

# Animal Models of Human Disease: Severe and Mild Lead Encephalopathy in the Neonatal Rat\*

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Inorganic lead produces cerebral dysfunction and clinically definable encephalopathies in man. To date there have been few studies on the biochemical changes in brain following exposure to inorganic lead. Studies correlating toxicity with behavioral and brain neurochemical changes following lead exposure have been hindered because adult laboratory animals are resistant to the central nervous system effects of lead poisoning. Such studies have been impeded by lack of suitable experimental models until Pentschew and Garro showed that brain lesions develop in neonatal rats when a pregnant rat newly delivered of her litter is placed on a 4% lead carbonate containing diet. Lead passes into the developing sucklings via maternal milk. Lead-poisoned newborns have pronounced retardation of growth and during the fourth week of life develop the severe signs of lead encephalopathy, namely, extensive histological lesions of the cerebellum, brain edema, and paraplegia. There is an approximate 85-fold increase in the lead concentration of both the cerebellum and cerebral cortex relative to controls, but edema and gross vascular changes are confined to the cerebellum. Ingested lead had little effect on RNA, DNA, and protein concentrations of developing rat cerebellum and cerebral cortex. However, there was a reduction of between 10 and 20% in the DNA content of the cerebellum around 3 weeks of age in the lead-exposed sucklings. This suggests a failure of cell multiplication in this part of the brain.

A critical evaluation of this experimental approach indicated that under similar dietary conditions experimental lactating rats eat 30% less food than controls resulting in: (a) sustained loss in body weight of nursing mothers and that (b) offsprings who develop paraplegia and cerebellar damage do so after gaining access to lead containing diet.

We have studied mothers' food consumption and body weight changes and blood, milk, and brain lead content; and newborns' body and brain weight changes, blood and brain lead content, and brain serotonin (5HT), norepinephrine (NE), dopamine (DA), and  $\gamma$ -aminobutyric acid (GABA). We have found that a lactating mother rat eating 5% lead acetate (2.73% Pb) produced milk containing 25 ppm lead. When the mothers' diet is changed at day 16 from 5% PbAc to one containing 25 ppm Pb, and

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neonates allowed free access to the solid 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 a less severe form of "encephalopathy" consisting of hyperactivity, tremors, and stereotype behavior. Pair-fed controls coetaneous to experimental groups do not display such activities. There was no change in brain 5HT, GABA, or NE, but a 15-20% decrease in brain DA. Change in DA relative to other monoamines suggests a relationship between CNS dysfunction due to lead and DA metabolism in the brain.

The experimental design as described provides a model of CNS dysfunction due to lead exposure without debilitating histopathologies. It is possible that our findings on increased motor activity and changes in brain dopamine may correspond to early responses to lead exposure before recognized overt signs of toxicity.

## Introduction

It has been known since ancient times that lead may cause poisoning in man (1), but only within this century have extensive studies of the problem been made.

The more commonly encountered insults to the nervous system originate from side B of the ecodiagram. A second area of exposure is lead added to our environment from industrial and automobile exhausts. A national inventory of the sources of lead emission in the United States by the Air Pollution Control Office shows that at a minimum, 184,316 tons of lead were emitted in 1968 and that 98% (181,000 tons) of inorganic lead emission was from the combustion of leaded gasoline.

It is now well recognized that inorganic lead produces not only clinically definable encephalopathies and neuropathies, but also various behavioral changes indicative of cerebral dysfunction. However, only within the past fifty years has attention been called to its effects in children (2, 3) in whom toxicity can easily be overlooked until clinically recognizable encephalopathy occurs (4).

The brain is exceptionally sensitive to the effects of lead poisoning (5), and it is the young—from birth to about 7 years of age—who show the most serious brain damage following lead poisoning. The symptoms of lead poisoning are well defined, and there is considerable knowledge about the histopathological condition of the brain tissue of those poisoned individuals who do not survive (6). In spite of the seriousness of the clinical effects of lead on the central nervous

system, less is known about lead-induced lesions of the nervous system than about renal (7) or hematopoietic (8) effects. Indeed, not all children dying of acute lead encephalopathy have discernible histologic changes. No apparent abnormality could be found by Pentschew (6) in three of twenty autopsied cases. In spite of a considerable body of literature giving detailed descriptions of human autopsy material, the pathogenesis of lead induced encephalopathy is not well understood and the cause of neuronal damage is not clear. In those poisoned individuals who do survive acute lead encephalopathy, the associated pathological changes may or may not be completely reversible (5). There is a high incidence of permanent brain damage which can lead to periodic convulsions, irritability, hyperactivity, retardation of normal development, emotional instability, behavioral disorders, low attention span, impaired motor development, and antisocial behavior.

A clinical estimate of permanent neurological effects of lead poisoning in 425 children between the ages of 9 months and 8 years has been reported by Perlstein and Attala (9). The incidence and nature of sequelae as related to the mode of onset of symptoms is shown in Table 1.

It is worthy of note that 80% of children with encephalopathy experienced some permanent neurological deficits, recurrent seizures and mental retardation being most common. Smith et al. (10) likewise report that at least 25% of the young children who survive an attack of acute lead encephalopathy sustain permanent brain damage. Subtle neurologic deficits and mental impair-

Table 1. Neurological sequelae of lead intoxication.

Sequelae	MODE OF ONSET (number)						
	Total (425)	Encephalopathy (59)	Seizures (43)	Ataxia (17)	Gastro-intestinal (232)	Febrile (16)	Asymptomatic (58)
None	61%	18%	33%	41%	69%	81%	91%
Mental retardation	22	38	33	29	19	19	9
Seizures	20	54	39	35	13	0	0
Cerebral palsy	2	13	0	6	0	0	0
Optic atrophy	1	6	0	6	0	0	0

\* Data of Perlstein and Attala (9).

ment are the more common, and include: sensory disorders, perseveration, distortion in the sense of form and proportion, impairment in motor coordination, impaired learning ability, short attention span, ease of distractibility, development of destructive behavior patterns such as hostility and aggressiveness, and hyperactivity. Functional brain damage associated with lead, relative to mental development, has been studied by Chisolm and Harrison (11), Byers and Lord (12), Moncrieff et al. (13), and, more recently by Gibson et al. (14), and Millar et al. (15) in patients with mental deficiency, and David et al. (16) in hyperactive children. These studies (9-16) were based on case histories of children who had ingested, intentionally or accidentally, large amounts of lead from house paint, peeling wall plaster, toys, or poorly fired ceramicware and other eating utensils.

The effect of relatively low level chronic exposure to lead on the brain of the young during their earliest period of life, when the brain is undergoing rapid growth and development into specialized function has not been investigated. Although there have been numerous detailed studies on the histopathology of brain following lead poisoning there has, unfortunately, been very little investigative effort directed towards the biochemical interaction of lead and brain neurochemical components.

Studies on the cellular etiology of cerebral dysfunction resulting from lead encephalopathy had been impeded by the inability to produce unequivocal neurological changes in laboratory animals comparable to human

clinical cases of lead encephalopathy. Adult animals show resistance to the central nervous system effects of lead poisoning. The major criticism of most experimental models is that very large doses of lead are required to produce even marginal toxicity and that often there is a paucity or even absence of clinical manifestations of central nervous system toxicity in these models. An experimental model exhibiting the morphological alterations similar to those occurring in humans with lead toxicity has been described by Pentschew and Garro (17), who showed that lesions of the central nervous system of neonatal rats can be produced by lead fed to the lactating mother rat being transmitted to the suckling young via the maternal milk. The developing neonates show no early adverse effects of being nourished by lead-containing milk other than a profound retardation in body growth. Paralysis of the hind legs occurred about 20-30 days after birth. The cerebellum of these animals contained numerous small petechial hemorrhages and was deeply pigmented. Abnormal vascular permeability was most striking in the corpus striatum, occipital cortex, cerebellum, and lower spinal cord. Constant and characteristic histopathological brain changes are produced by this form of experimental lead poisoning thus enabling the study of the sequence of events that gives rise to cerebral dysfunction following lead poisoning. Similar findings have been reported by Lampert et al. (18), Thomas et al. (19), Michaelson (20), and Clasen et al. (21) in the rat, and by Rosenblum and Johnson (22) in the mouse. The lead-exposed

suckling rat has proven to be a reasonable model for those interested in the histopathological lesions of lead poisoning. However, histopathological studies are not the sole investigative approach, and indeed there are human cases of fatal lead encephalopathy without neuropathologic changes (6). In other toxicities cerebral dysfunction has also been observed in the absence of morphological damage to neurons (23). Virchow is quoted to the effect that "Cellular pathology is not at an end if one cannot see any alterations in the cell. Chemistry brings the clarification of living processes nearer than does anatomy. Each anatomical change must have been preceded by a chemical one" (6).

The constant and characteristic histopathological brain changes produced by experimental lead poisoning of the suckling rat (17) could conceivably permit the biochemical study of the sequence of events that give rise to cerebral dysfunction in lead poisoning. To date there have been no definitive published reports on this problem.

As cited above, there are well documented clinical cases of survivors of lead encephalopathy with sequelae of behavioral disorders. Therefore, the relationship of lead poisoning to developing cerebral deficits resulting in learning deficits and abnormal behavior warrants examination. It would appear that the suckling rat is a reasonable model for such a study.

We have successfully reproduced Pentschew and Garro's (17) model and studied biochemical changes produced in brain of the suckling rat when lead is added to the maternal diet. We studied the effects of lead on (a) body growth, (b) brain growth, (c) concentrations of RNA, DNA, and protein, (d) water content of cerebellum and cerebrum in lead-exposed suckling neonatal rats relative to control animals of the same age, and (e) some aspects of amino acid metabolism. We believe that the observed biochemical effects correspond to the severe forms of lead encephalopathy. However, maintaining a constant dietary exposure to lead (25 ppm) from birth through the fourth week of life leads to a mild form of encephalo-

pathy characterized by hyperactivity, aggressiveness, tremors, and stereotyped repetitive self-grooming. There is no change in brain serotonin,  $\gamma$ -aminobutyric acid, or norepinephrine, but a decrease in dopamine in the lead-exposed animals. Changes in dopamine relative to other monoamines plus development of abnormal behavior suggests a relationship between disruption in normal brain function due to lead and dopamine metabolism in the brain.

## Materials and Methods

The rats used in our study relating to severe forms of lead encephalopathy were selected experienced breeder albino rats of the Porton strain, bred at the Medical Research Council Laboratories, Carshalton, Surrey, England. Each pregnant rat was housed in an individual cage and fed normal laboratory chow and tap water *ad libitum*. As soon as each female gave birth to her young she was started on powdered chow containing 4.5% lead acetate and tap water. Comparable litters that received normal powdered laboratory chow and tap water were studied concurrently as controls. The day on which the newborn rats were first found was designated as day zero, their real age may have been 0 to 12 hr more. At 5 days of age each litter was reduced to six animals. The mother and the individual offspring were weighed twice weekly.

