Effects of prenatal lead exposure on visual discrimination in sheep

by

Thomas Lee Carson

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INTRODUCTION

Since 4000 years before Christ when man first formed this soft gray metal into tools and vessels, the deleterious effects of lead poisoning have been part of man's history. Even in the early decades of the industrialized 20th century lead poisoning was a constant hazard to workers in lead associated industries.

Today even with our increased awareness of its manifestations, lead poisoning is a wide-spread 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 pealing 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. 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 neurologic effect of subclinical lead exposure was investigated in this study by a comparative medicine approach utilizing an animal model and methods of behavioral toxicology.

The study reported here was conducted to determine if changes in behavioral capabilities as measured by performance on a visual discrimina-

tion task existed in the offspring of ewes ingesting subclinical levels of lead throughout gestation.

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.

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 non-food 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 was emphasized. Alterations in the brain parenchyma were 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. These lesions suggest that vascu-

lar damage is a major factor in the pathogenesis of cerebral disorder (Popoff et al., 1963).

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 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 μg/100 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 μg/100 ml. Out of a second group of 40 children which were diagnosed as having encephalitis, 12 children had blood leads of 38 μg/100 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 μg/100 ml.

Another study (Millar et al., 1970), however, reported no children with blood lead levels above 40 μg/100 ml in a group of 27 mentally retarded children.

Byers and Lord (1943) presented a follow-up study on 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 sub-

ject'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 has 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 interfers 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 corticalatrophy, 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. And 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 μg/100 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 μg/100 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 then 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, p. IV-2).

Lead readily crosses the placenta from the maternal to the fetal circulation (Barltrop, 1969; Cantarow and Trumper, 1944). Blood lead levels in newborns are dependent upon and correlate positively with maternal blood levels (Haas, 1972). 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 μg/100 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 break-down 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 electronmicroscopic studies that vascular damage was the primary dysfunction in lead encephalopathy in rats.

Thomas et al. (1971) also produced encephalopathy in suckling rats by adding 4.5% lead carbonate in the feed and 1% lead acetate in 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 the direct action of lead.

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.

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 (ALA-dehydratase) 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 ALA-dehydratase 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 levels and blood ALA-dehydratase 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 using behavioral toxicologic techniques to assess the effects of lead or lead compounds on the function of the nervous system have been reported.

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 fron 8 days to 5 weeks of age at the time of lead exposure.

However, 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 lead/kg daily for the first 20 days following parturition (Brown, 1973).

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).

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 μg/100 ml for the last 21 weeks of the study.

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 tox-

icosis. 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.

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.

Visual Discrimination in Sheep

Seitz (1951) found that the East Prussian prairie sheep performed well on shape discrimination tasks involving circles, 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 interferred 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 mammals. 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.

METHODS

Production of Prenatally Lead Exposed Lambs

Thirty Columbia-Rambouilet 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 μg/100 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 lower lead feeding levels in the present project was to maintain blood lead levels of approximately 30 μg/100 ml and 15 μg/100 ml in the "high" and "low" lead exposed groups, respectively, thereby avoiding clinical manifestations of lead toxicosis. Therefore, finely divided metallic lead was incorporated into the pelleted ration of the high and low lead exposure groups at 1000 and 550 parts per million, respectively. The exposed groups received 225 gm of the respective lead-containing concentrate per day 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 high and low lead exposed ewes, respectively, for a 35-day period before breeding and during the 150 days of gestation.

Blood lead residues were determined biweekly during the period of lead exposure by the atomic absorption method of Hessel (1968). During gestation, the mean $\pm$ standard deviation blood lead levels were 4.7 $\pm$ 0.9, 18.6 $\pm$ 2.9, and 34.8 $\pm$ 9.9 μg/100 ml for the control, low, and high lead exposed 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.

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

Husbandry and Behavioral Experience of Young Lambs

The lambs nursed the ewes until they were weaned at 3 months of age. Within approximately 6 weeks of parturition and the termination of lead ingestion, the blood lead levels in the exposed ewes returned to within normal limits of below 10 μg/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. It should be noted that 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. Mean blood lead for

control lambs at 2 to 4 weeks of age was 6.0 μg/100 ml, while the low and high exposure lambs averaged 17.0 and 25.0 μg/100 ml, respectively. At 10 and 12 weeks, mean blood lead levels were 4.0, 9.0, and 14.0 μg/100 ml for the control, low, and high lambs, respectively. Throughout the study, no clinical manifestations of elevated lead exposure were observed in the lambs.

