# Hyperactivity: A Lead-Induced Behavior Disorder

by E. K. Silbergeld* and A. M. Goldberg*

**Mice were exposed to lead from birth by substituting solutions of lead acetate for the drinking water of their mothers. The suckling mice were thus exposed to lead through their mother's milk and, at weaning, directly through the drinking water. Controls received equal concentrations of sodium acetate. No deaths of offspring or mothers occurred during the first 90 days of exposure. It has been suggested recently that lead exposure may account for some incidences of behavior disorders in children. Levels of motor activity of individual offspring were measured from weaning until 70 days of age in specially designed activity cages. Lead-treated mice were more than three times as active as age-matched or size-matched controls.**

**Treated and control animals were administered drugs currently used in the treatment and diagnosis of hyperactivity in children. All control animals responded as expected to all drugs used in this study. However, lead-treated mice responded paradoxically to *d*- and *l*-amphetamine, methylphenidate, and phenobarbital. That is, the CNS stimulants suppressed their hyperactivity while phenobarbital exacerbated the lead-induced hyperactivity. These findings suggest that lead produces an animal model of hyperactivity which may have clinical relevance and which may explain some cases of hyperactivity in children.**

Clinical experience has shown a relationship between lead intoxication and behavioral disorders in children (*1–4*). These disorders are manifest as irritability, restlessness, and aggressiveness, but no experimental evidence has been previously advanced to support or deny the clinical experience. Although animal studies employing high levels of lead exposure have produced significant organ damage (*5*), encephalopathies (*6, 7*), and neuromuscular impairment (*8, 9*), no behavioral dysfunction due to lead has been induced. This report presents and characterizes the results of a study demonstrating a significant behavior disorder in mice resulting from chronic ingestion of lead.

## Methods

The animal model of chronic lead poisoning developed in this study follows a design similar to that first devised by Pentschew and Garro (*10*) and Rosenblum and Johnson (*6*). The route of exposure in these studies, and the present one, is indirect. Lead is administered to the mother and through her to nursing offspring which are the subject of study. In this research, lead acetate was dissolved in boiled distilled water and

*Department of Environmental Medicine, Johns Hopkins University School of Hygiene and Public Health, 615 N. Wolfe Street, Baltimore, Maryland 21205.

supplied for drinking water to pregnant CD-1 mice (Charles River Lab) upon parturition. Three concentrations of lead were used: 2, 5, and 10 mg/ml. Controls received equal concentrations of sodium acetate. Litters were normalized to six animals 48 hr after birth. Upon weaning, the offspring were separated by sex and housed by litter. At that time, six litters of mice (36 animals) from mothers on lead solutions, (two from each treatment level) were placed on tap water, while the others were continued on the same solutions which had been supplied to their mothers. Growth and development of the offspring were monitored from birth to 60 days of age.

Between 30 and 150 days of age, the offspring were individually tested for levels of motor activity. Motor activity was measured by placing a grid arrangement of 2 in square electrosensitive plates under a cage similar to those in which the animals were housed (*9*). Basically, the unit of activity measured was the crossing by the mouse from active to ground square. In all experiments offspring from control and treated mothers were simultaneously tested. In the first 21 experiments, animals were measured for activity for 3 hr on four consecutive days. After having established a pattern of activity, subsequent experiments were run for 3 hr consecutively on only one day.

The data to be presented were obtained on over 150 animals from over 30 litters developed at four different times during the calendar year.

## Results

Growth and development were significantly affected in offspring of mothers given lead solutions to drink. A dose-related depression in growth (Fig. 1) from day 5 through day 60 was observed in mice treated with 2, 5, and 10 mg/ml lead acetate. Sauerhoff and Michaelson (*11*) reported that this may be due to decreased food intake by the mother. However, in this study we observed no weight loss by the mothers during the suckling period. At weaning, 12 animals from

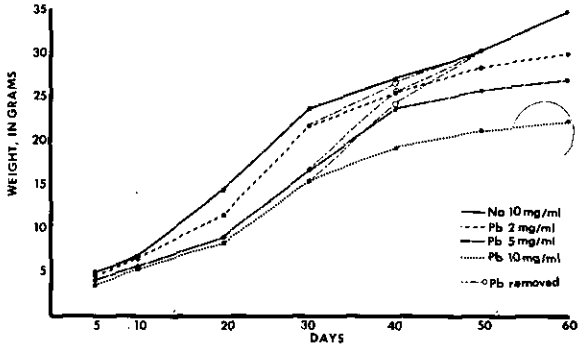


FIGURE 1. Effects of lead on growth of mice. Points are means for 30–40 animals. Differences in weight for all doses and controls are significant from day 20 to day 60 ($P<0.01$) except for controls and 2 mg/ml animals on day 40. Data of Silbergeld and Goldberg (*9*).