The rats used in our study on the less severe form of lead encephalopathy (minimal brain dysfunction) were timed-pregnant Sprague-Dawley rats obtained from ARS/Sprague-Dawley Co., Madison, Wisconsin. Daily food consumption was measured after parturition. As soon as each experimental female gave birth to her young she was started on powdered chow containing 5% lead acetate and tap water. Nursing mothers and individual litters were weighed each day between 10 and 11 A.M. The food consumption of experimental mothers was measured each day, and the average amount eaten was provided in the form of normal chow to pair-fed control nursing mothers. Ordinarily,

the only source of nutrition for newborn animals up to about 18 days of age, is maternal milk. They are then capable of climbing into the maternal food containers and thus obtain lead from both the solid diet (27,300 ppm) and the maternal milk (25 ppm). In order to circumvent this sudden change in lead exposure at 16 days of age the mother's diet was changed to one containing 25 ppm lead prepared as described below.

The animals were kept at a temperature of  $24 \pm 1^\circ\text{C}$  in a room having a 12 hr light and dark cycle commencing at 7:00 A.M. and 7:00 P.M., respectively.

### Preparation of Diet

Lead acetate (Reagent, A.C.S.) was pulverized by hand in a mortar with a pestle and added to the powdered diet to give a final concentration of 4.5% or 5% lead acetate (2.73% Pb, 27,300 ppm). This was then thoroughly mixed in an electrically operated commercial Hobart Mixer (N-50, setting 1) for 45 min. Uniform mixing was assumed; no attempt was made to assay random samples for its lead content. Diet (125 g) was kept in food jars sitting within a jar which served to catch scattered food. The same procedure was used in preparation and serving of diet containing 25 ppm lead.

### Chemical Analysis of Tissues (RNA, DNA, and Protein)

Suckling rats were sampled at 5, 10, 16, 21, and 26 days of age. In each instance five rats, irrespective of sex, were taken from the group exposed to lead and five rats from the corresponding control. Each rat was killed by decapitation, and the brain was quickly excised and immersed in cold ( $4^\circ\text{C}$ ) saline. Cerebellum and cerebral cortex separated from hippocampus and caudate nucleus were placed on individual preweighed aluminum foil squares sitting on a block of solid carbon dioxide. This operation was completed within 2 min. Tissue wet weights were recorded and the tissues were placed in homogenizing tubes ( $15 \times 110$  mm) containing 5 ml of cold ( $4^\circ\text{C}$ ) 0.2N perchloric acid. After homogenization and centrifugation, the supernatant was discard-

ed and the acid insoluble pellet was dissolved in 2 ml of 0.3N KOH by incubating at  $37^\circ\text{C}$  for 60 min. A suitable aliquot was removed for determination of protein (24, 25) and the remainder was used for nucleic acid analysis (26). The RNA separated from DNA was estimated spectrophotometrically and the DNA was measured by a modification (27) of the diphenylamine reaction (28). Standard curves for protein (bovine serum albumin) and DNA (Sigma, highly polymerized DNA) were made with each new series of determinations.

### Tissue Water Content

The cerebellum and cerebral cortical tissue from comparable groups of animals, isolated as described above, was weighed in tared vials and allowed to remain in a drying oven ( $110^\circ\text{C}$ ) for 2 days. The samples were then placed in a desiccator (*in vacuo*) for an additional 2 days and then reweighed. The difference between the wet and dry weights indicated the tissue water content.

### Lead Analysis

In those studies on severe forms of lead encephalopathy, dried tissue samples were used for determination of lead content of cerebellum and cerebrum. In subsequent studies, tissue and biological fluids were used without additional processing other than as described below. Estimation of lead was carried out by flameless atomic absorption spectroscopy with a Perkin-Elmer model 403AA fitted with a Heated Graphite Atomizer (HGA-70). The volume to be analyzed ( $10 \mu\text{l}$ ) was injected into the carbon rod and the oven programmed for 80 sec drying time at  $60^\circ\text{C}$ , 3 min ashing at  $470^\circ\text{C}$ , and 16 sec atomization at  $1560^\circ\text{C}$ . The lead lamp was a high intensity lamp, operated at 8 MA with a slit opening of 3 mm, at wavelength 3833 in the ultraviolet band, with a final attenuation at 0.25. New standard curves, ranging from 0.3 to 0.9 ng of lead, were made for each new set of assays. The concentration of lead in each test sample was estimated from the standard curve (29, 30).

## Collection of Milk

The milking device is a conical type graduated centrifuge tube with a ground zone for a standard tapered stopper. The stopper is replaced by a ground-in glass pipet stopper with a flared top similar to that found in a standard dropping bottle, but without the rubber bulb. A side-tube was adapted to the centrifuge tube and the former shaped to hold rubber tubing leading to a vacuum gauge, connected to a needle valve which was in turn connected to a water pump. Lactating mother rats were separated from their young prior to milking and injected intraperitoneally with 0.5 ml (100  $\mu$ g/ml) synthetic oxytocin having a reported activity of 181 international units/mg. Milk was collected 30 min after oxytocin administration by applying the flared-top pipet stopper of the milking device to the teat. The needle valve on the vacuum line was adjusted to 10 to 11 in. of Hg (negative pressure) resulting in collapse of the tissue around the teat permitting milk to flow from the end of the test into the collecting tube.

## Preparation of Milk and Brain for Lead Analysis

**Milk:** A 25- $\mu$ l portion of milk was transferred to nitric acid (10%)-washed small conical centrifuge tubes, frozen, and stored at  $-20^{\circ}\text{C}$  until analyzed for its lead content. The sample was then thawed, 50  $\mu$ l of tetraethylammonium hydroxide (24% in methanol) were added, thoroughly mixed and heated at  $45^{\circ}\text{C}$  for 2 hr or until the sample was dissolved. This was then further diluted by the addition of 125  $\mu$ l of deionized water. An eightfold dilution of the milk was sufficient for the adequate analysis for lead by atomic absorption spectroscopy.

**Brain:** Tissue was transferred to nitric acid (10%)-washed 25 ml glass stoppered graduated cylinders. To each gram of tissue (v/w) 2 ml of tetraethylammonium hydroxide (24% in methanol) was added and heated at  $60^{\circ}\text{C}$  for 4 hr or until the tissue was dissolved. The mixture was then diluted

with deionized water to a volume equivalent to 100 mg tissue/ml of solution (w/v).

## Spontaneous Activity

The degree of spontaneous activity was estimated by using a selective activity meter. This particular meter uses radiofrequency electromagnetic proximity sensors to detect the movements of the animal(s) residing in a plastic cage sitting on top of the instrument. Six sensors are located under the plastic top plate of the instrument. The sensors form a part of a resonant circuit. When an animal enters the magnetic field surrounding a sensor, the magnetic coupling between the animal and the sensor reduces the voltage in the resonant circuit, producing an impulse which is counted.

At selected ages an entire colony of six experimental or control sucklings were removed from their mother and placed in a cage on the selective activity meter. Food and water were provided. Testing of experimental and control groups was done on alternate days. The study was started at 7:00 A.M., and the sum of activity was recorded every 3 hr until 7:00 P.M. The last recording was a 12 hr count through the night.

## Administration of Radioactive Substrates

These studies were carried out with Porton strain rats, and the developing young rats were used at 7, 13, and 24 days of age. Suckling control and experimental rats received a subcutaneous injection of 20  $\mu$ Ci (0.2 ml)/100 g body weight of [ $^{14}\text{C}$ ] glucose (specific radioactivity 309 mCi/mole, Radiochemical Centre, Amersham). The animals were decapitated 10 min after the injection.

## Separation of Tissue Constituents

Previous methods described (31, 32) were followed with only minor modifications. Briefly, the amino acids present in the neutralized acid soluble fraction were separated from other constituents with the help of a cation-exchange resin (Dowex AG-50, X8,

H<sup>+</sup> form). The acidic amino acids, glutamate and aspartate, were adsorbed on a column (0.55 × 20 cm, 5 ml capacity) of anion-exchange resin (Dowex AG-1, X8, acetate form), and the resin was eluted in three steps with: (a) 20 ml of 0.05*N* acetic acid, (b) 30 ml of 0.2*N* acetic acid, and (c) 35 ml of 0.3*N* acetic acid. Under these conditions glutamate and aspartate were eluted in fractions b and c respectively. The material not adsorbed on this column was hydrolyzed with 4*N* HCl for 2 hr at 100°C and the resulting glutamate from glutamine was separated, as described above, on a column of Dowex AG-1 (acetate form) resin. The amino acids were determined by the ninhydrin procedure in a Technicon Autoanalyser by the sample-plate technique after the samples had been desiccated.

#### Determination of Radioactivity

Radioactivities of the samples were determined in a Nuclear-Chicago Scintillation Spectrometer (Mark II). The counting fluid contained 0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis(5-phenyloxazol-2-yl)benzene in toluene-2-ethoxyethanol (7:3, v/v), and 2-ethoxyethanol was used to ensure a one-phase system. Correction for quenching was applied either by the external standardization technique or by the addition of [<sup>14</sup>C] toluene internal standard.

#### Statistical Analysis

The results were transformed logarithmically and were analyzed statistically by using analysis of variance with a two-way classification (experimental or control versus age or versus time). The standard errors were calculated from the residual mean square in the appropriate way (33).

#### Brain Catecholamines

At the completion of the test periods for motor activity, the animals were decapitated and their brains rinsed in ice-cold deionized water, separated longitudinally into two halves, frozen on solid CO<sub>2</sub> and weighed. One half of the brain was used for lead

analysis, and the second half of the brain was analyzed for norepinephrine and dopamine content (34).

## Results

### General Appearance

The appearance of hair, the opening of eyes, locomotion, and the ability to eat solid food were delayed by up to 2 days in the lead-poisoned sucklings as compared to the controls. Thomas et al. (19) found no such delay in their experiments, using comparable amounts of lead carbonate. In addition to a pronounced retardation in growth rate (described below) the neonate exposed to lead had a ruffled coat and the hind legs protruded on either side of the body as the animal assumed a broad-based stance. Similar conditions have been produced in mice (22). At 19 to 21 days after birth the experimental animals developed a weakness of the hind limbs, incontinence, and lethargy leading to frank paraplegia (Fig. 1). Other investigators (17, 19) have reported similar findings at a later age, namely from 23 to 29 days. Autopsy at the stage of the hind limb paralysis revealed an extensive reddish-brown appearance of the cerebellum (Fig. 2). Whereas Pentschew and Garro (17) referred to imbuement with some pigment, not yet identified, Thomas et al. (19) cite extensive hemorrhages in the cerebellar cortex which become maximal on the day of paralysis. Preliminary studies on our rats by F. De Matteis (unpublished data) suggest that the pigmentation is primarily due to hemoglobin. No attempt was made to reverse the debilitating effects of lead or study improvements in mortality by feeding lead-free diet after weaning.

