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AUDITORY AND VISUAL DISCRIMINATION LEARNING
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Auditory and visual discrimination learning in sheep
prenatally and postnatally exposed to lead

by

Thomas Lee Carson

A Dissertation Submitted to the
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The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

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Major: Veterinary Pathology (Veterinary Toxicology)

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DEDICATION

To my wife and daughter

INTRODUCTION

Few metals have a longer history of use by man than lead, a soft gray metal which was first formed into tools and vessels over 6,000 years ago. There are few metals besides lead with a greater documentation of untoward effect on man and animals. Although lead poisoning was still a constant hazard to industrial workers in the early 1900's, the number of deaths attributable to acute lead poisoning has decreased to a very low figure during this century as a result of increased awareness of industrial hygiene. This improvement in health standards for workers was accomplished during a time when the industrial use of lead was on the increase.

Today, even with our increased awareness of its manifestations, lead poisoning is a widespread public health problem affecting thousands of young children living in older substandard housing. Most cases of childhood lead poisoning have involved children 1 to 3 years old who had ingested chips of lead-based paint peeling from rotting window sills, woodwork, and crumbling plaster walls.

Acute encephalopathy, one of the most serious manifestations of acute lead poisoning in children, has left many of its young victims with some degree of apparently permanent brain damage. However, all children which have ingested chips of lead-based paint have not suffered from clinical toxicosis. This observation introduces the concept of subclinical lead exposure, that is, a population which has been exposed to elevated levels of lead but that does not display clinical signs of classical lead toxicosis. The question then arises: if acute clinical lead toxicosis can produce permanent mental retardation in the young child, would not lower

subclinical lead exposure cause some less severe, yet permanent mental deficits in a susceptible nervous system?

The question, whether environmental lead levels are likely to have any long-term effects, can, in part, be examined by considering the effects of sublethal doses of lead in animals (Waldron and Stöfen, 1974). In addition, Ruffin (1963) proposed the use of functional testing for behavioral toxicity in experimental environmental toxicology.

Therefore, the neurologic effects of subclinical lead exposure were investigated in this study by a comparative medicine approach utilizing an animal model and methods of behavioral toxicology.

This study was part of an ongoing investigation of the neurologic and behavioral effects of low level lead exposure conducted by the Behavioral Toxicology Laboratory, Iowa Veterinary Diagnostic Laboratory, Iowa State University. The early phases of this investigation involved the production of prenatal lead exposed lambs and their subsequent testing on a series of visual discrimination problems (Carson, 1973; Carson et al., 1974a, 1974b).

The study reported here was conducted to determine if changes in learning capabilities as measured by auditory and visual discrimination tasks occurred in the offspring of ewes which had ingested subclinical levels of lead throughout gestation and in lambs which ingested lead early in life.

REVIEW OF LITERATURE

Neurologic Sequelae of Lead Poisoning in Children

Lead poisoning remains a significant public health problem especially in children 1 to 5 years of age living in older urban housing where peeling lead based paint and lead contaminated dust can be ingested (Environmental Protection Agency (EPA), 1972; Jacobziner, 1966; Jacobziner and Raybin, 1962; Lin-Fu, 1970). Eighty-five percent of recognized cases of childhood lead poisoning occur in the 1 to 3 year age range in which pica, the habit of eating nonfood substances, is especially prevalent (American Academy of Pediatrics, 1971).

Lead toxicosis can have adverse effects on several body systems, including severe involvement of the central nervous system (CNS). Prolonged high exposure can result in fulminating encephalopathy characterized by intractable convulsions, coma, and sometimes death (Chisolm, 1971).

An extensive description of the gross and histologic changes observed in the brain tissue of 22 children with fatal lead encephalopathy was presented by Blackman (1937). The sporadic presence of perivascular serous exudate, sometimes extending into adjacent tissue, and evidence of recent damage to vessel walls were emphasized. Alterations in the brain parenchyma was thought to be secondary to the accumulation of extravascular fluid.

Similar accounts of the morphology of lead encephalopathy in children presented by Pentschew (1965), Popoff et al. (1963), and Smith et al. (1960) describe vascular damage and serous exudation followed by endothelial, microglial, and astrocyte proliferation.

Brain damage has been further documented by abnormal electroencephalograms which have been reported in a high percentage of children with lead encephalopathy (Smith et al., 1963; Tanis, 1955; Thurston et al., 1955).

Though the neurologic involvement of acute lead poisoning has been recognized, a number of workers have reported residual mental impairment and neurologic deficits in children a few months to several years after apparent recovery from acute encephalopathy.

Perlstein and Attala (1966) reported a survey of follow-up examinations of a total of 425 children with lead poisoning. Evidence of neurologic sequelae in 39% of these children was observed 6 months to 10 years after being hospitalized for acute lead poisoning. Mental retardation, observed in 22% of the children, and recurrent seizures, observed in 20% of the children, were the most common and persistent findings reported. Perlstein and Attala also reported that of 232 children in this study with symptoms of lead poisoning characterized initially by gastro-intestinal complaints, but not by evidence of neurologic damage, 19% were later found to be mentally retarded and 13% to have convulsive disorders. They also reported that of 58 children in this study who had previously been treated for asymptomatic lead poisoning, five were found during follow-up studies to be mentally retarded. In some of the children in this study, Perlstein and Attala reported that brain damage was minimal with learning blocks, usually of a visual-perceptual type, being the only sign of impaired learning.

Moncrieff et al. (1964) associated elevated blood lead levels with neurologic disability by comparing three groups of children with a sample of 80 normal children who did not display pica and were not retarded. Only

two children in this normal group had blood lead levels above $37 \mu\text{g}/100 \text{ ml}$. The first group of 120 subjects studied by Moncrieff et al. were mentally retarded or had a history of behavioral disturbances. Fifty-five of the children in this first group had blood lead levels greater than $38 \mu\text{g}/100 \text{ ml}$. In a second group of 40 children which were diagnosed as having encephalitis, 12 children had blood levels of $38 \mu\text{g}/100 \text{ ml}$ or greater. In a third group of 50 children with anemia, pica, vomiting, or abdominal pain, 28 children had blood lead concentrations greater than $36 \mu\text{g}/100 \text{ ml}$.

Another study (Millar et al., 1970), however, reported no children with blood lead levels above $40 \mu\text{g}/100 \text{ ml}$ in a group of 27 mentally retarded children.

Byers and Lord (1943) presented a follow-up study of 20 children who had experienced lead poisoning during infancy or early childhood. Nineteen of the 20 did not progress satisfactorily in school, but the usual correlation between low intelligence quotient and the ability to learn in school was not supported by data from these children. However, sensorimotor defects were demonstrated in most cases by the inability to copy simple figures, such as crosses, triangles, and squares, and by poor performance on the Ellis Visual Designs Test (Bronner et al., 1927; Wood and Shulman, 1940), the Pintner-Cunningham Test Number 7 (Pintner and Cunningham, 1922), the Wechsler-Bellvue Test (Wechsler, 1939), or the Wood Picture Completion Test (Wood, 1940). All of these tests were designed to measure the subject's ability to deal with shape, direction, space, and projected imagery, all areas of the utmost importance for success in schoolwork.

Thurston et al. (1955) examined 11 children who had been treated for lead poisoning 5 to 10 years earlier. Mental retardation was not always obvious, and physical and laboratory tests in general did not reveal abnormalities. Overall intelligence as measured by the Stanford-Binet (Terman and Merrill, 1937) intelligence quotient, primarily a verbal test, remained intact. However, specialized tests of visual motor performance indicated subtle brain damage in the majority of cases. After repeated testing on the Graham-Kendall Visual Motor Test (Graham and Kendall, 1946), only two of the 11 children were rated as normal with the other nine rated as either borderline or in the brain damaged category. Only one of the 11 children was rated as normal on the Bender-Gestalt Visual Motor Test (Bender, 1938), while the rest showed rotation of designs, perseverations, difficulty with angulation, and substitution of primitive loops and lines for dots which have been described as characteristic of children with brain damage. Performance on the Goodenough Draw-a-man Test (Goodenough, 1926), primarily a test of visual motor functioning, was also generally low. The performance on these visual motor tasks was similar to that of children with brain damage from cerebral anoxia. In summary, organic brain damage was apparent only after repeated specialized psychological testing.

Bradley and Baumgartner (1958) also observed a prominent visual motor deficit when the Goodenough Draw-a-man Test and the Bender-Gestalt Visual Motor Test were administered to 18 children 3 to 5 years of age after recovery from acute lead encephalopathy. As in the previously cited study, the Stanford-Binet Test revealed no significant residual mental impairment.

Based on the performance of simple drawing tests such as copying a circle, Mellins and Jenkins (1955) found 14 of 15 children markedly

retarded in some way 4 to 6 months after recovery from lead encephalopathy. Fine muscle coordination and perceptual-motor skills were specifically mentioned as being impaired.

Forty-six children surviving acute lead encephalopathy were followed for 1 year or longer by Chisolm and Harrison (1956), and 23 were classified as having severe permanent damage to the brain.

In another follow-up study, Cohen and Ahrens (1959) reported that of 28 children recovering from lead poisoning, 13 were judged by psychologic testing to have residual neurologic impairment. Only four of the 13 were felt to have had some brain damage prior to the lead poisoning.

A more recent study (Smith et al., 1963) reported abnormal electroencephalograms as well as residual behavioral disorders in children 4 to 9 years after episodes of acute lead encephalopathy.

Others (Byers, 1959; White and Fowler, 1960; Woods and Walters, 1964) have also associated residual psychologic defects with prior history of lead poisoning.

Hardy (1966) hypothesized that subclinical lead exposure interferes with brain enzyme systems provided the exposure occurs during the period of central nervous system development in early childhood. This brain damage would be manifested as behavioral disorders when the child was 6 to 7 years old.

In reviewing reports of brain damage following early childhood lead poisoning, Chisolm and Kaplan (1968) observed that severe acute encephalopathy characterized by cortical atrophy, hydrocephalus, severe convulsive disorder, idiocy, and blindness were becoming increasingly rare. They concluded that subtle neurologic deficits were the more common outcome such as

perseveration and the lack of sensory perception, despite an apparently normal intelligence quotient on the Stanford-Binet Test. Further, such affected children tend to break a drawing down into its component parts rather than recognize the design as a whole, integrated unit. They reported that form and proportion are distorted for these children.

Although it is generally accepted that some degree of mental retardation results from severe lead encephalopathy, Chisolm (1965), while reviewing the incidence of lead poisoning and diagnostic criteria, raised the issue as to whether even a minor degree of elevation in blood lead concentration for a long period of time would be associated with future neurologic malfunction.

In concluding a recent review of the reported psychologic sequelae of lead ingestion in children, Wiener (1970) points out that none of the studies provided sufficient data to determine if mental deficiency is associated with asymptomatic or subclinical lead exposure. This question has far reaching importance since many children have been reported to have blood lead levels above 40 $\mu\text{g}/100\text{ ml}$ (Fine et al., 1972; Guinee, 1972; Lin-Fu, 1972), which by current diagnostic standards (HSMHA, 1971) reflects excessive lead exposure. In consideration of the increased susceptibility of children to lead, a lower acceptable maximum blood lead for children of 35 $\mu\text{g}/100\text{ ml}$ has recently been proposed (Zielhuis, 1972).