each treatment level were placed on tap water. These offspring reached near control weights within 10 days after removal of lead. Offspring continued on the same lead solutions as supplied to their mothers prior to weaning remained significantly smaller than coetaneous controls. Development landmarks were greatly retarded in mice receiving lead (Table 1). Eye opening, full incidence of body hair, coordinated walking, and weaning were delayed by as much as 8 days in the lead-treated offspring as compared to either controls or strain data.

At 30 days of age, offspring maintained after weaning on solutions of 5 and 10 mg/ml lead acetate began to show motor deficits and the suggestion of behavioral disorders due to lead. Some of these animals developed noticeable peripheral ataxia of their hind limbs and a characteristic splayed gait (Fig. 2). Observation of these animals also indicated a heightened reactivity and an increased frequency of fighting as determined by the incidence of bites on littermate males housed together.

Ingestion of lead significantly increased levels of motor activity in mice (Fig. 3). This figure shows results of three consecutive days of activity testing in 21 mice (40–60 days old) maintained before and after weaning on 10 mg/ml lead acetate, as com-

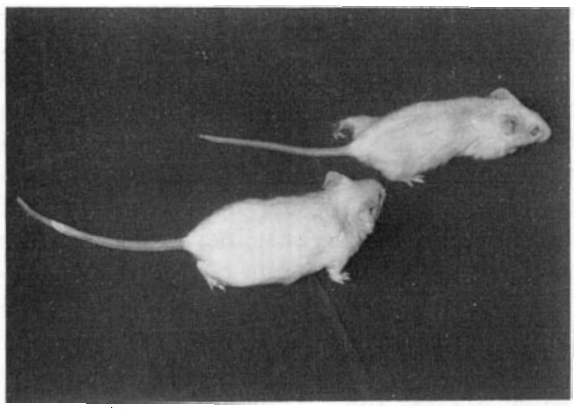

FIGURE 2. Peripheral neuromyopathy of lead. At top of photograph is a 40-day old mouse maintained before and after weaning on 10 mg/ml lead acetate. Lower animal is coetaneous control.

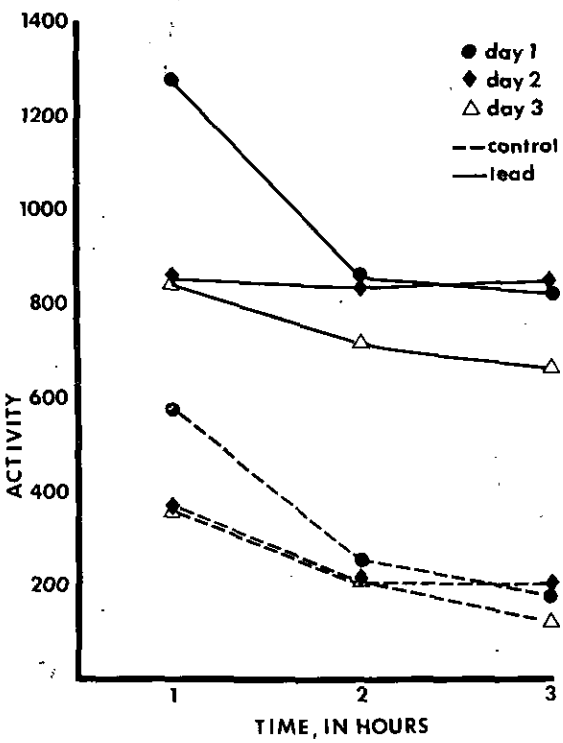


FIGURE 3. Effects of lead on motor activity (counts per hour) of 40- to 60-day-old mice given 10 mg/ml lead acetate before and after weaning. Results are for 21 treated and 21 control mice tested on three consecutive days. Differences between controls and treated are significant at $P<0.001$. Data of Silbergeld and Goldberg (*9*).

pared to 21 coetaneous controls. During the first hour of activity measurement of day 1, the treated mice had activity levels above 1200 counts/hr. Control mice had motor activity levels less than 600 counts/hr during the same measurement period. Both groups habituated comparably to the measurement conditions, as demonstrated by a sharp decrease in motor activity over the second hour of measurement. Nevertheless, lead-treated animals remained significantly more active than controls during the second hour and, indeed- for the rest of the measurement periods over which they were tested. In fact, treated mice were slightly more than three times as active as controls.

