Pathobiological and Behavioral Effects of Lead Intoxication in the Infant Rhesus Monkey

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When infant rhesus monkeys were exposed to lead via the addition of lead acetate (0.5–9 mg/kg body weight) to their formula or by the consumption of lead particles from lead-based surrogate mothers, they developed symptoms of lead intoxication within 6 weeks. Seizures, muscular tremors, and altered social interaction were the predominant changes. Visual impairment was also apparent in the more severely affected animals. In the animals showing obvious symptoms lead levels varied between 300 to 500 μg/100 ml of blood. Even in those animals having blood lead levels below 100 μg, hyperactivity and insomnia were observed. When the exposure to lead was eliminated, seizures subsided and visual impairment was reduced; however, the abnormal social interaction persisted. These animals also experienced a gradual decline in hematocrit and hemoglobin values during the period of examination. Liver and kidney biopsies obtained from these lead-exposed animals revealed characteristic intranuclear inclusions.

When adolescent and adult monkeys were exposed to doses of lead acetate similar to those employed in the infant experiments, lead levels in excess of 200 μg/100 ml of blood were recorded. However, there were no obvious behavioral abnormalities observed. There were, however, numerous lead inclusion bodies in kidney biopsy specimens from these animals.

These data suggest that, like man, the infant nonhuman primate is much more susceptible to lead intoxication than is the adult. The clinical and behavioral changes recorded in these infant rhesus monkeys suggest their use as an experimental model to evaluate lead intoxication.

The injurious effects of excessive exposure to lead by man and lower animals have been known since ancient times. In spite of recent widespread educational campaigns, lead intoxication remains a major health problem. Lead-based paints, lead-glazed earthenware, illicitly distilled whiskey, and industrial exposure to lead continue to give rise to lead intoxication in the human population. Water, food, and air have been shown to contain increasing levels of lead, particularly in urban areas. The increased concern about lead in the environment is well

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founded when one considers that from car exhausts alone, over 200,000 tons of lead are released into the air yearly (*1*).

Although lead intoxication has been recognized as a health problem for many years, there is a great deal to be learned concerning the mechanism of action of lead on various body tissues and organelles. One of the major problems encountered by the researcher is the establishment of a suitable experimental animal model with which to study lead intoxication. In this report, observations concerning the pathobiological and behavioral changes that develop in rhesus monkeys exposed to lead are presented. Neurological disturbances, including convulsions, blindness, muscular tremors, and altered behavioral patterns developed in infant rhesus monkeys. Since the changes observed in the infant rhesus monkey following exposure to lead are quite similar to those reported to occur in children, it is suggested that the rhesus monkey is a potentially suitable animal model with which to evaluate lead intoxication.

Experimental Observations

The response of the rhesus monkey to lead intoxication has been under investigation in our laboratory during the past 3 years. In the initial experiment, infant rhesus monkeys were housed for 3 months with surrogate mothers having a lead base. (A surrogate mother is a cloth covered metal cylinder used as a substitute mother after the infant is removed from its natural mother.) Throughout this period the infants scratched, licked, and rubbed these lead bases. Within two months they began to have recurring convulsions of short duration. In addition, there was a constant blinking of their eyelids and indications of confusion. During this period there also was a decrease in the physical activity and social interaction of these monkeys. One of the affected animals developed visual impairment. His pupils were unresponsive to light and moving objects. He moved around the cage with his arms extended, using his hands to feel the way.

Systematic observation of the behavior of these animals was conducted. This evaluation consisted of measuring the frequency and duration of animal behaviors in nine categories (*2*). A description of the behaviors encompassed in each category follows.

Ventral cling: contact of own ventral body surface with another animal.

Self-clasp: clasping of any part of own body with hand(s) and/or feet.

Self-mouth: oral contact to own body.

Rock and huddle: self-clasping accompanied by stereotypic rocking.

Locomotion: any self-induced change in physical location of self.

