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Development of Resistance to Lead Encephalopathy During Maturation in the Rat Pup

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Abstract. The purpose of this study was to determine the maturational period during which the rat pup becomes resistant to the toxic effects of lead on the brain. Pups were fed lead, as lead acetate, by esophageal catheter for 14 days beginning at various ages between 14–24 days. The daily lead doses, which produced a hemorrhagic cerebellar encephalopathy in at least 50% of pups, were 400 μg Pb/g body weight for animals fed from 14 days of age, 800 μg/g for animals fed from 16 days, and 1600 μg/g for animals fed from 18 days. In contrast, pups fed even higher lead doses beginning at 20 days showed only a patchy cerebellar edema by light microscopy while pups fed from 24 days had normal cerebellums by light microscopy. The encephalopathic lead doses in the younger pups resulted in the same cerebellar lead concentrations (about 30 μg/g protein) as the higher lead doses fed pups beginning at 20 or 24 days. When corrected for blood lead concentrations, the cerebellar lead concentrations were 20–25% higher in the encephalopathic compared to the older encephalopathy-resistant animals. This difference may be accounted for by cerebellar hemorrhages in the younger animals. Polarographic studies showed inhibition of respiration in cerebellar slices from animals fed lead from 14 days of age but not in animals fed from 20 or 24 days of age. Our results suggest that, during the encephalopathy-sensitive age period, a critical cerebellar concentration of lead is associated with the encephalopathy. Resistance to lead encephalopathy in older animals, with similar cerebellar lead concentrations, may be related to a capacity to sequester lead in new cellular locations away from its site of action on aerobic energy metabolism.

Key Words: Brain edema, Cell respiration, Encephalopathy, Lead.

INTRODUCTION

The rat pup fed inorganic lead has been studied extensively as an animal model for human lead encephalopathy (1–9). The lead-fed pup develops an often fatal encephalopathy, marked by hemorrhage and edema in the cerebellum, while lead feedings in the adult rat do not result in encephalopathy (1, 2). From the results of previous studies, we proposed that effects of lead on cellular aerobic energy metabolism are important in the pathogenesis of lead encephalopathy (9–11). Consistent with this hypothesis is the observation that lead concentrations in cerebellar mitochondria from lead-fed adult animals are lower than in cerebellar mitochondria from pups fed inorganic lead (9).

In order to test these proposed mechanisms of lead toxicity in the immature brain and of resistance to that toxicity in the mature brain, we have determined more precisely the age period during which the rat becomes resistant to lead en-

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cephalopathy. Pups were fed lead by esophageal catheter once daily for 14 days beginning at various ages between 14–24 days. During the period of lead feedings, animals were studied for light microscopic evidence of cerebellar encephalopathy and for associated changes in weight gain, packed red blood cell volume (PCV), blood lead concentration, brain water content and lead concentration, and respiration in cerebellar slices.

MATERIALS AND METHODS

Materials. Oligomycin and dinitrophenol (DNP) were obtained from Sigma Chemical Co. (St. Louis, MO). Inorganic chemicals and solvents were the highest grade available (J. T. Baker Chemical Co., Phillipsburg, NJ). The standard laboratory chow (Ralston Purina Co., St. Louis, MO) contained 1% calcium, 0.74% phosphorous, and 3.3 IU vitamin D/g.

Lead feeding. Sprague-Dawley albino rats were used. Pregnant females were obtained before delivery to allow accurate dating of litters. Each litter was adjusted to eight pups on the day of birth and maintained with one litter plus mother per cage. Litters were designated to receive either Na acetate (NaAc) or Pb acetate ($Pb(Ac)_2$) to begin at 14, 16, 18, 20, or 24 days of age. During the feeding period all pups were weighed once daily and the appropriate quantity of NaAc or $Pb(Ac)_2$ in about 0.25 ml H_2O was administered by an esophageal tube. Pups were observed each day for general activity, gait changes, hair loss, and other signs of morbidity.

Pups were killed by decapitation after 2, 7, or 14 days of feedings. Blood was drawn into microcapillary tubes and the packed red blood cell volume (PCV) was determined by centrifugation. The brain was removed, observed for evidence of swelling or discoloration, and then prepared for further study as described below. In parallel experiments whole blood was collected for analysis of lead concentration.

