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Studies concerning the effects of prenatal lead exposure on visual discrimination in rats

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by

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INTRODUCTION

Lead is a known toxic substance for which no beneficial biological role has yet been demonstrated. Effects of severe lead toxicosis have been recognized for centuries, however, symptoms of mild lead toxicosis are less definitive. Surveys, clinical studies and follow-up studies suggest that neurologic damage in humans can result from persistent exposure to low levels of lead. Children exposed to lead have shown deficits of visuomotor performance and visual-cognitive functioning. In addition, both clinical (David et al., 1972) and experimental data (Silbergeld and Goldberg, 1973) have demonstrated hyperactivity as another behavioral disturbance associated with lead exposure.

Although the immature central nervous system of children has been shown to be more vulnerable to the toxic effects of lead than that of the adult, little work has been done to study the effects of lead on fetal nervous tissues or any possible behavioral deficits which might result. Animal models have only recently been developed to study this area of behavioral toxicology.

Sheep have been used successfully by Carson et al. (1974) to show visuomotor deficits in lambs prenatally exposed to lead. However, the long gestation period of sheep as well as the usual single progeny per mating are severely limiting factors in the development of this species as a test model. In contrast, the rat has a short gestation period of 22 days and usually has litters of 8 to 12 pups.

If the rat could be developed as a test model, considerable latitude would be gained in studying the exact location and mechanism of action of

lead on the central nervous system. Further, safe levels of fetal lead exposure could be determined.

This research was designed to investigate the use of the rat as an animal model to study visuomotor behavioral deficits produced by prenatal exposure to lead. The suitability of the species, the dosages of lead used and the apparatus and protocol designed to detect behavioral deficits were evaluated.

REVIEW OF LITERATURE

General Aspects of Childhood Lead Toxicosis

Although lead is one of the most ubiquitous trace elements in nature, there has been no biological function ascribed or even suggested for this element. At present, the level of lead in human and animal tissues is used as a monitoring device for determining the level of environmental contamination of this proven toxic material. The toxic effects of lead on human and animal life in general have been known and investigated since ancient times. Lead-containing earthenware and lead-containing liquor distilling and storage containers were common sources of lead exposure to ancient Romans (Guinee, 1972). In more modern times, the incorporation of lead into interior and exterior paints has led to a high lead exposure in children, especially those living in inner city slums and dwellings. Cracking and peeling paint and plaster as well as higher concentrations of lead in house dust and soils surrounding these dwellings have proven to be a serious health hazard to children one to five years of age, the ages when hand to mouth behavior is highest (Berg and Zapella, 1964; Lin-Fu, 1973a, 1973b; Sayre, et al., 1974). Those children in which pica has developed represent a particularly serious problem because the likelihood of ingesting paint chips is greatly increased.

The encephalopathologic findings as well as the effect of lead on other systems of the body have been studied by countless numbers of investigators. Human research, however, is hampered by a lack of control and monitoring of the many independent physical and social variables

associated with lead toxicosis. Most studies involve the examination and evaluation of hospital records and statistics in retrospect. Controlled studies in which precise dosages of lead are administered, blood and tissue levels of lead constantly monitored and histologic and behavioral findings interpreted within the confines of a well-planned experimental design are impossible in human oriented research. It is with these limitations in mind that the following works concerning the effects of lead on children must be evaluated.

Although the peripheral nervous system of adults appears to be most vulnerable to the toxic effects of lead, the central nervous system of children is most often clinically affected (Pentschew, 1965; Seppalainen et al., 1975). Some recent work in both adults and children have shown this to be a very loose generalization since peripheral nerve effects in children as well as encephalopathies in adults have been demonstrated (Feldman et al., 1973; Whitfield et al., 1972). The more serious central nervous system effects of this metal in children have been attributed to a less well-developed blood brain barrier to lead as well as its effects on the continuing development and maturation of the nervous system during the formative years. A higher rate of lead absorption from the intestine and retention in the body tissues in the young is also given as a plausible explanation for the more serious effects in this age range (Kostial and Harrison, 1973).

Diet is also known to influence the metabolism of lead. Kostial et al., (1971), Mahaffey et al., (1973), and Six and Goyer (1970, 1972) have shown that lead absorption and tissue storage is enhanced when

dietary levels of calcium and iron are low. Deficiencies of calcium and iron are often noted in the diets of poorly nourished children in economically deprived areas. In the adult, storage of lead as an inactive material in compact bone functions to compartmentalize this element and to lessen the toxic effects on soft tissues. A relatively lower ratio of compact bone in the young child also predisposes this age group to lead toxicosis. The more massive and acute exposure that occurs during childhood because of increased hand to mouth behavior may also contribute to the greater central nervous system involvement as suggested by Chisolm and Harrison (1956).

Further evidence that the young animal is more susceptible to the toxic effects of lead than the adult has been demonstrated in rodents. Momcilovic and Kostial (1974) have shown an 8-fold increase in the concentration of lead in the brain of suckling rats compared to adult rats which indicates that the young have an increased capacity for accumulating lead in this tissue or a decreased capacity for actively excreting this element from the brain after it reaches this site.

Clinically the signs of childhood lead toxicosis are anorexia, nausea, vomiting, colic, and constipation with possible fever in the mild forms. Signs of ataxia, seizures, increased intracranial pressure with possible coma and death are often present in the more advanced cases (Jacobziner, 1966; Perlstein and Attala, 1966). Early clinical recognition of this syndrome, especially when evaluating children of probable high exposure, along with new chelating agents that have been successfully used in treatment, have led to a decrease in the number of deaths from lead toxicosis in recent years.

The possible long-term sequelae of lead toxicosis, especially in cases of clinical encephalopathy, have progressed unabated. The most common sequelae reported include mental retardation, recurrent seizures, cerebral palsy and optic atrophy (Chisolm and Harrison, 1956; Smith et al., 1963; Thurston et al., 1955). Children with a history of having had acute encephalopathy often showed deficits in vocalization and in performing visuomotor tasks requiring eye and fine muscle movement coordination. Often other standard intelligence measurements were not significantly altered. Perlstein and Attala (1966) conducted a follow-up study of children that had been treated initially for lead toxicosis. In those children who had exhibited severe encephalopathy, neurologic sequelae persisted in four out of five. With a decrease in the severity of the initial onset, a decrease in the number of children with sequelae was also noted. In those in whom the presenting symptoms were gastro-intestinal only, sequelae occurred in less than one out of three.

Pueschel et al. (1972), using hair samples, identified 98 of 705 children sampled as having an increased lead burden. Fifty-six of the 98 patients were studied in detail. During the neurological examination, 13 of 56 children exhibited unsteadiness, clumsiness and fine-motor dysfunctions. In comparison with a matched group of children without increased lead burden, only five out of 56 yielded equivalent neurological symptoms ($P<.001$).

Cohen and Ahrens (1959) reported that 45 percent of 29 children who survived acute lead toxicosis showed evidence of damaged psychic residuals. In a follow-up study of 20 children, none of whom

demonstrated striking evidence of encephalopathy upon initial admittance to the hospital as infants, Byers and Lord (1943) found only one to be progressing normally in school. The patients were most often found to be deficient in tests designed to bring out their ability to deal with shapes, direction, space and projected imagery. Sensorimotor deficits were demonstrated in nearly all of those tested. Landrigan et al. (1975) have investigated the association between low-level lead absorption and neurophysiological function. Forty-six symptom-free children with blood lead level concentrations of 40-68 µg/100 ml were compared with 78 ethnically and socioeconomically similar controls with levels less than 40 µg/100 ml. Testing with Wechsler intelligence scales for schoolchildren and preschool children (W.I.S.C. AND W.P.P.S.I.) showed age adjusted performance IQ to be significantly decreased in the group with higher lead levels. Children in all ages in the lead groups also had significant slowing in the finger-wrist tapping test. Full scale IQ, verbal IQ, behavior, and hyperactivity ratings did not differ.

In a recent study, Beattie et al., (1975) measured water-lead levels in the homes of 77 mentally retarded children and 77 nonretarded children and in the homes occupied by their mothers during pregnancy. The water-lead concentration was significantly higher in the homes of the retarded group.

Jenkins and Mellins (1957) studied 33 of 46 surviving children previously afflicted with lead encephalopathy. Twenty-seven were found to be mentally retarded, and in twenty the retardation was directly traceable to lead poisoning. Speech was impaired in 18 cases. Besides mental

retardation, most children developed emotional instability and became distractable. Study of the successes and failures on the tests (scatter analysis) showed that these patients had greatest difficulty in tasks calling for naming of objects, visual memory, and simple conceptualizing. Although visual memory was poor, these children did comparatively well on auditory memory. After recovery from the acute phase of poisoning, emotional instability, as evidenced by temper tantrums, frequent whimpering, fighting or excessive fearful withdrawal was reported. Extreme distractability was also reported. Neither during play nor testing could they maintain attention for any period of time.

Although an association between mental retardation and pica has been drawn by many workers studying the problem of lead toxicosis (Smith et al., 1963), the question of a cause-effect relationship remains largely unanswered. Does pica lead to ingestion of large amounts of lead which result in mental retardation or does mental retardation predispose children to pica thereby increasing the body burden of lead in children so afflicted? The difficulty of answering this question and many others concerning lead toxicosis in children has lead Wiener (1970) to the conclusion that appropriate animal models must be developed in order to answer this important and far-reaching question.

Because of the above evidence that subtle neurologic damage may occur as a sequela to clinical and subclinical exposure to lead, it is important that safe maximum non-toxic blood lead levels be established. These blood lead levels have not been unequivocally established for any age of any species. The influence of dietary factors such as calcium

and iron on the metabolism of lead make it particularly difficult to establish a maximum tolerable level of exposure. The proposed Environmental Protection Agency (EPA) guidelines for upper acceptable blood lead levels are 40 µg/100 ml for adults and children and 30 µg/100 ml for expectant mothers, the fetus and newborn (EPA, 1972).

In studies conducted by Byers (1959) and Perlstein and Attala (1966) approximately 25 percent of children tested, based on several hundred thousand children sampled, had blood lead levels equal to or greater than 40 µg/100 ml. Other workers have also found significant numbers of children with blood lead levels of 40 µg/100 ml or greater (Fine et al., 1972; Guinee, 1972; Lin-Fu, 1972). This clearly shows the magnitude of the potential number of children, especially in urban areas, with elevated lead exposures. The neurological effects of this exposure are largely unknown.

The unborn fetus as well as children may be exposed to increased body burdens of lead. The consumption of illicit liquor as well as the inhalation of lead-laden fumes demonstrate possible sources of lead for the mother (Angle and McIntire, 1964; Palmisano et al., 1969). Work that has been done on transplacental movement of lead in humans indicates that the lead content of umbilical cord blood is approximately equal to that of the mother, demonstrating the possibility of exposure of the developing fetal nervous system to detrimental levels of lead (Gershanik et al., 1974; Harris and Holley, 1972).

Pathogenesis of Lead Encephalopathy

Although the effects of lead on the peripheral nervous system of the adult have been studied for years, a suitable model for studying the encephalopathic syndrome characteristic of children was not available until Pentschew and Garro (1966) produced central nervous system (CNS) lesions in neonatal rats nursing mothers on a diet containing 4 percent lead carbonate. Pups nursing these mothers were exposed to lead via the maternal milk. The encephalopathy that was produced was characterized by capillary activation, glial proliferation, areas of transudation and spotty hemorrhages. It was the opinion of these investigators that the neurologic damage produced was secondary to changes in the vascular walls, leading to edema and a state of tissue hypoxia. No direct damage to the nervous system by the lead ions was thought to occur.

In addition, encephalopathic lesions in the mouse were produced by Rosenblum and Johnson (1968) by a similar technique. In this species, the most prominent lesions were abnormally large numbers of fibrous intercapillary strands in several cerebral loci and astrocytosis of the hippocampus. Krigman et al., (1972) have also reported that lead-intoxicated rats showed gliosis and a decreased number of terminal boutons on neurons, associated with a decreased thickness of the somato-sensory cortex.

Thomas and Thomas (1974) have also produced encephalopathic lesions in the brain of newborn rats. The lesions consisted of alterations in

capillary endothelial cells, the formation of platelet thrombi and scattered areas of hemorrhage and edema especially affecting the cerebellum.

Hopkins and Dayan (1974) have produced encephalopathy in adult and juvenile baboons after lead intoxication. The main pathological findings were widespread cerebral edema and focal cortical necrosis.