Environmental exploration: tactual and/or oral manipulation by the monkey of inanimate objects, for example, towel, toy, cage, lock, etc.

Social explore: tactual and/or oral contact with another monkey other than ventral clinging.

Vocalization: any sound emitted by the animal.

Sex and play: any sexual advances or posturing directed toward another animal and/or any type of socially directed play activity (including rough-and-tumble, approach-withdraw, and noncontact type play).

The lead-exposed animals showed significant differences from control animals in the above mentioned behavioral categories (Figs. 1–5).

Lead blood levels determined on these animals following two months of exposure to the surrogate mothers ranged between 160 and 400 μg/100 ml blood as compared to the 10–20 μg in the control animals (*3, 4*).

Liver and kidney biopsies on these animals revealed numerous intranuclear lead inclusions in the tubular epithelium of the kidney (Fig. 6) and an occasional inclusion in the parenchymal cells of the liver.

Following the removal of the surrogate mothers, the convulsions gradually subsided. However, the animals continued to show reduced social interaction and abnormal behavioral patterns. Persistent vomiting, sucking on their toes and genitalia, self clasping,

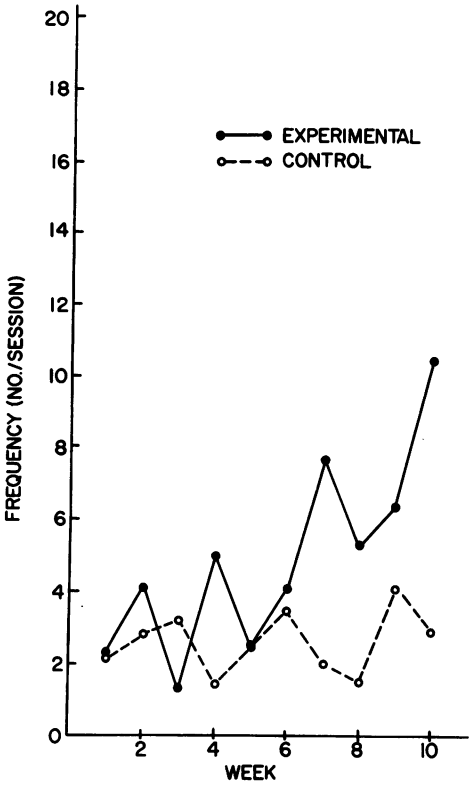

FIGURE 1. Frequency of ventral cling in lead-exposed infant rhesus monkeys: (●) experimental; (○) control.

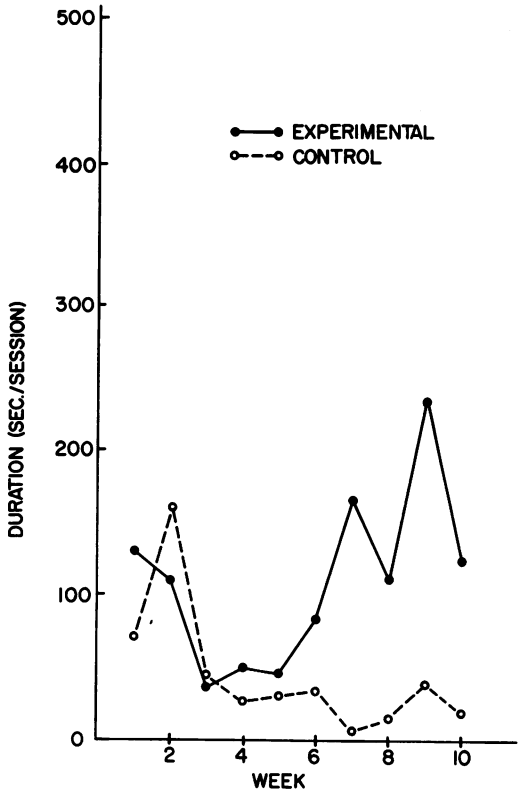

FIGURE 2. Duration of ventral cling in lead-exposed infant rhesus monkeys: (●) experimental; (○) control.

and hovering in the corner of their cages were common features of these animals. The infant monkey that suffered visual impairment showed some improvement in his eyesight; however, his general activities suggested a permanent decrease in vision.