Brain water content. Immediately after removing the brain, the cerebrum and cerebellum were separated from the brain stem, blotted dry, and weighed. Each tissue was dried in an individual crucible at 105°C for 4 hours (h). In initial studies, comparison of weights after four and 24 h at this temperature showed that at each age drying was complete after four h. The percentage of water in each region was (wet weight − dry weight/wet weight) × 100%.

Lead analysis. Lead contents of whole blood and of cerebral and cerebellar homogenates were analyzed by graphite furnace atomic absorption spectrophotometry (12). After removal of the brain, the cerebrum and cerebellum were separated from the underlying brain stem and homogenized in deionized distilled water. An aliquot of homogenate (0.25 ml) was dissolved in 25% tetramethylammonium hydroxide in ethanol (0.5 ml) and diluted with distilled water (0.5–2.0 ml). This solution was injected into the graphite furnace attachment (Perkin Elmer Model 2000) to the atomic absorption spectrophotometer (Perkin Elmer Model 206). Blood lead concentrations were measured in a similar manner using a Varian Techtron carbon rod atomizer (Model 61) and atomic absorption spectrophotometer (Varian Techtron Model AA-5). All solutions used in preparation of tissues for lead analysis were tested for lead contamination at regular intervals. Final lead concentrations of brain tissue are expressed as a fraction of the homogenate protein (13).

Tissue slice respiration. Immediately after removal of the brain, the cerebellum was separated from the underlying brain stem. Ten slices (300 μm thick) were taken from each hemisphere. After teasing away white matter, the slices were cut into thin strips by hand and suspended in 3 ml of a phosphate-buffered medium (pH 7.3) containing 0.9 m*M* $CaCl_2$, 2.0 KCl, 1.5 m*M* KH_2PO_4, 0.5 m*M* $MgCl_2$, 137 m*M* NaCl, and 4.3 m*M* $NaHPO_4$. About 0.3 ml of this suspension was placed in a 2 ml water-jacketed polarographic chamber (Gilson Co., Madison, WI). The chamber was filled with air-saturated medium. Temperature was maintained at 37°C. Glucose was added to a final concentration of 50 m*M*. O_2 concentration was measured with a Clarke platinum cathode assembly, polarized to −0.8 V, connected to a Gilson oxygraph.

In most experiments, the respiratory rate became stable within a few minutes (min). If the

TABLE 1

Effects of $Pb(Ac)_2$ Feedings for 14 Days Beginning at Various Ages in the Rat Pup

Age at start of feedings (days)	Daily lead dose (μg Pb/g body weight)	Deaths (%)	Encephalopathy* (%)	Change in body weight†	PCV‡ (%)
14	0§	12.5	0 (16)	++	34.9 ± 0.9 (9)
	→400¦	62.5	62.5 (24)	0	27.9 ± 0.7¶ (7)
	800	75	62.5 (8)	0	—
	1,200	100	100 (8)	−	—
16	0§	25	0 (16)	++	35.1 ± 0.6 (12)
	400	12.5	0 (8)	++	29.2 ± 1.1 (7)
	→800¦	38	63 (24)	0	23.9 ± 0.5¶ (14)
	1,600	100	75 (8)	0	—
18	0§	0	0 (8)	++	36.3 ± 0.5 (8)
	400	0	0 (8)	++	30.4 ± 0.8 (8)
	800	25	25 (8)	+	28.3 ± 1.3 (6)
	→1,600¦	46	63 (24)	0	20.6 ± 0.6¶ (15)
	2,400	87	75 (8)	0	—
20	0§	0	0 (8)	++	38.2 ± 0.5 (12)
	1,600	0	0 (24)	0	26.5 ± 1.0 (22)
	2,000	6.25	0 (24)	0	25.4 ± 0.6 (19)
	2,400	12.5	0 (8)	0	17.0 ± 1.3 (4)
24	0§	0	0 (16)	++	38.5 ± 0.7 (13)
	2,400	0	0 (8)	+	29.0 ± 0.7 (5)
	3,200	0	0 (16)	+	26.7 ± 1.1 (12)

* Animals were considered encephalopathic if they showed at least one of the following characteristics: hindleg paresis, brownish discoloration of the cerebellum, or cerebellar swelling. The results shown are the number of autopsy-verified encephalopathic animals as a percentage of the number of animals fed the respective lead doses (shown in parentheses).

