine	Urine ALA mg/100 ml	Comments
11-19-69	10.7	31.5	5,400	---[a]	0.10	--	--	Bred 12/27/69.
11-25-69	11.0	33.5	4,150	---	N.T.**	N.T.	--	
12-2-69		Blood clotted				0.78	--	Reduced appetite: 2/8/70,
12-9-69	9.3	29.0	7,300	8.95	--	--	N.T.	2/9/70.
12-19-69	9.5	30.0	7,200	---	--	N.T.	N.T.	
12-31-69	8.5	29.0	7,200	11.16	0.13	N.T.	N.T.	Gave birth to normal female lamb weighing 6 lbs. 5/19/70.
1-15-70	10.0	30.0	5,900	---	N.T.	N.T.	N.T.	
2-5-70	10.8	33.0	5,700	12.06	N.T.	N.T.	N.T.	Reduced appetite and depression: 5/20/70, 5/21/70, 5/23/70.
3-5-70	12.0	34.0	3,800	10.14	N.T.	N.T.	N.T.	
4-9-70	8.8	26.0	5,500	8.52	N.T.	N.T.	N.T.	
5-7-70	9.8	29.5	6,725	8.79	N.T.	N.T.	N.T.	Died 5/23/70

Postmortem examination of sheep revealed pleuritis, pericarditis, pneumonia, and exudate in the bronchi.

Bacteriological examination of lung resulted in the isolation of Pasteurella multocida.

Chemical analyses of maternal specimens for lead revealed:

Liver--0.14 ppm	Spleen--0.19 ppm
Kidney--0.45 ppm	Aorta--Negative to test
Brain--N.T.	Heart--Negative to test
Rumen contents--Negative to test	

[a] Data not available.

**Negative to test.

APPENDIX B: CHANGES IN RED BLOOD CELLS MORPHOLOGY AND DIFFERENTIAL COUNTS IN THE EXPOSED AND UNEXPOSED SHEEP

Table 37. Changes in RBC morphology[a] and differential counts in animal 343

Days of exposure	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	A few	No	13	10	--	32	45
-6	No	No	No	No	10	5	3	39	43
-13	No	No	No	No	13	11	9	23	49
1	No	No	No	No	13	--	9	27	51
3	No	No	No	No	17	1	9	21	52
5	No	No	No	No	7	29	3	36	25
8	No	No	No	No	20	3	8	11	58
11	Yes	No	Yes	No	12	24	1	23	40
16	Yes	Yes	Yes	No	10	9	11	13	51
23	Yes	No	Yes	Yes	8	24	10	33	25
31	Yes	No	Yes	Yes	10	24	7	19	40
38	Yes	No	Yes	Yes	6	34	13	25	22
45	Yes	Yes	Yes	Yes	6	25	22	13	34
52	Yes	No	Yes	Yes	11	10	4	30	45
59	Yes	Yes	Yes	Yes	3	28	2	2	65
66	Yes	Yes	Yes	Yes	10	28	16	10	36
73	Yes	Yes	Yes	Yes	4	23	28	18	27
80	Yes	Yes	Yes	Yes	10	13	16	14	47
87	Yes	Yes	Yes	Yes	6	16	16	16	46
94	Yes	Yes	Yes	Yes	8	4	20	13	55
101	Yes	Yes	No	Yes	10	7	15	18	50
108	Yes	Yes	No	No	10	13	13	12	52
115	No	No	No	No	4	5	8	43	40
122	No	No	No	No	14	3	9	30	44

[a]No changes were observed in RBC morphology in any animal after day 122. This applies to Tables 37-53.

Table 38. Changes in RBC morphology[a] and differential counts in animal 350

Days of exposure	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	2	9	0	11	78
-6	No	No	No	No	2	1	2	29	66
-13	No	No	No	No	5	2	6	38	49
1	No	No	No	No	9	0	6	34	51
3	No	No	No	No	13	0	2	45	40
5	No	No	No	No	3	3	3	34	57
8	No	No	No	No	4	1	5	24	66
11	No	No	No	No	8	1	7	27	57
16	No	No	No	No	5	11	3	32	49
23	No	No	No	No	6	2	18	15	59
31	Yes	Yes	No	No	7	2	11	17	63
38	Yes	Yes	No	No	5	5	31	5	54
45	Yes	Yes	No	No	7	3	5	38	47
52	Yes	Yes	No	No	3	4	1	52	40
59	Yes	Yes	Yes	No	8	4	2	23	63
66	Yes	Yes	No	No	9	2	4	31	54
73	Yes	Yes	Yes	No	5	5	0	48	42
80	Yes	Yes	No	No	1	6	0	34	59
87	Yes	Yes	No	Yes	3	8	1	27	61
94	No	No	No	No	4	6	1	33	56
101	No	No	No	No	2	6	1	26	65
108	No	No	No	No	3	6	1	14	76
115	No	No	No	No	5	14	1	24	56
122	No	No	No	No	2	6	5	19	68