### Body Weight

The rate of body growth was significantly retarded in the sucklings exposed to lead relative to the controls. Whereas the normal animals gained about 3 g/day, the experimental animals gained on the average 1.2g/day. Figure 3 illustrates the changes of body

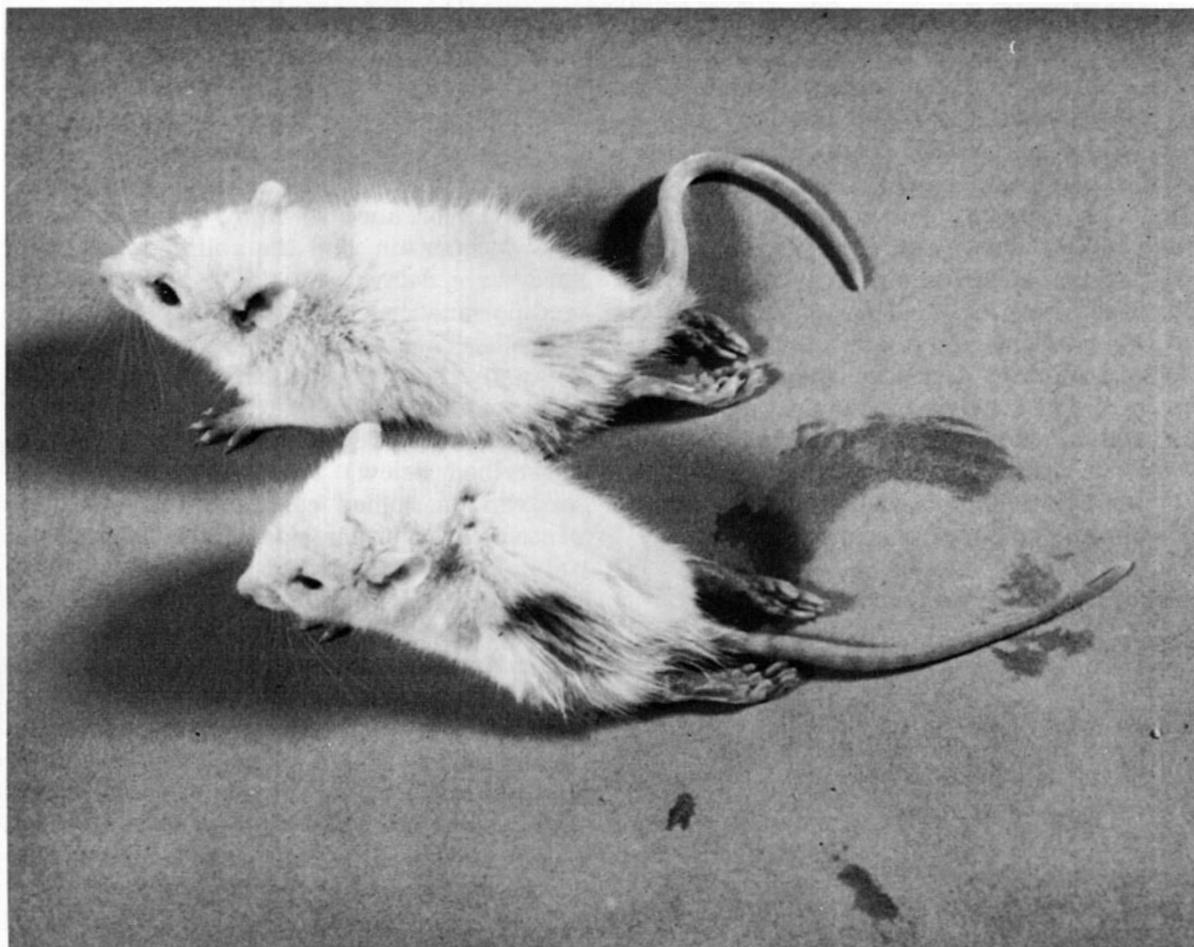


FIGURE 1. Rats, 19 to 21 days old, showing ruffled coat and paraplegia.

weight with age in poisoned and control animals. We could see no differences in the behavior of the mother toward her suckling rats between poisoned and control groups.

In these initial studies and those by previous investigators (17, 19) no attempt was made to measure the amount of milk obtained during suckling in order to account for the 60% reduction in growth rate. It was observed, however, that after suckling the stomachs of both poisoned and control neonates were generally distended by milky-white material, easily visible through the body wall. In spite of the apparent successful feeding by the neonates exposed to lead,

their weight at 26 days was like that of a 14-day-old control suckling rat.

#### **Brain Weights (Cerebellum and Cerebral Cortex)**

The wet weights of cerebellum and cerebral cortex from control and treated animals at various ages is illustrated in Figure 4. There is reduction in the respective tissue weights in the animals exposed to lead as compared to controls of similar age. The reduction in cerebral hemisphere weight does not appear until after 10 days, whereas in the cerebellum the retardation appears much earlier. By

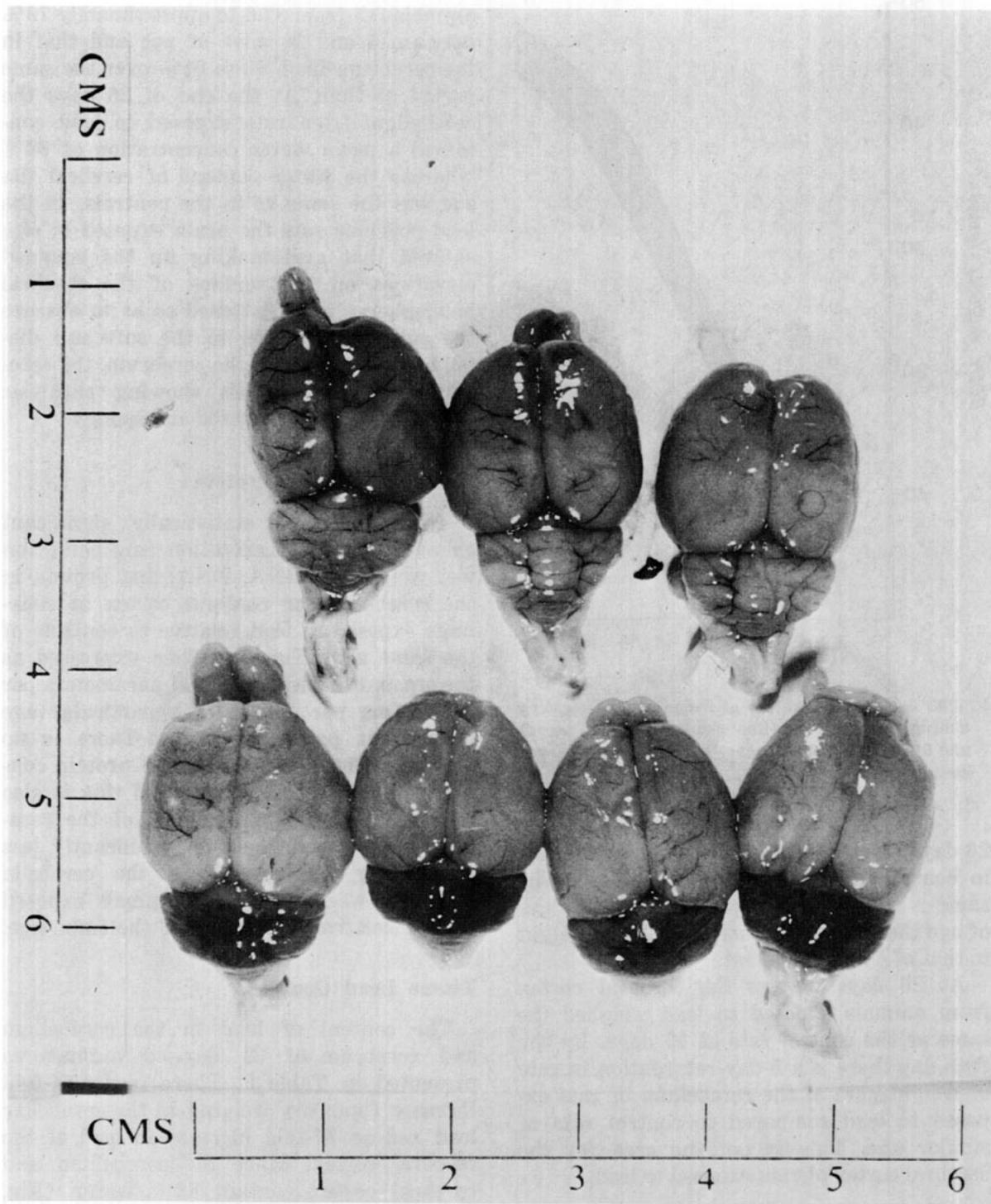


FIGURE 2. Brains from control (upper) and paraplegic rats (below). Note swollen and darkened appearance of the cerebellum.

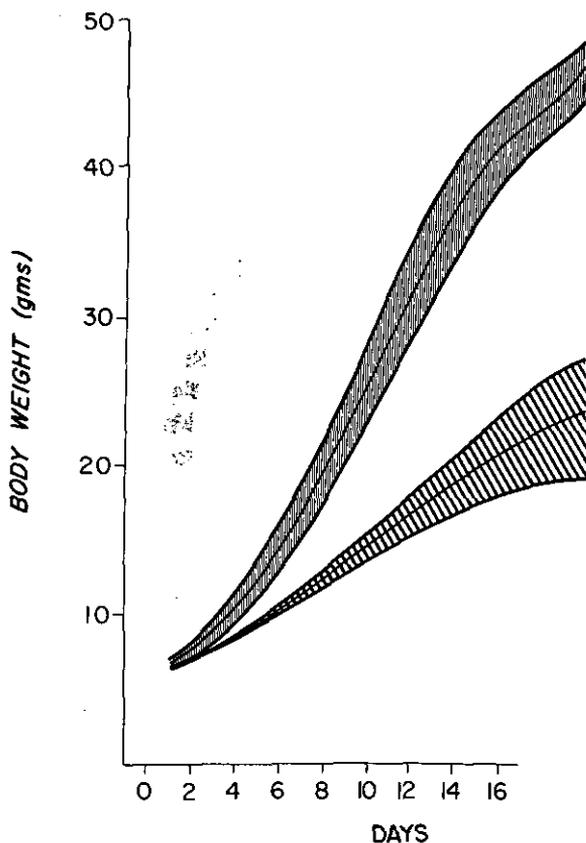


FIGURE 3. Growth (grams) of neonatal rats suckling mothers on normal (fine shading, upper curve) and 5% lead acetate (coarse shading, lower curve) containing diets.

26 days cerebral tissue weights are the same in control and rats treated with lead. The same is not true for cerebellum. At 26 days of age the cerebellum corresponded in weight to that of a 21-day-old rat.

At 26 days of age the cerebral cortex from animals exposed to lead weighed the same as the control rats at 19 days. By the 26th day there is a 5-day retardation in rate of weight gain of the cerebellum of rats exposed to lead compared to control rats of similar age. This is not the case for the cerebral cortex of rats exposed to lead.

#### Tissue Water Content

Figure 5 shows the percentage water in the cerebellum and cerebrum of control and

experimental animals relative to age. The water content of cerebellum in control animals fell from 87.6 to approximately 79% between 5 and 26 days of age and that in the cerebrum from 88 to 81% over the same period of time. At the end of 26 days the cerebellum from rats exposed to lead contained a mean water concentration of 82% whereas the water content of cerebral tissue was the same as in the controls. In the lead poisoned rats the brain exposed *in situ* showed that gyri making up the rounded elevations on the surface of the cerebral hemispheres were flattened so as to obscure the sulci. In addition to the soft and distended appearance of the cerebrum, the cerebella of those animals showing paralysis were invariably pigmented and spongy.