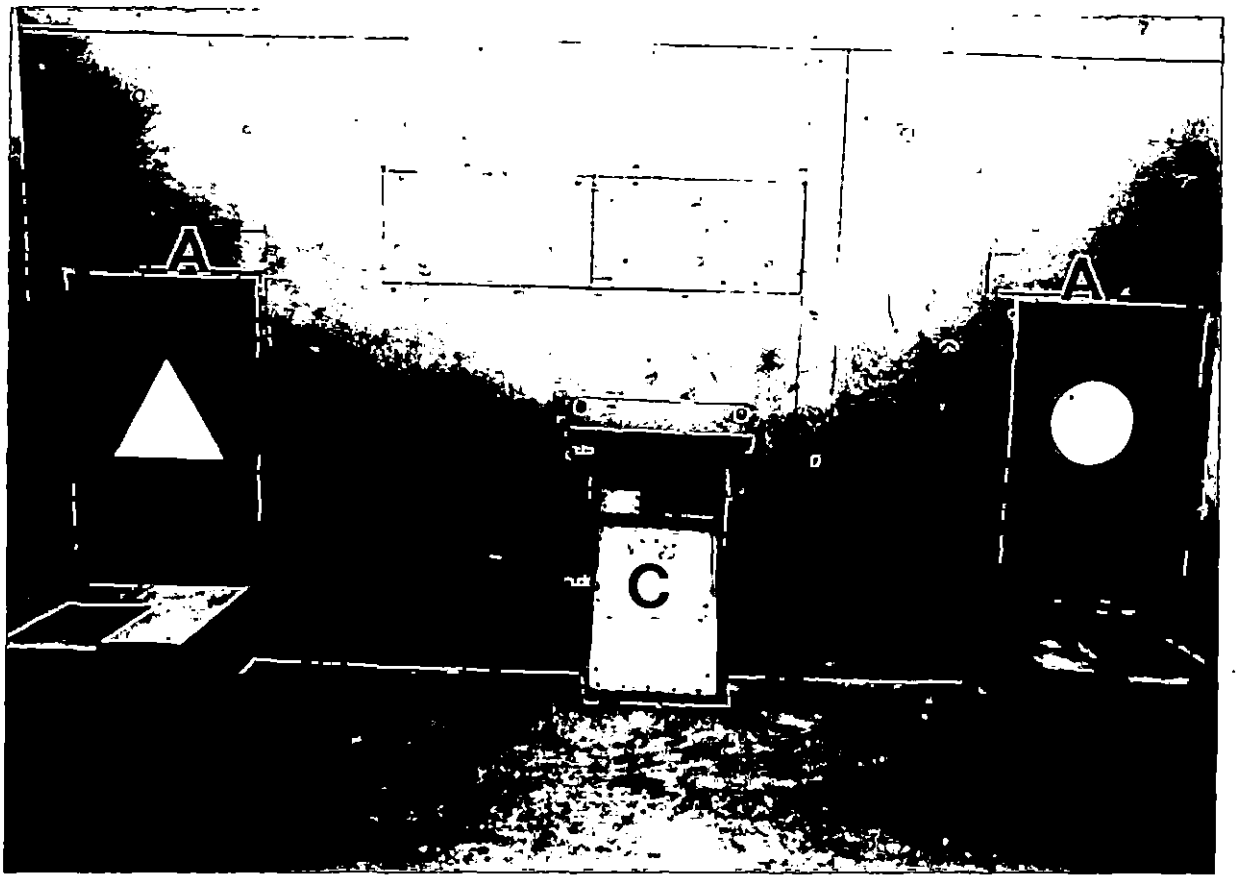
From 10 days to 3 months of age, the lambs were tested on a series of 16 closed-field maze problems. Significant differences in performance between control and exposed groups were not observed (Buck, 1972).

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 spurious causes during the course of neonatal care. One control lamb never became acclimated to the test chamber, an initial criterion for further training, and, therefore, was not included in this study. When 5 months old, four control, eight low-exposed, and six high-exposed lambs were trained to perform on a simultaneous two-choice, non-spatial visual discrimination task.

Apparatus

Operant behavioral training and subsequent discrimination testing was conducted in an operant chamber constructed in the Behavioral Toxicology Laboratory, Iowa State University. The specific details of the chamber have been previously described (Schnorr, 1972). Briefly, the chamber was an approximately 2.3m square room with two back projection screens and a retractable food hopper on the front wall (Figure 1). The front half of

Figure 1. Front wall of operant behavior chamber during a trial in problem 1. A) back projection screens with stimuli presented, B) response devices, C) retractible food hopper for presentation of corn reinforcement


A
A
C

the floor of the chamber was elevated 10.5 cm by a wooden platform to facilitate access to the food hopper by the small lambs.

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 $\pm$ 3 decibels as measured on the C scale of a sound level meter.[1]

Ventilation of the chamber was maintained by a small electric fan and ventilation duct near the ceiling.

A television camera mounted on the upper rear part of the chamber enabled closed-circuit television monitoring of the lambs' activity.

A slide projector[2] 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 modules.[3] A flow diagram of the program used to record and control the visual discrimination testing is presented in Figure 2.

Training of Lambs

Training was conducted on a one session per day, 5 days per week schedule. The 12 training steps used in shaping the naive lambs to perform on a two-choice visual discrimination problem are described below.