Vulnerability of the Developing Nervous System

The major impact of human lead poisoning occurs in young children during a period of rapid neurologic development. There is abundant clinical evidence that children are more prone than adults to suffer damage to the

central nervous system from lead exposure, since the younger the child the more vulnerable the brain to lead toxicosis (Perlstein and Attala, 1966). Gibson et al. (1967) observed that the growing brain of young animals in general is more susceptible to damage by lead than that of adult animals.

In a recent position statement, the EPA stated:

"In recognition of the possibility that young children may be more susceptible to lead than older children and adults, the newborn and the fetus would be expected to be especially vulnerable to lead. Exposure of the developing central nervous system in utero to lead, an established neurotoxic agent, should thus be kept to a minimum" (EPA, 1972, pp. IV-2).

The ability of lead to cross the placental barrier was inferred from its efficacy at producing abortion and was demonstrated many years ago (Oliver, 1911; Bell, 1924; Aub et al., 1925; Hamilton, 1929; Hansmann and Perry, 1940; Cantarow and Trumper, 1944). Umbilical cord blood has been shown to contain lead in concentrations approximating those found in maternal blood (Scanlon, 1971; Harris and Holley, 1972; Rajegowda et al., 1972; Haas, 1972). There is a significant correlation between the two concentrations, and the lower concentrations found in cord blood may be an indication that the fetal tissues are removing lead from the maternal blood at a rate faster than it can be replaced (Barltrop, 1969). The blood of newborns contains lead in concentrations similar to that in umbilical cord blood (Robinson et al., 1958; Kubasik and Volosin, 1972).

It has been noted that the fetus is most susceptible to the effects of lead during the phase of rapid growth, and it has been postulated that human fetal tissues may be able to concentrate lead at least during the first 16 weeks of gestation (Karlog and Møller, 1958). In this context the high levels of lead found in the brains of stillborn infants are of consid-

erable importance since they may well reflect generally high levels in the nervous system (Schroeder and Tipton, 1968).

It has been recognized that children born of mothers with excessive lead exposure develop more slowly than normal and may show evidence of neurologic disturbance (Cantarow and Trumper, 1944; Hamilton and Hardy, 1939; Palmisano et al., 1969). The EPA (1972) suggested that a blood lead level of 30 $\mu\text{g}/100\text{ ml}$ and above in an expectant mother may represent a potential hazard to the unborn child.

Neurologic Involvement of Lead Poisoning in Animals

Pentschew and Garro (1966) reported that when neonatal rats were poisoned by adding 4% lead carbonate to the maternal diet, neurologic changes developed which culminated in paraplegia and death. A profound breakdown in the blood brain barrier, as indicated by focal staining of the brain with trypan blue, was noted in paraplegic rats. The cerebellum appeared especially susceptible to this alteration. Cerebral edema was present in intoxicated rats and was believed to play an important role in producing marked histologic abnormalities and neurologic deficits.

Lampert et al. (1967), employing the same protocol as the previous study, concluded from his electron microscopic studies that vascular damage was the primary lesion in lead encephalopathy in rats.

Thomas et al. (1971) also produced encephalopathy in suckling rats by adding 4.5% lead carbonate to the feed and 1% lead acetate to the drinking water of the maternal diet. They observed by light and electron microscopy endothelial swelling, edema, and abnormalities in Purkinje cells.

Neonatal mice were poisoned by Rosenblum and Johnson (1968) by the addition of 1% and 5% lead carbonate to the maternal diets. Intoxicated suckling mice displayed faulty growth and development. Histopathologic examination of the brains of nine lead exposed mice revealed abnormally large numbers of fibrous, intercapillary strands in several cerebral foci and astrocytosis in the hippocampus. No histologic evidence of cerebral edema was reported.

Neurotoxic Action of Lead on the CNS

Chisolm (1971) stated that the deleterious action of lead on the CNS is poorly understood. From clinical observations and pathologic studies of lead poisoned patients and experimental animals, he observed that two mechanisms appear to be involved in lead encephalopathy. First, by an obscure mechanism capillaries in brain tissue increase in permeability allowing fluid to escape into the adjacent parenchyma (edema). Severe swelling of the brain from this edema in the closed space of the skull destroys tissue of the CNS. Secondly, some cells of the brain may be impaired, or their function inhibited, by direct action of the lead.

Popoff et al. (1963) suggested that vascular damage was a major factor in the pathogenesis of the neurological disorder of lead poisoning. The reports of Pentschew and Garro (1966) and Lampert et al. (1967) have substantiated this view. The way in which lead apparently interferes with permeability of the capillaries of the brain is not clear, but it may be the result of an interference with some energy-regulating system which differentiates blood-brain barrier capillaries from less-differentiated capillaries (Waldron and Stöfen, 1974).

Tang et al. (1968) postulated that periodic acid-Schiff positive bodies observed in the perivascular spaces and the cytoplasm of adventitial cells, glia, and neurons in brains with lead encephalopathy may represent a glycoprotein denatured by lead and thus constitute evidence of direct injury to these cells by lead.

From observing changes in the tubular system of the axon in animals with lead neuropathy, Schlaepfer (1969) has made the suggestion that the neuropathy of lead poisoning might be caused by initial damage to the supporting cells of the nervous system.

Recent studies concerning the subcellular effects of lead on the CNS have been reported. Gibson and Goldberg (1970) reported impaired activity of the enzyme δ -aminolevulinic acid dehydratase (ALAD) in the brains of 3.5 kg rabbits which received daily subcutaneous injections of lead acetate in aqueous solution in doses of 10, 30, 150, and 200 mg of lead acetate per week. They reported that this enzyme inhibition was due largely to interference of lead with the sulfhydryl groups of the enzyme.

Millar et al. (1970) reported significant reduction in ALAD activity in the brains and blood of suckling rats when 4% lead (as the acetate) was added to the maternal diets. Millar also reported a negative correlation ($r = -0.81$) between blood lead levels and blood ALAD activity in 57 children. Millar concluded that these results suggest that even modest elevations of blood lead may be associated with biochemical abnormalities in the brain.

Behavioral Effects of Lead in Animals

Several studies have investigated the effects of lead on the locomotor activity of mice. Terzin and Vujkov (1969), using juvenile female mice, were able to demonstrate that sublethal doses of lead acetate administered intraperitoneally resulted in elevated levels of locomotor activity. This finding was substantiated by Silbergeld and Goldberg (1973), who showed that mice exposed to lead from birth via maternal milk were three times more active than age-matched controls when tested at 40 and 60 days of age. This behavioral effect is similar to that observed in children who have been described as hyperactive in cases of asymptomatic lead poisoning (Oliver et al., 1972).

In addition to changes in activity levels following lead exposure, several investigators have examined the abilities of animals to learn various behavioral tasks following lead exposure.

Doses of 15 to 20 mg tetraethyl lead administered intraperitoneally did not affect the learning ability of 150 gm rats on a water T-maze (Bullock et al., 1966).

Brown et al. (1971) reported that either three or four daily doses of 111 mg lead acetate/kg given intraperitoneally did not significantly alter learning and memory in rats tested in a water T-maze. The rats ranged from 8 days to 5 weeks of age at the time of lead exposure.

Snowdon (1973) demonstrated that lead injected into weanling or adult rats failed to produce a learning impairment in the Hebb-Williams maze despite the fact that signs of lead poisoning were produced.

When 10- to 15-month-old rhesus monkeys were administered lead acetate at 0.05, 0.50, or 5.00 mg/kg for 30 months, Goode et al. (1973) reported no

effect on performance of: 1) a conditioned response test used to evaluate the acquisition and retention of learned behavior and 2) a delayed response test which examined short-term memory and sensorimotor response. Blood lead residues of the high exposure group ranged from 45 to 60 $\mu\text{g/ml}$ for the last 21 weeks of the study.

Gusev (1960, as cited by Waldron and Stöfen (1974)), however, has shown that changes in the patterns of conditioned reflex behavior occur in animals exposed to low concentrations of atmospheric lead.

Residual learning disabilities in a T-maze were demonstrated in 8- to 10-week-old rats which had nursed lead exposed mothers for the first 3 weeks of life. The maternal rats received 17.5, 25.0, or 35.0 mg/kg daily for the first 20 days following parturition (Brown, 1973).

It has also been shown that the performance of lead-exposed rat pups in a Sidman avoidance task was significantly poorer than controls at various ages following lead exposure (Krigman et al., 1972). Snowdon (1973) injected lead acetate into lactating female rats and studied the effects on offspring in a Hebb-Williams maze. The exposed animals took more trials to reach the criterion level of performance and made significantly more errors than did controls. Thus there is some evidence supporting the hypothesis that pre- or postnatal low level lead exposure will produce deficits in performance of certain behavioral tasks.

Weir and Hine (1970) reported that goldfish exposed to sublethal lead concentrations of 10 ppm or less in their aquatic environment for 24 and 48 hr showed impaired performance on a conditioned avoidance task.

Van Gelder et al. (1973) reported increasingly poor performance on an auditory signal detection behavioral task by mature sheep receiving daily

oral doses of 100 mg lead/kg. The fewer number of correct responses by the lead exposed sheep was believed to be a manifestation of clinical lead toxicosis. Performance after 4 weeks of exposure was significantly less stable in the exposed sheep than in the controls. Van Gelder et al. (1973) also reported that daily oral doses of 120 and 230 mg lead for 27 weeks did not alter the performance of mature sheep on a fixed interval schedule of reinforcement behavioral task.

The above findings indicate that the neonatal developing nervous system is more susceptible to lead than is the adult nervous system. This tentative conclusion was the basis for a study in our laboratory of the effect of prenatal lead exposure in sheep. In documenting the first part of this study, Carson (1973) and Carson et al. (1974b) reported that lambs born to ewes with mean blood lead levels during gestation of $34 \mu\text{g}/100 \text{ ml}$ learned visual discrimination problems significantly slower than lambs from ewes with mean blood levels of 18 or $4.7 \mu\text{g}/100 \text{ ml}$ or controls. The exposure to lead during embryologic neural development was felt to account for the slowed learning in the 10- to 15-month-old lambs.

In summary, there is evidence indicating that low-level exposure to lead, either prenatally or neonatally, in man and animals results in behavioral deficits and neurophysiologic and structural changes in the nervous system.

Visual Discrimination in Sheep

Seitz (1951, as cited by Schnorr (1972)) found that the East Prussian prairie sheep performed well on shape discrimination tasks involving cir-

cles, squares, triangles, and crosses when they received a food (bread, lettuce, turnips) reinforcement.

Maland (1968) reported the effect of dieldrin on the performance of a two-choice visual discrimination task by sheep. Two geometric shape stimuli were presented simultaneously in a Y-maze apparatus. A mild electric shock served as a negative reinforcement when an animal approached the incorrect shape. A circle versus triangle discrimination was learned by all animals in 30 days. Daily dosing of exposed animals with 10 mg dieldrin/kg interfered with relearning of simple form discriminations and increased the latency to respond.