Lead acetate at varying concentrations was administered to infant rhesus monkeys in their formula (Similar, Ross Laboratories, Columbus, Ohio). Animals received either 3–4 mg or 9–10 mg lead acetate/kg body weight per day. Blood lead levels of 500 μg/100 ml blood were attained within 3 weeks at the higher concentration and within 5 weeks at the lower level. When blood lead levels approached 300 μg the animals developed intermittent clonic convulsions. In addition, their interaction with other animals was reduced, they exhibited abnormal jerky movements, and they screeched incessantly. After 3 and 5 weeks the lead was removed from the formula of the high and low lead animal groups, respectively. The average total lead intake during this period was 85 mg for the low level group and 90 mg for the high level group. Even though the convulsions disappeared within ten days, abnormal behavioral patterns persisted. These animals also exhibited a gradual decline in the levels of hemoglobin and hematocrits which began while the animals were on the lead-supplemented died and continued to decrease after the lead was eliminated (Table 1). Four weeks after lead was removed from the diet, blood lead levels of these animals remained elevated, exceeding 100 μg/100 ml blood (Table 1).

In a subsequent experiment, infants given 0.5 mg lead acetate/kg of body weight per day developed blood lead level of 60–100 μg lead/100 ml blood within 4 weeks. At these levels, the animals did not have convulsions;

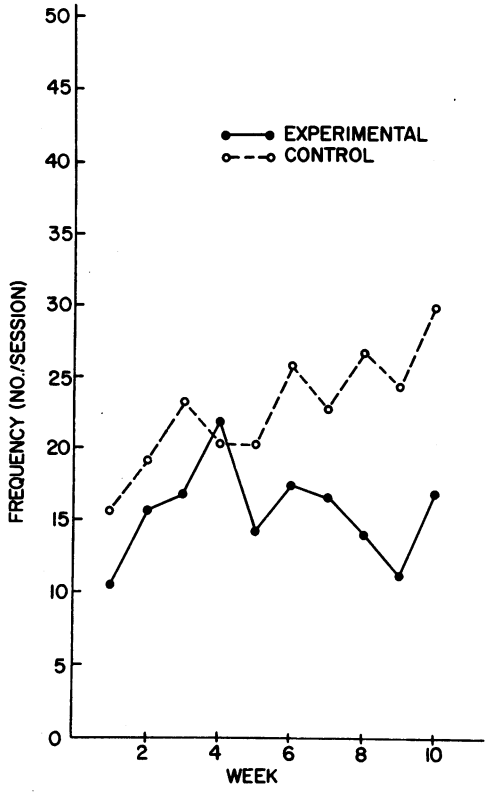

FIGURE 3. Frequency of social explore in lead-exposed infant rhesus monkeys: (●) experimental; (○) control.

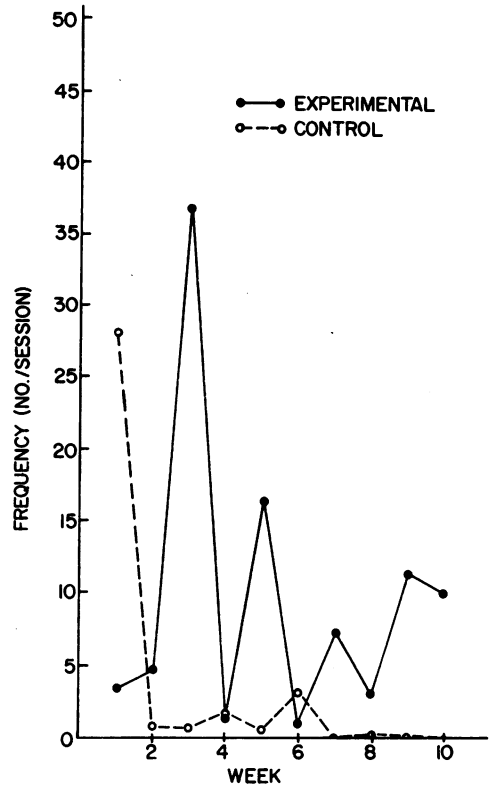

FIGURE 4. Frequency of vocalization in lead-exposed infant rhesus monkeys: (●) experimental; (○) control.

however, they displayed hyperactivity, insomnia, and a gradual decline in hemoglobin and hematocrit values.