† The changes in body weights during the two weeks of $Pb(Ac)_2$ feedings are described qualitatively in comparison to control animals. ++ indicates an increase in body weight of at least 100%; + indicates a gain of about 25–50% of original body weight; 0 indicates mean body weights changed less than 20%; − indicates a weight loss greater than 30% of original body weight.

‡ Packed red blood cell volumes (PCV) are expressed as the mean ± standard error of the mean (SEM) of the number of determinations shown in parentheses for each age and lead dose.

§ All control animals received NaAc (1,200 μg/g body weight in about 0.25 ml H_2O).

¦ The encephalopathic doses, defined as the lowest tested daily dose of $Pb(Ac)_2$ which produced the cerebellar encephalopathy in at least 50% of the pups in the respective age group, are indicated by an arrow (→).

¶ PCVs in animals receiving the minimum encephalopathic doses of $Pb(Ac)_2$ beginning at 16 and 18 days of age were significantly less than the PCVs of animals receiving 400 μg $Pb(Ac)_2$ from 14 days of age ($p < .001$).

cephalopathic dose for pups fed lead from 18 days of age, the 20-day-old animals showed weight loss and decreased PCVs similar to or greater than those seen in the younger encephalopathic pups. Pups fed these high lead doses from 24 days of age also showed similar decreases in PCVs. Unlike the younger animals, the pups fed lead from 24 days of age continued to gain weight, but at rates much less than those of control animals. Pups fed 2,400 or 3,200 μg Pb/g body weight for 14 days beginning at 24 days of age showed a 30% mean weight gain compared to greater than 100% mean weight gain in control pups ($p < .001$). Pups fed lead from 20 and 24 days of age did not develop hair loss.

Light microscopy. The brains of at least two pups were studied by light microscopy after receiving the encephalopathic lead doses (indicated by arrows in Table 1) for six and 14 days beginning at 14, 16, or 18 days of age (Fig. 1). Results were compared to two NaAc-fed control animals studied at each of these ages. The cerebrums of pups begun on daily lead feedings between 14–18 days were either normal or showed minimal patchy edema. Cerebellar changes were extensive in these animals after both six and 14 days of lead feedings. Large areas of edema were present in the molecular, granular, and Purkinje cell layers and in the white matter of more than 90% of the pups. Purkinje cells were spared even in the presence of extensive edema. Focal hemorrhages were observed frequently in the molecular and granular layers. Cell necrosis was evident in the granular and molecular layers and in the white matter of about half the lead-fed pups. In a few instances, proliferation of astrocytes was found in the granular layer and in the white matter.

Pups fed higher daily doses beginning at 20 days of age showed much less extensive lesions in the cerebellum. Animals fed 1,600 μg Pb/g body weight for 14 days showed slight patchy edema throughout all layers of the cerebellum. Of two pups fed 2,000 μg Pb/g body weight for 14 days, one appeared normal and one exhibited only focal edema in the molecular layer. The cerebellums from three of six pups fed 2,400 μg Pb/g body weight for six or 14 days were normal. The other three cerebellums showed slight patchy edema in the molecular, granular, and Purkinje layers with occasional focal pericapillary hemorrhage and necrosis in the molecular layer and white matter. At the highest dose, 3,200 μg Pb/g body weight, two pups showed minimal patchy edema in the molecular layer and white matter after six days. After 14 days of receiving this dose, cerebellums from two animals were normal while three showed changes similar to those seen after six days.

Four animals were studied after receiving 3,200 μg Pb/g body weight for 14 days beginning at 24 days of age. There were no lesions in the cerebellums of these animals.