In another investigation of the effects of dieldrin on behavioral performance of sheep, Schnorr (1972) employed a two-choice operant visual discrimination task using a positive food reinforcement for correct responses. The sheep were reported to demonstrate a visual ability comparable to other higher animals. Significant behavioral decrements were observed in animals exposed to 5.0 mg dieldrin/kg. The most sensitive behavioral index was the days to criterion for each problem.

Use of Auditory Stimuli with Sheep

Studies conducted at the Behavioral Toxicology Laboratory, Iowa State University, have reported behavioral tasks employing auditory stimuli to be sensitive to the detection of mild CNS impairment in sheep.

Sandler (1968) trained sheep to bar press within 5 seconds after the presentation of a 0.1 second 5 KHz tone of 6 db in order to gain access to food. A daily oral dose of 20 mg/kg technical dieldrin produced a marked

decrement in the percent of correct responses by each sheep on this vigilance task.

Elsberry (1972) employed a similar auditory detection task in which sheep were trained to respond within 5 seconds after the presentation of a 0.1 second 5 KHz tone in order to gain a 3.5 second access to cracked corn. A decrement in performance followed daily oral exposure of 15 mg/kg technical dieldrin.

METHODS

The study reported here is comprised of three basic parts. The first two parts involve further examination of the prenatally lead-exposed lambs which had previously been tested on a visual discrimination task (Carson, 1973; Carson et al., 1974b). These prenatally lead-exposed sheep were tested first on an auditory discrimination task and secondly retested on a visual discrimination task. The third part utilized different animals which were postnatally exposed to lead and were tested on a visual discrimination task.

Production of Prenatally Lead Exposed Sheep

Thirty Columbia-Rambouillet crossbred yearling ewes were randomly divided into three groups of 10 animals (Carson et al., 1973). Two groups were fed lead while the other group served as an unexposed control. The ewes were maintained on a ration of pelleted ground corn and soybean oil meal, chopped alfalfa hay, dicalcium phosphate, trace mineral salt, and water.

A previous study (Sharma, 1971) reported blood lead levels approaching 60 $\mu\text{g}/100\text{ ml}$ and overt lead toxicosis after feeding 12 ewes an average of 11.8 mg lead/kg/day for approximately 45 days. The goal of the present project was to maintain blood lead levels of approximately 30 $\mu\text{g}/100\text{ ml}$ and 15 $\mu\text{g}/100\text{ ml}$ in the "higher lead" and "lower lead" groups, respectively, thereby avoiding clinical manifestations of lead toxicosis. Therefore, finely divided metallic lead was incorporated into the pelleted ration of the higher and lower lead groups at 1,000 and 550 parts per million (ppm), respectively. The exposed groups received 225 gm daily of the respective

lead-containing concentrate 7 days per week, while the control group received 225 gm of concentrate containing no added lead. The ewes in each group were fed together allowing for the possibility of unequal exposure among animals within a group. Daily oral exposure of approximately 4.5 and 2.3 mg lead/kg body weight was maintained for the higher and lower lead ewes, respectively, for a 35-day period before breeding and during the 150 days of gestation. Only one ram was used to breed the 30 ewes.

Blood lead residues were determined biweekly during the period of lead exposure by an atomic absorption method (Hessel, 1968) and are presented in the Appendix. During gestation, the mean \pm standard deviation blood lead levels were 4.7 ± 0.9 , 18.6 ± 2.9 , and 34.8 ± 9.9 $\mu\text{g}/100$ ml for the control, lower lead, and higher lead groups, respectively. These mean blood lead residues of the three groups differed significantly from each other at the $P < .05$ level by the Scheffé (1953) comparison between groups (Carson et al., 1973).

Lead ingestion by the exposed ewes was terminated at parturition. Nine, eight, and seven single lambs were born to the ewes in the control, lower lead, and higher lead groups, respectively.

Husbandry and Early Behavioral Experience of in utero Lead Exposed Sheep

The lambs nursed the ewes until they were weaned at 3 months of age. Within approximately 6 weeks after parturition and the termination of lead ingestion, the blood lead levels in the exposed ewes returned to within normal limits of below 10 $\mu\text{g}/100$ ml. However, randomly collected milk samples from the ewes early in lactation revealed milk lead levels approximately equal to the concurrent blood lead level in the respective ewe.

Therefore, the lambs from lead exposed ewes were not only exposed to lead prenatally when lead crossed the placenta but also neonatally when they ingested lead containing milk from their lactating mothers. The three groups of lambs and their dams were quartered separately so that control lambs did not have the opportunity to nurse lead exposed ewes.

The blood lead level was determined once for each lamb between 2 and 4 weeks of age and again between 10 and 12 weeks of age (Appendix). Mean blood lead for control lambs at 2 to 4 weeks of age was $6.0 \mu\text{g}/100 \text{ ml}$, while the low and high exposure lambs averaged 17.5 and $27.7 \mu\text{g}/100 \text{ ml}$, respectively. At 10 and 12 weeks, mean blood lead levels were 4.0 , 8.5 , and $15.8 \mu\text{g}/100 \text{ ml}$ for the control, low, and high lambs, respectively. Throughout the study no clinical manifestations of elevated lead exposure were observed in the lambs.

From 10 days to 3 months of age, the lambs were tested on a series of 16 closed-field maze problems. Significant differences in performance between control and exposed groups were not observed (Carson et al., 1974a).

Following weaning the lambs were maintained on a ration of cracked corn, soybean oil meal, chopped alfalfa hay, dicalcium phosphate, trace mineral salt, and water. Four lambs in the control and one lamb in the high exposure group succumbed from bacterial enteritis and/or pneumonia during the course of neonatal care. One control lamb never became acclimated to the behavioral testing chamber, an initial criterion for further training and, therefore, was not included in this study.

When 13 months of age, these three groups of sheep were tested on a simultaneous two-choice, nonspatial visual discrimination task. The ani-

mals from ewes with 34 $\mu\text{g}/100\text{ ml}$ mean blood lead level during gestation learned the problems significantly slower than did the control and lower-lead exposed lambs (Carson, 1973; Carson et al., 1974b).

Part of the study reported herein involves the further examination of these prenatally lead-exposed sheep first on an auditory discrimination task followed by relearning of the visual discrimination task reported by Carson et al. (1974b) and finally learning of two new visual discrimination problems (Figure 1).

Production of Postnatally Lead Exposed Sheep

Twenty-five normal, healthy Columbia-Rambouillet crossbred lambs born to the resident breeding flock over a 77-day period were randomly assigned to one of five treatment groups. Lambs in groups 1, 2, 3, 4, and 5 received 0, 2, 4, 8, and 16 mg lead/kg body weight, respectively. Oral dosing of the lambs with the solubilized lead acetate started at 5 days of age and continued on a 5 day per week basis until the lambs were approximately 12 weeks old or a total of 60 dosage days. Blood lead levels of the five groups of lambs were determined eleven times during the first 252 days of life (Table 1).

Husbandry and Early Behavioral Experience of Postnatally Lead Exposed Sheep

The five groups of lambs nursed their dams until they were weaned at 3 months of age. Between 10 days and 3 months of age, these lambs were tested on a series of 10 closed-field maze problems. No difference in performance was measured between the control and lead exposed lambs (Carson et al., 1974a).

Figure 1. Geometric form stimuli of the eight visual discrimination problems used to test the sheep with prenatal lead exposure

problem

correct

incorrect

1



2



3



4



5



6



7



8



Table 1. Group mean blood lead levels of postnatally lead exposed lambs. Groups I, II, III, IV, and V ingested 0, 2, 4, 8, and 16 mg lead/kg body weight, respectively

Age (weeks)	Group				
	I ^a	II	III	IV	V
1	11	12	15	14	15
2	10	47	66	66	108
4	12	63	106	112	163
8 _b	15	57	81	123	162
12 ^b	16	39	42	97	93
16	6	18	38	54	48
20	5	8	21	26	25
24	7	25	28	32	32
28	5	9	19	15	20
32	7	18	26	30	28
36	5	14	21	24	26

^aControl group.

^bLead exposure was terminated during the twelfth week.

Following weaning the lambs were maintained on a ration of cracked corn, soybean oil meal, chopped alfalfa hay, dicalcium phosphate, trace mineral salt, and water.

One lamb in group II and one animal in group III succumbed from causes unrelated to lead exposure. Three lambs in group V (16 mg lead/kg body weight) died from lead poisoning before behavioral testing was begun.

At five months of age these five groups of lambs were trained to perform on a simultaneous two-choice, nonspatial visual discrimination task. The protocol of this training period has been previously described (Carson, 1973). Between 10 and 15 months of age these five groups of postnatally lead exposed lambs were tested on a series of nine visual discrimination problems (Figure 2).

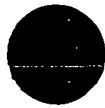
Figure 2. Geometric form stimuli of the nine visual discrimination problems used to test the sheep with postnatal lead exposure

problem

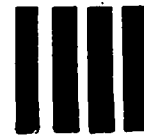
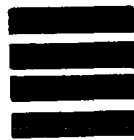
correct

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1



2



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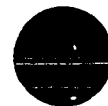
4



5



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7



8



9



Apparatus

Operant behavioral training and subsequent auditory and visual discrimination testing of both prenatal and postnatal lead exposed sheep were conducted in operant chambers constructed in the Behavioral Toxicology Laboratory, Iowa State University. The specific details of the visual discrimination chamber have been previously described (Schnorr, 1972).

Briefly, the chamber was an approximately 2.3m square room with two back projection screens, 2 response devices (Elsberry, 1972), and a retractable food hopper (Sandler et al., 1971) on the front wall (Figure 3). Reinforcements throughout the training and testing period consisted of the food hopper in the test chamber swinging up to make a small portion of cracked corn available to the animal being tested.