In order to compare the response of infant and juvenile monkeys to lead exposure, the latter were given lead acetate in their drinking water at a sufficient level to provide 20 mg lead/kg body weight per day. This level was sufficient to establish a mean blood level of 135 μg lead/100 ml blood within 4 weeks. During this period, the animals consumed a total of approximately 2.5 g of lead. Throughout the 30-day trial period, food intake, physical status, and behavioral patterns of these animals remained unaltered. Hemograms and serum chemistry determinations conducted on a weekly basis throughout the trials were not modified (Table 2). After 30 days the monkeys were sacrificed and the tissues evaluated grossly, microscopically, ultrastructurally, and biochemically. Grossly, all organs appeared normal. Microscopically and ultrastructurally, the only morphological change was an occasional intranuclear lead inclusion body in the renal tubular epithelium similar to that shown in Figure 6. Biochemically, there were no appreciable changes in the level of DNA, RNA, and protein of the liver homogenates obtained from the experimental animals (Table 3). In addition, microsomal protein, RNA, phospholipid, and cholesterol levels were quite similar in both the control and experimental livers (Table 4). Hepatic microsomal enzyme activity of aniline hydroxylase, *N*-demethylase, nitroreductase, glucose-6-phosphatase, and esterases were also not appreciably affected by the oral ingestion of lead acetate (Table 5). The procedures employed in these determinations have been previously reported (*5*).

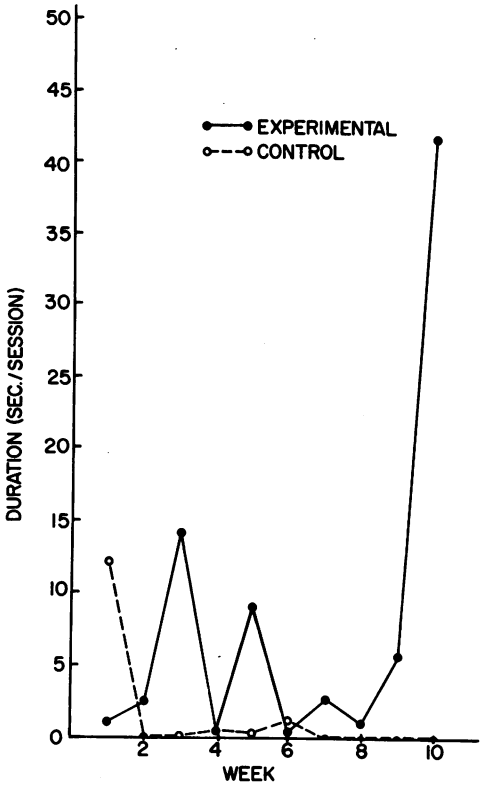

FIGURE 5. Duration of vocalization in lead-exposed infant rhesus monkeys: (●) experimental; (○) control.