Table 39. Changes in RBC morphology[a] and differential counts in animal 351

Days of exposure	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	5	1	0	34	60
-6	No	No	No	No	0	2	4	34	60
-13	No	No	No	No	8	4	1	44	43
1	No	No	No	No	0	0	0	37	63
3	No	No	No	No	8	6	0	32	56
5	No	No	No	No	6	4	3	38	49
8	Yes	No	No	No	5	1	1	54	40
11	Yes	Yes	No	No	2	3	0	26	69
16	Yes	Yes	No	No	2	1	0	53	44
23	Yes	Yes	No	Yes	0	1	0	45	54
31	Yes	No	Yes	Yes	0	2	3	21	74
38	Yes	Yes	Yes	Yes	2	4	5	28	61
45	Yes	Yes	Yes	Yes	2	6	4	32	56
52	Yes	Yes	No	Yes	1	2	2	32	63
59	Yes	Yes	No	Yes	2	2	1	36	59
66	Yes	Yes	Yes	Yes	3	1	1	41	54
73	Yes	Yes	No	No	1	0	3	67	29
80	No	No	Yes	No	1	3	2	17	77
87	No	No	No	No	1	4	1	36	58
94	No	No	No	No	4	6	1	30	59
101	No	No	No	No	1	2	2	28	67
108	No	No	No	No	0	4	7	45	50
115	No	No	No	No	2	3	5	50	40
122	No	No	No	No	0	5	0	35	60

Table 40. Changes in RBC morphology[a] and differential counts in animal 353

Days of exposure	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	10	0	0	36	54
-6	No	No	No	No	0	3	0	34	63
-13	No	No	No	No	7	6	1	49	37
1	No	No	No	No	0	0	0	37	63
3	Yes	No	No	No	6	6	0	36	52
5	Yes	No	No	No	6	4	3	38	49
8	Yes	No	No	No	4	1	1	54	40
11	Yes	No	No	No	5	2	2	51	40
16	Yes	No	No	No	4	2	0	31	63
23	Yes	Yes	No	Yes	2	3	0	26	69
31	Yes	Yes	No	Yes	2	3	1	55	39
38	Yes	Yes	No	Yes	2	1	0	53	44
45	Yes	Yes	Yes	Yes	0	1	0	45	54
52	Yes	Yes	No	Yes	1	1	0	31	67
59	Yes	Yes	No	Yes	0	2	3	21	74
66	Yes	Yes	Yes	Yes	2	4	5	28	61
73	Yes	Yes	Yes	Yes	2	6	4	32	56
80	Yes	Yes	Yes	Yes	3	5	4	22	66
87	Yes	Yes	Yes	Yes	1	2	2	32	63
94	Yes	Yes	Yes	Yes	2	2	1	36	59
101	No	No	No	No	1	6	0	33	60
108	No	No	No	No	4	8	5	24	59
115	No	No	No	No	3	1	1	41	54
122	No	No	No	No	5	6	2	45	42

Table 41. Changes in RBC morphology[a] and differential counts in animal 354

Days of exposure	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	5	1	1	12	81
-6	No	No	No	No	6	1	0	35	58
-13	No	No	No	No	3	0	1	36	60
1	No	No	No	No	3	0	1	36	60
3	No	No	No	No	0	3	1	30	66
5	No	No	No	No	0	6	0	42	52
8	No	No	No	No	3	0	1	40	56
11	Yes	Yes	Yes	Yes	7	0	2	47	44
16	Yes	Yes	Yes	Yes	5	0	1	41	53
23	Yes	Yes	Yes	Yes	1	2	0	14	83
31	Yes	Yes	No	No	3	2	1	28	66
38	Yes	Yes	Yes	Yes	1	3	0	47	52
45	Yes	Yes	Yes	Yes	2	1	1	51	45
52	Yes	Yes	No	No	1	3	0	45	51
59	Yes	Yes	No	No	4	3	6	32	55
66	Yes	Yes	No	Yes	1	4	0	29	66
73	Yes	Yes	Yes	Yes	2	1	0	29	68
80	Yes	Yes	Yes	Yes	1	1	1	31	67
87	Yes	No	No	Yes	1	4	2	40	53
94	Yes	No	No	Yes	3	1	0	50	46
101	Yes	No	No	No	0	2	1	3	94
108	No	No	No	No	1	6	2	35	56
115	No	No	No	No	0	10	0	17	73
122	No	No	No	No	1	0	0	26	73