A constant level of white noise was maintained in the chamber through a loudspeaker on the ceiling. The white noise plus the ambient noise brought the sound level in the chamber to 72 ± 3 decibels as measured on the C scale of a sound level meter.¹

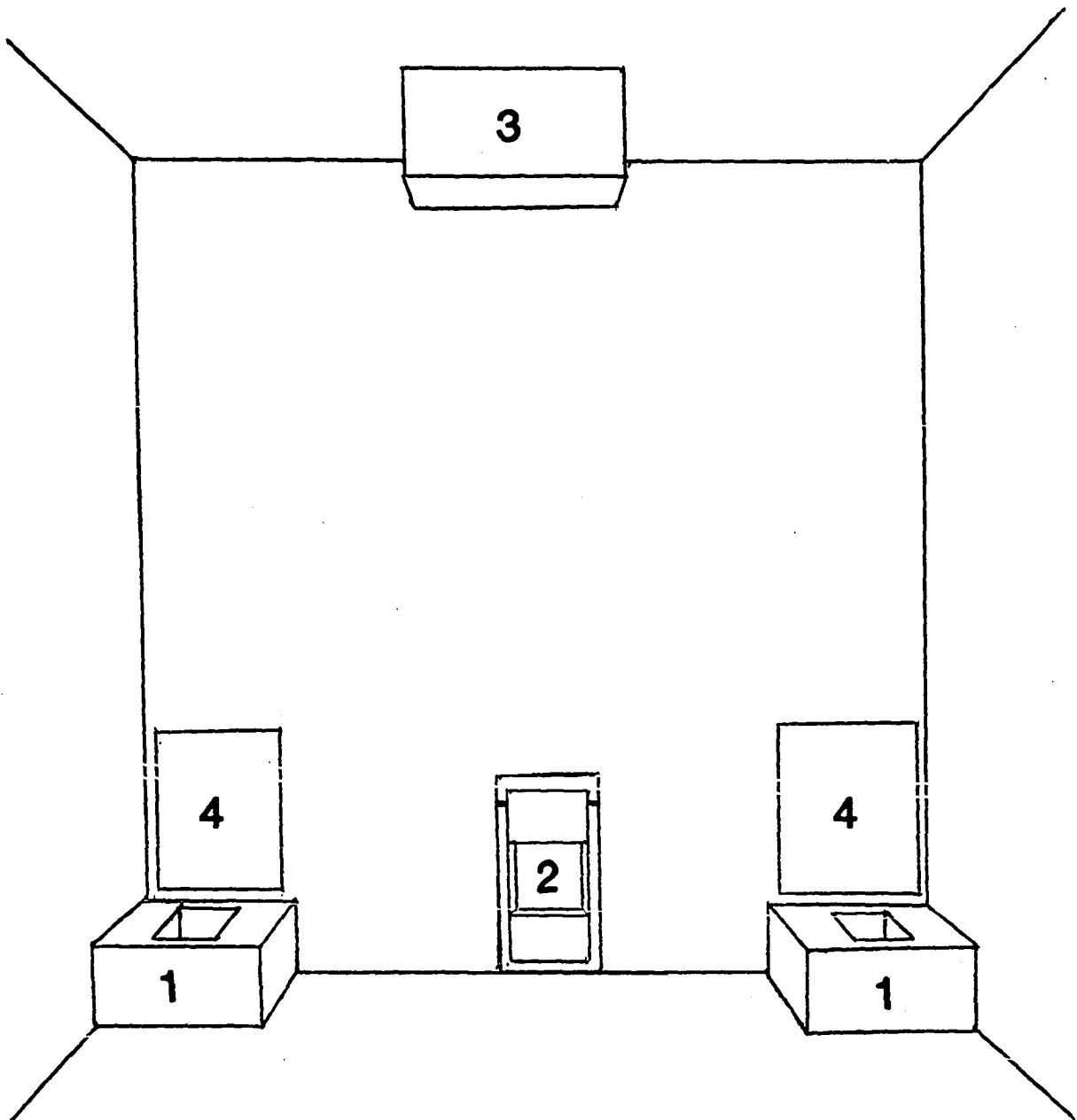
A slide projector² and mirror arrangement on the outside of the chamber was used to project the stimuli on the back projection screens.

The programming of stimuli presentation, reinforcement presentation, and recording of the data was automated using solid state logic control

¹1551-C Sound-level Meter, General Radio Company, Concord, Massachusetts.

²Kodak Carousel AV900 Projector, Eastman Kodak Company, Rochester, New York.

Figure 3. Sketch of front wall of operant chamber for visual discrimination testing: 1) response devices, 2) food hopper, 3) ceiling mounted loudspeaker, 4) back projection screens



modules.³ A flow diagram of the program used to record and control the discrimination testing is presented in Figure 4.

The behavioral chamber used in the auditory discrimination testing was very similar to the visual discrimination chamber. However, no back projection screens were present. The chamber was equipped with three loudspeakers for the presentation of tone stimuli, one on the center of the ceiling and one on each side wall approximately 100 cm from the front wall of the chamber (Figure 5). The white noise background speaker was also present in this chamber. The ambient sound plus the white noise brought the sound level in the chamber to 70 ± 1 decibels.

The tone stimuli used in the auditory testing added 3 to 5 decibels to the chamber sound level depending upon location within the chamber. The speakers were arranged within the chamber to minimize this spatial variation in sound level. The tone stimuli were electronically produced with an oscillator and amplifier.

The response devices in the two front corners of the chamber and the food hopper in the front wall for the presentation of cracked corn were similar to those in the visual discrimination chamber. Reostatically adjustable spotlights were located on the ceiling above the response devices. Solid state logic control modules were utilized for the programming of tone stimuli presentation, reinforcement presentation, and recording of data.

³ Massey Dickinson Company, Inc., Saxonville, Massachusetts.

Figure 4. Flow diagram of the logic used to control the auditory and visual discrimination testing paradigm

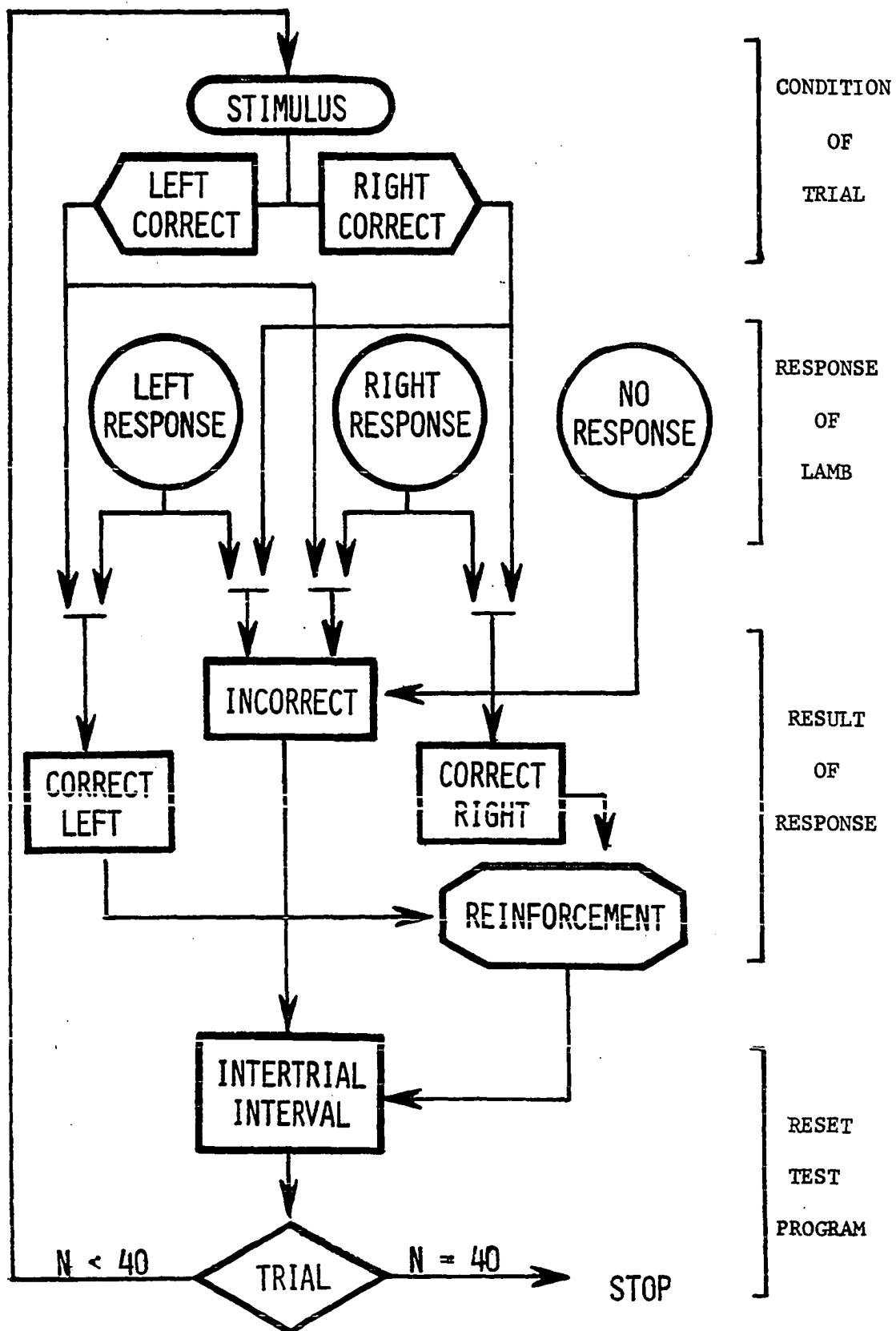
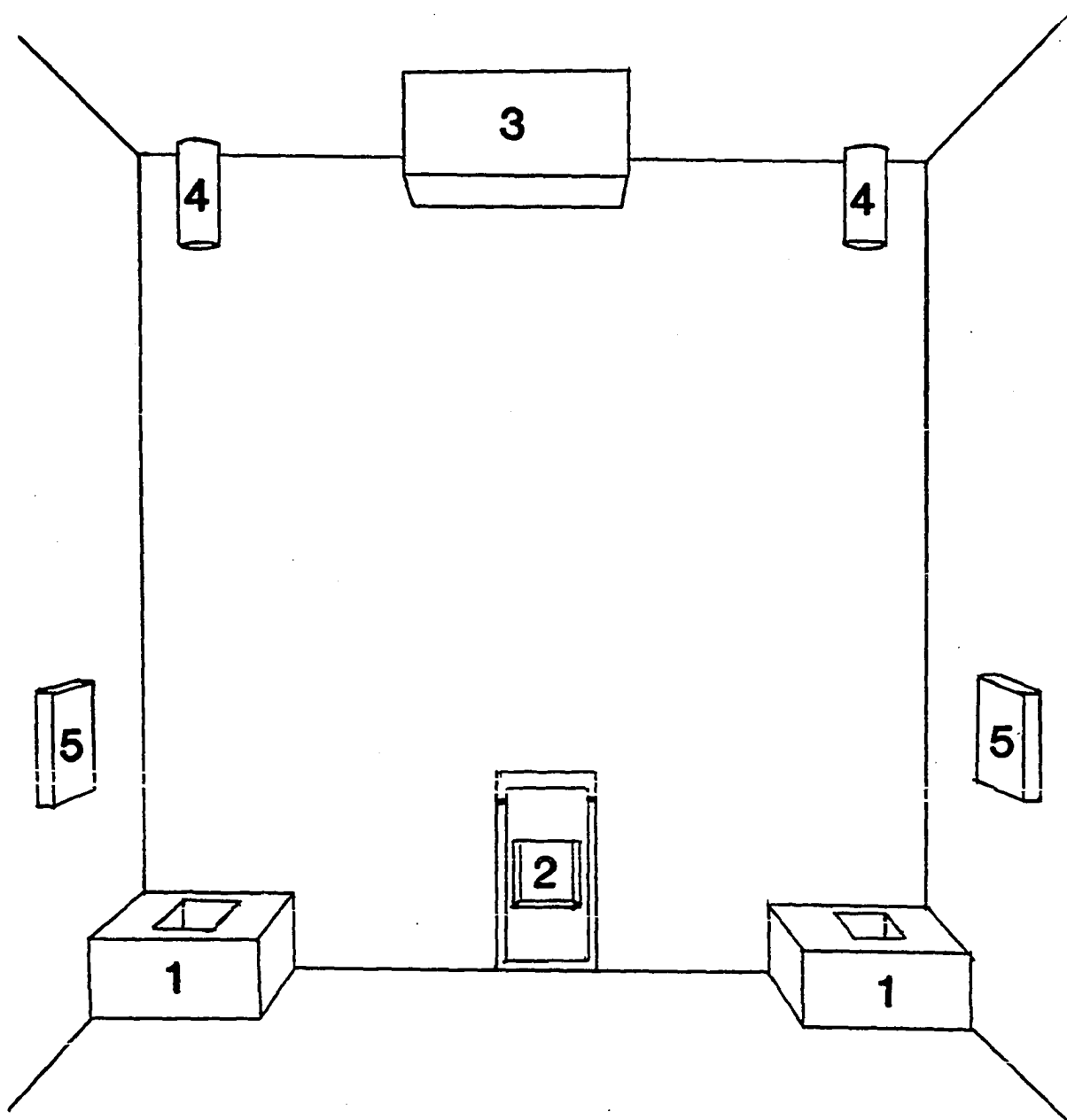


Figure 5. Sketch of front wall of operant chamber for auditory discrimination training and testing: 1) response devices, 2) food hopper, 3) ceiling mounted loudspeaker, 4) spotlights, 5) side wall mounted loudspeakers



Training for Auditory Discrimination

Subsequent to completion of the initial visual discrimination testing as described by Carson (1973), the two groups of prenatally lead exposed and control sheep were given a two-week rest period during which time no behavioral testing was done. Training for the auditory discrimination task was conducted on a one session per day, 5 days per week schedule. The three groups of sheep were approximately 15 months of age at the onset of this training period.

