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Growth, Behavior, and Brain Catecholamines in Lead-Exposed Neonatal Rats: A Reappraisal

Abstract. *Daily oral administration of lead to newborn rats has no adverse effect on their body growth. Lead-treated rats were more active than age-matched controls. Endogenous levels of brain dopamine were unchanged, whereas norepinephrine was increased, suggesting a possible relationship between lead exposure during earliest developmental periods, increased motor activity, and brain norepinephrine, and not brain dopamine as previously postulated.*

Neurological manifestations of lead poisoning can be induced in suckling rats by feeding a diet containing 4.5 percent lead carbonate to the lactating mother rats. Lead is transmitted to the young via the maternal milk (*1, 2*). There is a pronounced retardation in growth rate in sucklings from lead-exposed mothers, and during the fourth week they develop paraplegia and extensive reddish-brown pigmentation of the cerebellum (*1–3*).

A lactating mother rat eating 5 percent lead acetate (2.73 percent lead) produces milk containing 25 parts of lead per million (ppm) (*4*). When the mother's diet is changed just prior to weaning from 5 percent lead acetate to one containing 25 ppm lead, and neonates are allowed free access to the same solid maternal diet, the sucklings still have retarded body growth but do not develop paraplegia or grossly apparent vascular damage of the cerebellum. However, during the fourth week these animals exhibit hyperactivity, tremors, and stereotyped behavior (*4–6*). It has also been reported that such behavior was manifest at a time when there is an eightfold increase in the concentration of lead in brain, and no apparent change in norepinephrine but a 20 percent decrease in dopamine relative to coetaneous controls (*6*).

The fact that the young, even after weaning to the lower lead diet, still have retarded body growth confounds the study by adding the factor of possible undernutrition to such an investigation.

We now report that the daily oral administration of lead acetate solution (1.09 mg of lead) to suckling rats does not influence growth relative to coetaneous controls and that lead-exposed animals show periods of increased motor activity. Unlike Sauerhoff and Michaelson, in their earlier report (*6*) from this laboratory, we are unable to detect a statistically significant lowering of brain dopamine levels, but we do find slight increases in norepinephrine.

The rats used in our study were timed pregnant Sprague-Dawley rats obtained from ARS/Sprague-Dawley Co., Madison, Wisconsin, and their offspring. The rats were housed as previously described (*4–6*). They were then divided into three groups, as follows:

Group A (controls): nursing mothers consuming normal powdered laboratory chow and tap water, and sucklings given daily oral doses of 0.1 ml of 2 percent sodium acetate and weaned on day 16 to normal diets;

Group B: nursing mothers consuming normal powdered laboratory chow and tap water, and sucklings receiving daily oral doses of 2 percent lead acetate (0.1 ml, 1.0 mg of lead) and weaned on day 16 to a diet containing 40 ppm lead;

Group C: nursing mothers consuming a diet containing 5 percent lead acetate, and tap water, changed at day 16 to a diet containing 40 ppm lead (*4*), and sucklings with no additional treatment, weaned on day 16 to a diet containing 40 ppm lead. Treatment of mothers with lead started on the day after birth of their offspring.

Nursing mothers and individual litters were weighed each day between 10:00 and 11:00 a.m. At 5 days of age each litter was reduced to six animals. Neonates of groups A, B, and C were allowed free access to food and water. This regimen was maintained until the end of the experiment.

Preparation of diets and analysis of brain and blood for lead were described earlier (*4, 6*). Spontaneous activity (*4*) and brain dopamine and norepinephrine (*7*) were analyzed as in the earlier report (*6*).

Body weight changes (growth) of newborn rats of the three groups are shown in Fig. 1. There was no difference in body weight gain of those young receiving daily oral doses of sodium acetate or lead acetate. They both had an average weight gain of 3 g per day from the time of birth until 34 days of age. A similar gain in daily weight has been observed in sucklings from mothers eating normal laboratory chow (*4, 5*). The newborns receiving nourishment from mothers eating a diet containing 5 percent lead acetate (group C), who are weaned to a diet containing 40 ppm lead, gain approximately 2.3 g per day, experiencing about a 30 percent depression in growth relative to groups A and B.