Postmortem examinations of rhesus monkeys after experimentally produced lead toxicosis showed that definite morphological evidence of disease was confined almost exclusively to the central nervous system. The principle change consisted of extensive edema involving the white matter of the cerebrum and cerebellum (Clasen and Hartman, 1973).

Tanis (1955) has reported that the basic pathological changes in human lead encephalopathy are edema, punctate hemorrhages, gliosis and focal areas of necrosis throughout the brain, the changes being undeniably irreversible. In one case on which he reported, the basal ganglia were seen to have pale swollen vacuolated parenchymatous brain tissue with congestion of the blood vessels. Neurons throughout showed toxic changes, especially in the basal ganglia, where they were undergoing chromatolysis. Blackman (1937) found lesions throughout the entire central nervous system of children, though most abundant in the cerebral hemispheres and cerebellum. Vascular changes were found including capillary necrosis and thrombi, abundant exudate, tissue damage and evidence of repair. Most injury to the nervous tissue seemed dependent on the accumulation of serous exudate. His findings also included multiple, small necrotic foci usually associated with vascular lesions.

Biochemical Aspects of Lead Toxicity

Heavy metals including lead show a strong affinity for ligands such as phosphates, cysteinyl and histidyl side chains of protein, purines, pteridines and porphyrins. They inhibit a large number of enzymes having functional sulfhydryl groups, bind to and affect the conformation of nucleic acids and disrupt pathways of oxidative phosphorylation (Vallee and Ulmer, 1972).

Lead has been shown by a number of workers to have a rather specific inhibitory action on the enzyme delta amino levulinic acid dehydratase (ALAD). This is an enzyme necessary to catalyze the union of two molecules of delta amino levulinic acid to form porphobilinogen. Porphobilinogen is a metabolite in the formation of heme and other porphyrin containing compounds, some of which are important in the cellular oxidative mechanisms (Gibson et al., 1967; Kao and Forbes, 1973). Reduced ALAD activity has been demonstrated in the brains of lead exposed rabbits (Gibson and Goldberg, 1970) and rats (Millar et al., 1970). Millar et al. (1970) have found a positive correlation between blood lead levels and ALAD activity in the blood and brain and suggest that even small increases in blood lead may be associated with biochemical abnormalities in the child's brain.

Recent work by Gordon and Shellenberger (1974) using rats supports the hypothesis that norepinephrine (NE) and dopamine (DA) are involved in the central regulation of motor activity. Sauerhoff and Michaelson (1973), using newborn rats exposed to lead via the mother's milk, have reported an eightfold increase in the concentration of lead in the

brain, no change in norepinephrine, but a 20 percent decrease in dopamine relative to controls, suggesting a relationship between CNS dysfunction due to lead and dopamine metabolism in the brain. Golter and Michaelson (1975) from the same laboratory have reported finding elevated levels of activity associated with unchanged levels of endogenous dopamine while norepinephrine levels were increased approximately 13 percent.

Because lead influences the activity of several enzymes, the biochemical basis of lead toxicity in the nervous system is likely to be complex. Nathanson and Bloom (1975) have found that very low concentrations of lead inhibit adenyl cyclase activity in mammalian brain tissue. Consequently, the marked inhibitory effect of low concentrations of lead on an enzyme thought to be intimately involved in the chemistry of brain tissues suggests that interference with adenosine 3',5'-cyclic monophosphate metabolism by this ion could be a factor in some of the neurological manifestations of lead toxicity (White et al., 1973). LeMay and Jarett (1975) add support to the above hypothesis by their finding that lead inhibits the adenyl cyclase activity of fat cell membranes and pancreatic islet homogenates in conditions used for histochemical localization of enzyme activity.

These reports indicate the need for further research into the complex biochemical interaction between catecholamines, enzyme concentrations and activity, brain lead concentration and possible related behavior during developmental and mature states. Versatile animal models will be required for these studies.

Neurologic Effects of Lead Toxicosis

Little is known concerning the direct effect of lead on the mature or developing nervous system. In vitro studies were done by Silbergeld (1973) using rat phrenic nerve-diaphragm preparations. Force of contraction and time elapsed between stimulation and beginning of contractions were measured. Concentrations of lead equal to or greater than 25μM produced significant reductions in the force of contraction and in the latency of response from stimulation to contraction. The effect produced appears to be at the presynaptic level. Electrical stimulation of muscle showed no significant changes in force of contraction or latency at any concentration of lead. Exogenous acetylcholine in submaximal concentrations produced equal responses in treated and untreated preparations in the absence of stimulations. The above findings indicate that the effect was not on muscle contractile mechanisms nor on the postsynaptic end-plate receptivity to acetylcholine.

Loop and Cooper (1974) have shown that lead depresses the monosynaptic reflex of the cat spinal cord by decreasing the electrical excitability of motoneurons. Further electrophysiological testing by these workers suggests that lead decreases the sodium conductance of the motoneuron membrane.

Lehrer et al. (1974) reported preliminary data indicating that total brain DNA and ganglioside levels are normal in 60 day old mice raised on lead-containing diets although total brain weights were reduced. Total brain cerebrosides were decreased indicating a relative

delay in myelination relative to cell proliferation and membrane growth, suggesting a specific effect of lead on the process of myelination.

Lampert and Schocket (1968) have demonstrated a process of demyelination and remyelination in the peripheral nerves of rats chronically exposed to lead. Proliferation followed by degeneration of Schwann cells occurred. The proliferated Schwann cells again underwent degeneration, leaving remnants of basal laminae. Further proliferation of Schwann cells growing along these membranes resulted in concentric layers of flattened cells around axons.

Krigman et al. (1974a) have investigated the effect of lead encephalopathy upon myelination in the developing rat. Lead encephalopathy was produced by adding lead carbonate (4% W/W) to the diet of nursing mothers. Lead levels in the brain of 30 day old rats were four times that of the mothers. The overall effect was retardation of growth of neural tissues without reduction of cell population. Formation of myelin was altered, axons were reduced in size and the number of myelin lamellae in the sheaths was also reduced. They concluded that the hypomyelination was due primarily to retarded growth and maturation of the neurons and not a reflection of a defect in the myelinating glial cells or a delay in the initiation of myelination.

Krigman et al. (1974b) also examined the effect of lead on cortical development. Lead intoxication resulted in an overall reduction of cortical grey matter characterized by astrocytosis and by preservation of the neuronal population. Neurons were, however, smaller and the

proliferation of neuronal processes was retarded with an apparent reduction in the number of synapses per neuron. They proposed that the deficit in neuronal maturation was a reduction in the dendritic field and a concomitant reduction in synaptic connections.

Another biochemical manifestation in tissues of various animals exposed to increased body burden of lead is the appearance of distinctive inclusion bodies. The exact role and metabolic significance of the acidfast, intranuclear inclusion bodies have never been elucidated. They appear particularly often in the proximal convoluted tubules of the kidney but they have been reported to occur in other tissues of the body including liver. Goyer et al. (1970) have demonstrated that they consist of a lead-protein complex. Their appearance seems to be one of the first biological indications of undue lead exposure. Quantitative analysis shows that lead is concentrated within the bodies. Relatively small amounts of lead are present within the cytoplasm and mitochondria. It is hypothesized by these workers that lead accumulates in the intranuclear inclusion body thereby sparing toxic injury to cytoplasmic organelles.

Although the cytological and biochemical aspects of lead toxicity are far from understood, these findings do suggest the very real possibility of a direct cellular insult to tissues including nervous tissue and that this possibility deserves further investigation.

Prenatal Effects of Lead

The vulnerability of the developing mammalian fetus to the toxic effects of lead has been documented by a number of workers. Gross anatomical defects have been produced in golden hamsters exposed to lead in utero (Ferm and Carpenter, 1967). In this species a rather specific effect on the developing tail bud has been noted (Carpenter et al., 1973). McClain and Becker (1975) have reported on the teratogenicity, fetal toxicity and placental transfer of lead administered intravenously to rats. A urorectocaudal syndrome was produced when lead was administered on day nine of gestation. Administration of lead on day 16 however produced hydrocephalus and hemorrhage of the central nervous system.

Green and Gruener (1974) have studied the kinetics of maternal-fetal transfer of lead in the rat. They reported lead being transferred at different stages of gestation and that lead transport was rapid so that the fetus was in equilibrium with the mother 24 hours after injection.

Lead is known to cross the human placental barrier (Karlog and Moller, 1958). There have been few human studies conducted to correlate maternal blood lead levels with newborn blood lead levels. Those reports which have been published dealt with low normal levels in the mother (Gershanick et al., 1974; Harris and Holley, 1972). These studies indicated that with maternal blood lead levels of 10 to 20 µg/100 ml, maternal and fetal levels were essentially equal. Studies correlating elevated maternal levels (40 to 80 µg/100 ml) with fetal levels were lacking in the literature. In summary, several studies have indicated that lead crosses the human placenta in significant amounts although the precise gradient is not known.

Few documented cases of elevated fetal exposure resulting in behavioral deficits in the offspring have been reported. Palmisano et al. (1969) have reported on a single case of apparent undue fetal exposure to lead. Neurologic examination of the infant revealed increased muscle tone in the lower extremities. The arms and legs exhibited frequent and spontaneous episodes of tremulous activity with some spontaneous ankle clonus. Stepping maneuvers appeared consistently spastic.

Angle and McIntire (1964) have reported a single case in which maternal blood lead levels were approximately 240 µg/100 ml during the latter part of the first 8 months of pregnancy. An apparently normal infant was born at term and follow-up studies of the child at 4 years of age revealed no mental or physical abnormalities.

Conflicting data and lack of controlled experimental conditions make evaluation of the potential danger of elevated lead exposure to the human fetus extremely difficult.

Lead and Behavior

In recent years, the ultrastructural and biochemical aspects of lead toxicity have received increased attention. However, any possible relationship between these subtle ultrastructural and biochemical changes and behavioral deficits has only recently been investigated. David et al., (1972) reported an association between raised blood lead levels and hyperactivity in children. Since that time animal studies have shown a cause-effect relationship between lead and hyperactivity in several species. Silbergeld and Goldberg (1973) have shown that mice receiving lead through maternal milk were more than three times as active as

age-matched controls when measured by horizontial body movements. These two workers later demonstrated in their animals the paradoxical response to amphetamines and phenobarbital typical of certain hyperactive, minimally brain damaged children. This work suggested a very good possibility of using mice as an animal model for studying some forms of childhood hyperactivity (Silbergeld and Goldberg, 1974).

Sobotka and Cook (1974), using neonatally lead exposed rats, demonstrated several important pharmacobehavioral characteristics similar to those of the minimally brain dysfunctioned child. Altered responsiveness to amphetamine, poor learning performance and alleviation of this performance deficit by amphetamine treatment were observed. These data support the suggestion that prenatal and early postnatal lead exposure may be etiologically related to some variants of minimal brain dysfunction. Using a water "T" maze, Brown (1973) has demonstrated learning deficits in the 8 to 10 week old rat nursed three weeks by lactating mothers exposed to 17.5, 25 or 35 mg/kg lead daily 1 to 20 days after parturition. Brown was unable to demonstrate similar effects when dams were exposed during the time period 10 to 20 days postpartum. This indicated that the first 10 days postpartum are particularly sensitive times in the development of the rat CNS.

Using eight day to six week old rats, Brown et al. (1971) were unable to demonstrate learning deficits in their subjects after intraperitoneal (IP) administration of clincially toxic doses of lead acetate. Behavioral tests included performance in the water "T" maze and shuttle box. In the latter study, however, all exposures were acute, definitely unlike the chronic, prolonged exposure typical of the human situation.

The most comprehensive work published to date concerning the prenatal effects of lead on learning behavior is that of Carson et al. (1974). Two groups of ewes were fed elemental lead several weeks before and during their gestation in amounts sufficient to maintain blood lead levels at 34 and 18 µg/100 ml in the high and low groups respectively. Lambs produced under these conditions had blood lead levels of 24 µg, 17 µg and 6 µg/100 ml, in the high, low and control groups respectively, when tested at 2 to 4 weeks of age. At 10 to 15 months of age the lambs prenatally exposed to maternal blood lead levels of 34 µg/100 ml required significantly more days to learn visual discrimination problems than did those lambs exposed to low dosage and control levels ($P<.005$).

Although the experimental evidence strongly suggests the increased susceptibility of the early developing nervous system to the toxic effects of lead, only the work of Carson using sheep has been directed toward evaluating the possible damage to the CNS *in utero*. Limitations of sheep as an experimental model include the relatively long gestation period and the fact that seldom more than one offspring is produced per gestation. If the rat could serve as an animal model for evaluation of *in utero* damage to the fetus from lead exposure, considerable latitude would be gained for the evaluation of structural, biochemical and behavioral abnormalities produced.