Table 42. Changes in RBC morphology[a] and differential counts in animal 355

Days of exposure	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	1	4	0	38	57
-6	No	No	No	No	2	2	0	46	50
-13	No	No	No	No	0	0	0	43	57
1	No	No	No	No	2	0	0	35	63
3	No	No	No	No	5	0	0	31	64
5	No	No	No	No	2	3	0	36	59
8	No	No	No	No	7	0	0	31	62
11	No	No	No	No	3	1	0	41	55
16	No	No	No	No	12	0	1	46	41
23	No	No	No	No	6	1	0	49	44
31	Yes	No	No	No	5	1	0	35	59
38	Yes	No	No	No	7	0	0	43	50
45	Yes	No	No	No	3	0	0	56	41
52	Yes	No	No	No	2	0	0	51	47
59	Yes	Yes	No	No	4	4	0	45	47
66	Yes	Yes	No	No	0	2	1	23	74
73	Yes	Yes	No	No	4	3	0	50	43
80	Yes	Yes	No	No	0	1	2	36	61
87	Yes	Yes	No	No	0	0	1	38	61
94	Yes	Yes	No	No	0	1	0	42	57
101	Yes	Yes	No	No	1	2	0	43	54
108	Yes	Yes	No	No	3	1	1	15	80
115	Yes	Yes	No	No	0	6	5	54	35
122	Yes	Yes	No	No	0	1	2	59	38

Table 43. Changes in RBC morphology[a] and differential counts in animal 358

Days of exposure	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	0	1	0	4	95
-6	No	No	No	No	10	3	1	53	33
-13	No	No	No	No	2	2	0	49	47
1	No	No	No	No	4	1	2	33	60
3	No	No	No	No	10	2	1	30	57
5	No	No	No	No	12	0	1	43	44
8	No	No	No	No	4	0	1	52	43
11	No	No	No	No	6		2	38	54
16	No	No	No	No	2	0	0	39	59
23	No	Yes	No	No	8	2	0	53	37
31	Yes	Yes	No	No	4	2	0	45	49
38	Yes	Yes	No	No	7	0	1	57	35
45	Yes	Yes	No	No	8	1	1	49	41
52	Yes	Yes	No	No	6	2	1	30	61
59									
66									
73									
80									
87									
94									
101									
108									
115									
122									

Table 44. Changes in RBC morphology[a] and differential counts in animal 363

Days of exposure	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	13	10	--	33	44
-6	No	No	No	No	11	4	3	40	42
-13	No	No	No	No	10	10	1	30	49
1	No	No	No	No	13	--	9	27	51
3	No	No	No	No	17	1	9	21	52
5	No	No	No	No	20	3	8	11	58
8	No	No	No	No	8	24	10	33	25
11	Yes	No	No	No	6	34	13	25	22
16	Yes	Yes	Yes	No	10	28	16	10	36
23	Yes	No	Yes	No	10	10	20	30	40
31	Yes	No	Yes	Yes	10	20	20	20	30
38	Yes	Yes	Yes	Yes	3	2	1	28	66
45	Yes	Yes	Yes	Yes	1	3	0	45	51
52	Yes	No	Yes	Yes	4	3	6	32	55
59	Yes	Yes	Yes	Yes	1	4	0	29	66
66	Yes	No	Yes	Yes	2	1	0	29	68
73	Yes	Yes	Yes	Yes	1	4	2	40	53
80	Yes	Yes	No	Yes	3	1	0	50	46
87	Yes	No	Yes	Yes	1	0	2	35	56
94									
101									
108									
115									
122									