The time needed for the sheep to acclimate to the auditory test chamber was reduced considerably due to the previous operant training experience. One or two training sessions proved sufficient.

The sheep were first trained to perform on an FR-1 (one reinforcement for each response) reinforcement schedule. Manually controlled reinforcement was given to initiate this response.

Once a successful FR-1 performance was achieved, each sheep was trained to perform equally well on both sides of the chamber. For each training session, only the responses on one side of the chamber were reinforced. In an effort to minimize position habits, the side designated as correct was alternated between the right and left sides on successive days of training.

When animals reached the stage in training where they performed equally well on both sides of the chamber, tone stimuli were introduced into the training protocol. For each trial one of two tone stimuli, either 200 Hz or 5,000 Hz, was presented into the chamber through the loudspeakers. The 200 Hz tone activated the right side as correct, and the 5,000 Hz tone activated the left side as correct. The tone was presented in the

chamber for a maximum of 15 seconds or until the correct response was made. Because the sheep had been previously conditioned to a visual stimulus, the spotlight over the designated correct response device was lighted concurrently with the presentation of the tone stimuli. As training continued, the intensity of the cue spotlight was progressively reduced. This visual cue to which the animals were attentive was used to transfer their attention to the simultaneously presented tone stimuli and thereby shortened the overall training period. Eventually when the transfer of the conditioned response from one stimulus to the other stimulus was complete, the spotlights were removed.

Auditory Discrimination Testing

With no spotlight cue, the animal was performing an auditory two-tone frequency discrimination task. Each of the seven auditory discrimination problems used in this study consisted of two tone stimuli, one of high frequency and the other of low frequency (Table 2). Only one tone stimuli of a problem was presented for each trial. The order of presentation of either the high tone or the low tone on consecutive trials within a testing session was on a random basis. The tone stimuli were presented in the chamber for one second. Correct performance consisted of responding on the right response device each time the low tone was presented and responding on the left response device each time the high tone was presented. In this manner the test animal had to discriminate between a high frequency or low frequency tone to receive reinforcement. The audible difference between the two tone stimuli of successive problems diminished because the low tone of each problem increased approximately 20% and the high tone decreased

Table 2. Auditory discrimination problems

Problem	Frequency of tone stimuli (Hz)	
	Correct left	Correct right
1	5,000	200
2	4,800	240
3	3,200	300
4	2,600	360
5	2,000	430
6	1,600	510
7	1,200	620

approximately 20% as compared to that of the previous problem. Therefore, on successively higher numbered problems it became more difficult to discriminate between the two tone stimuli. A 15-second intertrial interval was allowed between the end of one trial and the beginning of the successive trial. A daily testing session consisted of 40 trials. Animals were tested on a 5 days per week schedule. A learning criterion on problem 1 of 70% correct or better for 10 successive days had to be achieved before testing was started on the second problem. The learning criterion on problems 2 through 7 was 70% correct or better for 5 successive days. The number of days of testing to reach criterion on each problem was recorded for each sheep.

Visual Discrimination Testing

The two groups of prenatal lead-exposed and control sheep were tested on a series of eight visual discrimination problems (Figure 1). The first six of this series are problems on which these three groups of sheep had been previously tested (Carson, 1973). It should be noted that although

these problems are the same, the order in which they appear in the test series is different from that described by Carson (1973). Therefore, the first six problems actually constitute a relearning experience. However, problems 7 and 8 were new problems for these sheep.

The four groups of postnatally lead-exposed and their control sheep were tested on a series of nine visual discrimination problems (Figure 2). The first six problems of this series were the same problems and presented in the same order that Carson (1973) utilized in the earlier study of the prenatally lead exposed sheep. Problems 7, 8, and 9 are repeats of three of the earlier six problems and thus constitute a relearning experience for the postnatally lead exposed sheep.

Each of the problems used for visual discrimination testing consisted of a set of two geometric form images or stimuli (Figures 1 and 2). One stimulus in each problem was designated as being correct. During a trial both stimuli of a pair were simultaneously presented with one stimulus projected on the left screen and the other stimulus projected on the right screen. The sheep received reinforcement when a response was made during a trial on the side of the correct stimulus. The position of the correct stimulus alternated between the right and left sides on consecutive trials of a testing session on a chance order (Appendix) (Gellerman, 1933). An incorrect response received no reinforcement and ended the trial. The intertrial interval was 15 seconds. The maximum trial length was 20 seconds. Forty trials comprised each testing session. Testing was conducted on a one session per day, 5 days per week schedule. The learning criterion for mastering a problem and advancing to the next problem was 70% correct or better for 3 consecutive days. Responses were recorded as being either

right correct, right incorrect, left correct, or left incorrect. The total number of responses, total reinforcements, and time length of the testing session were recorded daily. The total number of testing days required to reach criterion was recorded for each sheep for each problem.

Analyses of Data

An analysis of variance was calculated for the mean number of days to reach criterion for sheep within groups, as well as for days to reach criterion for individual problems.

Student's t test was calculated on group means in problems where a significant group difference in speed of learning existed.

Correlation coefficients were calculated for the total days to reach criterion by prenatal lead exposed sheep on the auditory discrimination and the visual discrimination problems and the mean blood lead levels of their respective dams during the first, second, and third trimester of gestation.

Necropsy and Collection of Tissues

At the termination of the study sheep in the prenatal lead exposed and control groups were stunned with electrical shock and euthanized by exsanguination.

Brains for histopathologic examination were collected and immersed in 10% buffered formalin.

Histologic Procedures

Tissues were fixed in 10% formalin, processed by routine parafin technique, sectioned at 6 microns, and stained with hematoxylin and eosin.

RESULTS AND DISCUSSION

Sheep with Prenatal Lead Exposure

Auditory discrimination

The mean number of testing days required for sheep within groups to reach learning criterion on the seven auditory discrimination problems is listed in Table 3 and represented in Figure 6. The analysis of variance calculated for these data is presented in Table 4. The overall treatment effect across all seven problems was significant at $P < .01$ ($F = 4.89$, $df = 2,125$). The effect of problems was significant at $P < .005$ ($F = 20.89$, $df = 6,125$).

This significant treatment effect demonstrated that the higher lead, the lower lead, and the control sheep differed significantly ($P < .01$) in the number of testing days required to master the seven auditory discrimination problems.

The mean number of days per auditory discrimination problem for sheep within prenatal lead groups to reach criterion were 11.1, 11.3, and 17.9 days for the control, lower lead, and higher lead groups, respectively.

Further analyses of variance to define the source of the above treatment effect revealed a significant difference attributed to treatment between the control and higher lead groups ($F = 5.24$, $df = 1,56$) and the lower lead and higher lead groups ($F = 7.26$, $df = 1,84$). No significant differences due to treatment were demonstrated between the control and lower lead groups.

When sheep group performance was examined on an individual problem basis, Student's t test showed a significant difference ($t = 3.69$, $df = 8$)

Table 3. Mean \pm standard deviation days required for sheep with prenatal lead exposure and controls to achieve learning criterion for auditory discrimination problems

Group	Discrimination problem						
	1	2	3	4	5	6	7
Control	32.0 \pm 10.4	5.0 \pm 0	5.0 \pm 0	7.2 \pm 3.9	8.5 \pm 3.5	5.0 \pm 0	15.0 \pm 14.0
Low	34.2 \pm 20.5	5.0 \pm 0	5.0 \pm 0	5.7 \pm 0.9	6.0 \pm 2.6	7.6 \pm 4.0	15.7 \pm 14.0
High	56.6 \pm 22.9	10.8 \pm 13.0	8.0 \pm 6.2	7.8 \pm 3.6	11.5 \pm 9.3	7.6 \pm 3.4	23.6 \pm 18.9