Increases in motor activity were seen in mice treated before and after weaning with 2, 5, and 10 mg/ml lead acetate. There were no differences in the degree of heightened motor activity of 40 to 60 day-old mice treated with any concentration of lead in this range (Table 2).

To see whether or not the maturity of the

**Table 1. Developmental landmarks in lead-treated and control mice.**

| Landmark | Day of occurrence: Strain Data[a] | Controls | Lead (10 mg/ml) |
|---|---|---|---|
| Eye opening | 14 | 13 | 17 |
| Full incidence of hair | 16–17 | 16 | 20 |
| Coordinated walking | 17–18 | 18 | 25 |
| Weaning | 21 | 22 | 30 |

[a] Information on CD-1 mice supplied by Charles River Laboratories. Data of Silbergeld and Goldberg (*12*).

**Table 2. Motor activity levels in mice treated with 2, 5, and 10 mg/ml lead acetate and controls.**

| Concentration of lead acetate in drinking water, mg/ml | Number | Motor activity, counts/hr (for hour 2, day 1)[a] |
|---|---|---|
| 2 | 6 | 845±126 |
| 5 | 20 | 942± 80 |
| 10 | 21 | 825± 40 |
| 0 | 21 | 310± 25 |

[a] Means ± standard errors of the mean. Data of Silbergeld and Goldberg (*12*).

offspring influenced activity, a series of experiments was performed on animals between the ages of 30 to 150 days. Offspring receiving 5 mg/ml lead acetate were tested for activity against coetaneous controls. All lead-treated animals were at least three times as active (Fig. 4). Further, the highest level of activity of either group was at 30 days. However, the level of activity of 30-day-old controls (556 ±75 counts/hr) was not as great as that in the lead-treated mice at any time period.

The drugs selected for study were chosen for their relevance in the treatment of what has been called minimal brain dysfunction hyperactivity in children (*13, 14*). All drugs were dissolved in distilled water and administered IP. In control mice, *d*- and *l*-amphetamine at 10 mg/kg produced significant increases in motor activity accompanied by stereotypic behavior such as chewing, gnawing, and drooling. Methylphenidate (40 mg/kg) also increased activity of control mice. The sedative drugs phenobarbital at 20 mg/kg and chloral hydrate (100 mg/kg) decreased motor activity as expected (Fig. 5).

In lead-induced hyperactive mice, paradoxical responses to CNS stimulants and the barbiturate phenobarbital were observed (Fig. 5). In these mice, *d*-amphetamine significantly suppressed motor activity to about 1/10 of the predrug baseline. Further, there were no observable signs of stereotypic behavior. Both methylphenidate and *l*-amphetamine also decreased the activity of lead-treated hyperactive mice, although to a lesser degree than *d*-amphetamine at the doses used. Phenobarbital produced a very marked increase in the level of activity of these mice. At 20 mg/kg, phenobarbital stimulated their already high levels of activity to 2000 counts/hr, the highest seem with any group on any treatment. Chloral hydrate depressed activity to the same degree in treated and control mice with the onset and duration of complete sedation the same in both groups. It is interesting to note that

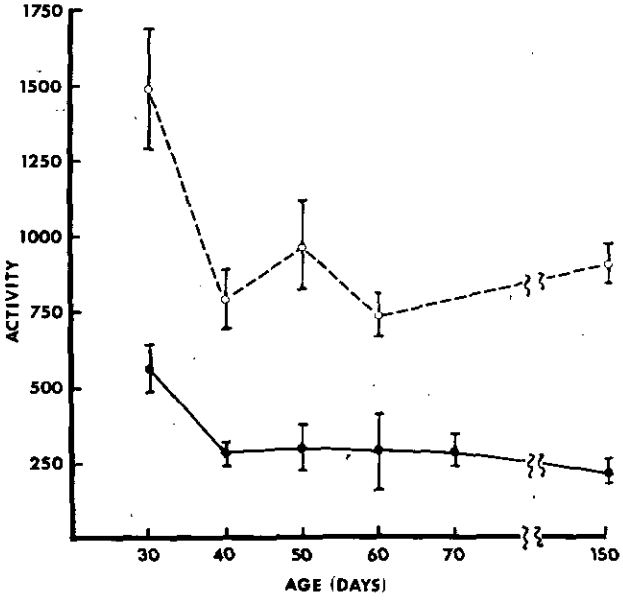


FIGURE 4. Effects of lead on motor activity (counts/hr) of 30- to 150-day-old mice given 5 mg/ml lead acetate before and after weaning. Results are for the second hour of measurement with each point representing the mean ± S. E. M. for 5–16 animals. Differences between controls (solid line) and treated are significant at $P<0.001$.