MATERIALS AND METHODS

Subjects and Parallel Animals

Twenty-four hooded Long-Evans strain female rats approximately 120 days of age were randomly selected from 30 recently purchased stock and randomly divided into three groups of eight animals each. From each group of eight animals, three were selected randomly to gather information concerning maternal blood and tissue levels of lead. These animals were exposed to lead, housed, and handled in the same manner as the dams of the experimental subjects. Two control animals selected for parallel studies expired approximately 3 weeks after the beginning of the experiment, one from urinary cystic calculi and its sequelae and the other following ether anesthesia. Two animals were randomly selected from the remaining purchased stock which had been housed, fed and handled in an identical manner to the experimental dams. This brought the number of control dams back to eight which was considered adequate for control purposes. Commercial lab blocks[1] were fed ad libitum and individual water consumption was measured for a period of one week. Lead in the form of lead acetate tri-hydrate was added to the drinking water of two groups of animals while the third group was maintained on distilled water. Lead exposure intended to be achieved by this method was 25 mg/kg for the low dosage group and 150 mg/kg for the high level group. The female rats used for the production of the test subjects readily consumed the lead-treated water. No significant differences ($P>.05$) in milliliters of water consumed per kilogram of body weight were

[1]Wayne Lab-BLOX, Allied Mills, Inc., Chicago, Illinois.

found in the control, low and high dosage animals (Appendix Table 6). The actual exposure to lead achieved by this route was 29.4 ± 2.5 and 144.5 ± 14.4 mg/kg (mean ± standard deviation) for the low and high dosage groups respectively based on volume of water consumed times percent lead concentrations of the drinking water (Appendix Table 6).

After a period of two weeks of lead exposure, daily vaginal smears were obtained from each female. Those females found to be in proestrus or estrus were placed with a male overnight. In the morning, females were examined for vaginal plugs and vaginal smears were again made. The presence of sperm indicated day 0 of pregnancy. Animals were housed, fed and watered individually during the gestation period. Exposure to lead was terminated at parturition. One of three animals originally intended for euthanasia to gather parallel statistics was salvaged and added to the experimental control group to replace a control dam which had a 23 day gestation period and delivered a litter of stillborn pups. This raised the net number of control dams with suitable litters to five from which the control subjects (Ss) were selected.

One of the low dosage dams failed to conceive and was eliminated from the experiment when this fact became evident. A second low dosage animal and her offspring were eliminated when the litter became severely afflicted with ringtail leading to a very uneven, stunted litter. A total of three dams in the low dosage group with suitable litters remained.

All five high dosage dams conceived, however, one female delivered very weak or stillborn pups after a 23 day gestation. The female and all offspring were dead 24 hours postpartum. Postmortem examination of the female revealed a perforated uterus and diffuse peritonitis. Thus four

high dosage dams with suitable litters remained.

When 48 hours of age, the pups were counted, sexed and culled. Litters were standardized at 8 pups each consisting of 4 males and 4 females when feasible. Litters were weighed at ages 48 hours, 1 week, 2 weeks, and 3 weeks, at which time pups were weaned. Pups from each mating were separated into two cages according to sex and remained in groups of two to five until experimental subjects were selected. From each of five control, three low dosage and four high dosage litters, two male offspring were randomly selected as experimental animals yielding ten control, six low dosage and eight high dosage subjects for a total of twenty-four male Ss.

No significant differences ($P>.05$) were found in the number of pups alive at 48 hours postpartum, or in the average weight of pups at 2 weeks and at the 3 week weaning period. The low dosage pups weighed significantly more ($P<.05$) than the control and high dosage animals when weighed at 48 hours and 1 week of age (Appendix Table 6). Subjects were weighed weekly and training began when the animals reached a mean weight of 564 $\pm$ 47 gms.

Apparatus

Training apparatus

The subjects were first trained to lever press for a food pellet reward. The apparatus consisted of a Skinner box with an operant panel on one end. A rodent lever was mounted in each of the two sides of the panel. Immediately above each lever a small back projection screen[2] was mounted on which

[2]Replacement Projector-A509-2A, Grason-Stadler Company, Inc., Concord, Massachusetts.

the visual patterns were presented. The food hopper into which the 20 mg Noyes pellet[3] discharged was equally spaced between the right and left levers. The panel was identical to that shown in Figure 2. The environmental chamber consisted of a plywood enclosure lined with foam rubber. A viewing window for observation of the subject was present in the hinged door. The pellet dispenser could be controlled by the experimenter with a remote switch. A house light was present in the environmental chamber that could be on, off or dimly lighted. A ventilation fan in one end drew fresh air into the chamber from the opposite end and also provided the chamber with sufficient white noise.

Testing apparatus

After the subjects became proficient at operating the levers in the training chamber, they were introduced to the testing chamber (Figure 1). This consisted of two chambers that were similar to and mirror images of the training chamber. An operant panel was present on each end of the apparatus and the subjects passed from one panel to the other through a centrally placed doorway between the chambers (Figure 2). Manipulation of one panel for a reward ended the presentation and automatically switched the program logic and presentation to the opposite side. The subject then turned around and proceeded through the opening to face the operant panel which had been previously activated. The programming of stimuli presentation, reinforcement presentation, and recording of the data was automated

[3]P. J. Noyes Company, Lancaster, New Hampshire.

Figure 1. Front view of two-sided behavioral testing chamber within the environmental chamber.

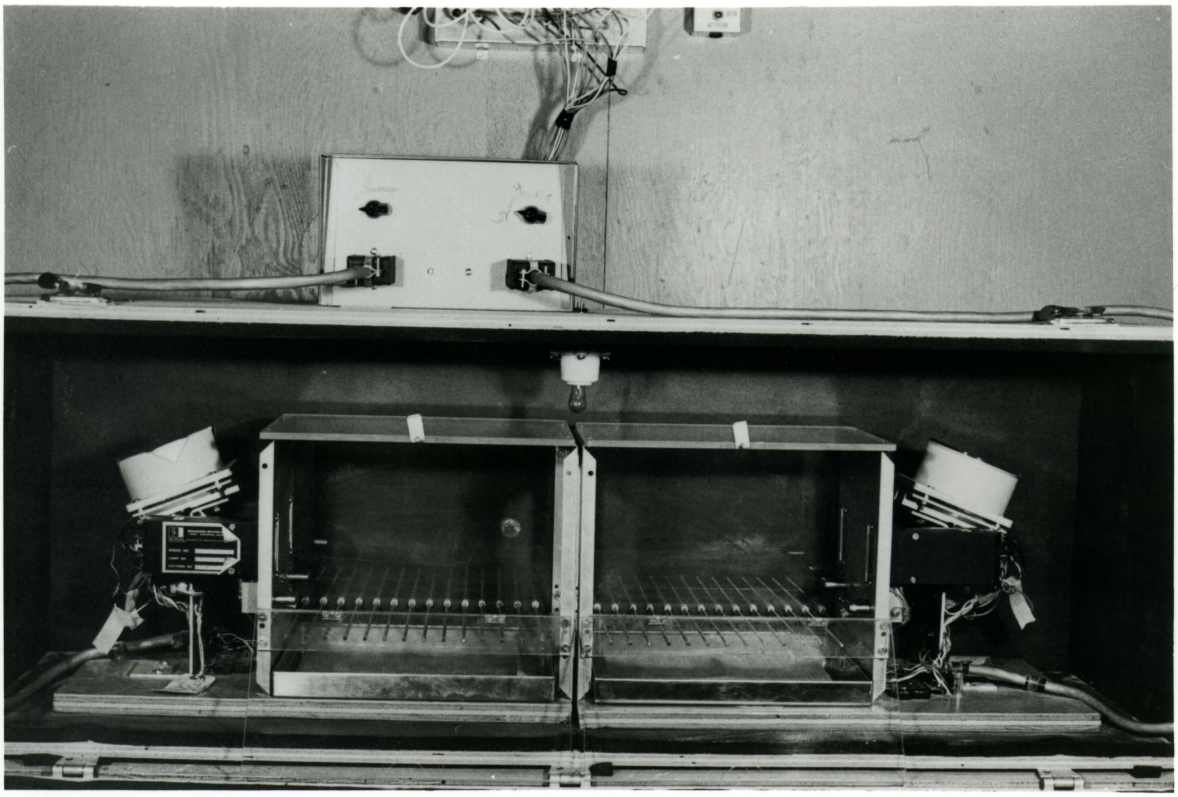
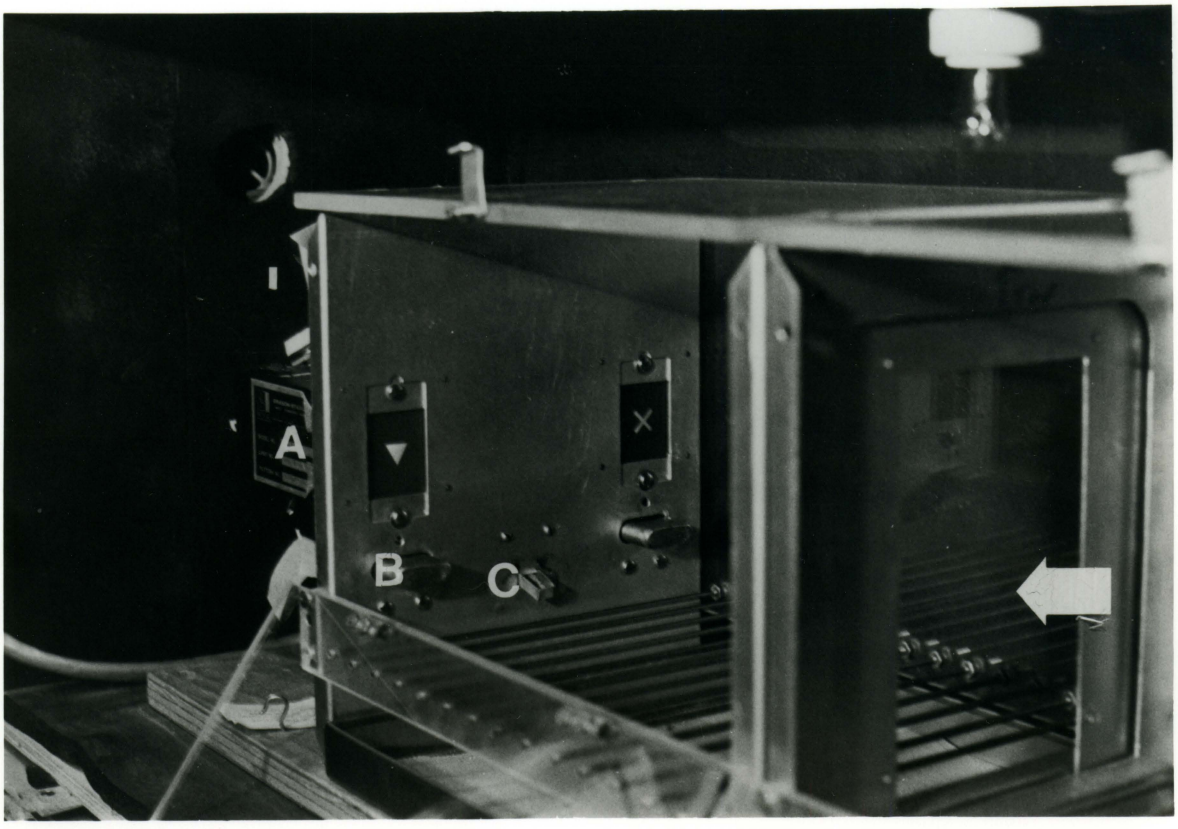


Figure 2. Left side of behavioral testing chamber showing operant panel during a trial in problem 4.
A) Back projector
B) Rodent lever below screen
C) Food hopper
Arrow indicates passageway through center door.


A
B
C

using solid state logic control modules[4]. A flow diagram of the program used to record and control the visual discrimination testing is presented in Figure 3.

Procedures

Deprivation schedule

Training began with the initiation of the feeding deprivation schedule. The ideal weight of the individual subject was calculated at 75% of the animal's weight at the beginning of the training period. Food was weighed individually and subjects were fed the calculated amount at the end of the daily training or testing session. After 30 days, subjects reached the weight at which they were maintained throughout the training and testing sessions. Subjects were weighed weekly throughout the duration of the experiment and increases or decreases were made in the weight of the food offered to maintain a close approximation to the previously calculated ideal weight.