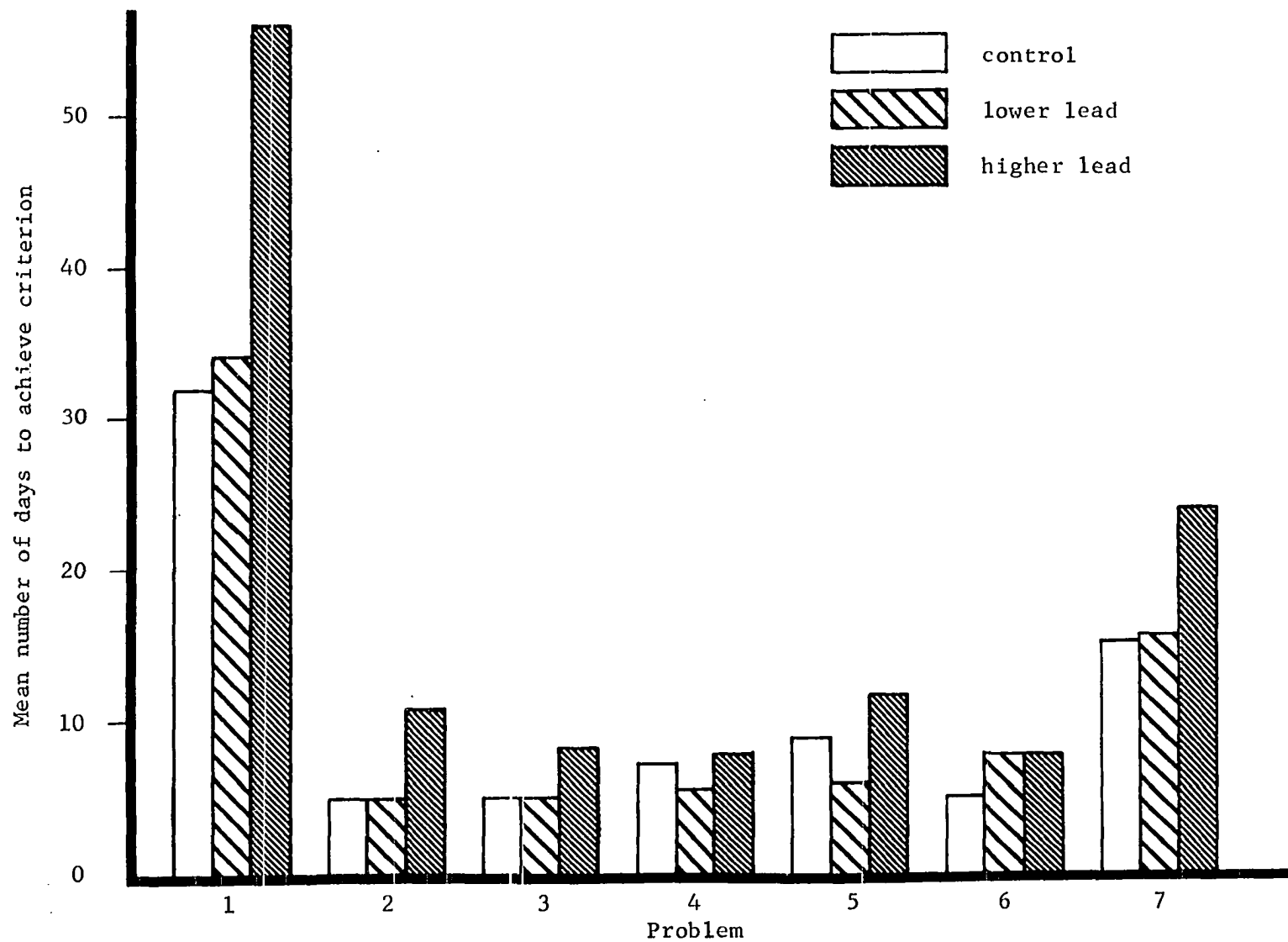


Figure 6. Mean number of testing days required for sheep with prenatal lead exposure and controls to achieve learning criterion on the seven auditory discrimination problems

Table 4. Analysis of variance for number of days to achieve learning criterion for sheep within groups across all seven auditory discrimination problems (prenatal lead exposure)

Source	d.f.	M.S.	F
Treatment	2	640.63	4.89*
Problem	6	2733.72	20.89**
Treatment x problem	12	121.74	0.93
Error	125	130.81	--

* $P < 0.01$.

** $P < 0.005$.

only between the control and higher lead groups in problem 1. No other significant differences were observed between groups within individual problems.

Problem 1 appeared to be the most difficult for all groups. This observation can probably be attributed to the high learning criterion for 10 days at 70% correct or better set for this problem while problems 2 through 7 had a criterion of only 5 days at 70% correct or better performance. In addition problem 1 was the first auditory discrimination problem on which these sheep were tested. Therefore, the high days to criterion observed in problem 1 could reflect difficulty with learning the task of auditory discrimination per se and not necessarily problem 1.

The total days to criterion for all seven auditory discrimination problems for each sheep and the mean blood lead levels of their respective dam during the first, second, and third trimester of gestation are pre-

sented in the Appendix. The correlation coefficients calculated for the total days to reach criterion for each sheep and their dam mean blood lead level during the first, second, and third trimester of gestation were 0.48, 0.32, 0.38, respectively.

The positive correlation ($r = 0.48$) between the total number of days required to reach criterion for the 7 auditory discrimination problems and the mean blood lead of the ewes during the first trimester of gestation may be an important finding. Organogenesis in sheep occurs during the first trimester of gestation (Bryden et al., 1972). It, therefore, appears that the chance of permanent neurologic damage is greatly increased if lead exposure occurs during the formative stages of a developing nervous system. This observation may, in part, explain the lack of neurologic deficits observed in the postnatal lead exposed lambs on the visual discrimination testing, as well as the minimal neurologic changes observed following sub-clinical lead exposure in previous animal behavioral studies (Brown et al., 1971; Bullock et al., 1966; Goode et al., 1973; Snowden, 1973; Van Gelder et al., 1973) where prenatal lead exposure was not employed.

Visual discrimination

The mean number of days required for sheep within groups to reach learning criterion on the eight visual discrimination problems is listed in Table 5 and represented in Figure 7. The analysis of variance calculated for these data is represented in Table 6. The overall treatment across all eight problems was significant at $P < .01$ ($F = 4.28$, $df = 2, 117$). The effect of problems was significant at $P < .005$ ($F = 25.8$, $df = 8, 117$).

Table 5. Mean \pm standard deviation days required for sheep with prenatal lead exposure and controls to achieve learning criterion on visual discrimination problems

Group	Discrimination problem							
	1	2	3	4	5	6	7	8
Control	3.0 \pm 0	3.2 \pm 0.4	3.2 \pm 0.4	3.7 \pm 0.8	5.2 \pm 2.7	23.0 \pm 13.8	29.0 \pm 6.4	4.5 \pm 1.1
Low	3.2 \pm 0.4	3.4 \pm 0.7	4.2 \pm 1.3	4.5 \pm 1.7	7.0 \pm 6.7	30.0 \pm 15.8	34.7 \pm 6.2	16.4 \pm 8.9
High	5.1 \pm 1.5	9.3 \pm 8.8	4.2 \pm 0.9	8.5 \pm 6.1	23.8 \pm 10.9	21.0 \pm 12.5	30.8 \pm 10.0	15.2 \pm 9.6

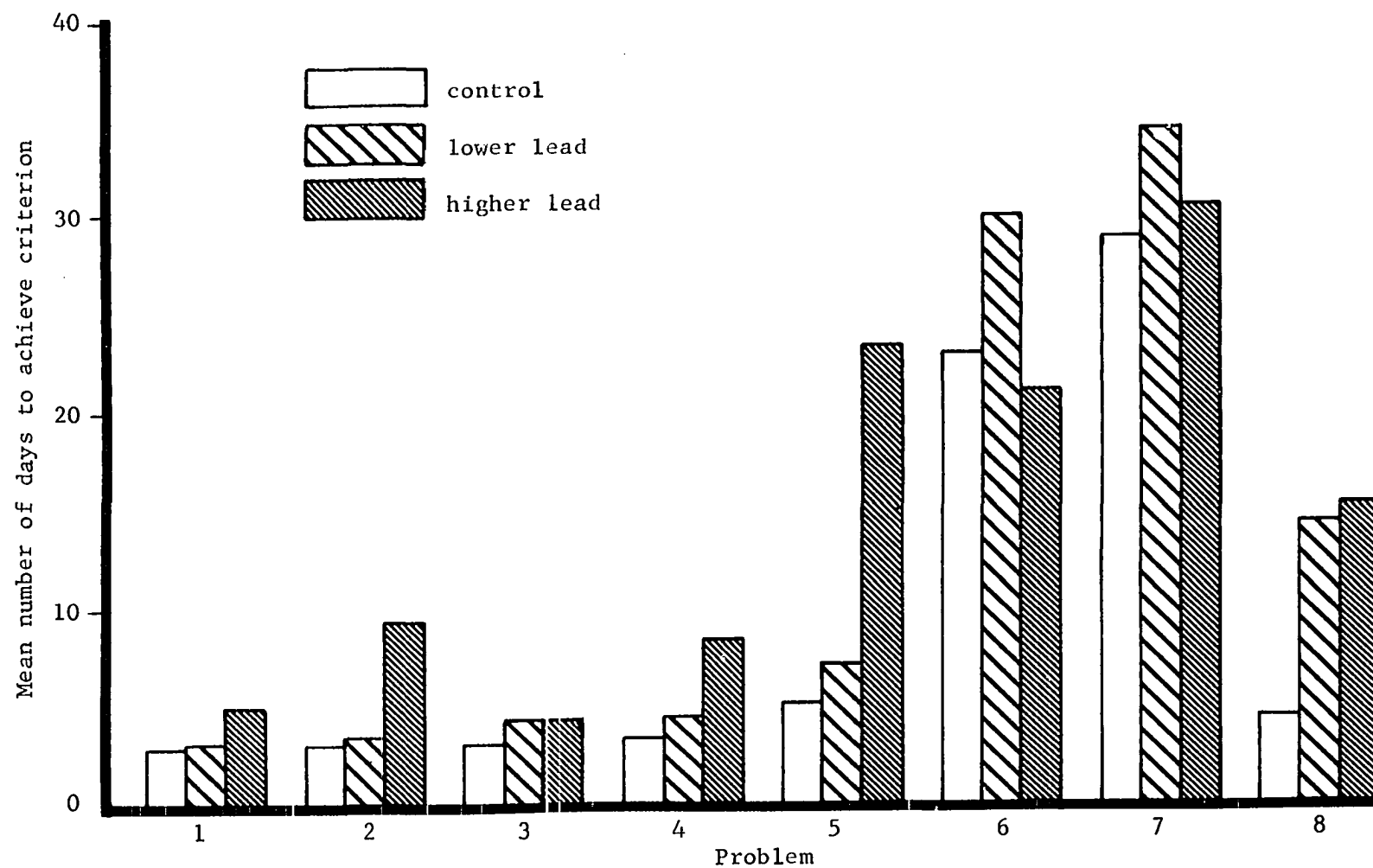


Figure 7. Mean number of testing days required for sheep with prenatal lead exposure and controls to achieve learning criterion on eight visual discrimination problems

Table 6. Analysis of variance for number of days to achieve learning criterion for sheep within groups across all eight visual discrimination problems (prenatal lead exposure)

Source	d.f.	M.S.	F
Treatment	2	253.18	4.28*
Problem	8	1529.34	25.88**
Treatment x problem	16	94.94	1.60
Error	117	59.09	--

* $P < 0.01$.

** $P < 0.005$.

This significant treatment effect demonstrated that the higher lead, the lower lead, and the control sheep differed significantly ($P < .01$) in the number of testing days required to master the eight visual discrimination problems.

The mean number of days per visual discrimination problem for sheep within prenatal lead groups to reach criterion were 9.3, 12.9, and 14.6 days for the control, lower lead, and higher lead groups, respectively.

Further analyses of variance to define the source of the above treatment effect revealed a significant difference due to treatment between the control and the lower lead sheep ($F = 5.16$, $df = 1,81$) and between the control and higher lead sheep ($F = 7.93$, $df = 1,63$). No significant group differences attributed to treatment were observed between the lower lead and higher lead groups ($F = 1.09$, $df = 1,90$).

Comparison between groups within individual problems of the mean days to reach criterion was made using Student's t test. Significant group differences were observed only between the control and higher lead sheep in problem 5 ($t = 7.29$, $df = 7$) and between the lower and higher lead groups in problem 5 ($t = 3.31$, $df = 10$).

Problem 6 was still quite difficult for all groups even though the sheep had had previous exposure to this problem (Carson, 1973; Carson et al., 1974b). It is interesting to note that although problems 7 and 8 were size discriminations similar to problem 6, the sheep had more difficulty with problem 7, while problem 8 proved to be less difficult. Again it appears as though the greatest difficulty was in mastering a new behavioral task; that is, size discrimination problems were a different type of task than the earlier problems of shape discrimination.