#### RNA, DNA, and Protein

There were no statistically significant changes in the concentration (mg per gram wet weight) of RNA, DNA, and protein in the cerebellum or cerebral cortex of sucklings exposed to lead relative to controls of the same age (Fig. 6). When expressed as the amount of these chemical parameters per organ (mg per organ) at a particular age a different pattern emerges. There is no change in the RNA, DNA, and protein content of the cerebral cortex, and this is also true of the RNA and protein of the cerebellum. However, there is significantly less DNA (mg per organ) in the cerebella around 3 weeks of age of animals exposed to lead relative to controls of the same age.

#### Tissue Lead Content

The content of lead in the cerebellum and cerebrum of 21 day-old animals is presented in Table 2. There is an 84-fold increase (ppm dry weight) in the cerebellar lead and an 87-fold increase in lead of the cerebral cortex. Since the cerebellum and cerebral cortex contain 82% water (Fig. 5), the wet tissue concentrations of lead are about 12.53 ppm and 5.98 ppm, respectively. This is similar to the concentration of lead in brain reported (35) in six

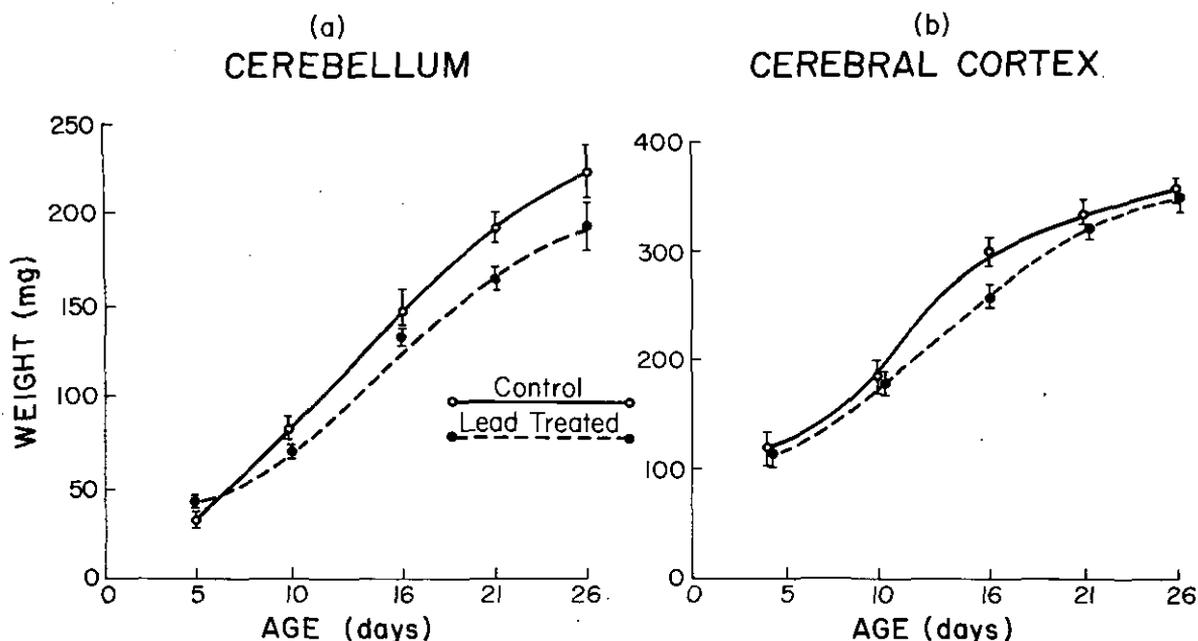


FIGURE 4. Wet weights of (a) cerebellum and (b) cerebral cortex of newborn suckling rats nourished by mothers on (○) normal diet and (●) on diet containing 4.5% lead acetate. Each point represents the mean weight of the organs from five animals; the vertical lines represent one standard deviation.

cases of human pediatric lead encephalopathy leading to death (12–27 ppm wet weight).

### Amino Acid

The conversion of glucose carbon into the amino acids of the cerebral cortex and cerebellum of rats of different ages is shown in Figure 7.

It has been shown previously for control

rats that as maturation proceeds there is an increased conversion of glucose carbon into forebrain amino acids (36, 37). The present results showed that the age curve of the conversion of glucose carbon into amino acids in the cerebellum was similar to that observed in the cerebral cortex.

In the young of lead fed mothers there was a significant decrease in the percentage of the total radioactivity in the acid-soluble material of the brain present in the amino

Table 2. Lead concentration in brain of 21-day-old rats.\*

Tissue	Conditions	Dry weight, mg	Concentration, ppm	Poisoned ppm / Control ppm
Cerebellum	Control	44.20 ± 4.17	0.83 ± 0.18	83.86
	Poisoned	38.43 ± 0.15	69.60 ± 27.89	
Cerebral cortex	Control	219.83 ± 10.59	0.38 ± 0.10	87.45
	Poisoned	187.50 ± 10.06	33.23 ± 4.13	

\* Nourished by mothers on normal diet (control) and diet containing 4.5% lead acetate (poisoned). Each value is the mean and standard deviation of tissue samples from three rats.

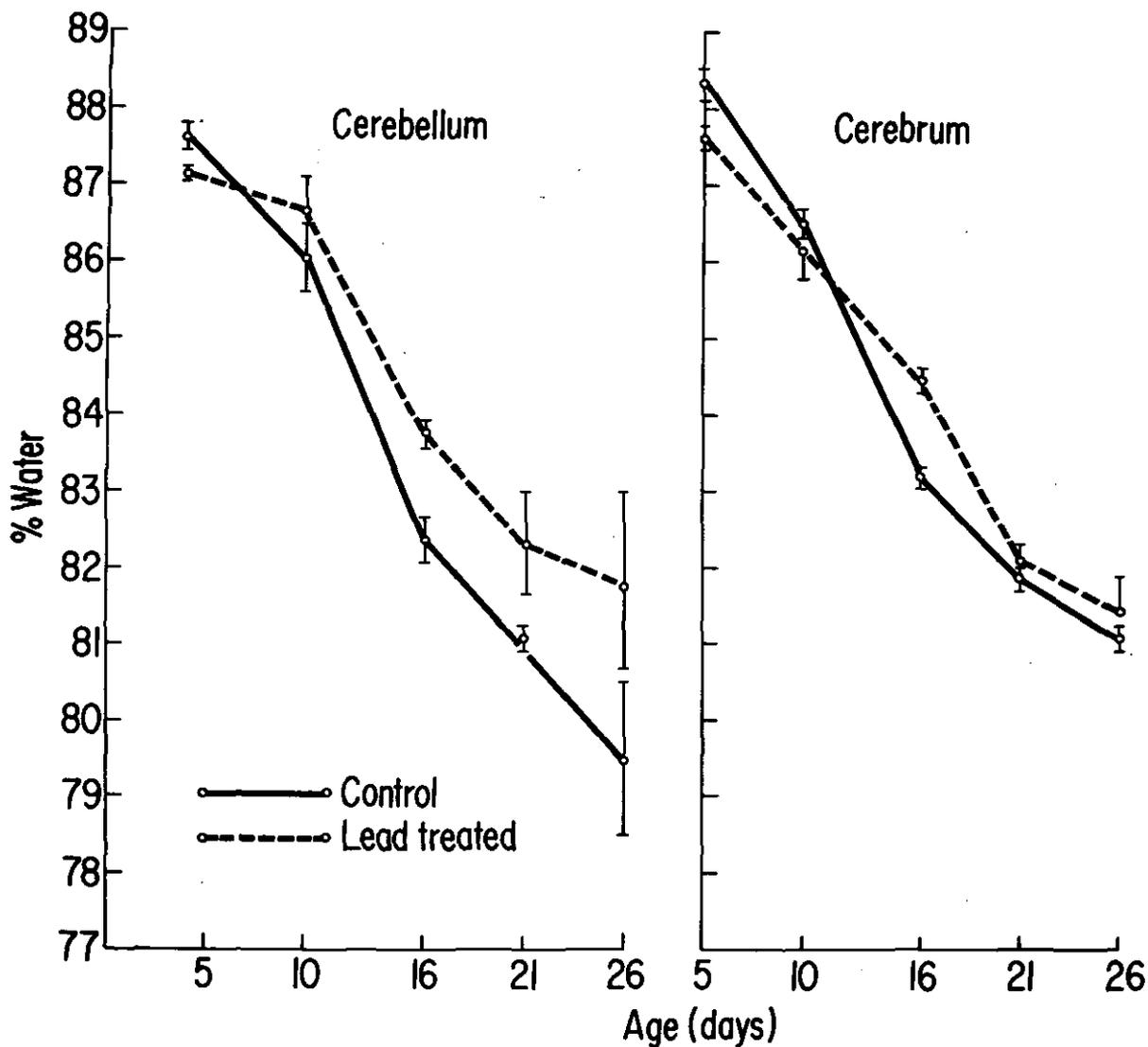


FIGURE 5. Changes in the water content of rat cerebellum (left) and cerebral cortex (right) from newborn sucklings nourished by mothers on normal diet (○—○) and diet containing 4.5% lead acetate (○—○). Each point represents the mean concentration of water in the organs of 5 rats and the vertical lines represent one standard deviation.

acid fraction compared with the young of mothers fed a normal diet. This decrease was evident for both the cerebral cortex and the cerebellum at the three different ages studied.