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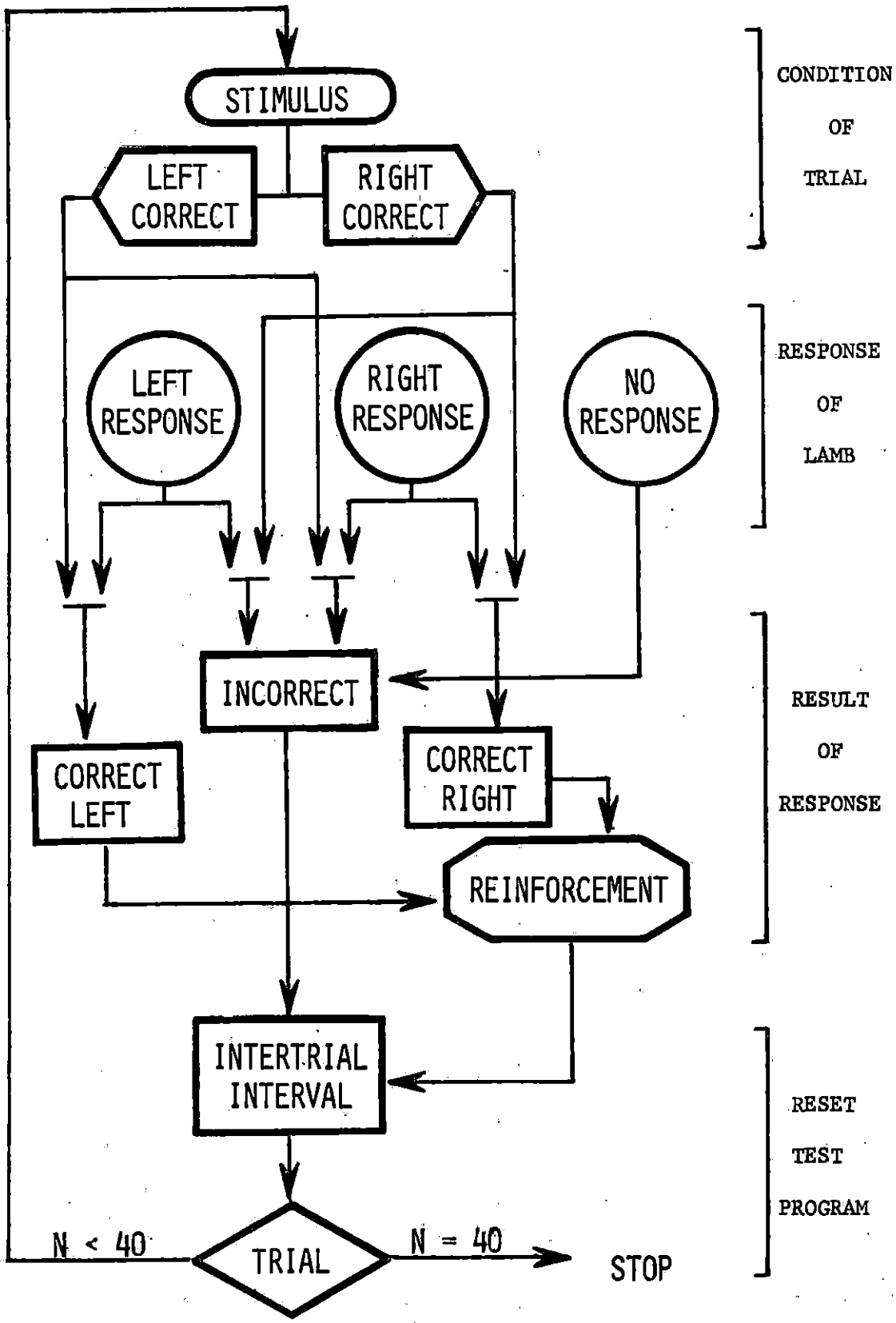
[1] 1551-C Sound-level Meter, General Radio Company, Concord, Massachusetts.

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

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

Figure 2. Flow diagram of the logic used to control the visual discrimination testing paradiagm


STIMULUS
LEFT CORRECT
RIGHT CORRECT
LEFT RESPONSE
RIGHT RESPONSE
NO RESPONSE
INCORRECT
CORRECT LEFT
CORRECT RIGHT
REINFORCEMENT
INTERTRIAL INTERVAL
TRIAL
N < 40
N = 40
STOP
CONDITION OF TRIAL
RESPONSE OF LAMB
RESULT OF RESPONSE
RESET TEST PROGRAM

In training step I (days 1 through 3), the lamb was acclimated to the operant chamber. The food hopper was in a stationary presented (extended) position; the lamb was allowed to eat corn from the hopper.

In training step II (days 4 through 8), the lamb was accustomed to the moving food hopper. A reinforcement was presented with the lamb being allowed to eat for approximately 10 sec before the hopper was retracted. Reinforcement was again presented after the lamb had moved away from the hopper. A session was ended after approximately 20 reinforcements.

After 1 or 2 days, the reinforcement was only given when the lamb approached a response device. The lamb was reinforced to make a response which automatically triggered additional reinforcement. All presentations of reinforcements were remotely controlled by the trainer.

In training step III (days 9 through 12), the lamb was allowed to respond on either response device and was reinforced on an FR-1 schedule. The food hopper was presented for 8 sec. The training session terminated after 20 reinforcements.

In training step IV (days 18 through 28), the lamb was given the opportunity to respond on only one response device on a given day while the other response device was covered. The side of the uncovered response device was alternated between the right and left side on consecutive days. Reinforcement was on a FR-1 schedule.

In training step V (days 29 through 39), the lamb was given the opportunity to respond on only one response device as in training step IV, but in addition the back projection screen above the uncovered response device was illuminated while the screen above the covered response device remained

dark. The position of the uncovered response device alternated between the right and left sides on consecutive days. An intertrial interval (ITI) of 6.0 sec was introduced in this step. The ITI occurred immediately after the food hopper was retracted. Responses occurring during the ITI were not reinforced. The maximum trial length was 90 sec. The session terminated after 20 reinforcements.

Training step VI (days 40 through 48) was the same as training step V with the exception that ITI was increased to 8.0 sec. The total number of responses, the total number of reinforcements, and the total elapsed time for the training session were recorded.

In training step VII (days 49 through 54), the lamb was given the opportunity to respond on either side as both response devices were uncovered. Only responses on the side of the illuminated screen (correct responses) were reinforced. Responses on the side of the unlighted screen (incorrect responses) were not reinforced. The position of the lighted screen (correct choice) alternated between the right and left sides on consecutive days of training. The ITI remained at 8.0 sec. The total number of responses, the total number of reinforcements, and the total elapsed time were recorded for each training session. The session ended after 20 reinforcements.

In training step VIII (days 55 through 63), the program was the same as for training step VII with the exception that ITI was increased to 9.5 sec. Total responses, total reinforcements, and total elapsed time for each training session were recorded.