Training procedures

Operant training began by acclimatizing the subjects to the behavioral chamber. Several food pellets were placed in the food hopper and subjects were allowed 15 minutes in the chamber. Exploratory behavior took place and animals were also introduced to the feeding hopper and to the 20 mg Noyes pellets used as the food reward.

After three days, pellets were discharged into the hopper as they were being eaten by the subjects. Rewards were then withheld until the animal

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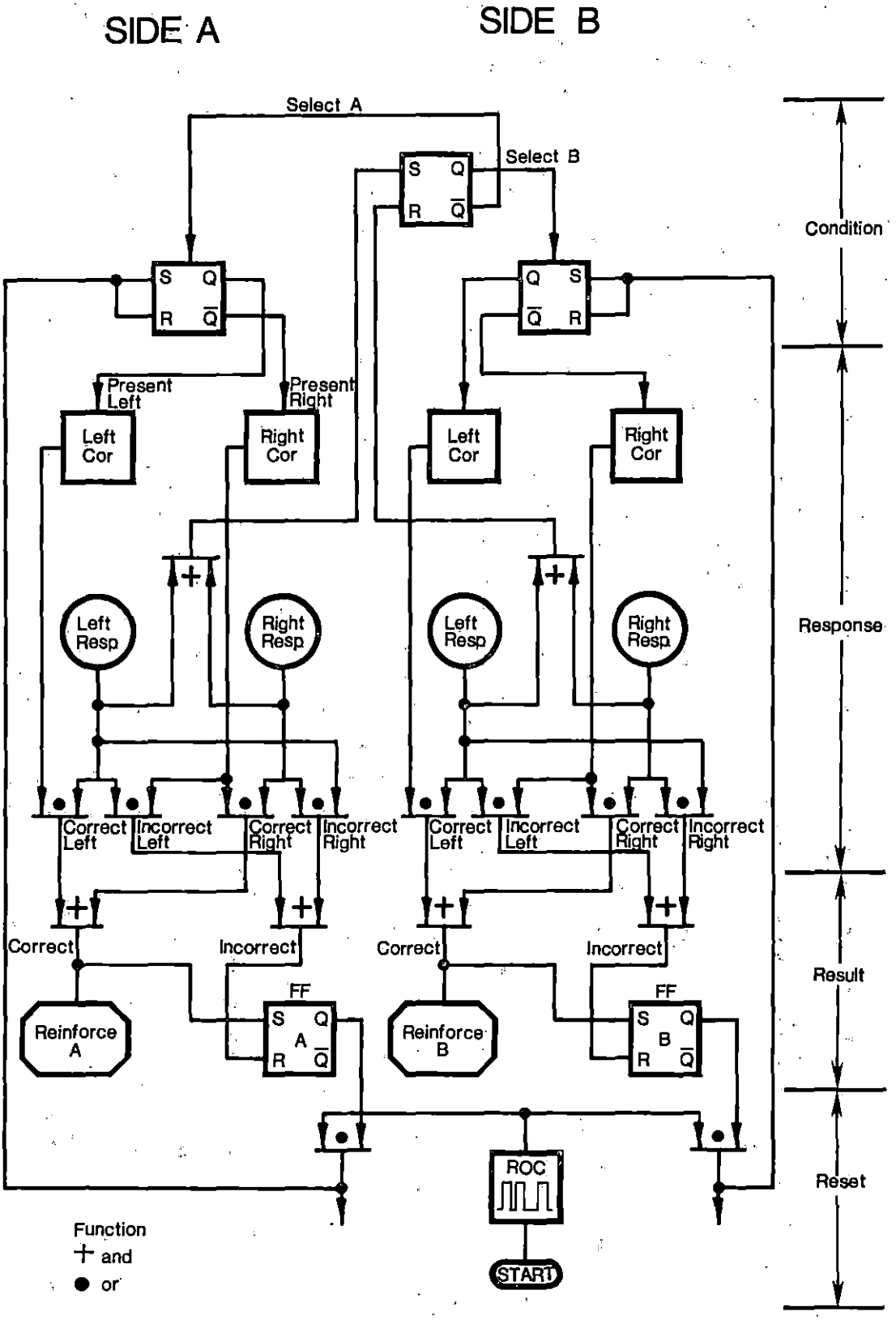
[4]Massey Dickinson Company, Inc., Saxonville, Massachusetts.

Figure 3. Flow diagram of the logic used to control the visual discrimination testing paradigm


SIDE A
SIDE B
Select A
Select B
S
Q
R
Q̄
Present Left
Present Right
Left Cor
Right Cor
Left Resp
Right Resp
Correct Left
Incorrect Left
Correct Right
Incorrect Right
Correct
Incorrect
Reinforce A
Reinforce B
FF
A
B
ROC
START
Function
+ and
● or
Condition
Response
Result
Reset

made successive attempts at exploring and later manipulating the levers. When the subject was able to reward himself frequently, no rewards were given manually by the experimenter. Animals were initially trained to operate both levers, however, if an animal became partial to one side, rewards were given manually to increase activity on the less favored side. The apparatus also allowed the experimenter to de-activate the favored side by means of a remote device during the initial training. When the animals were proficient at operating both right and left levers for a food reward, a series of alternating activation and deactivation between the right and left side control logic was initiated. This allowed the animals success and nonsuccess at both levers and resulted in proficient manipulation of both levers without the development of a strong positional habit.

All experimental animals adapted to the training apparatus well. Proficiency at manipulating both levers for a food reward was attained after approximately 6 to 8 daily sessions of 10 minutes each. By the sixth session, all animals had demonstrated the ability to manipulate either lever for a reward, however, right or left sided preferences were detectable in one control and one high dosage subject. The seventh and eighth sessions were used to discourage positional habits and to allow Ss to become more proficient at lever manipulation. By the end of the eighth session, all animals were ready for the testing procedures.

Originally the training boxes were intended to serve as testing chambers. The first problem involved making a choice between a lighted square and an unlighted side, the lighted side being correct. The

stimulus was presented for 7.5 seconds with a 7.5 second intertrial interval (ITI) when the panel was unlighted and levers were inactive. A total of 80 trials per session, consisting of a twenty-minute session with four trials per minute, were presented each day. Failure to respond during the 7.5 second trial period was recorded as an incorrect response.

The experiment was conducted for eleven days. Results showed extreme variability among the Ss. Several subjects attained scores as high as 85 to 95% while others remained at little above chance levels. Those animals receiving low scores were generally unwilling to wait through the ITI until the stimulus was again presented. Manipulation of the levers during the ITI often resulted in an immediate response at the beginning of a trial. This reward for manipulating an apparently unlighted lever reinforced this behavior making further testing under these conditions futile.

Daily sessions were discontinued while the testing chamber used during the rest of the experiment was designed and constructed. Following a three session acclimatizing period, testing began.

Testing procedure

Subjects were acclimatized to the testing chamber by placing them inside for three 15 minute daily sessions. During this time both right and left sides of the panel were lighted and a pellet was given following a response on either side. Both sides of the opposite panel were then automatically lighted and the subject would then pass through the center doorway and make a response. This activity was allowed to progress unabated during the 15 minute acclimatizing session. After three daily sessions, subjects moved freely between the chambers and testing sessions began.

Each daily session consisted of 40 trials, 20 on each side of the chamber. At the end of 40 trials or 15 minutes, the session was ended. Testing was conducted on a one session per day, seven days per week schedule. Responses were recorded as being right correct, left correct, right incorrect or left incorrect. The total time required to complete each daily session was recorded for each animal. The total percent correct response was calculated from the total number of correct choices out of 40 total responses.

A series of pattern form discrimination problems were proposed (Figure 4). A satisfactory criterion level of performance was selected for each problem depending on the degree of difficulty encountered in solving the problem. After reaching the prescribed percentage correct for three out of four days, the animals were advanced to the next problem.

Tissue Analysis

Blood samples were obtained from the orbital sinus under ether anesthesia. Blood lead analysis was performed by atomic absorption spectrophotometry according to the method described by Hessel (1968). Parallel dams were euthanized on day 21 of pregnancy. Maternal liver and kidney tissues were harvested at this time. The fetuses in the uterus were counted. Pups 48 hours of age were culled from experimental litters when litters were standardized at 8 pups each. Pups were homogenized in a Waring blender and wet tissue lead levels were determined. Tissue lead analysis was performed on maternal liver and kidney, and homogenates of pups 48 hours of age. Tissue was weighed on a wet-weight basis, dehydrated

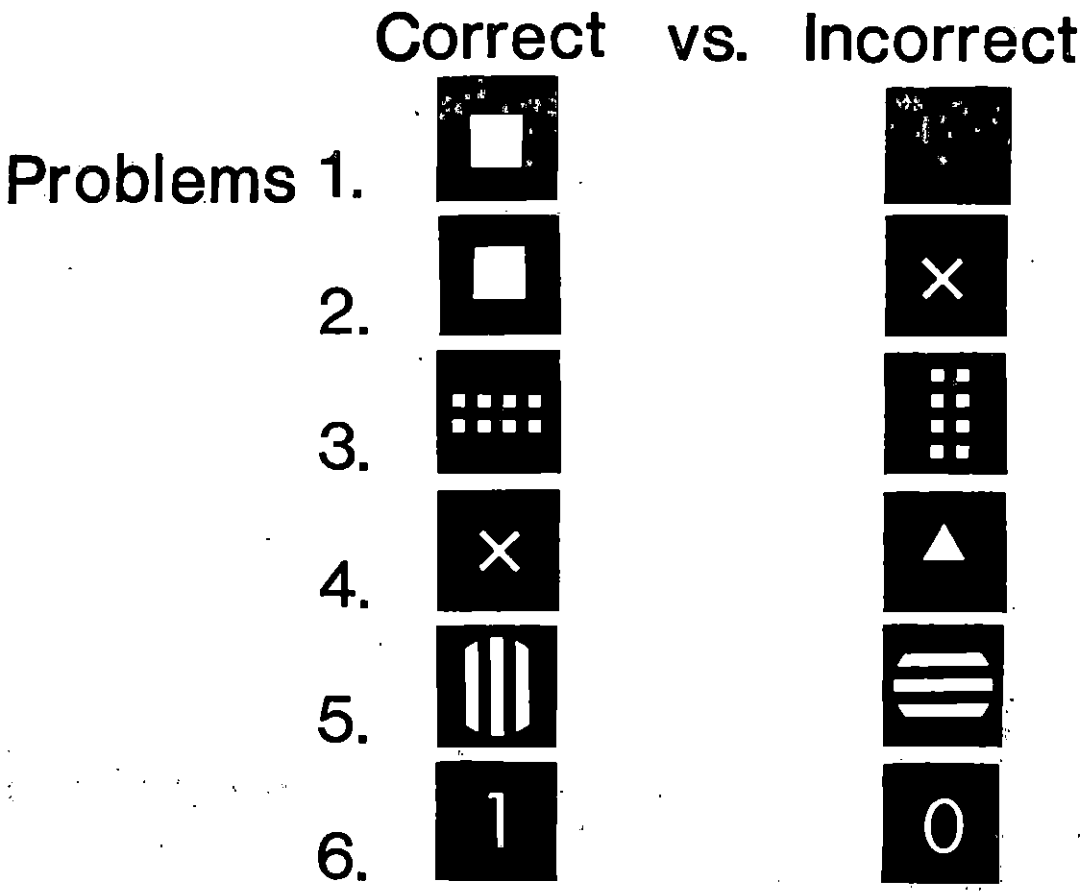


Figure 4. Geometric form stimuli of the six visual discrimination test problems

and ashed at 500°C using 20 mg Magnesium Acetate solution/g sample as an ashing aid. The ash was then diluted in 2N HCl and dissolved in an appropriate volume of water. The analysis was done on a Perkin-Elmer 303 Atomic Absorption Spectrophotometer. (Veterinary Diagnostic Laboratory, c1975, p 3.7)

Data Analysis

Subjects and parallel animals data

Maternal and litter data were subjected to Student's t-test to determine significance of dosage group mean differences. Planned comparisons were made between the following groups: control versus low, low versus high, control versus high, and control versus treated (combined low and high dosage groups).

Visual discrimination testing

Days to criterion The mean number of days required to reach criterion level of performance was calculated for each dosage group on each problem satisfactorily completed. Group means were subjected to Student's t-test and planned comparisons as described above were made where differences were found.

Four day means of percent correct score The percent correct score was calculated daily for each animal. From these values, consecutive four day means were calculated. When appreciable group differences between these 4 day averages were found, planned comparisons, as described above, were made using Student's t-test.