The total days to criterion for all eight visual discrimination problems for each sheep and the mean blood lead levels of the respective dam during the first, second, and third trimester of gestation are presented in the Appendix. The correlation coefficients calculated for the total days to reach criterion for each sheep and their mean ewe blood lead during the first, second, and third trimester of gestation were 0.28, 0.17, and 0.19, respectively.

Histopathologic examination

No histologic changes compatible with those previously associated with lead encephalopathy were observed in the brain tissue sections from the prenatal lead exposed and control sheep. No lesions of chronic enterotoxemia were observed.

Sheep with Postnatal Lead Exposure

Visual discrimination

The mean number of testing days required for sheep within groups to reach learning criterion on each of the nine visual discrimination problems is listed in Table 7 and represented in Figure 8. The analysis of variance calculated for these data is presented in Table 8. The overall treatment effect across all nine problems was not significant ($F = 1.40$, $df = 4, 188$). The effect of problems was significant at $P < .005$ ($F = 19.87$, $df = 8, 188$).

This lack of a significant treatment effect demonstrated that the four groups of postnatally lead exposed and the control sheep did not differ significantly ($P < .01$) in the number of testing days required to master the nine visual discrimination problems. The mean number of days per visual discrimination problem for sheep within the postnatal lead exposure groups were 10.8, 8.6, 8.4, 11.3, and 11.5 days for groups I (control), II, III, IV, and V, respectively.

Problem 6, two solid circles of unequal size, again proved to be the most difficult problem for all of the sheep.

General Comments

The significant effects of problems ($P < .005$) (Tables 4, 6, and 8) observed in all three of these experiments indicated that the problems within an experiment differed from one another in their degree of difficulty. This difference in degree of difficulty was not unexpected in view of the differing complexity of both pairs of form stimuli utilized in the discrimination problems and the pairs of tone stimuli used in the auditory discrimination testing. The difference in degree of difficulty between

Table 7. Mean \pm standard deviation days required for sheep with postnatal lead exposure to achieve learning criterion on nine visual discrimination problems

Group	Discrimination problem								
	1	2	3	4	5	6	7	8	9
I	8.3 \pm 2.5	5.8 \pm 2.3	4.3 \pm 2.8	6.6 \pm 2.0	23.1 \pm 17.3	31.1 \pm 14.5	5.8 \pm 4.3	3.3 \pm 0.8	9.5 \pm 10.6
II	10.8 \pm 7.1	6.2 \pm 2.2	5.0 \pm 2.4	6.0 \pm 3.6	13.4 \pm 4.6	26.2 \pm 10.0	3.8 \pm 1.7	3.4 \pm 0.5	3.2 \pm 0.4
III	12.0 \pm 3.4	5.0 \pm 1.0	5.0 \pm 1.7	8.0 \pm 7.8	13.6 \pm 8.5	13.6 \pm 7.7	3.6 \pm 1.1	3.0 \pm 0	12.0 \pm 15.5
IV	11.2 \pm 3.1	8.6 \pm 4.5	4.2 \pm 0.8	7.2 \pm 6.1	24.4 \pm 11.6	25.0 \pm 16.0	5.2 \pm 3.0	5.4 \pm 3.7	10.6 \pm 10.7
V	13.5 \pm 3.5	6.5 \pm 3.5	4.5 \pm 0.7	10.5 \pm 9.1	16.5 \pm 9.1	38.5 \pm 9.1	7.0 \pm 2.8	3.0 \pm 0	3.5 \pm 0.7

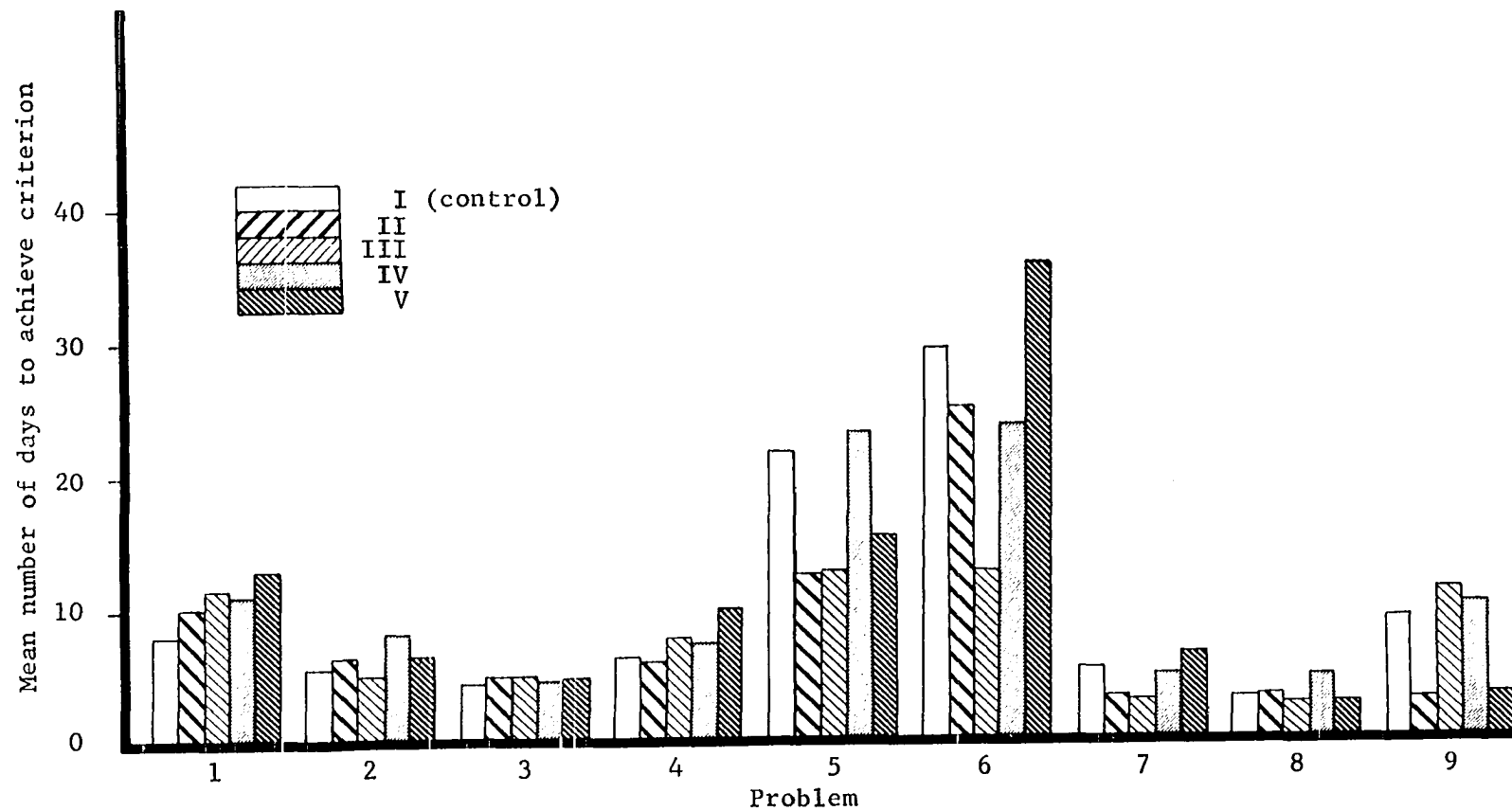


Figure 8. Mean number of testing days required for sheep with postnatal lead exposure and controls to achieve learning criterion on nine visual discrimination problems

Table 8. Analysis of variance for mean number of days to achieve learning criterion for sheep within groups across all nine visual discrimination problems (postnatal lead exposure)

Source	d.f.	M.S.	F
Treatment	4	75.47	1.40
Problem	8	1052.55	19.87*
Problem x treatment	32	48.19	0.89
Error	188	53.87	--

* $P < 0.005$.

problems within an experiment was further demonstrated by the fact that the mean days to reach criterion of the control group varied between problems within an experiment. Had the problems been of equal difficulty, one would have expected the mean number of testing days required to reach criterion on consecutive problems to be similar or actually decrease slightly after the task per se had been mastered (Schnorr, 1972). However, the mean days required to reach criterion by the control groups did differ between problems (Tables 3, 5, and 7) indicating a difference in degree of difficulty of the problems within an experiment.

Several studies, however, have reported neurologic or behavioral changes in a species of rodent following exposure to either oral or injected lead compounds (Silbergeld and Goldberg, 1973; Brown, 1973; Krigman et al., 1972; Snowden, 1973; Pentschew and Garro, 1966; Lampert et al., 1967). These reports, in view of the lack of behavioral changes seen in the postnatally lead exposed lambs, suggest a species difference

between rodents and sheep in their susceptibility to postnatal lead exposure and consequent neurologic involvement. Perhaps this difference phenomenon is related to the relative maturity of the respective animals at the time of parturition.

The behavioral deficit associated with prenatal lead exposure demonstrated in this study is consistent with the residual learning disabilities reported by Brown (1973) in 8- to 10-week-old rats which had nursed lead-exposed mothers for the first 5 weeks of life.

The results of this study are also consistent with the earlier reports (Carson, 1973; Carson et al., 1974b) of slowed learning in these prenatally lead-exposed sheep and are evidence for the permanence of the neurologic deficit which was demonstrated in these animals.

These results are compatible with clinical reports of the residual neurologic effects of lead in children. Perlstein and Attala (1966) reported that minimal brain damage 6 months to 2 years after recovery from acute lead poisoning involved learning blocks, usually of the visual-perceptual type. Byers and Lord (1943) found through the use of specialized psychological tests that the ability to deal with shape, direction, space, and projected imagery was impaired in children who had recovered from lead poisoning several years earlier. Thurston et al. (1955) and Bradley and Baumgartner (1958) demonstrated by tests of visual-motor function prominent visual-motor deficits in children several years after acute lead poisoning. Mellins and Jenkins (1955) reported impairment of fine muscle coordination and perceptual-motor skills in young children 4 to 6 months after recovery from lead encephalopathy. Jenkins and Mellins (1957) reported that lead poisoned children had the greatest difficulty

with tasks calling for the naming of objects and simple conceptualizing. None of these clinical studies of children, however, reported involvement of auditory-motor or auditory perceptual skills in the syndromes of neurologic impairment.

Significance of the Study

The demonstration of behavioral deficits in the offspring of ewes maintained with a mean blood lead during gestation of 34.8 $\mu\text{g}/100\text{ ml}$ supports recent Environmental Protection Agency (1972) guidelines that for pregnant women the upper acceptable blood lead level should be no more than 30 $\mu\text{g}/100\text{ ml}$. No clinical manifestations of overt toxicosis were observed in the ewes during the period of lead exposure. However, this study did demonstrate visual and auditory learning deficits in 1½- to 2-year-old sheep whose blood lead level at 2 to 4 weeks of age had been 25 $\mu\text{g}/100\text{ ml}$, a level measurably below the 40 $\mu\text{g}/100\text{ ml}$ recommended by the Environmental Protection Agency (1972) as probably safe for children. One must be cautious, however, in equating the significance of blood lead levels across species lines especially when the setting of health guidelines for humans is concerned. Therefore, a species of animal phylogenetically closer to humans than sheep should be employed in future prenatal lead exposure studies. A species of subhuman primate should be used to more fully assess the effect of subclinical in utero lead exposure on the developing nervous system.