Training step IX (days 64 through 74) was the same as training step VIII with the exception that the ITI was increased to 11.0 sec. Total

responses, total reinforcements, and total time for each training session were recorded.

The program in training step X (days 75 through 82) was the same as for training step IX with the exception that the ITI was 13.0 sec and the maximum trial length was shortened to 60 sec. Total responses, total reinforcements, and total time per training session were recorded.

In training step XI (days 83 through 87), the program was the same as for training step X with the exception that the lighted screen (correct response) alternated between the right and left sides on consecutive trials within a training session, the ITI was 15.0 sec, and maximum trial length was 40 sec. Total responses, total reinforcements, and total time for each training session were recorded. The training session was terminated after 25 reinforcements.

In training step XII, the position of the illuminated screen (correct response) alternated between the right and left sides on a chance order on consecutive trials within a training session (Gellerman, 1933) (Appendix). The ITI was 15.0 sec and the trial length was 20 sec. As a change from previous steps, an incorrect response aborted a trial and was recorded as an incorrect response on the left side or an incorrect response on the right side. Correct responses were also recorded as to their side of origin. The training session ended after 40 trials. The lamb advanced to the first visual discrimination problem after three consecutive days of 70% or more correct responses.

Visual Discrimination Testing

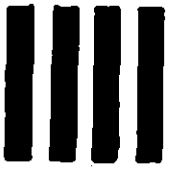
Each of the six problems used for visual discrimination testing consisted of a set of two geometric form images or stimuli (Figure 3). One stimulus in each problem was designated as being correct. During a trial, both stimuli of a pair were simultaneously presented with one stimulus on the left screen and the other stimulus of the pair on the right screen. The lamb 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 side 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 ITI was 15 sec. The maximum trial length was 20 sec. Forty trials comprised each testing session. Testing was conducted on a one session per day 5 days per week schedule. The criterion for mastering a problem and advancing to the next problem was 70% or more correct performance for 3 consecutive days. Lambs were tested on problems 1 through 6 in consecutive order. 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 lamb for each problem.

Analyses of Data

Training steps

Performance on each of six training steps (VI-XI) was examined individually. Each of the three parameters of performance on a problem (total

Figure 3. Geometric form stimuli of the six visual discrimination test problems

problem	correct	incorrect
1		
2		
3		
4		
5		
6		

responses, total reinforcements, and total time for the training session) were compared between groups independently of the other parameters.

A linear regression, a quadratic regression, and a common y-intercept (α) were calculated for total responses, total reinforcements, and total time of the training session for each of the three groups of lambs for each of the examined training steps. The calculated regression coefficients for the linear regressions, the quadratic regressions, and the y-intercepts were subjected to an analysis of variance to determine differences between groups.

Visual discrimination testing

Days to criterion An analysis of variance was calculated for the mean number of days to reach criterion for lambs within groups, as well as for days to reach criterion by lambs for individual problems. Student's t test was calculated for problems where a group difference existed.

A correlation coefficient was calculated for the total days to reach criterion for all six problems for each lamb and the mean blood lead level of the respective dam during the first, second, and third trimester of gestation.

Learning curve A comparison of the manner of learning as reflected in the shape of the learning curve was assessed by calculating a quadratic regression ($y = \alpha + \beta_1 x + \beta_2 x^2$) for the number of correct responses on consecutive days within a problem by each lamb. Four measures ($\hat{y}$'s) were derived for each lamb on each problem from four X's equally spaced over the number of days of testing on that problem. An analysis of variance was calculated on the four measures.

RESULTS

Training Steps

The regression coefficients for mean total responses, mean total reinforcements, and mean total time of training session for training steps VI through XI are presented in Tables 1, 2, and 3, respectively. Significant differences between groups were observed only for mean total reinforcements for the intercepts in training step IX ($F = 4.02$, $df = 2,15$) and for the quadratic coefficients for training step X ($F = 4.45$, $df = 2,15$). No other significant group differences were demonstrated in performance on the training steps.

Visual Discrimination Testing

Days to criterion

The mean number of testing days required for lambs within groups to reach criterion on the six visual discrimination problems is listed in the Appendix and represented in Figure 4. The analysis of variance calculated for these data is presented in Table 4. The overall treatment effect was significant at $p<.005$ ($F = 10.19$, $df = 2,90$). The effect of problems ($F = 18.41$, $df = 5,90$) and the treatment by problem interaction ($F = 3.16$, $df = 10,90$) were significant at $p<.005$.

The analysis of variance calculated on days to criterion for individual problems is presented in Table 5. A significant treatment difference at $p<.02$ ($F = 5.48$, $df = 2,15$) was observed for problem 6, two circles of unequal size (Table 5). Student's *t* test showed a significant difference between the control and the high lead groups at $p<.013$ ($t = 2.89$, $df = 8$) and between the low and the high lead groups at $p<.001$ ($t = 3.03$,

Table 1. Regression coefficients for mean total responses for lambs within prenatal lead exposure groups for training steps VI through XI

Training step	Group	Linear	Quadratic	Intercept
VI	control	4.73	1.34	14.33
	low	1.96	1.44	16.37
	high	4.30	1.15	13.70
VII	control	6.12	-1.43	20.48
	low	1.70	-1.27	26.32
	high	0.94	-0.92	23.47
VIII	control	12.27	-0.93	31.21
	low	1.60	-0.59	35.87
	high	3.70	-0.51	32.34
IX	control	3.39	-1.34	54.63
	low	7.46	-0.62	38.00
	high	6.62	-0.35	41.46
X	control	1.04	2.21	71.05
	low	6.89	1.86	48.11
	high	9.57	2.62	47.71
XI	control	-1.12	6.30	81.15
	low	-8.53	4.90	66.94
	high	-12.18	2.63	68.33

df = 12) in problem 6. It should be noted that although four of the high lead and one of the low lead lambs did not learn problem 6 in the 35 days maximum testing period, 35 days were used in the statistical analyses. The most time required by remaining animals to learn problem 6 was 20 days.