Learning curves Whenever learning curves for a problem appeared to differ appreciably, the slope (β) of the learning curve was calculated for each subject and the β values were subjected to Student's *t*-test to determine significant differences in the means of the between group planned comparisons as described above.

RESULTS

Parallel Animals

Significant differences ($P<.05$) in the parallel maternal blood lead values were found between the three groups. No difference between the two periods, beginning of breeding and the end of the gestation were found (Table 1). The standard deviation of a level mean was 0.037. A difference between the level means of 0.17 was declared significant ($P<.05$), which made all comparisons except control vs. low significant and even that one was close to being significantly different.

No significant differences were found in the liver tissues of parallel dams when killed near the time of parturition (Table 2). The standard deviation of a mean was 1.51. A difference of 6.81 was required for significance ($P<.05$). The greatest difference found between the low and high dosage groups was 2.75 indicating that liver lead levels did not correlate with lead dosage levels.

Very large and significant differences were found in the kidney lead values of the parallel dams (Table 3). Kidney tissue from high dosage animals contained significantly more ($P<.01$) lead than the low or control groups. The low dosage group also was significantly increased ($P<.05$) above the controls indicating the ability of the rat kidney to concentrate lead. The standard deviation of a mean was 1.27. A difference of 5.71 was declared significant ($P<.05$) and a difference of 10.50 was declared highly significant ($P<.01$).

No significant differences were found in the number of fetuses *in utero* when the parallel dams were killed on day 21 of pregnancy. The

Table 1. Blood lead levels of parallel dams at breeding and parturition (ppm)

	Control	Low	High	mean
Breeding	0.15	0.22	0.40	0.26
Parturition	0.07	0.30	0.48	0.28
mean	0.11	0.26	0.44	0.27

Standard deviation of a level mean = 0.037

Table 2. Lead levels in liver tissue of parallel dams near time of parturition (ppm)

	Control	Low	High
Liver (mean)	4.25	3.50	6.25

Standard deviation of a level mean = 1.51

Table 3. Lead levels in kidney tissue of parallel dams near time of parturition (ppm)

	Control	Low	High
Kidney (mean)	3.47	11.62	20.88

Standard deviation of a level mean = 1.27

standard deviation of a level mean was 1.08. A difference of 4.65 was required for significance (Table 4). The greatest difference found was 2.5 between the low and high dosage groups.

Lead levels in homogenates of 48 hour pups showed close to significant differences between control and high dosage groups (Table 5). However, without more replication of the high level, the results should be interpreted cautiously. The trend toward higher levels with higher dosages seems evident and consistent with other measures taken in the experiment.

Experimental Animals

Testing results

Problem #1 Twenty-two of the 24 Ss attained a score of 90% or better during the first three days. No animal required more than five sessions to reach criterion level of performance (Appendix Table 8).

The criterion level of performance for this problem was attained so rapidly that appreciable differences of group means were not found. Neither was a learning curve produced which could be examined for group differences.

Problem #2 A performance level of 90% correct on three out of 4 consecutive days was reached in a mean number of days of 12, 18 and 16 for the control, low and high groups respectively ($P>.05$).

The variability within a group was quite high as shown in Appendix Table 8. Three control animals met criterion in 5 days, however, 39, 26, and 22 days were required for two low and one high dosage animals respectively. A statistical evaluation of days to criterion for this problem is presented in Appendix Table 9.

Table 4. Number of fetuses *in utero* on day 21 of pregnancy

	Control	Low	High
n	2	2	2
mean ± S.E.	12.5 ± 2.1	10.5 ± 0.7	13.0 ± 1.4

Standard deviation of a level mean = 1.08

Table 5. Lead levels in homogenates of 48 hour pups (ppm)

	Control	Low	High
n	4	2	1
mean ± S.E.	0.81 ± 0.23	1.13 ± 0.32	2.50 ± 0.45

The percent correct daily score for each subject was averaged for the first 4 days on the experiment. Group means of these scores were 79, 74, and 75 for the control, low and high dosage animals. A statistical evaluation of these scores is presented in Appendix Table 10.

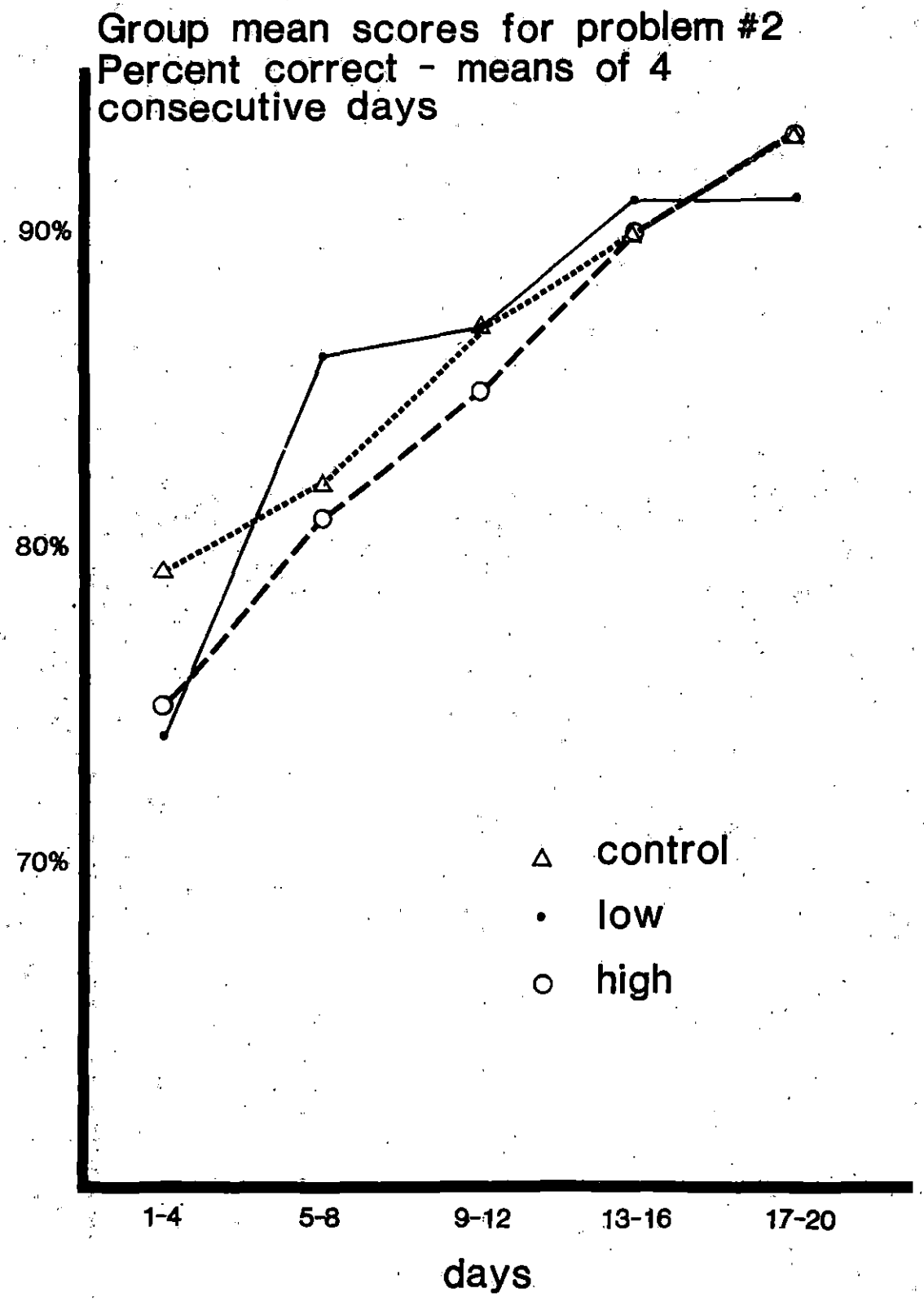
The learning curves generated by the experimental groups were unremarkable (Figure 5).

Problem #3 After fifty sessions, only 3 subjects had attained a score of 80% or better for three out of 4 sessions. The group which achieved criterion level was comprised of a control and two low dosage animals (Appendix Table 8).

Soon after problem 3 began, many subjects developed positional habits. Remote, manual deactivation of favored levers became necessary to prevent irreversible position preferences. Individual scores attained during sessions in which manual control became necessary were unsatisfactory for statistical purposes. After several weeks it was apparent that some animals would probably never learn the problem under the existing circumstances. A decision to discontinue problem 3 was made.

The logic for the behavioral apparatus was modified somewhat for the continuation of problems 4 through 6. For these problems, an incorrect choice resulted in a repeat of the same presentation. This prevented the animal from using one side continuously and eventually encouraged the subject to use the alternate lever. This change in logic also biased the score in favor of a higher percent correct since the animal was now able to use previous results to aid in predicting the correct choice.

Figure 5. Learning curve for the first 20 days of problem 2


Group mean scores for problem #2
Percent correct - means of 4 consecutive days
90%
80%
70%
control
low
high
1-4
5-8
9-12
13-16
17-20
days

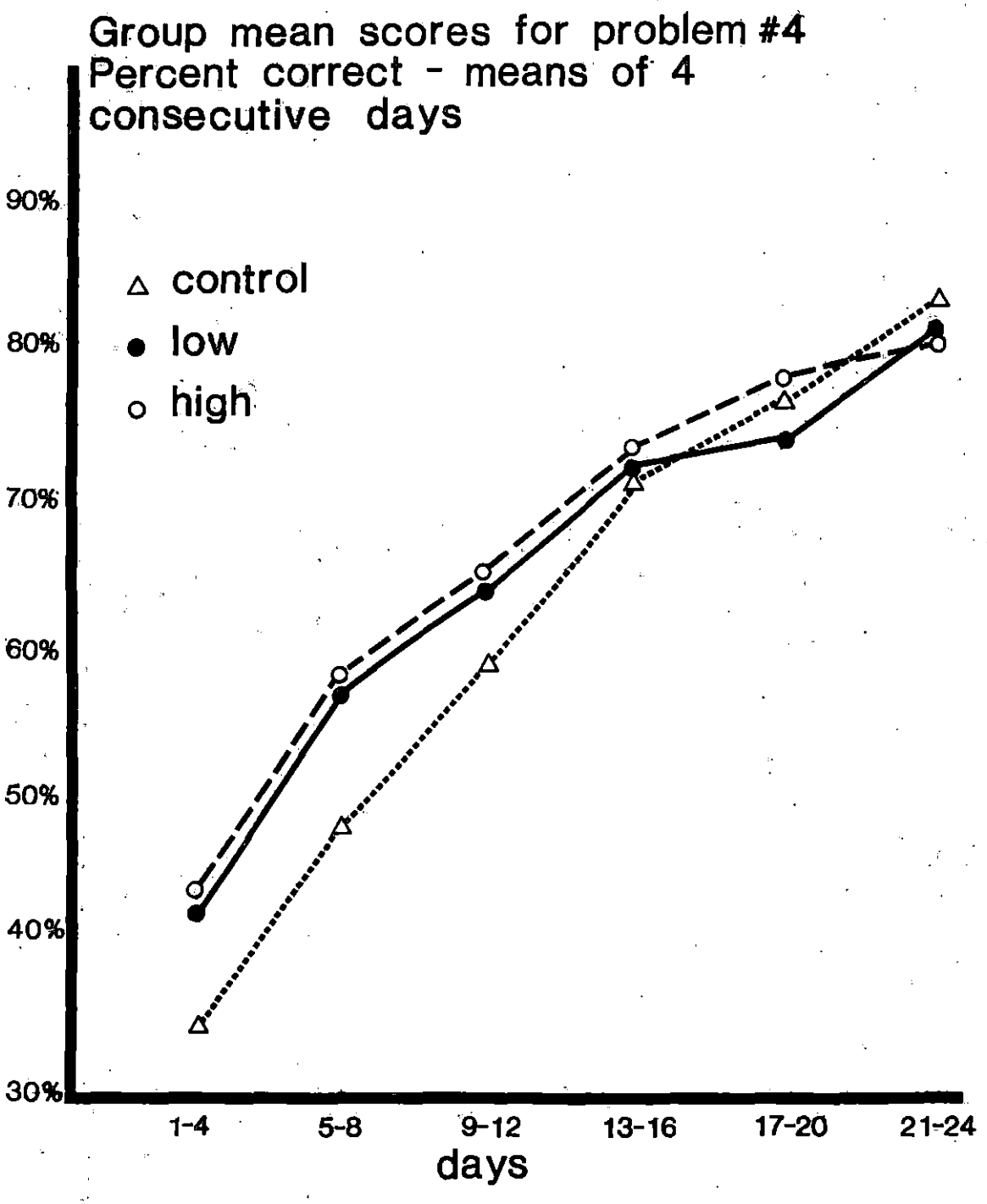
Problem #4 A criterion performance level of 80% correct on three out of 4 days was achieved by all animals after forty days had elapsed. The least number of days in which any animal reached criterion level of performance was ten. Group means were within plus or minus one day of each other (Appendix Table 8).