Brain water content. Cerebral and cerebellar water contents in pups fed an encephalopathic lead dose from 14 days of age and fed higher lead doses from 20 and 24 days of age were compared to age-matched NaAc-fed controls (Table 2). These results support the light microscopic observations. A two-factor analysis of variance indicates that lead feedings begun at 14 days of age resulted in small increases in the water content of the cerebellum ($p < 0.05$) but not the cerebrum. A similar analysis shows no significant increase in the cerebral or cerebellar water content in pups fed lead from 20 days of age, consistent with the minimal, patchy cerebellar edema seen by light microscopy. In animals fed lead from 24 days of age, there also was no change in cerebral or cerebellar water content.

Lead concentrations. Cerebellar lead concentrations in pups fed encephalopathic lead doses beginning at 16 or 18 days of age were about 30 μg Pb/g protein (Table 3). The concentrations of lead were similar in the cerebrum and cerebellum at each of

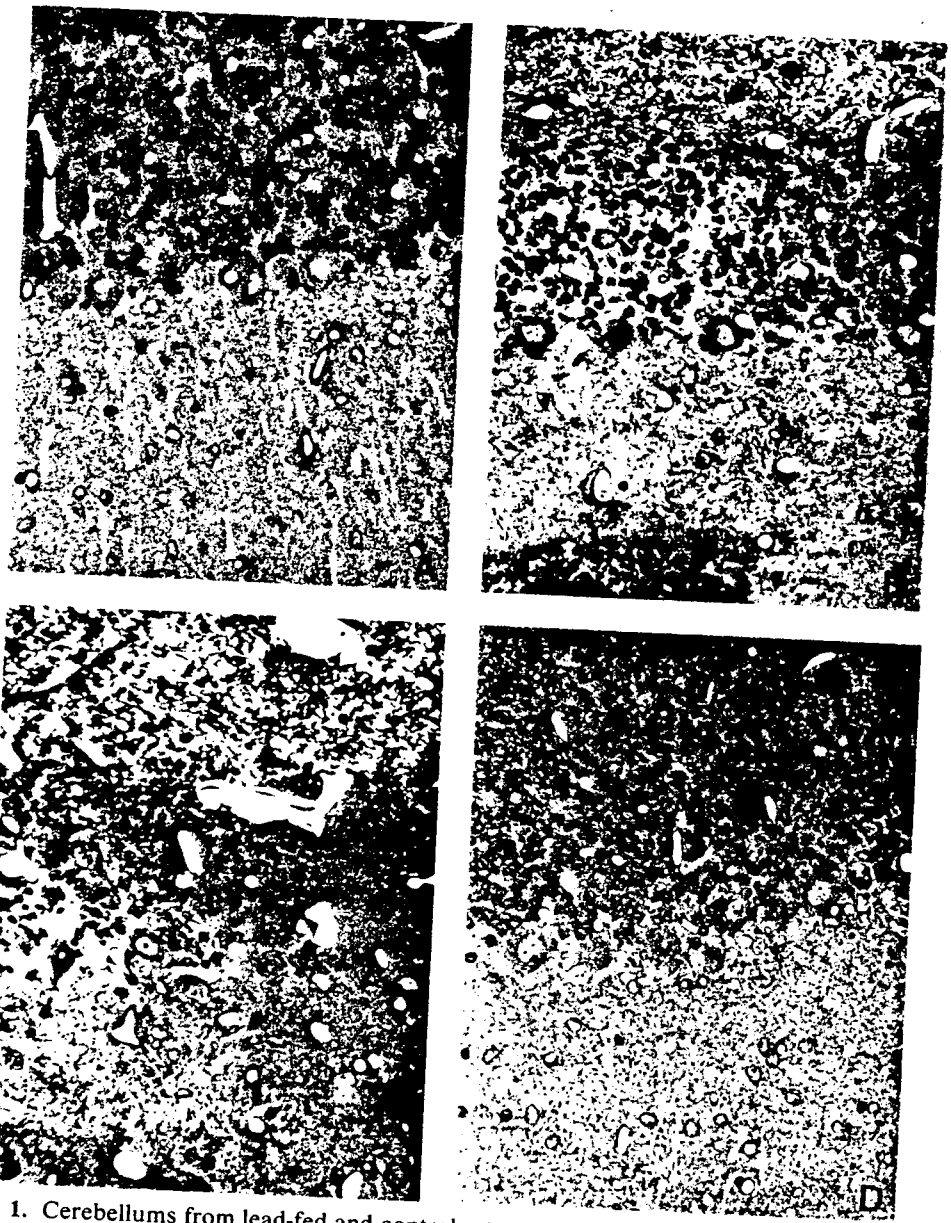

Fig. 1. Cerebellums from lead-fed and control rat pups sectioned at 1 μm and stained with toluidine blue-Azure II. A. Control rat pup fed NaAc (1,200 μg NaAc/g body weight) daily for 14 days beginning at 16 days of age. ×250. B. Rat pup fed 800 μg Pb/g body weight as $Pb(Ac)_2$ daily for 14 days beginning at 16 days of age. There was marked edema in the granular and molecular layers and focal hemorrhage in the molecular layer. Neuronal necrosis was evident in the granular layer. ×250. C. Rat pup fed 1,600 μg Pb/g body weight as $Pb(Ac)_2$ daily for 14 days beginning at 