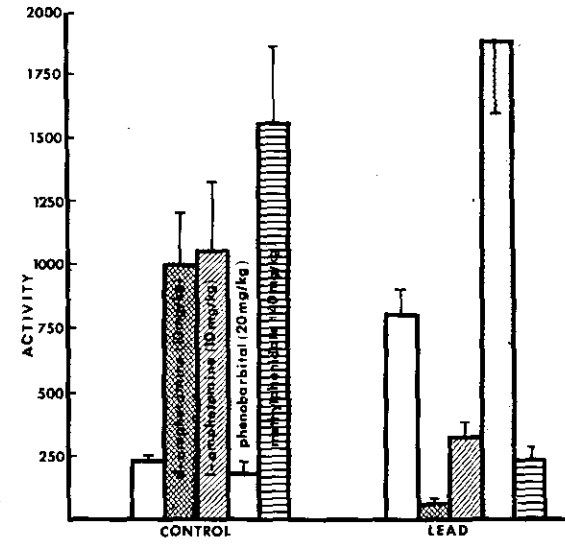


FIGURE 5. Effects of drugs on motor activity (counts/hr) in control and treated mice. Open bars represent predrug baselines in both groups. Results are expressed as means ± S.E.M. of at least seven animals. Differences in response to all drugs between control and treated mice were significant at $P<0.001$. Data of Silbergeld and Goldberg (*15*).

chloral hydrate is the sedative of choice in childhood hyperactivity (*13*).

Figure 6 shows the results of a dose–response study for *d*-amphetamine on controls and treated mice. In control animals, *d*-amphetamine produced a dose-related increase in motor activity with stereotypic behavior apparent at the higher doses. In the lead-induced hyperactive mice, *d*-amphetamine at doses of 2 and 5 mg/kg slightly increased levels of motor activity. At 10 mg/kg, *d*-amphetamine completely suppressed motor activity with no observable signs of stereotypic behavior.

## Discussion

These experiments demonstrate that chronic ingestion of lead, supplied through the drinking water, can produce a significant behavioral disorder in mice, i.e., a significantly increased level of motor activity. This hyperactivity was seen in animals exposed to lead from birth and persisted for at least 150 days. All animals tested were chosen for lack of any overt signs of neuromuscular damage, although they were significantly smaller than controls of the same age.

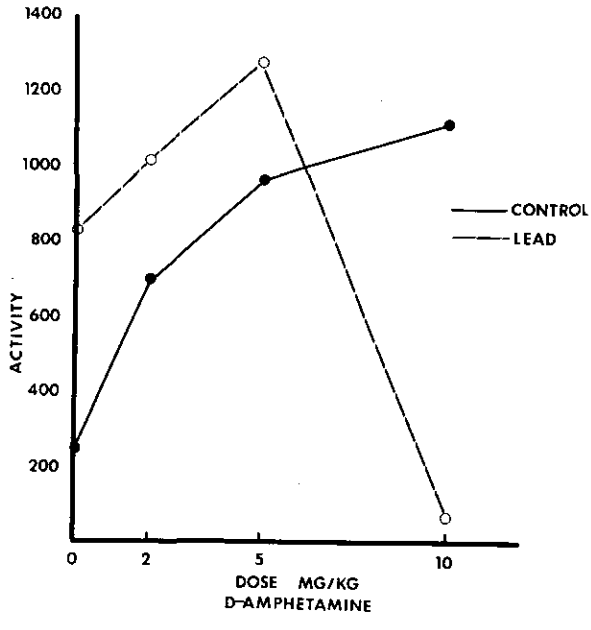


FIGURE 6. Motor activity response, (counts/hr) to *d*-amphetamine in control and treated mice. Each point is the mean of seven animals. Data of Silbergeld and Goldberg.