Table 45. Changes in RBC morphology[a] and differential counts in animal 364

Days of exposure	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	13	0	0	47	40
-6	No	No	No	No	10	3	0	37	50
-13	No	No	No	No	3	1	0	48	50
1	No	No	No	No	11	0	1	52	36
3	No	No	No	No	4	4	2	25	65
5	No	No	No	No	7	0	0	54	39
8	Yes	No	No	No	5	0	5	50	40
11	Yes	Yes	Yes	Yes	9	1	0	34	56
16	Yes	Yes	Yes	Yes	7	1	0	53	39
23	Yes	Yes	Yes	Yes	5	5	1	27	62
31	Yes	Yes	Yes	Yes	8	4	0	44	44
38	Yes	Yes	Yes	Yes	10	0	0	46	39
45	Yes	Yes	Yes	Yes	3	1	0	56	40
52	Yes	Yes	Yes	Yes	9	2	0	51	38
59	Yes	Yes	Yes	Yes	10	0	0	46	44
66									
73									
80									
87									
94									
101									
108									
115									
122									

Table 46. Changes in RBC morphology[a] and differential counts in animal 367

Days of exposure	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	0	1	5	55	39
-6	No	No	No	No	0	0	3	68	29
-13	No	No	No	No	0	0	1	69	30
1					2	0	0	56	42
3	No	No	No	No	13	0	0	56	31
5	No	No	No	No	8	1	0	57	34
8	No	No	No	No	5	0	1	54	40
11	No	No	No	No	5	0	2	55	38
16	Yes	Yes	No	No	10	0	1	24	65
23	Yes	Yes	No	No	9	0	0	41	50
31	Yes	Yes	No	No	0	0	5	36	59
38	Yes	Yes	No	No	2	0	0	33	65
45	Yes	Yes	No	No	3	0	0	63	34
52	Yes	Yes	No	No	4	0	0	59	37
59	Yes	Yes	No	No	0	1	0	53	46
66	Yes	Yes	No	No	2	0	0	71	27
73	Yes	Yes	No	No	1	0	0	56	43
80	Yes	Yes	No	No	3	0	0	59	38
87	Yes	Yes	No	No	1	0	0	49	50
94	Yes	Yes	No	No	6	2	0	56	36
101	Yes	Yes	No	No	2	0	1	50	47
108	Yes	Yes	No	No	6	0	0	48	46
115	Yes	Yes	No	No	3	0	0	50	47
122	Yes	Yes	No	No	3	0	0	58	39

Table 47. Changes in RBC morphology[a] and differential counts in animal 344 (control)

Days of experiment	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	10	3	0	40	47
-6	No	No	No	No	6	0	0	31	63
-13	No	No	No	No	21	0	0	32	47
1	No	No	No	No	7	0	0	28	65
11	No	No	No	No	12	0	0	39	49
23	No	No	No	No	19	0	0	29	52
38	No	No	No	No	19	0	1	34	46
59	No	No	No	No	8	2	0	32	58
87	No	No	No	No	10	0	1	29	60
118	No	No	No	No	9	0	0	24	67
150	No	No	No	No	16	0	0	17	67

Table 48. Changes in RBC morphology[a] and differential counts in animal 346 (control)

Days of experiment	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	0	3	0	3	94
-6	No	No	No	No	6	0	1	40	53
-13	No	No	No	No	8	0	0	44	48
1	No	No	No	No	9	0	0	51	40
11	No	No	No	No	9	0	0	45	46
23	No	No	No	No	10	1	0	43	46
38	No	No	No	No	6	2	0	39	53
59	No	No	No	No	0	6	0	1	93
87	No	No	No	No	5	1	0	44	50
118	No	No	No	No	17	2	0	46	35
150	No	No	No	No	12	4	0	44	40

Table 49. Changes in RBC morphology[a] and differential counts in animal 349 (control)

Days of experiment	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	1	3	0	29	67
-6	No	No	No	No	1	1	1	36	61
-13	No	No	No	No	6	3	0	28	63
1	No	No	No	No	7	1	0	28	64
11	No	No	No	No	9	3	0	39	49
23	No	No	No	No	3	0	0	30	67
38	No	No	No	No	8	0	1	18	73
59	No	No	No	No	7	0	0	20	73
87	No	No	No	No	4	0	0	20	76
118	No	No	No	No	6	6	0	36	52
150	No	No	No	No	2	0	0	42	56

Table 50. Changes in RBC morphology[a] and differential counts in animal 359 (control)