18 days of age. Neuronal necrosis was widespread throughout the molecular and granular layers. Edema was evident in all layers of the cerebellum. ×250. D. Rat pup fed 2,400 μg Pb/g body weight as $Pb(Ac)_2$ daily for 14 days beginning at 24 days of age. All layers of the cerebellum were similar to those of control animals. ×250.

TABLE 2
Water Content in Cerebrums and Cerebellums of Rat Pups Fed $Pb(Ac)_2$ or NaAc Beginning at Various Ages*

Age at start of feedings (days)	Duration of feedings (days)	Water content (% H_2O)			
		Cerebrum		Cerebellum	
		NaAc	$Pb(Ac)_2$	NaAc	$Pb(Ac)_2$
14 (400 μg Pb/g body weight)	2	83.46 ± 0.23 (4)	83.48 ± 0.14 (4)	83.10 ± 0.34 (4)	82.67 ± 0.22 (4)
	7	82.11 ± 0.04 (7)	82.25 ± 0.10 (7)	81.51 ± 0.12 (7)	82.85 ± 0.57 (7)
	14	80.95 ± 0.08 (9)	80.93 ± 0.11 (9)	80.67 ± 0.11 (9)	81.13 ± 0.27 (9)
20 (2,000 μg Pb/g body weight)	2	80.86 ± 0.12 (4)	81.34 ± 0.04 (4)	80.16 ± 0.24 (4)	80.88 ± 0.11 (4)
	7	81.18 ± 0.10 (4)	81.10 ± 0.09 (4)	80.95 ± 0.16 (4)	80.56 ± 0.09 (4)
	14	80.00 ± 0.14 (4)	79.99 ± 0.10 (4)	80.07 ± 0.04 (4)	80.39 ± 0.69 (4)
24 (3,200 μg Pb/g body weight)	2	81.65 ± 0.05 (4)	81.37 ± 0.15 (4)	81.34 ± 0.17 (4)	81.23 ± 0.22 (4)
	7	81.16 ± 0.16 (4)	80.86 ± 0.13 (4)	80.78 ± 0.13 (4)	80.76 ± 0.28 (4)
	14	79.34 ± 0.08 (4)	79.77 ± 0.19 (4)	79.30 ± 0.23 (4)	79.51 ± 0.38 (4)

* Water contents are expressed as the percentage of water, calculated as (wet weight − dry weight/wet weight) × 100 (see Methods). Each value is the mean ± SEM of the number of measurements shown in parenthesis.

TABLE 3
Concentration of Lead in Cerebrums and Cerebellums of Rat Pups Fed NaAc or $Pb(Ac)_2$ for 14 Days Beginning at Various Ages