Treatment with sodium acetate (group A) did not have any effect on lead content of blood (0.9 μg per gram of packed cells) and brain (0.16 μg/g, wet weight) relative to that found previously in normal animals in this laboratory (*4–6*). As expected, exposure to lead results in increased concentrations of lead in blood as well as brain. Animals suckling lead-exposed mothers (group C) experienced a thirteen- and fourfold increase in the lead content of blood and brain respectively. Newborns fed daily with 1.0 mg of lead (group B) had five- and threefold increases in lead in blood and brain, respectively.

Table 1. Norepinephrine and dopamine concentrations (mean ng/g, wet weight, ± standard error of the mean) in brains of rats eating lead-containing diet (400 ppm lead, except for the 8-day-old rats) and pair-fed controls. Number of animals in parentheses.

Age (days)	Norepinephrine			Dopamine		
	Control (A)	Leaded (B)	Ratio (B/A)	Control (D)	Leaded (E)	Ratio (E/D)
8	189 ± 5 (6)			187 ± 7 (6)		
19	315 ± 9 (7)	330 ± 5 (7)	105	497 ± 14 (7)	445 ± 14 (7)	93
24	368 ± 9 (7)	386 ± 10 (7)	105	487 ± 15 (7)	488 ± 10 (7)	100
33	399 ± 11 (7)	450 ± 15 (7)	113*	616 ± 32 (7)	658 ± 20 (7)	107
51	448 ± 13 (6)	453 ± 12 (7)	101	686 ± 16 (6)	734 ± 28 (7)	107
65	470 ± 9 (6)	476 ± 7 (7)	101	787 ± 24 (6)	797 ± 21 (7)	101
81	520 ± 13 (7)	543 ± 10 (7)	104	881 ± 46 (7)	894 ± 30 (7)	101

* Student's t-test, $P = .05$.

At 5 and 6 weeks of age, six siblings from group A and six siblings from group B were tested for relative degree of spontaneous motor activity over a 24-hour period encompassing both dark and light cycles (12:12). Increases in motor activity of rats receiving lead acetate relative to those receiving sodium acetate were found in most instances.

Hyperactivity has likewise been seen by other investigators (*8*, *9*).

Brain concentrations of norepinephrine and dopamine were determined in half of each brain in control and lead-exposed, 40- and 41-day-old rats. The ingestion of lead from the time of birth until 41 days of age appeared to have no statistically significant effect on either brain norepinephrine or brain dopamine.

An earlier report from this laboratory (*4*) has shown that lead-exposed, as distinct from poisoned, developing rats are hyperactive and aggressive, and display stereotyped repetitive behavior. It was reported by Sauerhoff and Michaelson (*5*, *6*) that lead exposure had no effect on the concentration of brain norepinephrine but resulted in a 20 percent depression in brain dopamine. The present study reaffirms the finding of increased motor activity in lead-exposed animals but was unable to demonstrate any statistically significant differences in norepinephrine and dopamine in these animals compared to controls of the same age.

Culliton (*10*) has recently raised the question, What does one do when, seemingly all of a sudden, one can no longer repeat experiments done in one's own laboratory? Our laboratory has repeated Sauerhoff and Michaelson's experiment under the same conditions as previously reported (*6*), and measured steady-state levels of norepinephrine and dopamine in brains of leaded and pair-fed control rats. In this instance, experimental rats were weaned to, and maintained on, a diet containing 400 ppm lead, for 81 days (Table 1).