A marked trend in the number of days required to reach criterion was also observed in problem 5 from the representation in Figure 4. An analysis of variance (Table 5) indicated that a significant treatment effect did exist in problem 5 at $p<.08$ ($F = 3.22$, $df = 2,15$). Student's t test

Table 2. Regression coefficients for mean total reinforcements for lambs within prenatal lead exposure groups for training steps VI through XI

Training step	Group	Linear	Quadratic	Intercept
VI	control	1.98	-1.80	16.83
	low	0.13	-0.89	16.72
	high	1.56	-0.99	15.73
VII	control	0.61	0.51	17.20
	low	-0.75	0.37	18.87
	high	-2.90	0.52	21.04
VIII	control	-1.74	0.26	23.28
	low	0.40	0.97	19.21
	high	-1.25	0.12	21.62
IX	control	-1.52	-0.49	21.82[a]
	low	0.31	0.17	19.79
	high	1.01	-0.38	18.76
X	control	0.28	-0.52[a]	19.60
	low	-0.12	0.73	20.38
	high	0.14	-0.13	19.88
XI	control	0.15	0.32	25.45
	low	-0.69	-0.26	24.54
	high	-1.00	0.95	25.00

[a]Coefficients in this group of three differed significantly ($P<.05$).

revealed a significant difference between the control and the high lead groups at $p<.08$ ($t = 2.13$, $df = 8$) and between the low and the high lead groups at $p<.08$ ($t = 2.01$, $df = 12$) in problem 5. One of the high lead lambs did not learn problem 5 in 29 days of testing, however, 29 days were used as a basis for the statistical analyses. The greatest number of days required to master problem 5 by any of the remaining lambs was 23.

Table 3. Regression coefficients for total time of training session for lambs within prenatal lead exposure groups for training steps VI through XI

Training step	Group	Linear	Quadratic	Intercept
VI	control	-0.35	0.15	3.32
	low	0.61	0.10	3.41
	high	-0.54	-0.77	4.22
VII	control	0.36	-0.26	2.98
	low	0.36	0.54	3.42
	high	0.29	-0.33	3.36
VIII	control	0.20	-0.86	4.34
	low	0.43	-0.48	4.49
	high	2.01	-0.31	2.46
IX	control	0.66	-0.76	5.40
	low	-0.11	-0.56	5.00
	high	-0.28	-0.52	5.41
X	control	0.12	0.26	5.68
	low	-0.26	0.93	5.83
	high	-0.34	-0.39	5.49
XI	control	(mechanical failure: time was not recorded)		
	low			
	high			

The total days to criterion for all problems for each lamb and the mean blood lead level of the respective ewe during the first, second, and third trimester of gestation is presented in the Appendix. The correlation coefficients calculated for the total days to reach criterion for each lamb and the mean ewe blood lead level during the first, second, and third trimester of gestation were 0.61, 0.45, and 0.52, respectively.