The average of the four daily percent correct scores attained during the first 4 days of problem 4 were evaluated. The only comparision that approached significance was the control vs. the treated group. The values obtained were 34, 41, and 43 percent, for the control, low, and high dosage groups respectively. A statistical evaluation of the group means are presented in Appendix Table 10.

The learning curve generated by the dosage groups is shown in Figure 6. The slope of the learning curve for the first 20 days of problem 4 was calculated for each subject. The β values obtained were examined for between group differences using Student's t-test. A significant difference was found between the control and treated group means (Appendix Table 10). The control animals began 8 percentage points below the combined low and high dosage groups. All groups showed a positive, gradual increase in level of performance. However, average percentage correct scores generated from days 21 through 24 revealed that the control group had attained the highest mean score.

Problem #5 The subjects performed at this task for a time period ranging from 59 to 80 days. A criterion level of performance of 75% was not attained by any of the subjects during this time. A graph of daily percent correct scores indicated that the level of performance

Figure 6. Learning curve for the first 24 days of problem 4


Group mean scores for problem #4
Percent correct - means of 4 consecutive days
90%
80%
70%
60%
50%
40%
30%
△ control
● low
○ high
1-4
5-8
9-12
13-16
17-20
21-24
days

remained static over several weeks (Figure 7). The percent correct score for subjects compiled for the first 10 consecutive days averaged 60, 58, and 59 for the control, low, and high dosage groups. The mean score for all three groups combined during the 30 to 40 day time period was 62 percent.

Problem #6 A learning curve for problem 6 is presented in Figure 8. The percent correct score for consecutive 4 day means was tabulated for each subject. Across group averages were also calculated. An average improvement of 1.22 $\pm$ .88% was attained at each 4 day interval from day 1 through day 28. The environmental chamber house light was turned off after 28 daily sessions on problem 6. An improvement of 4.1 percentage points was obtained by the subjects during the 29 to 32 day interval. This value lies greater than 2 standard deviations away from the mean of the previous improvement increments. Thus, a significant positive effect ($P<.05$) on performance was produced by eliminating the house light.

The shape of the learning curve indicated that a criterion level of performance of 75% might conceivably be reached, however, no group differences were evident after 32 days. A decision was made to terminate the problem.

Figure 7. Learning curve for the first 40 days of problem 5

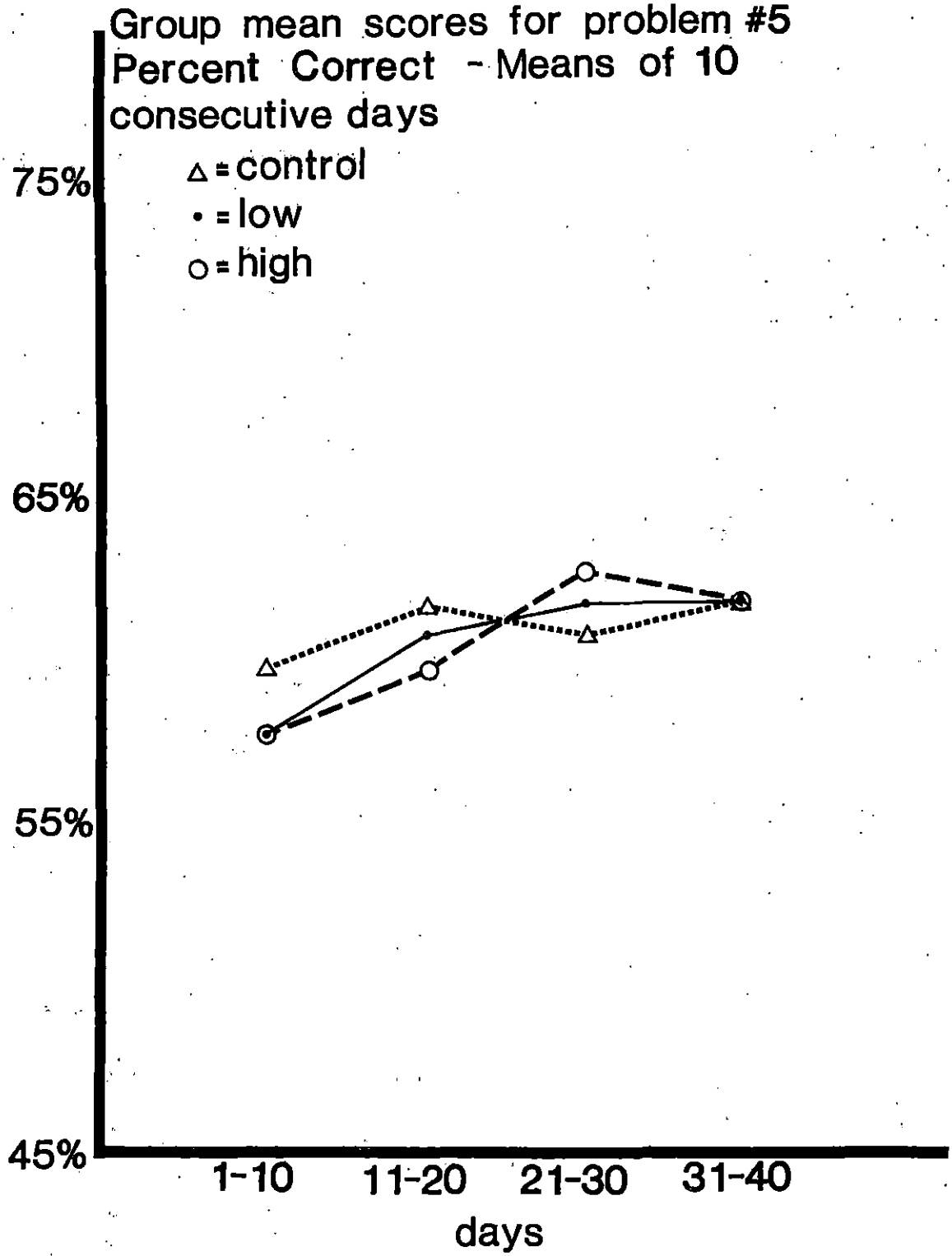
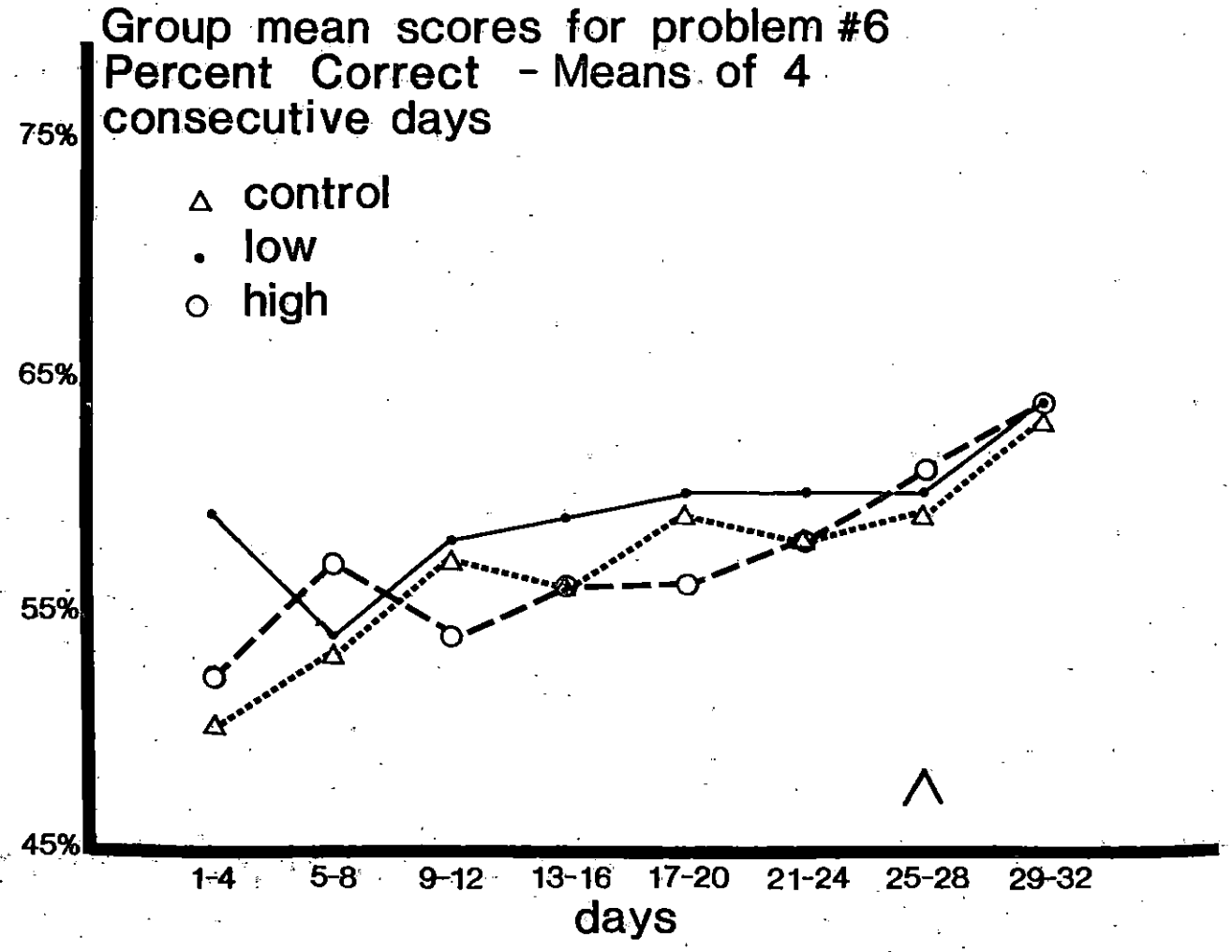

Group mean scores for problem #5
Percent Correct - Means of 10 consecutive days
△ = control
• = low
○ = high
75%
65%
55%
45%
1-10
11-20
21-30
31-40
days

Figure 8. Learning curve for the first 32 days of problem 6

Arrow at the bottom indicates time when houselight was extinguished.


Group mean scores for problem #6
Percent Correct - Means of 4 consecutive days
75%
65%
55%
45%
control
low
high
1-4
5-8
9-12
13-16
17-20
21-24
25-28
29-32
days

DISCUSSION

Subjects and Parallel Animals

The number of females chosen to make parallel studies and to produce the experimental subjects could justifiably have been increased. Reasons why some of the females initially chosen were eliminated from the experiment included failure of some to concieve, early loss of entire litters and dams at parturition and death related to anesthetic procedures required in order to obtain blood samples for lead analysis. One female died as a result of urolithiasis. The required number of three animals per treatment group to make parallel studies and five animals per treatment group to produce the experimental litters as provided for in this study made very small allowance for unforseen problems. Three times the initial number of animals at the beginning of the experimental period would have been desirable. Offspring from those females that were to be behaviorally tested could have been randomly selected from appropriate groups at the time training and testing began.

Also, larger numbers of animals would add strength to the applied statistics. When the effect of prenatal exposure of toxicants is the variable to be measured, litter effect is inseparably associated with treatment effect. The genetic influences of the dam and sire, the *in utero* environmental effects, as well as the behavioral effects of postnatal fostering by the dam are distributed alike to the offspring within a litter. The toxicant administered to the dam is also distributed similarly to the placentae via the maternal blood supply. Consequently the offspring within a litter are not randomly distributed independent

observations with lead dosages as the only dependent variable (Weil, 1970).

In this study, however, no correlation between subjects within a litter was found on performance of problems 2 and 4 ($P>.30$). Therefore all measurements of the Ss on these problems were treated as independent observations. If a significant correlation had been found between subjects within a litter, only litter means could have been treated as independent observations. For this reason it would have been desirable to have more litters with preferably only one subject per litter represented as the experimental unit.