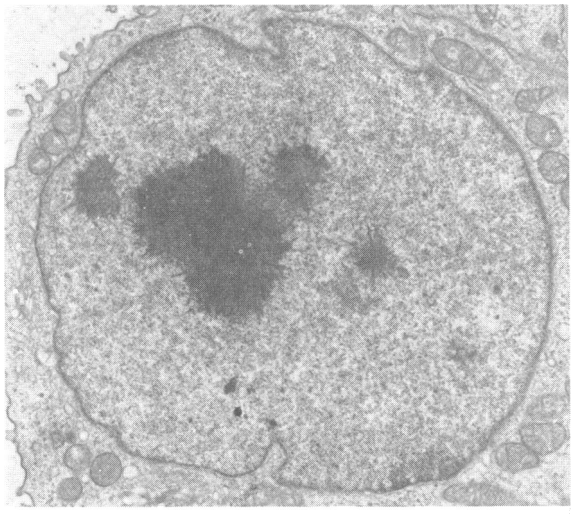

FIGURE 6. Intranuclear lead inclusions similar to that depicted in this figure were present in the renal tubular epithelium of monkeys exposed to lead based surrogate mothers for 2 months. Tissue obtained by a renal biopsy from an animal having convulsions and showing signs of visual impairment. Uranyl acetate stain; ×10,000.

Discussion

These data indicate that infant rhesus monkeys, like children, are more susceptible to the toxic effects of lead than are adolescents or adults. Infant monkeys exposed to a block of lead in their cage developed sev-

Table 1. Effects of lead ingestion on blood lead and hematocrit levels of infant rhesus monkeys.

Age, weeks	Control		Low lead (3–4 mg/kg-day)		High lead (9–10 mg/kg-day)	
	Blood lead, μg/100 ml	Hematocrit, %	Blood lead, μg/100 ml	Hematocrit, %	Blood lead, μg/100 ml	Hematocrit, %
1	17	48	20	47	11	48
2	20	38	74	38	113	40
3	20	37	87	36	400	34
4	12	41	247	34	508	33[a]
5	15	42	500	35	530	32
6	14	43	520	33[a]	130	29
7	11	38	183	35	120	30
8	11	40	94	34	92	32
9	14	39	102	33	95	32
10	15	40	100	33	90	34
11	17	39	74	34	85	35
12	12	40	65	35	80	35

[a] Lead removed from formula.

Table 2. Hemogram and serum chemistry of adolescent rhesus monkeys exposed to lead for 1 month.

Analysis	Pretreatment levels [a]	Terminal levels [a]
Hemoglobin, g-%	12.6±0.9	12.2±0.7
Hematocrit, %	36.9±2.3	37.8±1.8
WBC×10³/mm³	6.8±1.5	6.6±1.1
Total protein, g-%	7.0±0.8	6.6±0.2
Albumin, g-%	4.4±0.3	4.5±0.2
BUN, mg-%	17.4±3.1	26.4±4.3
SGPT	24.0±7.9	35.8±6.8

[a] Values expressed as means ±1 standard deviation.

Table 3. Effect of lead on monkey liver.

	Control [a]	Experimental [a,b]
No. of animals	5	8
DNA, mg/g liver	2.99±0.38	3.11±0.24
RNA, mg/mg DNA	1.72±0.13	1.59±0.19
Protein, mg/mg DNA	94.50±15.0	110±10

[a] Values expressed as means ±1 standard deviation.

[b] Difference with controls not statistically significant.

Table 4. Effect of lead on liver microsomes.

	Control [a]	Experimental [a]
No. of animals	5	8
Protein, mg/g liver	19.4±4.4	14.9±3.3
RNA, μg/mg protein	76.6±23.1	107.4±10.8
Phospholipid, μg/mg protein	538±92	668±43
Cholesterol, μg/mg protein	15.5±4.6	23.8±3.2 [b]

[a] Values expressed as means ±1 standard deviation.

[b] Difference with controls statistically significant: $P<0.01$.

ere symptoms of lead intoxication within two months. It was also shown that infant rhesus monkeys become hyperactive and develop insomnia when given doses as small as 10 mg lead over a period of 30 days. Convulsions and altered behavioral patterns were recorded when less than 100 mg of lead was added to the diet of infant monkeys for one month.