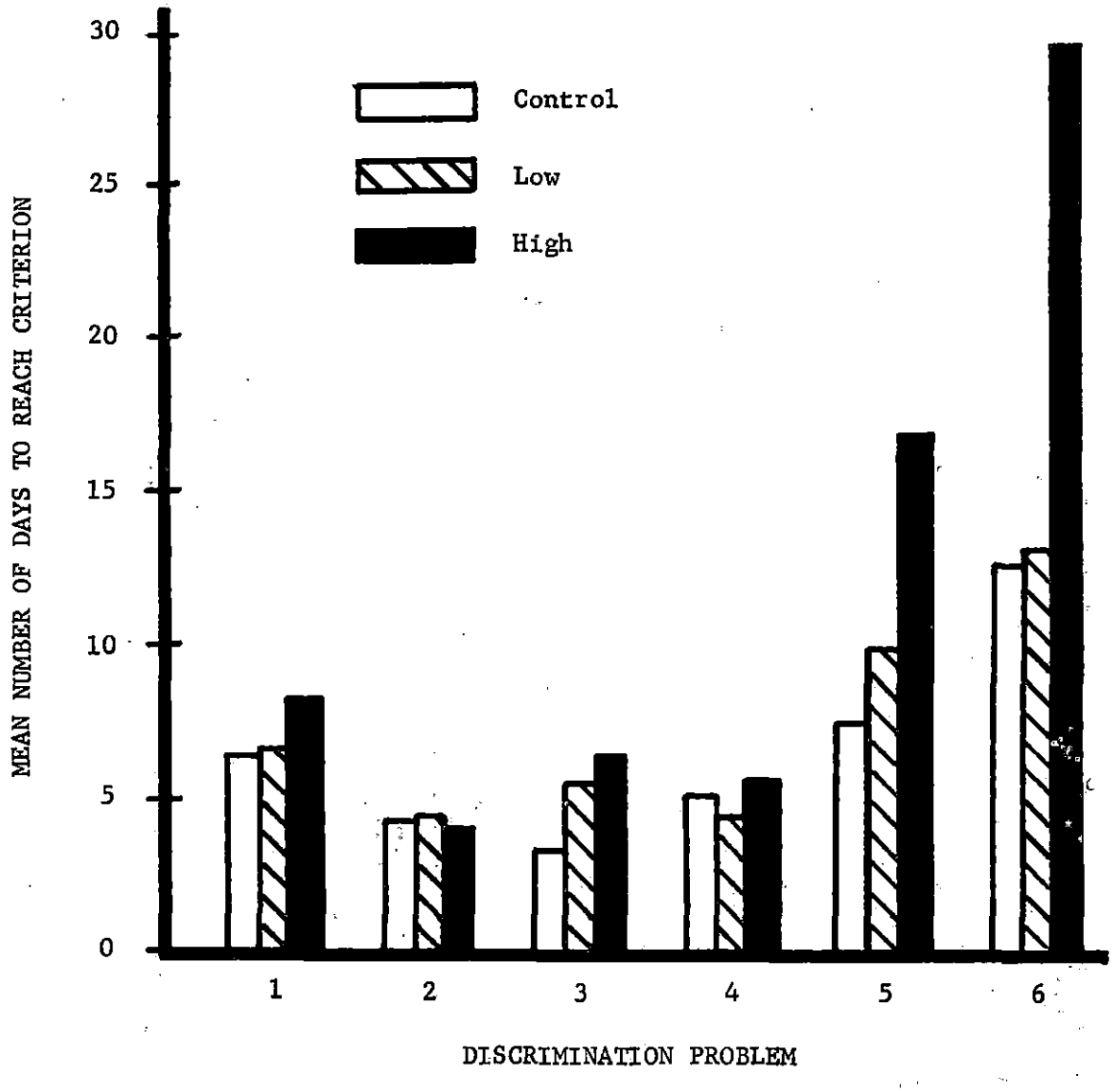


Figure 4. The mean number of days of testing required for lambs within the control and lead exposed groups to reach criterion on the six visual discrimination problems

Table 4. Analysis of variance for mean number of days to reach criterion for lambs within groups across all six visual discrimination problems

Source	d.f.	M.S.	F
Treatment	2	276.59	10.19[a]
Problem	5	499.76	18.41[a]
Treatment x Problem	10	85.87	3.16[a]
Error	90	27.15	

[a] $p < .005$.

Table 5. Analysis of variance for mean number of days to reach criterion for lambs within groups for individual visual discrimination problems

Problem	MSB	MSW	F[a]
1	8.02	4.39	1.82
2	0.24	0.84	0.29
3	11.76	14.67	0.80
4	1.57	1.73	0.91
5	128.46	39.84	3.22[b]
6	555.48	101.37	5.48[c]

[a] df = 2,15.

[b] $p < .08$.

[c] $p < .03$.

Learning curve

The analysis of variance calculated on the four measures derived from the quadratic regression of the learning curve on each problem is presented in Table 6. No significant effect of treatment group was demonstrated. However, a significant effect of problems ($F = 11.30$, $df = 4,60$) and of measure ($F = 779.41$, $df = 3,45$) was observed at $p<.005$. The problem by measure interaction was also significant ($F = 10.61$, $df = 12,180$) at $p<.005$ and is represented in Figure 5.

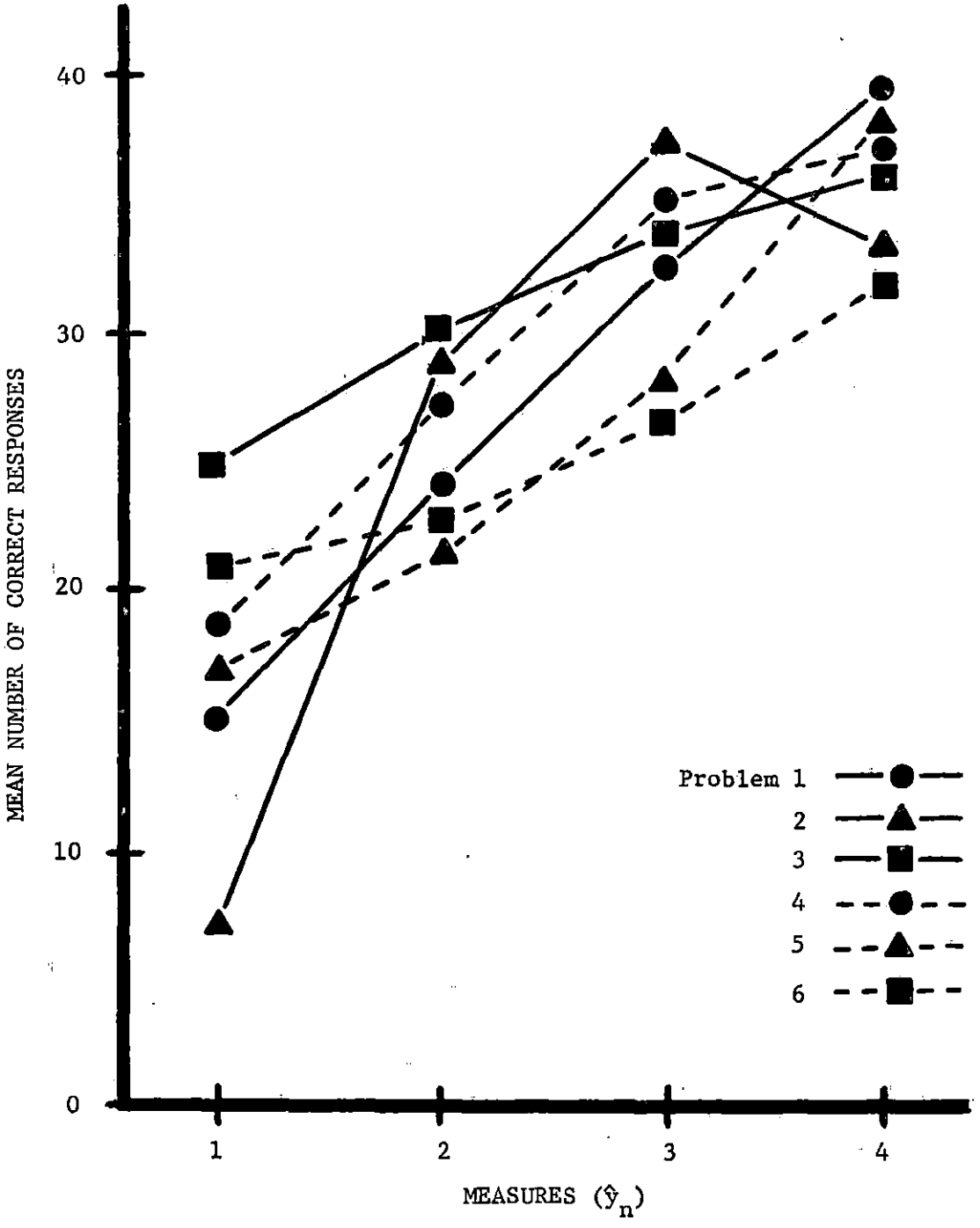
Table 6. Analysis of variance for measures for lambs within groups for visual discrimination problems[a]