#### <sup>14</sup>C Labeling of Amino Acids

A more detailed analysis of the labeling of those individual amino acids associated with the tricarboxylic acid cycle was carried out. The specific radioactivities of gluta-

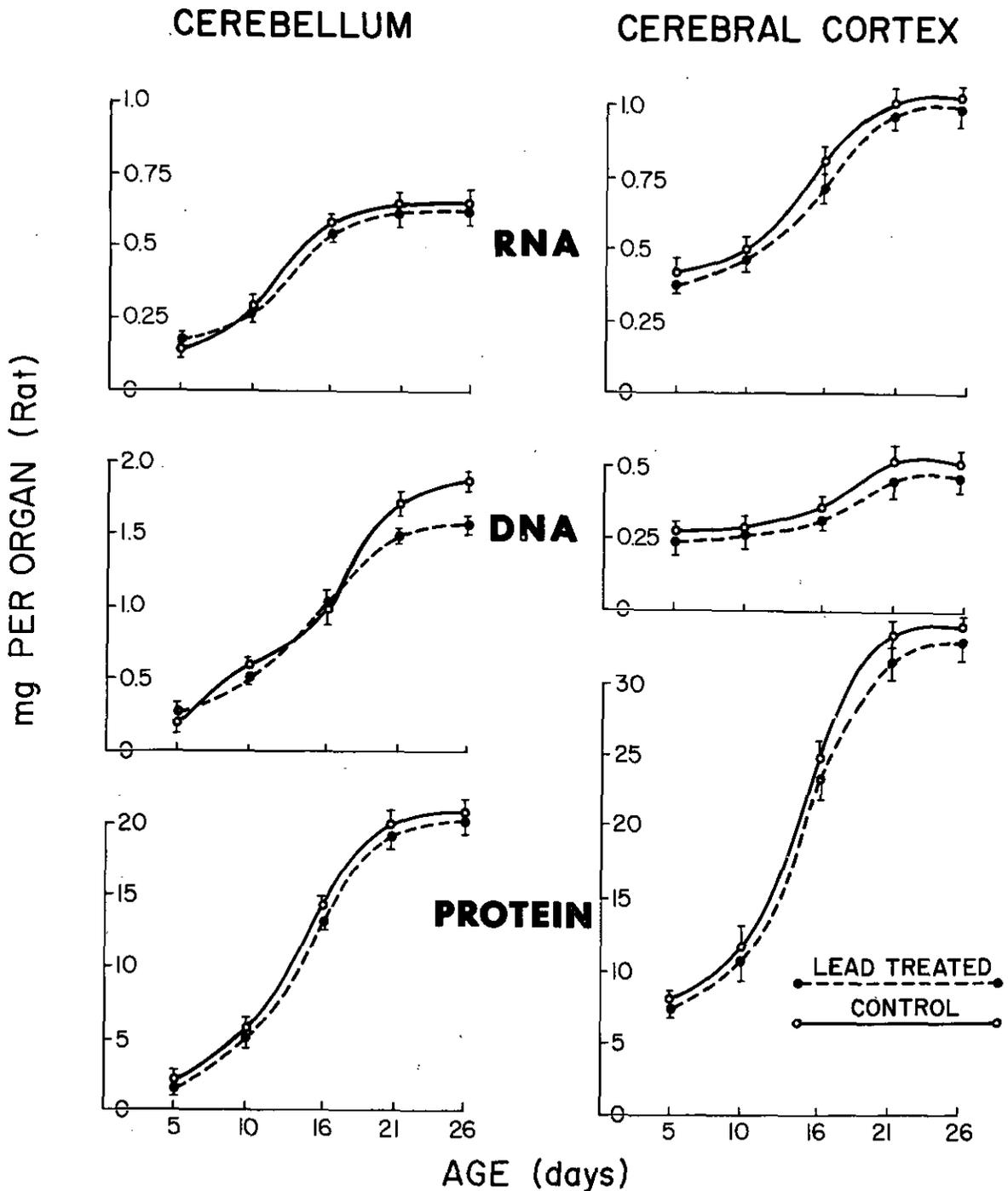


FIGURE 6. Changes in the RNA, DNA, and protein content of cerebellum (left) and cerebral cortex (right) of newborn sucklings nourished by mothers (○) on normal diet and (●) on diet containing 4.5% lead acetate. Each point represents the mean content of organs from five rats; the vertical line represents one standard deviation.

mate, aspartate, and glutamine, are shown in Table 3, which demonstrates that the amount of labeling was markedly lower in the experimental group than the corresponding values for the control animals at all ages and in the two brain parts studied.

However, differences in the amounts of the amino acids in the brains of the control and the lead-treated groups of animals could influence the distribution of  $^{14}\text{C}$  within the brain. Amino acid concentrations were, therefore, measured in the cerebral cortex and the cerebellum of rats of different ages (Table 4). The developmental increase in the concentrations of glutamate and aspartate was retarded in the lead treated animals. This was evident in both brain parts studied. On the other hand, the concentration of glutamine was significantly increased in the cerebellum but not in the cerebral cortex. In general, the changes in the concentrations of amino acids were relatively smaller than changes in the specific activities and a pattern of change that might have been consistent with the labeling data (compare Tables 3 and 4) did not emerge.

The results indicate that the effects of lead treatment on a particular parameter of biochemical maturation of the brain can be demonstrated. The main effect is that the utilization of carbon from glucose metabolism for synthesis of dicarboxylic amino acids associated with the tricarboxylic acid cycle is reduced by exposure to lead.

In the study described above, as well as

that of Pentschew and Garro (17), Thomas et al. (19) and Michaelson (20), it was found that suckling rats ingesting inorganic lead contained in the milk of mothers fed a lead containing diet results in a pronounced retardation in growth of the young and gross discolorization of the cerebellum. The low body weight of those animals suckling from mothers fed lead acetate, and some of the cerebellar changes, are similar to those seen in undernutrition. Furthermore, Michaelson (20) made the observation that the appearance of paraplegia and cerebellar pigmentation occurred shortly after the time when the neonates are capable of gaining access to the 4.5% lead acetate containing diet.

The question was raised (20) whether access to relatively greater amounts of lead contained in the maternal solid food (24, 579 ppm Pb) compared to maternal milk (40 ppm Pb) (17) could account for the histopathology observed in these experiments. In addition, the contribution of undernutrition to the experimental findings was not ascertained by any of the earlier workers (17-20).

#### Food Consumption and Body Weight Changes

In the present study, scattered food and that remaining in the food jar were sieved free of non-chow material, combined, and weighed to calculate the amount of

Table 3. Effect of ingested lead on the incorporation of glucose carbon into brain amino acids associated with the tricarboxylic acid cycle.

	7-day-old			13-day-old			24-day-old		
	Lead (A)	Control (B)	(A/B) $\times 100$	Lead (D)	Control (E)	(D/E) $\times 100$	Lead (G)	Control (H)	(G/H) $\times 100$
Cerebral cortex dpm/ $\mu\text{mole}$									
Glutamate	197	437	45	2135	4316	49	3778	7260	52
Aspartate	—	—	—	1765	3236	55	3438	5160	67
Glutamine	—	—	—	624	1831	34	1328	4473	30
Cerebellum, dpm/ $\mu\text{mole}$									
Glutamate	256	625	41	1460	2720	54	3272	5837	56
Aspartate	—	—	—	1535	2315	66	3650	5124	71
Glutamine	—	—	—	285	1228	23	833	3506	24

Table 4. Effect of lead ingestion on the concentration of brain amino acids during development.

	7-day-old			13-day-old			24-day-old		
	Lead (A)	Control (B)	(A/B) × 100	Lead (D)	Control (E)	(D/E) × 100	Lead (G)	Control (H)	(G/H) × 100
Cerebral cortex amino acid concentration, $\mu\text{mole/g}$									
Glutamate	3.91	4.58	85	6.85	7.66	89	8.84	10.77	82
Aspartate	1.76	2.10	84	2.39	2.71	88	2.03	3.27	62
Glutamine	2.10	2.78	76	3.00	3.10	97	2.55	2.44	105
Cerebellum amino acid concentration, $\mu\text{mole/g}$									
Glutamate	3.65	4.34	84	6.32	7.40	85	8.44	9.45	89
Aspartate	1.87	1.92	97	1.97	2.13	92	1.67	2.48	67
Glutamine	3.71	3.47	107	4.05	2.96	137	4.51	2.79	162

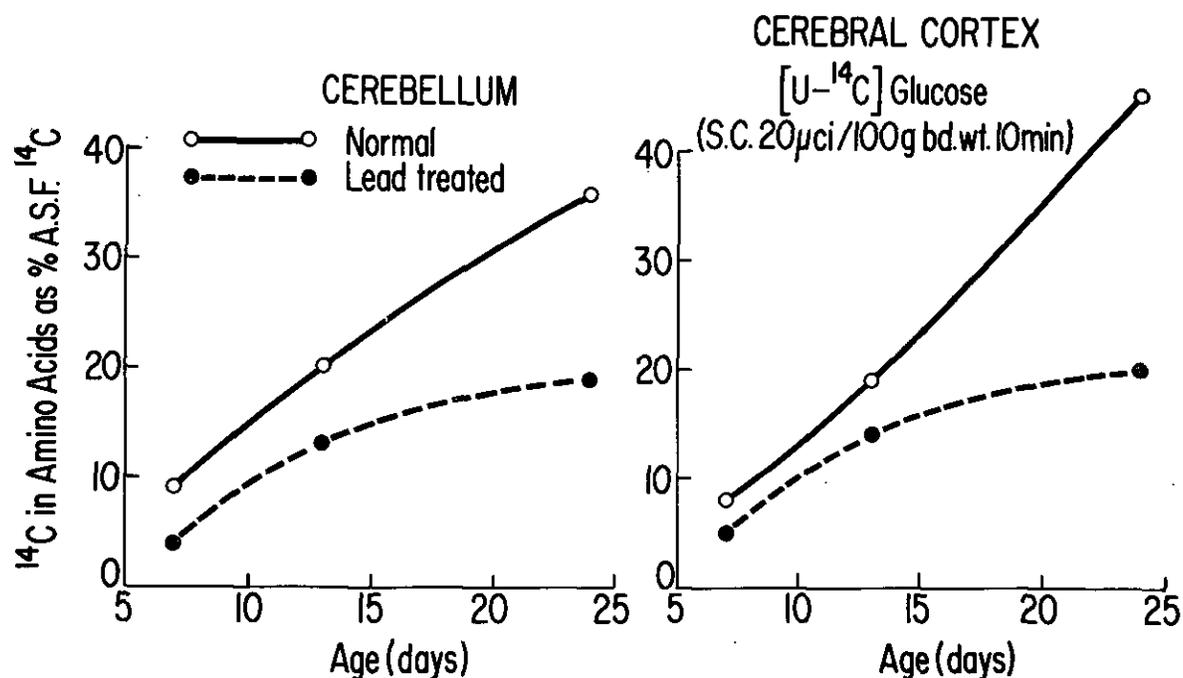


FIGURE 7. Percentage of the total acid soluble radio-activity in the amino acid fraction of cerebellum (left) and cerebral cortex (right) of developing rats suckling mothers (○) on normal diet or (●) on diet containing 4.5% lead acetate.

food consumed during the previous 24-hr period. Body weights of mother rats and newborns were recorded each day. The amount of food consumed and changes in body weight of lactating nursing rats eating powdered normal laboratory meal and 4.5% lead acetate containing diet is illustrated in Figure 8.

Female rats newly delivered of their young consumed approximately  $21 \pm 5$  g of food during the first 24 hr post-parturition. The experimental group offered 5% lead acetate containing diet at  $2.8 \pm 2.8$  g over the same time period. This represents 14% of that consumed by the control group. Both control and experimental groups increased

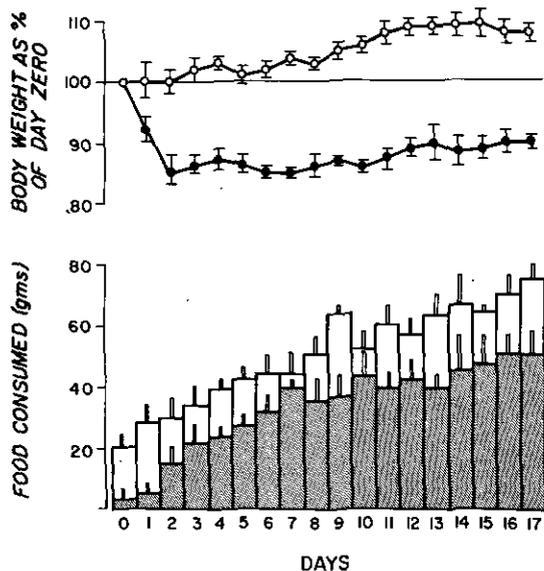


FIGURE 8. Plots of (bottom) food consumed and (top) relative percentage body weights of lactating rats eating (□, ○) normal diet and (□, ●) diet containing 4.5% lead acetate.

their food intake each day relative to the previous 24-hr period. From day 4 onwards, lactating rats on normal diet increased their food intake at an average rate of 2.5–3 g/day, whereas those on lead acetate showed a comparable rate of increased food consumption but starting two days later, namely on day 6. Throughout the period of days 6 through 17, lactating rats on lead acetate containing diet ate 30–35% less food than those on normal diet.