Age at start of lead feedings (days)	Daily lead dose (μg Pb/g body weight)	Lead concentrations*		
		Cerebrum (μg/g protein)	Cerebellum (μg/g protein)	Whole blood† (μg/ml)
14–24†	0	5.04 ± 1.67 (9)	5.78 ± 1.81 (8)	1.16 ± 0.16 (13)
14	400‡	26.82 ± 8.33 (4)	51.58 ± 17.03 (5)	11.31 ± 0.84 (14)
	800	22.40 ± 9.86 (2)	16.20 ± 3.91 (4)	
16	800‡	22.11 ± 5.33 (3)	32.64 ± 5.09 (3)	12.48 ± 1.02 (8)
18	400	5.74 ± 0.60 (4)	6.21 ± 0.63 (4)	
	800	14.75 ± 3.78 (3)	15.79 ± 0.18 (2)	
	1,600‡	22.66 ± 2.68 (2)	29.90 ± 7.16 (2)	9.94 ± 0.52 (8)
20	1,600	17.99 ± 3.96 (10)	27.74 ± 5.19 (12)	
	2,000	27.65 ± 5.04 (11)	30.75 ± 2.78 (12)	
	2,400	41.28 ± 17.72 (3)	27.55 ± 2.90 (4)	16.75 ± 1.18 (8)
24	2,400	19.83 ± 1.80 (4)	29.14 ± 3.05 (5)	
	3,200	18.94 ± 1.90 (5)	27.65 ± 5.51 (5)	16.36 ± 1.16 (13)

* Each value for lead concentration is the mean ± SEM of the number of measurements shown in parentheses.

† The results for control animals receiving no lead (0) were pooled for all animals fed NaAc (1,200 μg/g body weight) for 14 days beginning at various ages from 14–24 days. The lead values were the same in the brains of animals fed NaAc from these various ages.

‡ These lead doses, begun at the particular ages, resulted in encephalopathy in at least 50% of the pups (see Table 1).

these ages. Higher lead concentrations in the cerebellums of encephalopathic pups fed from 14 days were probably due to the presence of red blood cell-bound lead resulting from the more extensive hemorrhages (16). Generally, the same lead doses (e.g., 400 or 800 μg Pb/g body weight) resulted in higher cerebellar lead concentrations in younger compared to older pups. In animals fed high daily doses of lead from 20 days of age, in which there was light microscopic evidence of only patchy edema, and in animals fed lead from 24 days, in which there was no indication of lead toxicity, cerebellar lead concentrations were the same as those found in encephalopathic younger pups.

The levels of lead in whole blood were about 30% higher in the older animals fed higher daily lead doses compared to the blood lead levels in the encephalopathic younger animals (Table 3). These values were used to correct for the blood lead in the cerebellums. Assuming a maximal blood volume of 5% (17) and brain protein concentration of 100 mg/ml (18), the lead in the cerebellar parenchyma may be as

TABLE 4

Respiration in Cerebellar Slices from Rat Pups Fed NaAc or $Pb(Ac)_2$ Beginning at Various Ages

Age at start of feedings (days)	Duration of feedings (days)	Basal respiration*		Oligomycin-inhibited respiration*		DNP-stimulated respiration*	
		NaAc	$Pb(Ac)_2$	NaAc	$Pb(Ac)_2$	NaAc	$Pb(Ac)_2$
14	2	17.5 ± 1.3 (8)	16.0 ± 2.1 (8)	8.5 ± 0.4 (3)	7.6 ± 0.6 (3)	26.3 ± 1.5 (5)	30.0 ± 1.4 (5)
(400 μg Pb/g body weight)	7	19.1 ± 1.4 (12)	19.8 ± 1.4 (12)	9.5 ± 0.8 (3)	7.5 ± 0.6 (3)	32.7 ± 2.4 (9)	33.8 ± 1.1 (9)
	14	18.6 ± 0.9 (12)	16.3 ± 0.8 (11)	9.5 ± 0.7 (3)	9.3 ± 0.5 (3)	33.8 ± 1.4 (9)	32.8 ± 1.0 (8)
20	2	15.9 ± 0.8 (8)	15.4 ± 1.1 (8)	11.1 ± 0.9 (4)	10.4 ± 0.5 (4)	27.6 ± 2.2 (4)	22.1 ± 2.3 (4)
(2,000 μg Pb/g body weight)	7	13.5 ± 0.8 (8)	14.1 ± 1.3 (8)	9.2 ± 0.8 (4)	8.8 ± 0.6 (4)	29.0 ± 2.2 (4)	30.1 ± 4.6 (4)
	14	13.6 ± 1.3 (8)	11.1 ± 0.6 (6)	8.2 ± 0.3 (4)	7.3 ± 0.4 (3)	24.3 ± 1.9 (4)	18.3 ± 2.0 (3)
24	2	13.5 ± 0.7 (8)	15.6 ± 1.0 (8)	8.5 ± 0.5 (4)	10.0 ± 1.0 (4)	26.5 ± 2.9 (4)	34.3 ± 1.8 (4)
(3,200 μg Pb/g body weight)	7	19.3 ± 1.8 (8)	16.8 ± 1.1 (8)	13.5 ± 1.6 (4)	11.8 ± 0.8 (4)	34.1 ± 1.2 (4)	35.0 ± 2.8 (4)
	14	15.0 ± 0.9 (8)	14.2 ± 1.5 (6)	9.7 ± 1.3 (4)	8.1 ± 0.9 (3)	29.0 ± 1.4 (4)	33.0 ± 2.9 (3)