Days of experiment	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	0	3	0	43	54
-6	No	No	No	No	10	2	0	38	50
-13	No	No	No	No	7	1	0	41	51
1	No	No	No	No	5	0	0	44	51
11	No	No	No	No	10	0	0	40	50
23	No	No	No	No	9	2	0	35	54
38	No	No	No	No	11	5	1	44	39
59	No	No	No	No	11	1	0	51	37
87	No	No	No	No	5	0	0	41	54
118	No	No	No	No	6	2	0	58	34
150	No	No	No	No	1	2	0	56	41

Table 51. Changes in RBC morphology[a] and differential counts in animal 360 (control)

Days of experiment	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	3	3	0	28	66
-6	No	No	No	No	7	0	0	40	53
-13	No	No	No	No	7	0	0	52	41
1	No	No	No	No	12	1	0	38	49
11	No	No	No	No	19	2	0	37	42
23	No	No	No	No	11	2	0	34	53
38	No	No	No	No	23	0	0	28	49
59	No	No	No	No	3	1	0	6	90
87	No	No	No	No	15	0	0	44	41
118	No	No	No	No	14	5	0	39	42
150	No	No	No	No	5	1	0	49	45

Table 52. Changes in RBC morphology[a] and differential counts in animal 362 (control)

Days of experiment	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	1	0	0	33	66
-6	No	No	No	No	3	1	0	26	70
-13	No	No	No	No	1	0	0	34	65
1	No	No	No	No	8	0	0	27	65
11	No	No	No	No	4	0	0	29	67
23	No	No	No	No	8	0	0	22	70
38	No	No	No	No	9	1	0	15	75
59	No	No	No	No	8	1	0	21	70
87	No	No	No	No	6	2	0	30	62
118	No	No	No	No	2	4	0	25	69

Table 53. Changes in RBC morphology[a] and differential counts in animal 366 (control)

Days of experiment	RBC morphology				Differential count				
	Aniso-cytosis	Poikilo-cytosis	Immature RBC	Basophilic stippling	Eosino-phil %	Mono-cytes %	Neutrophil % Band	Neutrophil % Segs	Lympho-cytes
-1	No	No	No	No	6	0	0	41	53
-6	No	No	No	No	5	0	0	42	53
-13	No	No	No	No	4	0	0	44	52
1	No	No	No	No	0	0	0	76	24
11	No	No	No	No	0	0	0	52	48
23	No	No	No	No	3	0	0	48	49
38	No	No	No	No	2	1	0	53	44
59	No	No	No	No	9	0	3	36	52
87	No	No	No	No	4	2	0	49	45
118	No	No	No	No	6	1	0	46	47
150	No	No	No	No	4	0	0	48	48

APPENDIX C: CORRELATION MATRIX

Table 54. Correlation matrix[b]

	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14
1[a]	1													
2	.332	1												
3	.146	.862	1											
4	.020	.398	.007	1										
5	-.028	.637	.674	.120	1									
6	.213	.002	-.037	.109	.107	1								
7	-.063	.030	.037	.017	.135	.220	1							
8	-.233	-.158	-.117	.100	-.045	.473	.387	1						
9	-.815	-.198	-.063	-.007	.038	.620	.176	.298	1					
10	-.298	.058	.079	.187	.084	-.020	-.042	.229	.260	1				
11	-.082	.023	.001	.097	.107	.022	.189	.255	.055	.221	1			
12	-.041	-.026	-.109	-.024	.003	.091	.213	.375	.093	.309	.456	1		
13	.035	-.051	-.012	.155	.012	.041	-.167	-.246	-.027	-.144	-.433	-.533	1	
14	.095	.043	.036	-.266	-.082	-.075	.036	-.041	-.091	-.344	-.198	-.071	-.689	1

[a]1 = day; 2 = hemoglobin; 3 = packed cell volume; 4 = white blood cells; 5 = red blood cells; 6 = blood lead; 7 = urine lead; 8 = ALA; 9 = lead in feed; 10 = eosinophils; 11 = monocytes; 12 = neut. - bands; 13 = neut. - segs; 14 = lymphocytes.

[b]Degrees of freedom for error was 161.

Table values for r with one independent variable and 150 degrees of freedom are 0.159 at 5% and 0.208 at 1% level of significance. Interpolation to 161 degrees of freedom results in values for r of 0.155 at 5% and 0.202 at 1% level of significance.