Although lead exposed sheep did learn both the auditory and the visual discrimination tasks significantly slower than the controls, the impact of this study would be greater had a larger number of animals been included in

each of the treatment groups. The control group in the prenatal lead study contained only four animals, and the lower lead group contained only eight animals. It should be acknowledged, however, that the production of prenatally exposed animals with the goal of equal sized groups of trainable and testable animals is not easily achieved.

If this study were to be repeated, I would recommend 1) an attempt to achieve larger group sizes; 2) early vaccination of all animals against clostridial enterotoxemia; 3) the involvement of electrophysiologic documentation of neurologic damage; 4) more complete utilization of experimental pathologic techniques including electron microscopic studies; and 5) closer monitoring of the body lead burden in the prenatally lead exposed animals.

CONCLUSIONS

It was concluded that: 1) slowed learning of auditory and visual discrimination tasks in sheep when they were $1\frac{1}{2}$ -2 years of age was associated with subclinical prenatal lead exposure and 2) postnatal oral lead exposure for the first 12 weeks of life did not affect performance of a visual discrimination task when they were $1-1\frac{1}{2}$ years of age.

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APPENDIX

Gellerman Series

Chance consecutive order for 20 left side correct trials and 20 right side correct trials within a testing or training session of 40 trials (Gellerman, 1933):

R R R L L R L R L L L R R R L L R L L R

R R L R L L R R L L L R R L R R L L L R

Table 9. Number of days required for prenatal lead exposed sheep to reach learning criterion for auditory discrimination problems

Sheep number	Discrimination problem						
	1 ^a	2 ^b	3	4	5	6	7
<u>Control</u>							
10	40	5	5	14	11	5	39
12	37	5	5	5	5	5	5
13	14	5	5	5	5	5	5
19	37	5	5	5	13	5	11
<u>Lower lead</u>							
30	30	5	5	5	5	5	5
31	10	5	5	5	5	5	21 ^c
32	79	5	5	7	5	7	50 ^c
35	40	5	5	7	13	15	6
36	10	5	5	5	5	5	17
37	25	5	5	5	5	5	5
38	41	5	5	7	5	14	9
39	39	5	5	5	5	5	13
<u>Higher lead</u>							
41	39	5	5	5	5	7	50 ^c
42	48	5	5	6	7	5	9
45	51	5	5	5	5	6	50
46	31	5	22	5	17	5	12
47	104 ^c	40	6	13	30 ^c	8	16
48	71	5	5	13	5	15	5

^aCriterion was 10 days at 70% correct.

^bCriterion was 5 days at 70% correct.

^cCriterion was not achieved.

Table 10. Number of testing days required for sheep with prenatal lead exposure to reach learning criterion on eight visual discrimination problems

Sheep number	Discrimination problem							
	1	2	3	4	5	6	7	8
<u>Control</u>								
10	3.0	3.0	4.0	5.0	4.0	18.0	22.0	3.0
12	3.0	4.0	3.0	3.0	10.0	32.0	39.0	6.0
13	3.0	3.0	3.0	4.0	3.0	3.0	25.0	4.0
19	3.0	3.0	3.0	3.0	4.0	39.0	30.0	5.0
<u>Lower lead</u>								
30	4.0	3.0	3.0	6.0	4.0	49.0	40.0 ^a	26.0
31	4.0	3.0	7.0	5.0	4.0	29.0	40.0 ^a	26.0 ^a
35	3.0	3.0	5.0	3.0	4.0	16.0	29.0	5.0
36	3.0	3.0	4.0	4.0	3.0	46.0	40.0 ^a	26.0 ^a
37	3.0	4.0	5.0	8.0	3.0	4.0	29.0	11.0
38	3.0	5.0	3.0	3.0	23.0	44.0	25.0	5.0
39	3.0	3.0	3.0	3.0	8.0	22.0	40.0 ^a	5.0
<u>Higher lead</u>								
42	5.0	5.0	3.0	4.0	27.0	19.0	35.0	15.0
45	5.0	27.0	4.0	14.0	33.0	31.0	40.0 ^a	26.0 ^a
46	8.0	7.0	6.0	18.0	35.0	39.0 ^a	40.0 ^a	26.0 ^a
47	3.0	3.0	4.0	4.0	19.0	5.0	14.0	6.0
48	5.0	5.0	4.0	3.0	5.0	11.0	25.0	3.0

^aCriterion was not achieved.

Table 11. Number of days of testing for sheep with postnatal lead exposure to reach learning criterion on nine visual discrimination problems

Sheep number	Discrimination problem								
	1	2	3	4	5	6	7	8	9
<u>I (control)</u>									
51	9	4	4	10	48	45	14	5	31
52	4	9	10	4	18	8	3	3	6
53	9	5	3	8	7	26	7	3	3
54	7	8	3	6	11	24	3	3	7
55	11	6	3	6	42	45	5	3	6
57	10	3	3	6	13	39	3	3	4
<u>II</u>									
61	5	4	3	4	15	9	3	4	3
62	11	6	5	3	8	27	3	3	4
63	8	5	3	7	18	28	3	3	3
64	23	10	9	12	17	34	3	4	3
65	7	6	5	4	9	33	7	3	3
<u>III</u>									
71	10	5	6	3	5	5	3	3	3
73	10	4	3	4	22	16	5	3	3
75	16	6	6	17	14	20	3	3	30
<u>IV</u>									
81	14	16	4	3	40	45	8	12	29
82	9	5	3	5	29	9	3	3	3
83	15	6	5	6	23	11	3	5	4
84	10	10	4	18	22	38	9	4	11
85	8	6	5	4	8	22	3	3	6
<u>V</u>									
93	11	4	5	4	10	45	5	3	4
95	16	9	4	17	23	32	9	3	3

Table 12. Total testing days to reach criterion for prenatal lead exposed sheep and blood lead level of their respective dam during gestation

Sheep number	<u>Total days to criterion</u>		<u>Mean dam blood lead ($\mu\text{g}/100\text{ ml}$)</u>		
	Auditory	Visual	First trimester	Second trimester	Third trimester
10	119	62	5	4	2
12	67	100	5	5	3
13	44	48	5	5	3
19	81	90	6	6	3
30	60	135	14	18	15
31	56	118	19	24	25
32	158	--	21	25	24
35	91	68	15	20	21
36	52	120	19	18	24
37	55	67	17	21	20
38	86	111	17	20	18
39	77	87	19	13	14
41	116	--	25	28	33
42	85	113	29	25	34
45	127	180	25	26	28
46	97	179	26	23	33
47	213	58	29	21	31
48	119	61	33	49	57

Table 13. Blood lead levels ($\mu\text{g}/100\text{ ml}$) of dams ingesting lead throughout gestation

Dam number	Week of gestation										
	1	3	5	7	9	11	13	15	17	19	21
<u>Control</u>											
10	0	4	8	6	6	0	4	5	6	0	0
11	8	5	6	8	6	5	18	5	6	5	4
12	5	3	7	5	6	0	8	5	8	0	0
13	6	3	7	4	6	1	8	5	7	0	2
14	6	3	8	0	6	0	9	5	8	7	6
15	0	3	4	5	6	0	9	5	6	3	3
16	5	4	4	4	6	0	14	5	12	0	2
17	0	3	6	5	6	0	8	5	8	1	0
18	6	3	7	7	6	2	6	5	7	0	4
19	4	4	6	9	6	3	10	5	8	0	0
<u>Lower lead</u>											
30	12	11	19	13	16	14	30	13	14	16	14
31	19	12	22	24	21	20	36	20	24	27	25
32	24	20	19	21	21	17	39	22	24	25	24
33	15	13	13	17	11	8	22	3	24	18	11
34	22	16	20	20	18	16	32	12	21	32	18
35	14	12	19	14	14	17	31	19	23	28	13
36	19	20	17	18	14	11	30	18	23	26	22
37	19	17	19	12	14	21	35	15	21	22	16
38	20	13	15	19	12	23	31	12	20	24	10
39	25	12	17	23	11	12	21	9	12	15	14
<u>Higher lead</u>											
40	33	34	39	47	27	44	72	65	62	86	63
41	22	17	26	33	19	26	42	24	34	32	32
42	34	26	24	31	21	25	36	19	34	36	32
43	34	24	35	42	20	36	44	37	48	70	50
44	28	--	34	29	17	22	36	20	37	38	31
45	32	17	26	24	18	26	34	24	26	36	22
46	31	23	24	27	17	17	32	24	30	36	32
47	35	24	28	28	14	18	32	20	31	38	25
48	30	22	34	44	27	51	62	57	51	67	53
49	47	49	45	51	27	47	59	47	60	61	44

Table 14. Blood lead levels of prenatally lead exposed sheep

Sheep number	Blood lead ($\mu\text{g}/100\text{ ml}$)	
	2-4 weeks of age	10-12 weeks of age
<u>Control</u>		
10	4	4
12	7	4
13	7	3
19	6	5
<u>Lower lead</u>		
30	24	17
31	20	0
32	16	8
35	15	0
36	16	7
37	19	15
38	16	17
39	14	4
<u>Higher lead</u>		
41	25	11
42	36	25
44	30	12
46	21	10
47	17	8
48	32	24
49	33	21

Table 15. Blood lead levels ($\mu\text{g}/100\text{ ml}$) of sheep with postnatal lead exposure

Sheep number	Age in weeks										
	1	2	4	8	12	16	20	24	28	32	36
<u>I (control)</u>											
51	15	12	13	20	19	11	3	7	5	8	4
52	16	6	12	10	9	--	5	8	5	8	5
53	5	7	16	23	12	3	--	5	5	7	5
54	14	13	14	20	13	1	5	--	5	8	5
55	7	16	8	8	19	--	5	--	5	7	5
56	5	14	17	7	--	--	--	8	--	--	--
<u>II (2 mg/kg)</u>											
61	15	63	66	90	55	21	9	22	16	20	17
62	19	41	46	55	23	35	15	29	5	18	12
63	8	56	70	--	--	85	---	--	--	--	--
64	12	34	36	25	35	8	2	--	5	14	11
65	6	44	105	50	33	15	9	--	10	21	15
<u>III (4 mg/kg)</u>											
71	17	100	92	95	55	20	17	27	16	27	20
72	18	44	70	70	50	60	19	23	13	23	20
73	21	47	120	80	45	50	29	35	20	28	22
74	12	82	108	70	died						
75	10	61	150	70	63	31	19	--	28	28	25
<u>IV (8 mg/kg)</u>											
81	12	87	61	110	80	35	21	27	22	25	19
82	20	44	108	120	100	65	29	35	32	34	24
83	24	44	176	155	135	75	32	38	30	40	38
84	9	63	105	60	53	35	33	31	25	28	17
85	6	95	--	160	40	18	15	33	12	26	21
<u>V (16 mg/kg)</u>											
91	10	85	139	160	100	85	died				
92	20	111	140	145	140	died					
93	23	38	280	215	115	65	25	34	20	31	32
94	14	150	140	150	died						
95	9	158	120	130	58	31	--	31	21	25	19