As in the earlier study, herein reported, there was no indication of lowered brain dopamine in lead-treated rats. On the contrary, relative to pair-fed controls our repeated findings suggest that there may be a small increase in catecholamines (*11*). It is worthy to note that Silbergeld and Goldberg (*12*) have found that steady-state levels of norepinephrine are increased 25 percent, while dopamine and acetylcholine levels are unchanged in lead-induced hyperactive mice. It is important to emphasize that the experimental animals are not suffering from lead intoxication in the usual sense of the word. Rather, this experimental design results in a lead-exposed animal leading to subtle changes in behavior. It is not unreasonable to suggest that one might suspect similar subtle changes in neurochemical components which might directly or indirectly be related to that behavioral parameter. A number of investigators have presented data supporting the hypothesis that norepinephrine and dopamine are involved in the central regulation of motor activity (*13*).

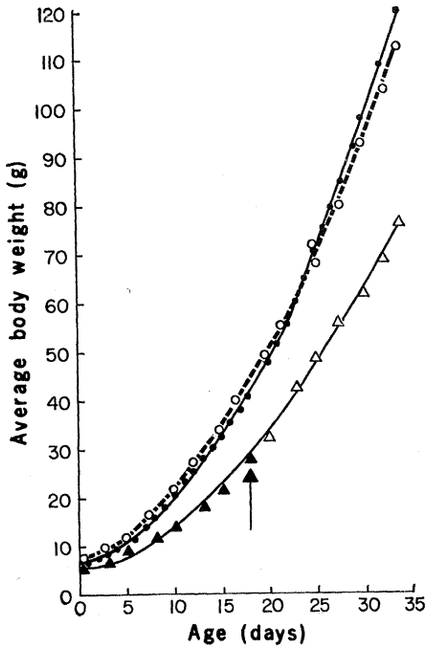

Fig. 1. Growth of young rats. Group A, receiving daily oral doses of sodium acetate and weaned to normal food (●——●); group B, daily oral doses of 1.0 mg of lead and weaned to a diet containing 40 ppm lead (○- - -○) compared to group C, suckling lactating mother rats eating 5 percent lead acetate (▲——▲) and weaned (at arrow) to a diet containing 40 ppm lead (△——△).

If a balance is the basis for a tendency toward equilibrium between the different but interdependent elements controlling a particular physiological function, then imbalance would lend to the unmasking of some aspect of that function. Therefore, one need not seek large changes in the concentration of neurotransmitter to account for behavior change. The intraventricular administration of norepinephrine produces an increase in locomotor activity, whereas dopamine is much less effective (*14*). Additional evidence indicates that dopamine, rather than norepinephrine, is associated with stereotyped behavior, while motility is more related to norepinephrine (*15*).

The observed 13 percent (this study) and 25 percent (*12*) increases in norepinephrine in brain within the period when animals have been observed as hyperactive are compatible with the reported observations.

One is not restricted to feeding lead to a lactating rat to achieve a mild

form of experimental lead encephalopathy in the young. Oral administration is feasible from the time of birth. The clinical significance of these studies is that a higher incidence of lead exposure in some hyperactive children relative to "normals" has been reported (*16*). It is possible that increases in brain norepinephrine and increased motor activity may be an early response to low-level lead exposure during early developmental periods and not a lowering of brain dopamine as previously postulated by Sauerhoff and Michaelson (*6*). It is worthy to note that similar findings of hyperactivity and increased brain levels of norepinephrine have been reported in lead-exposed mice (*8, 9, 12*). The experimental design as described provides a means to study the biological effects of lead exposure on neonatal developing rats without debilitating histopathologies (*1*) or excessive depression in body weight (*1–6*), thereby eliminating factors such as malnutrition, which could conceivably confound the results. This permits better-controlled investigations into the relationship between behavioral sequelae of lead poisoning and its neurochemical mechanisms.