Source	df	M.S.	F
Treatment	2	82.9	0.66
Animal/Treatment	15	126.5	
Problem	4	770.2	11.30[b]
Treatment x Problem	8	82.2	1.21
Animal x Problem/Treatment	60	68.1	
Measure	3	4897.0	779.41[b]
Treatment x Measure	6	48.9	0.78
Animal x Measure/Treatment	45	62.8	
Problem x Measure	12	384.6	10.61[b]
Animal x Problem x Measure/Treatment	180	36.2	

[a]Problem 2 was not included in this analysis.

[b]$p<.005$.

Figure 5. Plot of the measure by problem interaction observed in the analysis of variance of measures (Table 6). Each curve is a plot of the four mean measures for all lambs on one problem. The units of measures are the number of correct responses during a testing session of 40 trials on a given day


40
30
20
10
0
MEAN NUMBER OF CORRECT RESPONSES
1
2
3
4
MEASURES ($\hat{y}_n$)
Problem 1
2
3
4
5
6

DISCUSSION AND CONCLUSIONS

Training Steps

Although statistically significant group differences ($p<.05$) were demonstrated for mean total reinforcements for the intercept in training step IX and for the quadratic coefficient for training step X, these differences were not a part of a consistent pattern through the series of training steps. Furthermore, no group differences were demonstrated for the remaining 34 sets of regression coefficients for mean total reinforcements. It would then appear that the demonstrated group differences were due to chance and not to a measurable consistent difference between total mean reinforcements of the lead exposed groups. Because no consistent group differences were demonstrated for regression coefficients for total mean responses, total mean time of the training session, and total mean reinforcements, it was concluded that the three groups of lambs did not perform training steps VI through XI in different fashions.

Visual Discrimination Testing

The significant treatment effect observed in the analysis of variance calculated for the mean number of days required for lambs within groups to reach criterion (Table 4) demonstrated that the three groups of lambs differred significantly ($p<.005$) in the number of days required to master the six visual discrimination problems. The mean number of days per problem for lambs within groups to reach criterion were 6.4, 7.2, and 11.6 days for the control, low, and high groups, respectively.

The significant effect of problems ($p<.005$) indicated that the problems differed from one another in their degree of difficulty. This differ-

erence in degree of difficulty was not unexpected in view of the differing complexity of the pairs of form stimuli utilized in the six discrimination problems. The difference in degree of difficulty between the six discrimination problems was demonstrated further by the fact that the mean days to reach criterion of the control group varied between problems. Had the problems been of equal difficulty, one would have expected the mean number of 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 group did vary between problems (Figure 3) and actually increased toward the end of the series of six problems, indicating an increased degree of difficulty in the last problems.

The analysis of variance calculated for the days to reach criterion for individual problems revealed that a significant effect of treatment was observed in problems 5 and 6. Student's *t* test revealed that the high lead lambs from ewes with mean blood lead levels during gestation of 34.88 μg/100 ml took longer to master problems 5 and 6 than did the lambs from ewes with mean blood lead levels during gestation of 18.6 (low) or 4.7 (control) μg/100 ml. Problems 5 and 6 were more difficult for all three groups. Even though all the lambs had considerable prior experience with the discrimination task, they required more days to master this problem than any of the five previous problems. Problem 6 differed in that it involved a size discrimination of two circles rather than a form discrimination.

Although it was clear that the high lead-exposed lambs required more days to learn the series of six visual discrimination problems, the possibility existed that the lambs in the lead exposed group actually learned

the problems in a different manner than did the unexposed controls. Therefore, the manner of learning as reflected in the shape of the learning curve, that is a plot of the number of correct responses on consecutive days of a problem, needed to be assessed. The analysis of variance calculated for the four measures derived from the learning curve revealed no significant effect of treatment, thus indicating that the three groups of lambs did not learn the problems in a dissimilar manner. The significant effect due to problems ($p<.005$) (Table 6) as in the case of the days to reach criterion analysis, was not unexpected because of the apparent difference in degree of difficulty between problems.