The average daily food consumption of lactating rats eating normal diet for the first 17 days following parturition was 53 g and those on lead acetate containing diet was 37g day. The latter group, therefore, consumed 30% less food than controls on an average daily basis. The influence of different quantities of food intake on the body weight of lactating rats is illustrated in Figure 8. When the daily weight changes are measured as a percentage of the body weight on the day of birth, it is found that the lactating rats on normal diet continue to gain weight. The experimental group on lead acetate and

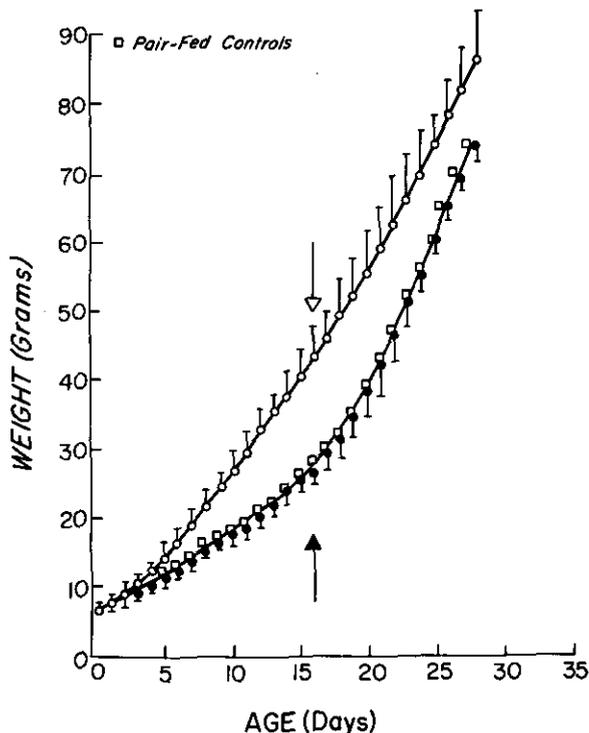


FIGURE 9. Growth (grams) of neonatal rats suckling mothers (○) on normal and (●) on diet containing 5% lead acetate followed by weaning to normal ( ) or 25 ppm ( ) diets; (□) control neonates suckling mothers pair-fed to experimental mother rats.

eating 30% less food experience a perceptible fall in body weight which remains below 90% of that on day zero throughout the 17 days illustrated (Fig. 8) and as long as 90 days thereafter. The influence of lowered food consumption and loss in body weight in lactating mother rats on growth of newborns was demonstrated in Figure 9.

### Paired Feeding

The diminished food consumption by lactating rats eating 5% lead acetate-containing diet and the slowed growth of their offspring relative to control groups implicates nutritional influences among the test group. In order to account for this, concurrent paired-feeding was carried out. In this instance the amount of 5% lead acetate containing diet consumed by the experimental group was firstly estimated and a comparable amount of normal food offered to the

pair-fed controls. The ability to match the growth rate of the lead acetate experimental group is illustrated in Figure 9.

### Lead Content of Milk and Brain

Milk was obtained from lactating rats (see methods) eating normal and 5% lead acetate diets at the 16th day of the experiment whereas brain tissue was obtained by sacrificing 25 day old rats. Milk and brain were prepared for lead analysis as described in Methods. Lactating rats eating 2.73% lead (5% lead acetate) for 16 days following birth of their young produce milk containing approximately 25 ppm lead. Milk from lactating rats eating normal diet contains less than 0.02 ppm lead. Rats 25 days old with a lifetime dietary exposure of 25 ppm lead in maternal milk and/or solid food have brains containing 0.5 ppm lead as compared to less than 0.10 ppm found in control animals.

### Diet Containing 25 ppm Lead

Developing newborn rats suckling milk containing 25 ppm lead are capable of climbing into the food jar by 18 days of age and thereby capable of consuming solid lead-containing diet. In order to keep the lead exposure of developing rats constant, on the 16th day the maternal diet was changed to one containing 25 ppm lead, comparable to that found in milk. The consequential acceleration of growth rate due to change from a liquid maternal milk diet to solid food in both control and experimental animals is shown in Figure 9. Prior to the 16th day control animals increased their body weight at 3 g/day, whereas those suckling milk (25 ppm lead) from a lead-exposed mother gain approximately 2 g/day. However, from 16 days onwards, access to solid diet accelerated the weight gain to approximately 5 g/day in both the control group eating normal diet and the experimental group eating diet containing 25 ppm lead.

### Spontaneous Activity

At 23, 24, and 25 days of age complete families of six siblings from pair-fed con-

trols or 25 ppm Pb lead-exposed groups were placed in a spontaneous activity metering device for 24 hr (water and respective diets *ad libitum*). Appropriate pair-fed controls and experimental group were tested on alternate days for a single 24 hr period. Neonatal rats exposed to 25 ppm dietary lead for the first 3½ weeks of life exhibit a 40 to 93% greater activity than coetaneous pair-fed controls (Table 5).

### Brain Catecholamines

In a concurrent study experimental and control rats were killed at 5, 13, 21, and 29 days of age and their brain treated for lead and catecholamine analysis as described in Methods. Brain lead and catecholamine content of experimental and control animals at various ages of development and during the period immediately following assessment of hyperactivity are shown in Table 6.

Exposure to lead via the regimen described leads to a significant increase in the amount of lead in brain within 5 days of the onset of exposure (compare columns A and E, Table 6). The brain lead content of exposed animals increases another 33% after an additional 3 weeks of exposure. It is interesting to note that there are no observable differences in the norepinephrine content between control and lead exposed animals (compare columns B and F, Table 6). Dopamine content is similar in 5- and 13-day-old experimental and control animals, however, there is a statistically significant decrease in dopamine at 21 and 29 days in the leaded animals relative to controls (compare columns C and G, Table 6). Further-

Table 5. Spontaneous Activity.\*

Age days	Spontaneous activity, average counts/hr (24 hr.).		
	Controls (A)	Lead-treated (B)	(B - A) × 100
23	1295	1805	139
24	1238	2394	193
25	1250	1750	140

\* Six neonate siblings.

Table 6. Lead, norepinephrine (NE), and dopamine (DM) concentrations ( $\mu\text{g/g}$  wet weight) in brains of developing rats eating leaded diet (40 ppm Pb) and pair-fed controls.<sup>a</sup>

Age, days	Pair-fed controls				Lead-exposed experimentals			
	Lead, $\mu\text{g/g}$ (A)	NE, $\mu\text{g/g}$ (B)	DM, $\mu\text{g/g}$ (C)	Ratio C/B (D)	Lead content, $\mu\text{g/g}$ (E)	NE, $\mu\text{g/g}$ (F)	DM, $\mu\text{g/g}$ (G)	Ratio G/F (H)
5	0.10 $\pm$ 0.01 (7)	0.156 $\pm$ 0.010 (7)	0.266 $\pm$ 0.049 (7)	1.70	0.60 $\pm$ 0.10 (7)	0.156 $\pm$ 0.020 (7)	0.261 $\pm$ 0.024 (7)	1.67
13	0.10 $\pm$ 0.01 (6)	0.168 $\pm$ 0.020 (6)	0.318 $\pm$ 0.026 (6)	1.89	0.70 $\pm$ 0.15 (6)	0.160 $\pm$ 0.020 (6)	0.300 $\pm$ 0.024 (6)	1.87
21	0.10 $\pm$ 0.01 (6)	0.230 $\pm$ 0.020 (6)	0.560 $\pm$ 0.020 (6)	2.43	0.78 $\pm$ 0.15 (6)	0.238 $\pm$ 0.020 (6)	0.467 <sup>b</sup> $\pm$ 0.046 (6)	1.96
29	0.10 $\pm$ 0.01 (6)	0.298 $\pm$ 0.020 (6)	0.560 $\pm$ 0.033 (6)	1.88	0.88 $\pm$ 0.11 (6)	0.293 $\pm$ 0.035 (6)	0.450 <sup>b</sup> $\pm$ 0.041 (6)	1.54

<sup>a</sup> Mean values  $\pm$  S.D.

<sup>b</sup> Student's (*T*) test *P* = 0.01.

more, a comparison of dopamine to norepinephrine ratios in experimental relative to control animals (compare columns D and H, Table 6) shows an approximate 20% depression of dopamine in the 21- and 29-day-old experimental animals, and this is within a time frame when increased motor activity is evident.

## Discussion

The present status of our knowledge concerning cerebral dysfunction produced by lead may reasonably be characterized as healthy confusion: there have been many good, suggestive experiments but the problem is so complex that many contradictions, uncertainties, and irrelevancies cloud the picture. At this point we cannot give an unqualified answer to even such a simple and basic question as, "does low-level lead poisoning produce mental deficiency?" In a recent review of the clinical literature, Wiener (38) concluded that "Those reports which claimed positive findings had either used too few cases from which to generalize or had not provided for controls for relevant variables . . .", thus precluding any definitive conclusion. On balance, of course, both the clinical and experimental literature indicate a "yes" answer to our question. The paucity of good information is probably largely attributable to the lack, until recently, of suitable animal experimental models.

Lead encephalomyelopathy of the suckling rat was considered by Pentschew et al. (17) to be a milestone in the experimental investigation of saturnism. It was felt that for the first time constant, spectacular and characteristic brain changes could be produced by a simple procedure. A systemized evaluation of lead encephalopathy in suckling rats (17, 19, 21) and mice (22) indicates that the animals, especially the rat, show lesions of the cerebellum and striatum. It is Pentschew's view (17) that the lesions are similar to those seen in human lead encephalopathy, and there is unanimity in the finding that it is the capillary vasculature which is primarily affected. Light and

electron microscope autoradiographs from rat cerebellar tissue removed 1 hr after the interperitoneal injection of radiolabeled/lead shows that the radioactivity localizes within the cytoplasm of capillary endothelial cells. The astrocyte foot processes are labeled at a latter stage, and at 72 hr the deposits of lead are associated with edema (39).

A morphometric analysis of the brain from lead poisoned developing rats revealed that myelin formation is delayed, that there are fewer myelin turns (40), the cortical mantle is thinner, the neurons are smaller, and there are fewer terminal boutons (41, 42). In studies employing adult rats (43, 44) and nonhuman primates (45) poisoned with lead, paralysis was absent but degenerative changes in myelinated and unmyelinated axons in rat (43, 44) and abnormalities in the brain and spinal cord characterized by vascular lesions and demyelination in the nonhuman primates (45) were evident.