Like the young child, the infant monkey becomes anemic following exposure to lead. The decrease in hemoglobin following a relatively short period of lead exposure persisted for many weeks in infant monkeys following the discontinuation of exposure. These reduced hemoglobin values could not be attributed to reduced food intake in that the animals continued to eat well and gained weight as rapidly as did the control animals. Attempts were made to determine if hemoglobin synthesis was related to altered aminolevulinic acid dehydratase (ALAD) activity. Unfortunately, when the usual procedures (*6*) are employed with monkey blood, the enzyme activity of ALAD in all rhesus monkeys regardless of their previous treatment is extremely low. As a result, these data were found to be of questionable value in evaluating effects of lead on hemoglobin synthesis. Similar observations have been made by others (*7*).

Table 5. Effects of lead on enzymatic activity of liver microsomes.

	Control [a]	Experimental [a]
No. of animals	5	8
Aromatic hydroxylase, nmole *p*-aminophenol/30 min	42.5±10.0	43.2±6.4
N-Demethylase, nmole formaldehyde/30 min	115±37	123.5±26.0
Nitroreductase, nmole *p*-aminobenzoate/hr	27.2±4.1	19.9±9.3
Glucose-6-phosphatase, μmole PO_4/15 min	1.89±.41	2.96±0.43 [b]
Esterase, μmole *p*-nitrophenol/min	4.45±1.90	5.45±1.20

[a] Values expressed as means ±1 standard deviation.

[b] Difference with controls statistically significant: $P<0.01$.

All of the behavioral studies conducted on the lead-exposed infant monkeys indicated a retardation of development and altered social interactions. In addition, the abnormal behaviors manifested during the neonatal period did not disappear as the animals grew older. Microscopic examination of the tissues of the central nervous system will establish if this retarded be-

havioral development was related to lesions in the brain similar to those reported to occur in man and lower animals (*8–12*).

When juvenile monkeys were given 2.5 g of lead for one month no change in the behavior or physical status of the animals was recorded. These observations were substantiated by light and electron microscopic evaluation of the tissues and biochemical studies of the liver of the lead intoxicated animals. Similar observations on the seemingly resistant nature of adult rhesus monkeys to lead intoxication have been reported. A recent report from the American Petroleum Institute (*13*) described adult male and female rhesus monkeys that were intubated with 1.25 and 25 mg lead acetate/kg daily for 22 months. These animals showed no gross changes in appearance, behavior, or body weights. Analyses of blood and urine were also normal. Goode and Calandra (*7*) fed rhesus monkeys 0.05, 0.5, and 5 mg lead acetate/kg body weight daily for 30 months and recorded no changes in the physiological and behavioral status of the monkeys.

There are reports indicating that under certain conditions the adult monkey suffers lead intoxication. Zook et al. (*12*) reported on 34 cases of lead intoxication in nonhuman primates, all but four of which were Old World monkeys. It is interesting that 14 of the affected primates were juveniles that varied in age from 6 to 30 months, while the remaining 20 animals were adults. Fisher (*14*) reported a case of lead poisoning in the gorilla, and Cohen et al. (*15*) produced convulsions in baboons following the administration of lead. In addition, McIntosh (*16*) and Hausman et al. (*17*) reported cases of lead intoxication in adult nonhuman primates.

The precise reason for the variation in response of the two age groups to lead intoxication remains unclear. Undoubtedly, greater absorption and tissue deposition of lead and increased tissue susceptibility to its deleterious effects occur in infants. Goyer and Mahaffey (*18*) have recently reviewed the many factors that may be instrumental in determining the degree of toxicity to lead.

It is noteworthy that the clinical and behavioral changes that developed in the infant rhesus monkey are quite similar to those that occur in children suffering from lead intoxication. Convulsions, sluggishness, restlessness, hyperirritability, insomnia, visual disturbances, muscular twitching, tremors, ataxia, and vomiting are commonly recorded in lead-intoxicated children and are readily produced experimentally in the infant monkeys. These findings are of particular importance in that there is a great need for one animal model which will enable both the behaviorist and pathophysiologist to evaluate lead intoxication.

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