* The respiratory rates are expressed in n at 0/mg protein min and were measured as described in the Methods. Each result is the mean ± SEM of the number of animals shown in parentheses.

cerebellar slices no increase in phosphorylation-independent respiration (oligomycin-insensitive respiration) early in the course of lead feedings is seen as it is in isolated mitochondria. The increase in maximal respiratory capacity in cerebellar slices from older nonencephalopathic lead-fed pups may be secondary to an increase in mitochondrial respiratory chain cytochromes, as found in lead-fed adult rat cerebellar mitochondria (20).

It is probable that lead acts upon the immature brain by several mechanisms. Studies demonstrating endothelial cell hypertrophy and increased capillary cellularity in response to lead suggest that the toxic effects of lead on immature capillary endothelium lead directly to thrombosis, hemorrhage, edema, and parenchymal cell necrosis (3–5, 8). We have proposed that lead primarily inhibits cellular energy metabolism, possibly in capillary endothelium as well as in parenchymal cells, in the developing brain (1, 6, 7, 9). Primary effects of lead on parenchymal and capillary cell respiration could alter the control of water and electrolyte concentrations, thus contributing to the genesis of cerebellar edema (21–24). Inhibitory effects of lead on both cortical microperfusion and on cell respiration could be additive in the pathogenesis of cell necrosis in the lead-induced encephalopathy.

The mechanism or mechanisms by which the mature brain is resistant to lead toxicity also are unclear at this time. The cerebrum appears to become resistant to lead toxicity at a younger age than the cerebellum, in spite of similar lead levels in the two regions (8, 25). From the present results, we confirm the observation that cerebellar lead levels remain as high in the older resistant animals fed higher lead doses as in the encephalopathic younger animals (8, 9). The mildly increased lead concentration in the cerebellar parenchyma of the younger animals probably can be accounted for by the hemorrhages in the encephalopathic cerebellums (16). The development of resistance to lead toxicity may be due to differences in the subcellular or cellular distribution of lead in the brain. Consistent with this hypothesis and the proposal that lead acts on mitochondrial respiration, higher concentrations of lead are found in cerebellar mitochondria from pups fed lead beginning at 14 days than in cerebellar mitochondria from lead-fed adults (9). A similar association of altered intracellular lead distribution and resistance to lead toxicity may occur in capillary endothelium, since abnormal brain capillaries from immature lead-fed pups have the same lead concentrations as normal-appearing capillaries from older lead-fed pups (26). The proposed mechanisms of resistance to lead toxicity in the rat brain will be studied further using the short age period of 18–24 days during which time the rat pup first exposed to lead changes from an encephalopathy-sensitive to an encephalopathy-resistant state.

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REFERENCES

1. Pentschew A, Garro F. Lead encephalo-myelopathy of the suckling rat and its implications on the porphyrinopathic nervous diseases. Acta Neuropathol (Berl) 1966;6:266-78
2. Clasen RA, Hartmann JF, Starr AJ, et al. Electron microscopic and chemical studies of the vascular changes and edema of lead encephalopathy. A comparative study of the human and experimental disease. Am J Pathol 1974;74:215-39
3. Goldstein GW, Asbury AK, Diamond I. Pathogenesis of lead encephalopathy. Uptake of lead and reaction of brain capillaries. Arch Neurol 1974;31:382-9
4. Thomas JA, Thomas IM. The pathogenesis of lead encephalopathy. Indian J Med Res 1974;62:36-45