Histochemical studies by Brun and Brunk (46) have shown that heavy metals are normal constituents of lysosomes and that in lead intoxication this metal accumulates in lysosomes of nerve tissue. They also provide histochemical evidence that lead poisoning leads to an apparent increase in acid phosphatase and decrease in alkaline phosphatase activity (47) suggesting a derangement in the metabolic role for lysosomes within the brain.

In studies using rats intoxicated with lead from the time of birth, Druse et al. (40) and Krigman et al. (41, 42) found that the delay in myelin formation was associated with decreased concentrations of neural lipids: phospholipids, cholesterol, cerebroside, plasmagin, and gangliosides particularly those which are incorporated in neuronal membranes and myelin.

In similarly intoxicated rats, Krall et al. (48) found that at 25 days the rats show tremors as well as hindquarter paralysis. It was further reported that the norepinephrine content of the cerebellum increased 100-400% above controls, while the monoamine oxidase activity was depressed by 50% and

mitochondrial phosphorylation completely inhibited. The suggestion was made that the primary effect of lead on the cerebellum is the inhibition of mitochondrial oxidative activity. In quite a different study, but with the suckling rat as an experimental model of lead encephalopathy, Millar et al. (14) found that when lead was fed to lactating rats there was a significant and commensurate reduction in both blood and brains-aminolevulinic acid dehydratase (ALA-D) activity in the suckling rat. A correlation of low ALA-D activity in the blood of mentally retarded children to the experimental findings suggests to the authors (14) that even a modest elevation of blood lead may be associated with biochemical abnormalities in the developing brain of the young child.

There is growing evidence that nerve tissue, including the brain, is more sensitive to many foreign substances than has hitherto been suspected and that toxic effects may be manifest as subtle disturbances in behavior long before any classical symptoms of poisoning become apparent.

Moreover, some behavioral disorders may be amplified by social interaction within a group. Information concerning behavioral effects of lead has been obtained from animal studies and from long-term psychological studies of children accidentally poisoned by lead (9-16).

Gusev (49) has studied the effects produced by inhalation of lead oxide aerosols on conditioned responses in adult rats and rabbits. Others (50) have shown that lead can influence rapid eye movement (REM) phase of sleep in rats. The effects were produced by rather high dosage levels, but in comparing effects on rats with those on humans one should note that adult rats absorb about 1% of ingested lead, whereas humans absorb about 10%, with more than threefold variations between individuals (51). In a study employing adult rats, Brown et al. (52) were unable to demonstrate any impairment of learning and memory following high doses of inorganic lead. However, another study (53) with lead-poisoned sucklings (17) suggests that the brains of

neonates are particularly sensitive to lead with effects on learning still present in the eight- to ten-week-old adult. On the other hand, chronic oral administration of lead to young adult rhesus monkeys was without effect on short-term memory and sensorimotor-response (54). When lead toxicity was studied in juvenile baboons, the onset of clinical symptomatology was noted after only 1 week of 5.0 mg/day (intravenous injection) culminating in convulsions and blindness after 40 days (55). The effects of prenatal and early postnatal lead exposure on the developing nervous system and later intelligence and behavioral development was tested in lambs (56). The results indicate that subclinical prenatal exposure to lead can retard learning of a visual discrimination problem. It is interesting to note that David et al. (16) have shown that in a selected population of hyperactive children possessing subtoxic levels of blood lead, 60% had urinary lead levels associated with toxic exposure to lead following a single provocative challenge with penicillamine. The suggestion is that these hyperactive children had a relative heavy body burden (bone lead level) of lead indicating a long-term low-level exposure (16).

The clinical symptomatology and sequelae of lead poisoning described above were derived mainly from case histories of children who ingested, intentionally or accidentally, large amounts of lead from house paints, peeling wall plaster, toys or poorly fired eating utensils. We are now becoming aware of a second area of exposure from industrial and automobile exhausts. These clinical studies are ominous in import and clearly demand systematic experimental investigations of chronic lead poisoning. For at present, there are no data available concerning what effect, if any, relatively low-level chronic lead exposure may have on the brain of the young during their earliest period of life when the brain is undergoing rapid growth and development. Our ignorance concerning this medical and social problem has stemmed in part from the lack of suitable experimental models.

The usual experimental animal is resistant to the brain effects of lead. However, it is now known, from work in a number of different laboratories, including our own, that if lead is given to newborn rats they develop many of the symptoms seen in children poisoned by lead. By adjusting the experimental conditions one can develop situations analogous to severe or mild forms of "pediatric" lead encephalopathy. Since lead is being taken in by the newborn rat each day, the conditions for a sustained metabolic interaction with lead are favorable. This is especially true in the brain of the newborn rat, since formation of new cells is rapid during the period immediately after birth. Dendritic growth begins after day 6 and is especially rapid after day 12. Although the long-axoned neurons are formed predominantly before birth, neurogenesis also takes place during the postnatal period; at that time it leads mainly to the formation of short-axoned new cells, microneurons, which are the most abundant cells in the cerebellum.

The actual size of the brain increases rapidly during development. In brains of normal rats the greatest postnatal cellular increase occurs in the cerebellum. During the first 3 weeks the weight of the rat cerebrum increases about 8-fold and that of the cerebellum 20-fold (57). Between the ages 5 and 17 days, DNA increases 2.5 times in the cerebrum but 8.5 times in the cerebellum (58). New cell formation is vigorous during the first 21 days after birth, and new cell formation accounts for 59% of the final number of cells in the cerebrum and 97% of the final number of cells in the cerebellum (59). Although the cerebellum represents only 10% of the wet weight of the whole brains of rats and humans, half the cells, as calculated from the DNA concentration, are located in the cerebellum of both species (60, 61). This provides an opportunity to study two adjacent organs in the brain differing in proliferative activity. There is a retardation in the rate of cerebellar growth in rats exposed to lead compared to controls of the same age. Using the DNA content

as an index of the number of cells, we find, in agreement with others, that the normal cerebellum undergoes greater replicating activity during its development than does the remainder of the brain. Between 5 and 21 days the total DNA in our normal rats increased ninefold in the cerebellum and twofold in the cerebral cortex, at a time when the weight of the former and latter increase six and three times, respectively. Not only is there a 5-7-day delay in the growth of the brain in animals poisoned with lead, but there is also a greater inhibition of new cell formation in the cerebellum compared to the cerebrum of these animals.

If DNA is indeed an index of cell number, then the decrease in DNA indicates a 15-20% deficit in the number of cells in the cerebella of the animals intoxicated with lead. The findings in this study of extensive edema of the cerebellum compared to the cerebrum in circumstances in which there is an 80-fold increase in the lead content of both tissues (dry weight basis) is also somewhat analogous to human clinical findings.

There are certain parallels between these observations and those described by Pentschew (6) in human lead encephalopathy. He cites conspicuous increases in brain volume with pronounced flattening of the convolutions. In addition the most constant microscopic finding in human lead encephalopathy is the activation of the intracerebral capillaries, the earliest stage of which is characterized by dilation of the capillaries with swelling of the endothelial cells.

Our results indicate that there is a significant retardation in brain growth of rats suckling from mothers ingesting lead. It is now well established that as normal maturation proceeds there is an increased conversion of glucose carbon into brain amino acids associated with the tricarboxylic acid cycle (36, 37). When uniformly radio-labeled glucose is administered to the young of lead-fed mothers there was a significant decrease in the percentage of the total radioactivity in the acid-soluble material of the brain present in the amino acid fraction

compared to normal animals of the same age. Our experimental findings indicate that the uptake of glucose from blood to brain was not affected in chronic exposure to lead and the decrease in the labeling of tricarboxylic acid cycle amino acids reflects a reduction in the utilization of glucose in the tissues (62, 63). This implies that lead has an action *per se* on the biochemical maturation of glucose metabolism in the developing brain as well as anatomical maturation.

Our laboratory has reported (64), as did Pentschew and Garro (17) and Thomas et al. (19), the precipitous advent of paraplegia and pigmented cerebellum during the fourth week of development in lead exposed neonatal rats. We noted that sucklings are able to gain access to the maternal solid lead containing diet at the end of the third and beginning of the fourth week. The question arose whether access to relatively greater amounts of lead contained in the maternal solid food, compared to maternal milk, account for the histopathology and paraplegia observed in these experimental animals. Although all three authors record the pronounced retardation in growth, no information was offered as to the amount of food consumed by the nursing mother rat. Furthermore, the contribution of undernutrition to the experimental findings was not ascertained by concomitant studies on pair-fed controls.

We have found that lactating mother rats eating 5% lead acetate raise offspring displaying all the symptoms as previously described by Pentschew and Garro (17). With this dietary regimen the maternal milk contained 25 ppm lead.

When the diet of the experimental group is changed at day 16 to one containing 25 to 40 ppm Pb (comparable to the leaded milk the sucklings had been consuming) and neonates allowed access to the solid diet there is neither paraplegia nor evident gross histopathology. However, these animals do exhibit hyperactivity, aggressiveness, tremors and preoccupation with self-grooming. Exposure to lead via the regimen described

leads to a significant increase in the amount of lead in brain within 5 days of the onset of exposure. The brain lead content of exposed animals increases another 33% after an additional three weeks of exposure. We found no observable differences in the serotonin,  $\gamma$ -aminobutyric acid, or norepinephrine content between brains of control and lead-exposed animals. Dopamine content was similar in 5- and 13-day-old experimental and control animals, however, there was a statistically significant decrease in dopamine at 21 and 29 days in the leaded animals relative to controls. Furthermore, a comparison of dopamine to norepinephrine ratios in experimental relative to control animals shows an approximate 20% depression of dopamine in the 4-week-old lead-exposed animals. It is of considerable interest to note that the observed abnormal behavior is associated with an eightfold increase in the concentration of lead in the brain and a disruption in catecholamine metabolism. It is a truism that pathology must be preceded by biochemical events. It may be that this valuable experimental model described by Pentschew and Garro (17) tends to obscure some of the subtle effects of lead on the CNS. In addition the experimental design we have described may offer a model of CNS dysfunction due to lead exposure without debilitating histopathology. It is possible that our findings on increased motor activity and changes in brain dopamine may correspond to early responses of lead exposure before the usual looked for overt signs of lead toxicity. This view is supported by the recent findings of Silbergeld and Goldberg (65), who administered lead solutions to lactating mice. It was found that suckling mice developed behavioral disorders such as hyperactivity and aggression. *d*-Amphetamine suppressed motor activity, whereas phenobarbital produced marked increase in the motor activity of the hyperactive mice. The suggestion is that lead-induced behavioral disorders in mice appears to have significant parallels with the pharmacology of "minimal brain dysfunction" hyperactivity in children.

We believe the lead-exposed prenatal and/or neonatal rat and mouse have proven themselves to be a reasonable model for those interested in the histopathological, biochemical, neurological, or behavioral aspects of childhood lead poisoning.