Lead-induced hyperactivity was not apparently dose-related, at least not in the range of doses used in these experiments. This would suggest that increases in motor activity may be an early symptom of lead poisoning associated with low level intoxication. Increases in the amount of lead to which animals are exposed do not change this symptom but rather may induce other sequelae such as peripheral ataxia, encephalopathies, and death. Aniamls treated with 10 mg/ml lead acetate did in fact begin to die by day 90.

The response to *d*-amphetamine in lead-induced hyperactivity is similar to that observed in childhood hyperactivity. In the latter, it has been observed that the dosage of amphetamine must be increased until an effective dose is reached. In these lead-induced hyperactive mice a dramatic decrease in motor activity is obtained at 10 mg/kg. The resulting level of activity after *d*-amphetamine is lower than in control animals in the absence of any therapy.

In the drug studies reported, all control animals responded to all drugs in the predicted manner. However, the lead treated mice responded paradoxically to central nervous system stimulants in an analogous manner to hyperactive children.

Epidemiological evidence has recently been presented by David et al. (*4*) for the coincidence of hyperactivity with a history of lead exposure in children. Correlations between lead poisoning and the widespread childhood behavior disorder, hyperactivity or hyperkinesia, are most provocative, since the biochemical lesions involved in either lead poisoning or hyperactivity are not known. The importance of demonstrating behavioral disorder resulting from lead ingestion is therefore, threefold: (1) the development in an animal model of a behavioral aspect of lead poisoning permits further investigations into neurochemical mechanisms and possible therapies; (2) the results of this study support a causal relationship between chronic,

sublethal lead exposure and a serious behavioral disorder; and (3) the lead-induced behavioral disorder appears to have significant parallels with the pharmacology of minimal brain dysfunction hyperactivity in children which may allow use of this model in the further study and testing of therapeutic drugs of this disease.

## Acknowledgements

Methylphenidate (Ritalin) was generously supplied by Ciba-Geigy. The work was supported in part by EHS Grants 00034 and 00454.

### REFERENCES

1. Byers, R. K., and Lord, E. E. Late effects of lead poisoning on mental development. Am. J. Dis. Child. 66: 471 (1943).
2. Chisolm, J. J., and Harrison, H. E. The exposure of children to lead. Pediatrics 18: 943 (1956).
3. de la Burdee, B., and Choate, M. S. Does asymptomatic lead exposure in children have latent sequelae? J. Pediatr. 81: 6 (1972).
4. David, O., Clark, J., and Voeller, K. Lead and hyperactivity. Lancet (1): 900 (1972).
5. Goyer, R. A., et al. Lead dosage and the role of the intranuclear inclusion body. Arch. Environ. Health 20: 705 (1970).
6. Rosenblum, W. I. and Johnson, M. G. Neuropathologic changes produced in suckling mice by adding lead to the maternal diet. Arch. Pathol. 85: 640 (1968).
7. Thomas, J. A., Dallenbach, F. D., and Thomas, M. Considerations on the development of experimental lead encephalopathy. Virchows Arch. Path. Anat. 352: 61 (1971).
8. Silbergeld, E. K. Effects of lead on neuromuscular function. Fed. Proc. 32: 275 (1972).
9. Silbergeld, E. K., Fales, J. T., and Goldberg, A. M. Evidence for a junctional effect of lead on neuro-muscular function. Nature 247: 49 (1974).
10. Pentschew, A., and Garro, F. Lead encephalomyelopathy of the suckling rat and its implications on the porphyrinopathic disease. Acta Neuropathol. (Berlin) 6: 266 (1966).
11. Sauerhoff, M. W., and Michaelson, I. A. Effect of inorganic lead on monoamines in brain of developing rat. Pharmacol. 15: 2, 55 (1973).
12. Silbergeld, E. K., and Goldberg, A. M. A. lead-induced behavior disorder. Life Sci. 13: 1275 (1973).
13. Wender, P. H. Minimal Brain Dysfunction in Children. Wiley-Interscience, New York, 1971.
14. Millichap, J. G. Drugs in management of minimal brain dysfunction. In Minimal Brain Dysfunction, F. de la Cruz, B. H. Fox, and R. H. Roberts, Eds. (Ann. N.Y. Acad. Sci. 205): New York Academy of Sciences, New York, 1973, pp. 321–334.
15. Silbergeld, E. K. and Goldberg, A. M. Lead-induced behavioral dysfunction: an animal model of hyperactivity. Expl. Neurol. 42: 146 (1974).