5. Press MF. Lead encephalopathy in children. Am J Pathol 1977;84:485-8
6. Bull RJ. Lead and energy metabolism. In: Singhal RL, Thomas JA, eds. Lead toxicity. Baltimore: Urban & Schwarzenberg, 1980:119-69
7. Cavanagh JB. Metallic toxicity and the nervous system. In: Smith WT, Cavanagh JB, eds. Recent advances in neuropathology. Edinburgh: Churchill Livingstone, 1979;1:247-75
8. Krigman MR, Mushak P, Bouldin TW. An appraisal of rodent models of lead encephalopathy. In: Roizin L, Shiraki H, Grcevic N, eds. Neurotoxicology. New York: Raven, 1977;1:299-302
9. Holtzman D, Herman MM, Hsu JS, Mortell P. The pathogenesis of lead encephalopathy. Effects of lead carbonate feedings on morphology, lead content, and mitochondrial respiration in brains of immature and adult rats. Virch Arch [Pathol Anat] 1980;387:147-64
10. Holtzman D, Hsu JS. Early effects of inorganic lead on immature rat brain mitochondrial respiration. Pediatr Res 1976;10:70-5
11. Holtzman D, Hsu JS, Mortell P. In vitro effects of inorganic lead on isolated rat brain mitochondrial respiration. Neurochem Res 1978;3:195-206
12. Gross SB, Parkinson ES. Analysis of metals in human tissues using base (TMAH [tetramethylammonium hydroxide]) digests and graphite furnace atomic absorption spectrophotometry. At Absorpt Newsl 1974;13:107-8
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurements with Folin phenol reagent. J Biol Chem 1951;193:265-75
14. Slater EC. Applications of inhibitors and uncouplers for a study of oxidative phosphorylation. In: Estabrook RW, Pullman MD, eds. Methods in enzymology. New York: Academic Press, 1967; 10:48-57
15. Olson J, Holtzman D. Respiration in rat cerebral astrocytes from primary culture. J Neurosci Res 1981;5:497-506
16. Kochen JA, Greener Y. Brain lead levels in hemorrhagic lead encephalopathy. (Abstract) Pediatr Res 1977;11:563
17. Fishman RA. Brain edema. New Engl J Med 1975;293:706-11
18. Hertz L. Energy metabolism of glial cells. In: Schoffeniels E, Franck G, Tower DE, Hertz L, eds. Dynamic properties of glia cells. An interdisciplinary approach to their study in the central and peripheral nervous system. An International Symposium held at Liege, Belgium, August 29–30, 1977. Satellite Symposium to the Sixth International Meeting of the International Society for Neurochemistry. New York: Perganon, 1978:121-32
19. Michaelson IA, Sauerhoff MW. An improved model of lead-induced dysfunction in the suckling rat. Toxicol Appl Pharmacol 1974;28:88-96
20. Holtzman D, Hsu JS, Desautel M. Absence of effects of lead feedings and malnutrition on mitochondrial and microsomal cytochromes in the developing brain. Toxicol Appl Pharmacol 1981;58:48-56
21. Bull RJ, Stanaszek PM, O'Neill JJ, Lutkenhoff SD. Specificity of the effects of lead on brain energy metabolism for substrates donating a cytoplasmic reducing equivalent. Environ Health Perspect 1975;12:89-95
22. Goldstein GW. Lead encephalopathy: The significance of lead inhibition of calcium uptake by brain mitochondria. Brain Res 1977;136:185-8
23. Holtzman D, Obana K, Olson J. Ruthenium red inhibition of in vitro lead effects on brain mitochondrial respiration. J Neurochem 1980;34:1776-8
24. Olson J, Holtzman D. Respiration and cell volume of primary cultured rat cerebral astrocytes in media of various osmolarities. Brain Res 1982;246:273-80
25. Press MF. Lead encephalopathy in neonatal Long-Evans rats: Morphologic studies. J Neuropathol Exp Neurol 1977;34:169-93
26. Toews AD, Kolber A, Hayward J, Krigman MR, Morrell P. Experimental lead encephalopathy in the suckling rat: Concentration of lead in cellular fractions enriched in brain capillaries. Brain Res 1978:147:131-78

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