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## REFERENCES

1. Major, R. H. Classic description of diseases. Charles C Thomas, Springfield, Ill., (1932).
2. Holt, L. E. Lead poisoning in infancy. *Am. J. Dis. Child.* 25: 299 (1923).
3. McKhann, C. F. and Vogt, E. C. Lead poisoning in children. *JAMA* 101: 1131 (1933).
4. Greenberg, M., et al. A study of pica in relationship to lead poisoning. *Pediatrics* 22: 756 (1958).
5. Goyer, R. A., and Rhyne, B. C., Pathological effects of lead. *Int. Rev. Pathol.* 2: 2(1973).
6. Pentschew, A., Morphology and morphogenesis of lead encephalopathy. *Acta Neuropathol.* 5: 133 (1965).
7. Galle, P., and Morel-Maroger, L. Les lesions renales du saturnisme humain et experimental. *Nephron* 2: 273 (1965).
8. Haeger-Aronsen, B. Studies on the urinary excretion of aminolevulinic acid and other heme precursors in lead workers and lead intoxicated rabbits. *Scand. J. Clin. Lab. Invest. (Sup. 47)* 12: 6 (1960).
9. Perlstein, M. A., and Attala, R. Neurologic sequelae of plumbism in children. *Clin. Pediatr.* 5: 292 (1966).
10. Smith, H. D., et al., 1963. The sequelae of pica with and without lead poisoning. *Am. J. Dis. Child.* 105: 609
11. Chisolm, J. J., Jr., and Harrison, H. E. The exposure of children to lead. *Pediatrics* 18: 943 (1956).
12. Byers, R. K., and Lord, E. E. Late effects of lead poisoning on mental development. *Am. J. Dis. Child.* 66: 471 (1943).
13. Moncrieff, A. A., et al. Lead poisoning in children. *Arch. Dis. Child.* 39: 1 (1964).
14. Millar, J. A., et al. Lead and  $\delta$ -aminolaevulinic acid dehydratase levels in mentally retarded children and in lead poisoned rats. *Lancet* (2): 695 (1970).
15. Gibson, S. L. M., et al. Blood lead levels in normal and mentally retarded children. *Arch. Dis. Child.* 42: 573 (1967).
16. David, O., Clark, J., and Voeller, K. Lead and hyperactivity. *Lancet* (2): 900 (1972).
17. Pentschew, A., and Garro, F. Lead encephalomyelopathy of the suckling rat and its implications on the porphyrinopathic nervous diseases. *Acta Neuropathol. (Berlin)* 6: 266 (1966).
18. Lampert, P., Garro, F., and Pentschew, A. Lead encephalopathy in suckling rats. In: *Brain Edema.* I. Klatzo and F. Seitelberger, Eds., Springer-Verlag, Berlin and New York, 1967.
19. Thomas, J. A., Dallenbach, F. D., and Thomas, M. Considerations on the development of experimental lead encephalopathy. *Virchows Arch. Path. Anat.* 352: 61 (1971).
20. Michaelson, I. A. Effects of inorganic lead on levels of RNA, DNA and protein on developing neonatal rat. *Toxicol. Appl. Pharmacol.* 26: 539 (1973).
21. Clasen, R. A., et al. Ultrastructural studies in experimental lead encephalopathy. *Am. J. Pathol.* 66: 1a (1972).
22. Rosenblum, W. I., and Johnson, M. G. Neuro-pathologic changes produced in suckling mice by adding lead to the maternal diet. *Arch. Pathol.* 85: 640 (1968).
23. Coper, H., and Herken, H. Schädigung des Zentralnervensystems durch Antimetaboliten des Nikotinsäureamides. Ein Beitrag zur Molekularpathologie der Pyridinnukleotide. *Deutsch. Med. Wsechr.* 88: 2025 (1963).
24. Lowry, O. H., et al. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265 (1951).
25. Oyama, V. I., and Eagle, H. Measurement of cell growth in tissue culture with a phenol reagent (Folin-Ciocalteu). *Proc. Soc. Exp. Biol. Med.* 91: 305 (1956).
26. Munro, H. N., and Fleck, A. The determination of nucleic acids. In: *Methods of Biochemical Analysis* Vol. 14, 5. Click, Ed., Interscience, New York, 1966, p. 113.
27. Giles, K. W. and Meyers, A. An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature* 206: 93 (1965).
28. Burton, K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* 62: 315 (1956).
29. Murthy, L., et al. Atomic absorption of zinc, copper, cadmium and lead in tissues solubilized by aqueous tetramethylammonium hydroxide. *Anal. Biochem.* 53: 365 (1973).
30. Gross, S., and Parkinson, E. Tissue metal analysis by flameless atomic absorption spectroscopy, in press.

31. Patel, A. J., and Balazs, R. Manifestations of metabolic compartmentation during the maturation of the rat brain. *J. Neurochem.* 17: 955.
32. Patel, A. J., and Balazs, R. Effect of thyroid hormone on metabolic compartmentation in the developing brain. *Biochem. J.* 121: 469 (1971).
33. Patel, A. J., Balazs, R., and Johnson, A. L. Effects of under-nutrition on cell formation in the rat brain. *J. Neurochem.* 20: 1151 (1973).
34. Anton, A. A. and Sayer, D. F. A study of the factors affecting the alumina oxide-trihydroxy-indole-procedure for the assay of catecholamines. *J. Pharmacol. Expt. Therap.* 138: 360 (1962).
35. Okazaki, H., et al. Acute lead encephalopathy of childhood. *Trans. Am. Neurol. Assoc.* 88: 248 (1963).
36. Gaitonde, M. K., and Richter, D. Changes with age in the utilization of glucose carbon in liver and brain. *J. Neurochem.* 13: 1309 (1966).
37. Cocks, J. A., et al. Effect of thyroid hormone on the biochemical maturation of rat brain: conversion of glucose carbon into amino acids. *J. Neurochem.* 17: 1275 (1970).
38. Wiener, G. Varying psychological sequelae of lead ingestion in children. *Pub. Health Repts.* 85: 19 (1970).
39. Thomas, J. A., Dallenbach, F. D. and Thomas, M. The distribution of radioactive lead ( $^{210}\text{Pb}$ ) in the cerebellum of developing rats. *J. Pathol.* 109: 45 (1973).
40. Druse, M. J., et al. Effects of lead intoxication and starvation on postnatal myelination in the rat. *Fed. Proc.* 30 (Abstr. 528): 288 (1971).
41. Krigman, M. R., et al. Morphological, neurochemical, and behavioral correlates of lead intoxication and undernourishment in developing rats. *Fed. Proc.* 31 (Abstr. 2536): 665 (1972).
42. Krigman, M. R., et al. Morphometric and sphingolipid composition of myelin in lead intoxicated and undernourished suckling rats. *Am. J. Pathol.* 66 (Abstr.): 1a (1972).
43. Schlaepfer, W. W. Ultrastructural and histochemical studies of a primary sensory neuropathy in rats produced by chronic lead intoxication. *J. Neuropathol. Exp. Neurology* 27: 111 (1968).
44. Schlaepfer, W. W. Experimental lead neuropathy: a disease of the supporting cells in the peripheral system. *J. Neuropathol. Exp. Neurology* 28: 401 (1969).
45. Sauer, R. M., Zook, B. C. and Garner, F. M. Demyelinating encephalopathy associated with lead poisoning in nonhuman primates. *Science* 169: 1091 (1970).
46. Brun, A., and Brunk, U. Histochemical indications for lysosomal localization of heavy metals in normal rat brain and liver. *J. Histochem. Cytochem.* 18: 820 (1970).
47. Brun, A., and Brunk, U. Histochemical studies on brain phosphatases in experimental lead poisoning. *Acta. Pathol. Microbiol. Scand.* 70: 531 (1967).
48. Krall, A. R., et al. Elevation of norepinephrine levels and inhibition of mitochondrial oxidative phosphorylation in cerebellum of lead intoxicated suckling rats. *Fed. Proc.* 31: (Abstr. 2537): 665 (1972).
49. Gusev, M. I. Limits of allowable lead concentrations in the air of inhabited localities. In: *Limits of Allowable Concentrations of Atmospheric Pollutants.* V. A. Ryanzanov, Ed. (Book 4, 1960). (Translated from the Russian). U.S. Dept. Commerce, Office of Technical Services, Washington, D.C., 1961.
50. Xintaras, C., Sobecki, M. F., and Ulrich, C. C. Sleep: changes in rapid eye movement phase in chronic lead absorption. *Toxicol. Appl. Pharmacol.* 10 (Abstr. 18): 384 (1967).
51. Bryce-Smith, D. Behavioral effects of lead and other heavy metal pollutants. *Chem. Brit.* 8: 240 (1972).
52. Brown, S., Dragann, N., and Vogel, W. H. Effects of lead acetate on learning and memory in rats. *Arch. Environ. Health* 22: 370 (1971).
53. Brown, R. D. Long term effects of lead on learning and organ development in the growing rat. *Toxicol. Appl. Pharmacol.* 24 (Abstr. 71): 55 (1973).
54. Goode, J. W., Johnson, S., and Calandra, J. C. Evaluation of chronic oral administration of lead acetate to rhesus monkeys. *Toxicol. Appl. Pharmacol.* 24 (Abstr. 70): 53 (1973).
55. Cohen, N., et al. The juvenile baboon as a model for studies on lead poisoning in children. *J. Med. Primatol.* 1: 142 (1972).
56. Van Gelder, G. A., Carson, T. L., and Buck, W. B. Slowed learning in lambs prenatally exposed to lead. *Toxicol. Appl. Pharmacol.* 24 (Abstr. 72): 55 (1973).
57. Mandel, P., and Bieth, R. étude comparée du développement biochimique du cerveau chez quelques espèces de mammifères. *C. R. Acad. Sci. (Paris)* 235: 485 (1952).
58. Winick, M., and Noble, A. Cellular responses with increased feeding in neonatal rats. *J. Nutr.* 91: 179 (1967).
59. Balazs, R., et al. Effect of thyroid hormone on the biochemical maturation of rat brain: postnatal cell formation. *Brain Res.* 25: 555 (1971).
60. Chase, H. P., Lindsley, W. F. B., and O'Brien, D. Undernutrition and cerebellar development. *Nature* 221: 554 (1969).
61. Fish, I. and Winick, M. Cellular growth in various regions of the developing rat brain. *Pediatr. Res.* 3: 407 (1969).
62. Patel, A. J., et al. The metabolism of [ $^{14}\text{C}$ ] glucose by the brains of suckling rats intoxicated with inorganic lead. *J. Neurochem.*, submitted.

63. Patel, A. J., et al. Changes within metabolic compartments in the brains of young rats ingesting lead. *J. Neurochem.*, submitted.
64. Michaelson, I. A., and Sauerhoff, M. W., 1973. An improved model of lead induced brain dysfunction in the suckling rat. *Toxicol. Appl. Pharmacol.*, submitted.
65. Silbergeld, E. K., and Goldberg, A. M. Lead poisoning: an animal model of hyperactivity. *Pharmacologist* 15 (Abstr. 143): 181 (1973).