The mean age of the subjects when training began was 182 days. This represents older animals than are often used in this type of behavioral testing. Silbergeld and Goldberg (1973) tested mice at 40 to 60 days of age. Sobotka and Cook (1974) examined rats for evidence of brain dysfunction at various times up to 55 days of age. However, Carson et al. (1974) have shown behavioral deficits in 15 month old sheep exposed prenatally to lead. The latter finding indicates that the effects produced by prenatal lead exposure are residual and probably remain into at least early adulthood.

An important finding in this experiment is the lack of a significant reduction in the weight of lead exposed subjects at various intervals during the early growth period. Often the addition of four percent lead acetate to the maternal diet results in significant reductions in the weight of lead exposed pups (Krigman et al., 1974a; Michaelson and Sauerhoff, 1974). This effect is believed due primarily to poor lactation of the female as a result

of reduced food consumption. Although the high dosage females in this study gained significantly less weight from breeding till parturition, no differences were produced in the weights of the pups at 2, 3, 8 and 26 weeks of age. The significantly ($P<.05$) higher weights of the low dosage pups at 48 hours and 1 week of age does not appear to be a direct effect of lead. The low dosage females drank more water than either the control or high dosage animals possibly because of the mild salinity of the solution. It is suggested that the increased water consumption had a positive effect on lactation especially in the early neonatal period. The initial increased weights of the low dosage pups above the control and high groups was not considered a seriously adverse finding. The experiment was designed to detect learning deficits resulting from lead exposure. Previous research has not indicated that increased neonatal growth and development will adversely affect later performance. Therefore, one could not logically attribute any learning deficit in the low dosage animals detectable at a later time to the elevated early neonatal weights.

These findings are interpreted to mean that lead exposure of the female before and during gestation did not adversely affect the early and later postnatal physical development of the subjects. Also the failure to find significant differences in the number of fetuses *in utero* when parallel dams were killed or in the number of pups per litter at 48 hours of age support the conclusion that neither females nor pups demonstrated observable signs of overt lead toxicosis. This observation is important if behavioral deficits produced are to be attributed to the immediate effects

of lead rather than indirect effects subsequent to poor nutrition and delayed, diminished physical development.

The significant difference in blood lead levels between control and high dosage groups and the nearly significant difference between control and low dosage groups is an important finding in this experiment (Table 1). This study is based on the premise that any effects produced are primarily the result of prenatal lead exposure. It was important therefore that significant differences in maternal blood levels were demonstrable.

It was also important to demonstrate relatively stable blood lead levels throughout the gestation period. This was indicated by the lack of significant differences between dosage group blood lead values at the time of breeding and parturition (Table 1).

It is admittedly unfortunate that the maternal blood lead values could not be obtained from the same females which produced the experimental litters. It was believed that the stress associated with anesthetic procedures required to obtain blood samples at breeding and especially at parturition would adversely affect the females concerning delivery and early postnatal care of their young. Again, a larger number of parallel animals would add statistical strength to the data generated in the above manner thus minimizing the objections to this approach.

The significantly different kidney lead levels is another indication that females in the control, low and high dosage groups were indeed exposed to and absorbed significantly different amounts of lead (Table 3).

Suitability of the Apparatus

Patterns chosen

The suitability of the testing apparatus used to evaluate pattern form discrimination is possibly open to criticism. Problem 1 involved selecting a lighted rather than a dark stimulus and was not intended to represent a two choice pattern discrimination task. In problem 2, however, the total surface area of the square was greater than the X. For this reason the square was considerably brighter. Consequently the animals were in all probability responding to a brightness and intensity difference similar to problem 1. Problem 2 was considerably more difficult to solve than problem 1, and less so than problem 4, as indicated by the average number of days required to solve each problem. In this respect, problem 2 served as an intermediate between the intensity-oriented stimuli of problem 1 and the pattern-oriented stimuli of problem 4.

The square used as the positive stimulus in Problem 2 was also the positive stimulus of Problem 1. This undoubtedly aided the subjects in rapidly solving problem 2. The fact remains, however, that the brightness stimuli to which the subjects responded were at least visual if not pattern oriented.

The failure of any animal to meet criterion level of performance on problem 5 may also be attributable to failures of the apparatus and pattern chosen. Although Lashley (1938) has used the vertically and horizontally oriented stripes successfully in a jumping stand apparatus to test visual discrimination in rats, the physical differences between the jumping stand and the apparatus used in this experiment may explain the failure of the rats to respond to these stimuli in this study.

The jumping stand utilizes stimuli cards five inches square upon which stripes ranging in size from 3 mm to 20 mm are located. The subject is placed on a stand, the front edge of which is 25 cm away from the cards, and jumps at the patterns. The correct pattern allows the animal to pass and receive a reward. The incorrect choice results in a sudden stop and fall to a net. By the design of the apparatus, the animal makes a choice from a distance and immediately bears the consequences of that choice.

The apparatus used in this experiment allowed the subject to approach either stimulus closely and to then make a choice, the choice being either to respond to the stimulus immediately in front of him or to abstain from any response. Observations of subjects that were performing the more difficult problems such as problems 3 and 5 were rarely seen approaching an incorrect stimulus and then proceeding to the opposite side and responding to the correct stimulus. During the performance of problems 2 and 4, subjects were observed moving toward the correct stimulus soon after passing through the center doorway. If the apparatus, as designed, is to function in a two-choice visual discrimination problem, the subjects must have the ability to visually resolve the pattern differences before they are required to make a physical advancement toward the individual stimuli. An apparatus and pattern combination which fails to provide the above requirements functions more to test the subject's willingness to leave an incorrect stimulus in search of the correct one than it does to test two-choice visual discrimination abilities.

Furthermore, there is evidence that the pattern chosen in problem 5 was at the extreme limits of the rat's visual discriminatory abilities.

Lashley (1938) trained his subjects to respond to 20 mm wide horizontal and vertical stripes. Stripes of decreasing width were used until the limits of the rat's vision were exceeded. Under these conditions, stripes of 3 mm width could be readily distinguished by nonalbino rats while animals consistently failed to discriminate stripes 1.5 mm wide. This places the visual limits of the rat somewhere between 3 and 1.5 mm. When the subjects were allowed to approach the stimuli closer than 20 cm, no appreciable change in the limits were found.

The pattern stripes in this study were 3 mm wide. The distance from the stimulus to the middle of the center doorway was 30 cm. It seems reasonable to assume that the conditions of the situation in problem 5 are close to the outside limits of the Ss' ability to discriminate. Also the rats in this work were not previously trained to respond to wide stripes as in Lashley's work. The subjects' first exposure to the pattern occurred during this problem which likewise could account for the poor response of these rats under the conditions of this experiment.

Size of stimuli panels and attention to stimuli

The overall size of the stimuli panels used in this apparatus is considerably less than the jumping stand previously discussed. Lashley's apparatus (1912) uses patterns on five inch square cards. The cards are spacially separated by two inches. The subjects' attention to the stimuli is enhanced by the size and proximity of the cards.

In the apparatus used in this experiment, attention to stimuli is enhanced by the lighted aspects of the pattern presentation. Problem 6 was conducted while the environmental chamber light was dimly lighted.

The contrast between the darkened surroundings and the lighted stimuli panels was reduced considerably. An examination of the data revealed that the across group means of the daily percent correct scores were increasing gradually. A significant improvement in performance was noticed after the house light was extinguished. Personal observations of the subjects while performing problem 6 with the house light on showed that the subjects approached the stimuli less promptly and less directly. After passing through the center doorway, the subjects appeared to demonstrate increased exploratory behavior. After the subjects had performed problem 6 for 29 days, the house light was extinguished and the subjects again approached the stimuli more directly.

Admittedly the behavioral observations reported here were subjective, however, the rats' actions appeared quite definite. These findings suggest that maximum contrast between stimuli and background should be maintained in order to enhance the learned response, presumably by increasing the subjects' attention to stimuli.

Formation of positional habits

The development of positional habits when the discrimination task was difficult, became evident during problem 3. Soon after this problem began, many subjects developed positional habits and remote, manual deactivation of favored levers became necessary to prevent irreversible position preferences. Individual scores attained during sessions when manual control became necessary were unsatisfactory for statistical purposes. After several weeks it was apparent that some animals would probably never learn the problem under existing circumstances and problem 3 was discontinued.

During problem 3 the program logic was completely randomized. Correct and incorrect choices were presented on the right or left side on a 1:1 ratio. An animal that continuously responded to the right lever or left lever was rewarded on a 1:2 ratio. This frequency of reinforcement was too great to discourage positional habits.

Before continuation of problem 4, the logic was modified so that an incorrect choice failed to activate the random output converter. Consequently, the patterns were again presented on the panels as they had been the trial before. Results during problems 4, 5 and 6 indicated that the re-designed logic was effective in preventing positional preferences.

Although the apparatus used in this experiment had some deficiencies the experimenter had the ability to alter the paradigm with a minimum of time and effort which gave the apparatus a great deal of versatility. Changes in the schedule of reinforcement, the ability to investigate negative reinforcement and variable delays were some of the means available to alter the paradigm. Total session times as well as intertrial intervals could have been changed and recorded. Gathering of experimental data was automated and could be easily computerized if proper instrumentation was available. The instrumentation also would have eliminated experimenter bias from the large amounts of data which could have been generated.

Analysis of Behavioral Testing Data

Number of days to criterion

The number of days to criterion proved to be an insensitive measure of learning performance in problems 1, 2, and 4 where criterion was met. Results showed a great deal of variability. In problem 2, values ranged

from 5 to 39 days and in problem 4, from 10 to 40 days (Appendix Table 8). In all three problems, the control means are less than either the low or high dosage means. Problem 2, however, is the only one of the three in which values of the control and treated groups even moderately suggest a difference ($0.2>P>0.1$) (Appendix Table 9). Because of the within group variability, unacceptably high numbers of animals would have to be tested under the experimental conditions in order to gain significance.

An inherent problem with using days to criterion as a measure is that some value for criterion must be established. If this value is too low, the set level of performance is reached very rapidly and insignificant data is generated as was the situation in problem 1 (Appendix Table 8). If the level is set too high, the individual's behavioral idiosyncrasies may unduly influence his performance. An example of this may be found in the performance of one low dosage animal during Problem 2. The animal attained a score of 78% for days 5, 6, and 7, values of 85% and 88% on days 8 and 11 and a score of 95% on day 13. For the remaining 23 days, values in the high 80's and 90's were attained, however, it was not until day 39 that a score of 90% or better was achieved for 3 out of 4 consecutive days. In this example, the high number of days required to meet criterion is more a reflection of the stability and consistency with which the animal performed than a measure of the subject's learning abilities. A graph of this animal's performance for the first 15 days would appear much the same or better than many of the other subjects. However, when days to criterion are used as the measure, the animal would appear to have a definite learning deficit. His value of 39 is 13 days above the next slowest animal to meet criterion. When a single value such as days to criterion is used

as the measure of performance, all other data generated by a subject to achieve that score is disregarded. For the above reasons, days to criterion under the conditions of this experiment, was demonstrated to be a less than satisfactory measure of performance.

Four day means of percent correct score

At the beginning of both problems 2 and 4, group differences in the means of the percent correct score for the first four daily sessions were close to significance. The control scores were considerably higher for the first four days in problem 2 while in problem 4, the control values were nearly significantly ($P<.05$) lower than the treated group values (Appendix Table 10). By this means the experimenter is able to evaluate several days of data generated by the subject. The mean values obtained tend to be less variable than individual daily scores and less variable than days to criterion as discussed above. This method of evaluating data is especially helpful after differences seem apparent and before sufficient data has been generated to evaluate learning curves as will be discussed below.

Learning curve

Evaluation of differences of the learning curve appeared to be the most sensitive measure of group differences. Possibly the most significant finding of the experiment was the relatively low scores achieved by the control group at the beginning of problem 4 and the significantly different learning curve slopes for the first 20 days between the control and treated animals (Figure 6). Means of the percent correct score for the first four days were 34 and 43 percent for the control and treated groups respectively ($P<.05$). The mean β values of the learning curves for the

control vs. the treated groups were also found to be significantly different ($P<.05$) (Appendix Table 10). This result was somewhat unexpected since the trend previously had been for controls to function equal or slightly superior to the other groups. By the twenty-first to twenty-fourth day, the mean percent correct score of the controls was again above that of the low or high dosage groups.