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Secretion of a Nerve Growth Factor by Primary Chick Fibroblast Cultures

Abstract. *Normal primary chick embryo fibroblast cultures produce a nerve growth–promoting factor which cross-reacts with monospecific antibody to pure male mouse submaxillary gland nerve growth factor (NGF). When taken together with the earlier demonstration that mouse L_2 cells and 3T3 cells also produce an NGF-like protein, these findings suggest that secretion of this factor may be a general property of fibroblasts.*

Cultured mouse L_2 cells, 3T3 cells, and SV 3T3 (simian virus 40 transformed) cells secrete a protein which, according to biological activity and immunological criteria, is indistinguishable from pure male mouse submaxillary gland nerve growth factor (NGF) (*1*). These findings suggest that secretion of NGF, or a protein closely similar to it, may be a general property of fibroblasts. However, L_2 cells are malignant fibroblasts, 3T3 cells are aneuploid, and SV 3T3 cells are transformed fibroblasts. We have now turned to primary normal chick embryo fibroblast cultures, and we find that these also secrete a biologically active NGF which reacts with antibody to pure mouse NGF.

Male mouse submaxillary gland NGF was isolated as previously described (*1*). The protein was pure as judged by gel electrophoresis in three solvent systems and by immunoelectrophoresis (*1*). Dorsal root ganglia of chick embryos 10 to 14 days old were used to estimate the neurite outgrowth–producing effect of culture supernatants by minor modifications of the methods originally described by Levi-Montalcini *et al.* (*2*) and Hier *et al.* (*3*). Ganglia were placed on collagen-coated cover slips in petri dishes; the nutrient culture medium contained 90 percent Eagle minimal essential medium with Earle balanced salt solution (MEM, Gibco) plus 10 percent heat-inactivated fetal calf serum (Gibco). Preparations were examined microscopically after incubation for 18 to 24 hours in a humidified atmosphere containing 5 percent CO_2 at 37°C. A highly sensitive immunoassay employing T_4 bacteriophage was used to measure concentrations of NGF as low as 1 ng/ml. The details of this immunoassay, as well as the preparation of antisera to mouse NGF, have been described elsewhere (*1*). The basic feature of this assay is that when NGF is covalently coupled to bacteriophage, the infectivity of the virus (for *Escherichia coli*) can be blocked by antibody to NGF. Free, uncoupled NGF competes with the phage-NGF conjugate in the antibody reaction, and this forms the basis for the immunoassay. [See (*4*) for other references to this method.]

The preparation of monolayer fibroblast cultures of cells from decapitated 12-day chick embryos has been described (*5*). Cells dispersed by trypsinization were plated at an initial density of approximately 5×10^4 cells per square centimeter in 32-ounce (~ 95-ml) glass bottles (monolayer surface, 110 cm^2). After 5 days growth in Eagle basal medium (BME) supplemented with 3 percent calf serum, the cells (confluent) were washed twice with sterile saline (100 ml per wash), drained, and fed with serum-free BME. *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (0.01*M*) was added in order to maintain the *p*H at approximately 7.4.

Examination of stained cells [May-Grünwald Giemsa stain (*6*)] from such cultures has revealed that 90 to 95 percent of the cells in the monolayer are fibroblasts, as determined by cell morphology, the presence of collagen fibers in electron microscope thin sections, and nuclear size and shape. The remaining cells are multinucleate muscle cells or pycnotic cells of unknown type. Transfer to serum-free medium for 24 to 48 hours eliminates virtually all cells of nonfibroblast morphology (*7*). Removal of serum arrests growth and net protein and RNA synthesis; the cells do not divide or make DNA (*8*).

To study the production of fibroblast NGF as a function of time, cultures were incubated at 37°C in serum-free BME; at intervals the culture fluid was removed, centrifuged to remove cells (2000*g* for 10 minutes), dialyzed exhaustively against 0.01*M* ammonium acetate at 4°C, and lyophilized. For ganglion assays, the dry powder from 60 ml of culture fluid was redissolved in MEM at 100 times the original concentration and dialyzed thoroughly against MEM at 4°C. One part (0.05 ml) of