The significant effect of measures ($p<.005$), which represent the number of correct responses on a given day of testing (Table 6), was produced by the fact that the daily performance of each lamb improved over the period of testing on a problem. In other words, the performance score, as measured by the number of correct responses on a given day of testing, was higher toward the end of a problem than at the beginning.

The problem by measure interaction ($p<.005$) (Table 6) resulted from the fact that problems varied in the amount of time required by all lambs to reach criterion, thus producing variation between the slopes of the mean learning curves for problems. It is interesting to note in the plot of the problem by measure interaction (Figure 4), that the plots for the more difficult problems, problems 5 and 6, appear as gradual upsweeping curves while the plots for the remaining and less difficult problems contain a more rapid initial incline followed by a leveling off or in one case even a small decline between the third and fourth measures.

Based on these results, it was concluded that subclinical prenatal lead exposure did slow learning of a visual discrimination task in lambs. It is significant to note that, 1) though the lead exposure was primarily prenatal, the slowed learning was demonstrated when the lambs were 10 to 15 months old; and 2) no clinical manifestations of lead toxicoses were observed in the lambs throughout the study.

The high positive correlation ($r = .61$) between the total number of days required to reach criterion for the six 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 producing 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 following subclinical lead exposure in previous behavioral studies (Brown et al., 1971; Bullock et al., 1966; Goode et al., 1973; Van Gelder et al., 1973) where prenatal or early neonatal lead exposure was not employed.

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 consistent with clinical reports of the residual neurologic effects of lead poisoning in children. Perlstein and Attala (1966) reported that minimal brain damage 6 months to 2 years after recovery from acute lead poisoning often involved learning blocks, usually of a 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, visual memory, and simple conceptualizing.

The demonstration of behavioral deficits in the offspring of ewes maintained with a mean blood lead during gestation of 34.8 μg/100 ml supports recent Environmental Protection Agency (1972) guidelines that for pregnant women the upper acceptable blood lead level should be no more than 30 μg/100 ml. No clinical manifestations of overt toxicosis were observed in the ewes during the period of lead exposure. However, this study did demonstrate visual learning deficits in lambs with blood lead levels at 2 to 4 weeks of age at 25 μg/100 ml, a level measurably below the 40 μg/100 ml recommended by the Environmental Protection Agency (1972) as probably safe for children.

SUMMARY

The effects of subclinical prenatal and neonatal lead exposure on the developing nervous system and later intellectual and behavioral development are poorly understood. The effects of *in utero* lead exposure on postnatal behavior in lambs was investigated by behavioral toxicologic methods. Two groups of ewes were fed lead sufficient to maintain mean blood lead levels of 34.8 and 18.6 μg/100 ml, respectively, throughout gestation. No clinical manifestations of lead toxicosis were observed in these animals. The mean blood lead level during gestation for control ewes was 4.7 μg/100 ml. Lambs from the two lead-exposed groups and the control group had blood lead levels of 25, 17, and 6 μg/100 ml at 2 to 4 weeks of age. Postnatal behavior in the lambs was evaluated using four lambs from the control, eight lambs from the low-lead, and six lambs from the high-lead groups. At 5 months of age, the lambs were trained on a two-choice visual discrimination operant task. Between 10 and 15 months of age, the lambs were tested on a series of six visual discrimination problems. When learning criterion was achieved on a problem, the lamb was advanced to the next problem. The average number of days per problem for lambs within groups to reach criterion were 6.4, 7.2, and 11.6 days for the control, low, and high groups, respectively. The high-lead lambs required significantly ($p < .005$) more days to learn these problems. The results indicate that subclinical prenatal lead exposure can slow postnatal learning of a visual discrimination problem.

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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 7. Number of days required to reach criterion for lambs for visual discrimination problems

Lamb	Discrimination problem 1	2	3	4	5	6
Control						
10	7	3	4	4	7	7
12	7	4	3	4	10	17
13	5	6	3	7	4	8
19	6	4	3	4	8	18
$\bar{X} \pm$ st. dev.	6.3±0.8	4.3±1.1	3.3±0.4	4.8±1.3	7.3±2.2	12.5±5.0
Low						
30	5	4	3	5	9	7
31	4	5	5	3	6	12
32	6	4	3	4	5	5
35	4	5	4	3	5	8
36	5	4	6	3	9	6
37	7	4	5	7	15	35[a]
38	9	3	13	6	17	11
39	11	6	5	4	12	20
$\bar{X} \pm$ st. dev.	6.4±2.3	4.4±0.9	5.5±3.0	4.4±1.4	9.8±4.3	13.0±9.4
High						
41	7	4	4	4	23	37[a]
42	9	4	4	6	29[a]	37[a]
45	10	3	17	5	21	11
46	7	5	3	6	10	35[a]
47	11	4	7	6	7	18
48	6	4	3	5	9	39[a]
$\bar{X} \pm$ st. dev.	8.3±1.8	4.0±0.6	6.3±5.0	5.3±0.7	16.7±8.5	29.5±10.9

[a]Criterion was not achieved.

Table 8. Total days to reach criterion for lambs and blood lead level of the respective ewe during gestation

Lamb number	Total days to criterion	Mean ewe blood lead during gestation (μg/100 ml)		
		First trimester	Second trimester	Third trimester
10	36	5	4	2
12	48	5	5	3
13	37	5	5	3
19	46	6	6	3
30	36	14	18	15
31	38	19	24	25
32	30	21	25	24
35	33	15	20	21
36	36	19	18	24
37	76	17	21	20
38	62	17	20	18
39	61	19	13	14
41	82	25	28	33
42	92	29	25	34
45	71	25	26	28
46	69	26	23	33
47	58	29	21	31
48	69	33	49	57