A possible explanation lies in the fact that problem 4 was a form of reversal of problem 2. In problem 2, the X was used as a negative symbol while in problem 4, the X was used as the positive stimulus when paired with a triangle. The triangle used as the negative stimulus on problem 4 should have been neutral since it was not used previously for any test. Forty-five to eighty days had elapsed between the end of problem 2 and the beginning of problem 4. Because of the reversal nature of problem 4, it would be expected that the animals which remembered the X best as a negative stimulus would score the lowest initially until the negative aspects of the X became extinguished and the problem was learned. Stated in another way, animals which maintained a less imprinted memory of the X as a negative stimulus would be expected to attain a higher initial percent correct score when tested on this problem. The data indicate that the control and treated groups responded in exactly this manner. Results similar to these would be expected if exposure to lead interfered with the subject's ability to recall a past experience, in this case with a negative stimulus. Further studies, using patterns and problems which rats are capable of learning coupled with similar reversal situations, with and without spacial time variations, are indicated by these findings.

CONCLUSION

The results in this experiment do not relate directly to the lead associated hyperkinetic syndrome suggested by the findings of David et al. (1972) or the proposed lead-associated mental retardation suggested by Landrigan et al. (1975). However, these data suggest the real possibility that early, low-level lead exposure may influence learning behavior at a later time and more specifically that the learning deficiency may be the result of long-term memory deficits.

The effect of lead on long-term memory found in this experiment is not in agreement with the finding of Brown et al. (1971). In Brown's experiment, rats ranging from 8 days to 5 weeks in age were used and lead was administered by intraperitoneal injections. Subjects were tested in a water-filled T-maze and a standard shuttle-box. Animals in this experiment showed overt signs of lead toxicosis, some dying soon after lead exposure. In contrast, the present experiment exposed subjects to subclinical levels of lead during prenatal and early postnatal development, a particularly susceptible period in the development of the rat brain as demonstrated by Brown (1973). In his experiment, females were exposed to as little as 17.5 mg of lead per kg of body weight for the first twenty days postpartum. Offspring from these females were behaviorally tested at eight weeks of age and were found to have a deficit in maze learning ability. Although pups exposed for the first ten days postpartum showed learning deficits, those animals exposed from day 11 to day 20 did not. This indicates the importance of early exposure if detectable damage is to be produced.

Also behavioral data generated over a time period of several weeks was evaluated in the present experiment. By examining data gathered over a period of several weeks, subtle effects may be found that otherwise may be overlooked. Brown et al. (1971) treated the subjects and later tested them for three daily sessions. The data reported from this experiment shows standard deviations equal to 50% or more of treatment group means. With this degree of variability, only gross differences in performance could be declared significant.

Although the present experiment did not demonstrate significant differences in days to criterion between treatment groups, the results do correlate with the work of Carson et al. (1974) by showing a trend for the controls to solve problems more rapidly than treated groups.

Significant group differences in the work of Carson et al. (1974) using sheep became apparent only in the problems which proved to be most difficult. Problems involving circles, triangles and parallel lines were solved with little apparent group difference. The more difficult problems involved size discrimination and the somewhat more abstract pattern of a horizontally oriented U open either to the left or right. It is suggested that rats are less visually oriented than sheep and therefore may be incapable of solving the more difficult visual problems and hence treatment group differences will not be demonstrated by this means of data evaluation.

The results reported here add support to the work of Jenkins and Mellins (1957). These investigators, evaluated and made follow up studies on 32 lead-poisoned children. Difficulty with simple descriptive or

enumerative responses and trouble in articulating words were noted in the more severely affected children. In the less severely affected, however, the outstanding disability was one of attention and visual memory. The latter behavioral deficit though subtle and difficult to evaluate, deserves the full attention of researchers if the young as well as the unborn are to be spared this malady.

The experimenter is unaware of any evaluation of available human data designed to detect subtle deficits in long-term memory caused from prenatal or childhood exposure to lead besides the work of Jenkins and Mellins (1957). Neither is the experimenter aware of any animal studies undertaken to investigate the effects of lead on long-term memory using problems which involve visual stimuli. The results of this work suggest that such a study should be done. The levels of lead which will cause behavioral changes as well as the exact mode of action on nervous tissue must be elucidated to better understand the pathogenesis of lead induced memory deficits. The results of this experiment indicate that the rat may serve as a versatile, inexpensive, readily available animal model for some aspects of this investigation.

SUMMARY

A study was done to determine the suitability of the rat as an animal model for the investigation of neurological and behavioral effects of prenatal low-level lead exposure.

Female Long Evans strain hooded rats were exposed to various levels of lead via the drinking water from three weeks before breeding till parturition. Two males from each of the test litters produced were randomly selected for behavioral studies.

Subjects were trained to lever press for a food pellet reward. Testing consisted of a two-choice visual pattern discrimination in an instrumental condition chamber. Animals were presented with several problems, each problem consisted of a separate pair of stimuli. When criterion level of performance was achieved on a problem, the subject was advanced to the next problem. Days to criterion, percent correct achieved on daily scores and learning curves were examined for individual and group differences.

The experimental data obtained from rats in this study suggested the possibility that prenatal and early postnatal exposure to very small amounts of lead interfered with the processes of memory and may have influenced the performance of rats in a reversal discrimination problem.

These behavioral effects were produced with no overt clinical signs of lead toxicosis in the experimental dams or subjects.

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APPENDIX

Table 6. Two-tail t-test comparisons of maternal and litter value means.

Measurement	Group Means $\bar{X} \pm$ st. dev.			Comparison	df	t-value
		Treated (Tr)				
	control (c)	low (L)	High (H)			
Water consumption of dams from 3 weeks before breeding till parturition (cc/kg)	108 ± 8	121 ± 10	111 ± 11	CvsL	6	2.14
				LvsH	5	1.22
				CvsH	7	0.56
				CvsTr	10	1.34
Lead exposure of dams from 3 weeks before breeding till parturition (mg/kg)	0 ± 0	29.4 ± 2.5	144.5 ± 14.4	CvsL	6	28.4***
				LvsH	5	13.4***
				CvsH	7	23.0***
				CvsTr	10	3.4**
Number of pups per litter alive at 48 hours postpartum	11.2 ± 1.8	10.0 ± 2.6	9.0 ± 1.6	CvsL	6	.78
				LvsH	5	.62
				CvsH	7	1.90
				CvsTr	10	1.58
Mean weight of pups at 48 hours (gms)	6.5 ± 1.0	8.0 ± 0.4	6.6 ± 1.3	CvsL	6	2.46*
				LvsH	5	1.75
				CvsH	7	0.13
				CvsTr	10	1.05

*** P<0.001.
** P<0.01.
* P<0.05.

Table 6 (continued).

Measurement	Group Means $\bar{X} \pm$ st. dev.			Comparison	df	t-value
	control (c)	Treated (Tr) low (L)	Treated (Tr) High (H)			
Mean weight of pups at 1 week of age (gms)	13.9 ± 2.0	17.8 ± 1.4	14.4 ± 2.3	CvsL	6	2.87*
				LvsH	5	2.18
				CvsH	7	0.35
				CvsTr	10	1.40
Mean weight of pups at 3 weeks of age (gms)	28.7 ± 2.8	32.7 ± 3.0	29.4 ± 4.0	CvsL	6	1.88
				LvsH	5	1.17
				CvsH	7	0.33
				CvsTr	10	1.07
Mean weight of pups at 2 weeks of age (gms)	49.0 ± 4.6	55.9 ± 2.4	52.1 ± 8.2	CvsL	6	2.35
				LvsH	5	0.75
				CvsH	7	0.72
				CvsTr	10	1.41
Weight gain of pups from 48 hours till 3 weeks of age (gms)	42.6 ± 3.8	47.9 ± 2.8	45.5 ± 7.6	CvsL	6	2.09
				LvsH	5	0.51
				CvsH	7	0.77
				CvsTr	10	1.35

Table 7. Two-tail t-test comparisons of experimental subjects' weight and age values.

Measurement	Group Means $\bar{X} \pm$ st. dev. control (c)	Treated (Tr) low (L)	Treated (Tr) High (H)	Comparison	df	t-value
Weight of Ss at 8 weeks of age. (gms)	258±35	287±25	268±32	CvsL	14	1.72
				LvsH	12	1.21
				CvsH	16	0.57
				CvsTr	22	1.31
Weight of Ss at beginning of training period. (gms)	555±39	584±29	559±64	CvsL	14	1.57
				LvsH	12	0.88
				CvsH	16	0.17
				CvsTr	22	0.76
Weight gain of Ss from 3 weeks of age till beginning of training period. (gms)	506±39	528±28	507±61	CvsL	14	1.21
				LvsH	12	0.78
				CvsH	16	0.04
				CvsTr	22	0.54
Age of Ss at beginning of training period. (days)	182±3	181±3	183±2	CvsL	14	0.97
				LvsH	12	2.23*
				CvsH	16	1.34
				CvsTr	22	0.19

* $P<.05$.

Table 8. Number of days required to reach criterion for visual discrimination problems.

Rat	Discrimination problem 1	2	3	4	5	6
Criterion	90%	90%	80%	80%	75%	75%
Control						
51	3	5	39	19	69[a]	34[a]
52	3	16	41[a]	25	63[a]	34[a]
55	3	14	43[a]	21	67[a]	34[a]
56	3	5	52[a]	27	61[a]	34[a]
59	3	14	52[a]	17	71[a]	34[a]
62	3	15	42[a]	27	61[a]	34[a]
63	3	21	36[a]	25	63[a]	34[a]
66	3	11	46[a]	14	74[a]	34[a]
67	3	14	43[a]	--[b]	--[b]	--[b]
$\bar{X} \pm$ st. dev.	3.0±0.0	12.0±5.4		21.9±4.6		
Low						
71	3	7	50[a]	25	63[a]	34[a]
72	4	39	18[a]	--[c]	59[a]	34[a]
74	3	14	43[a]	20	68[a]	34[a]
76	3	26	31[a]	13	75[a]	34[a]
79	3	11	33	19	69[a]	34[a]
80	3	9	46	39	49[a]	34[a]
$\bar{X} \pm$ st. dev.	3.2±0.4	17.7±12.4		23.2±9.8		
High						
82	3	12	65[a]	16	72[a]	34[a]
85	3	14	43[a]	--[b]	--[b]	--[b]
87	3	22	35[a]	27	61[a]	34[a]
90	3	16	41[a]	15	73[a]	34[a]
92	3	17	40[a]	10	78[a]	34[a]
93	3	9	48[a]	40	48[a]	34[a]
94	3	18	39[a]	21	67[a]	34[a]
96	5	17	40[a]	29	59[a]	34[a]
$\bar{X} \pm$ st. dev.	3.3±0.7	15.6±4.0		22.6±10.2		

[a]Subject failed to achieve criterion in alloted time.
[b]Subject expired.
[c]Data not gathered because of recording error.

Table 9. Two-tail t-test comparisons of visual discrimination testing results. (Days to Criterion)

Measurement	Group Means $\bar{X} \pm$ st. dev.			Comparison	df	t-value
	control (c)	Treated (Tr) low (L)	Treated (Tr) High (H)			
Days to criterion Problem 1	3.0 ± 0.0	3.2 ± 0.4	3.3 ± 0.7	CvsL	14	1.32
				LvsH	12	0.26
				CvsH	16	1.13
				CvsTr	22	1.16
Days to criterion Problem 2	12.0 ± 5.4	17.7 ± 12.4	15.6 ± 4.0	CvsL	14	1.27
				LvsH	12	0.44
				CvsH	16	1.57
				CvsTr	22	1.49
Days to criterion Problem 4	21.9 ± 4.6	23.2 ± 9.8	22.6 ± 10.2	CvsL	12	0.35
				LvsH	10	0.12
				CvsH	14	0.18
				CvsTr	19	0.27

Table 10. Two-tail t-test comparisons of visual discrimination testing results.

Measurement	Group Means $\bar{X} \pm$ st. dev.			Comparison	df	t-value
	control (c)	Treated (Tr) low (L)	Treated (Tr) High (H)			
Percent correct mean scores for first four sessions of problem 2	78.6 ± 10.1	74.2 ± 6.7	75.3 ± 6.5	CvsL	14	0.95
				LvsH	12	0.31
				CvsH	16	0.81
				CvsTr	22	1.14
Percent correct mean scores for first four sessions of problem 4	34.0 ± 8.8	41.0 ± 8.6	42.6 ± 9.2	CvsL	12	1.44
				LvsH	10	0.30
				CvsH	14	1.90
				CvsTr	19	2.08[a]
β values of learning curve for first 20 days of problem 4	10.49 ± 2.80	7.98 ± 1.94	8.37 ± 1.50	CvsL	12	1.77
				LvsH	10	0.39
				CvsH	14	1.80
				CvsTr	19	2.35*

[a] t-value of 2.09 required for significance ($P<.05$).
* $P<.05$.