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Cerebrospinal Fluid Pressure in Weanling Rabbits with Chronic Plumbism

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Abstract

In chronic plumbism of children, elevated cerebrospinal fluid pressure has been reported. In an attempt to simulate this abnormality in an experimental animal, weanling rabbits were fed, in addition to a basal ration, lead intakes ranging from 0 to 100 mg/kg body weight/day for periods of either 10 or 12 weeks. Cerebrospinal fluid pressure was unaffected by lead intake. Concentrations of lead in the blood of the rabbits fed the highest lead intake were three times those in the blood of rabbits fed no lead. Similarly liver lead concentration was twelve times greater. As reported elsewhere, rabbits fed lead intakes ≥ 25 mg exhibited red blood cell stippling, elevated red blood cell protoporphyrin concentrations, elevated urinary delta-aminolevulinic acid and phorphobilinogen concentrations and lowered erythrocyte delta-aminolevulinic acid dehydratase activities. Since these biochemical alterations also occur in children exhibiting plumbism, it can be tentatively concluded that the weanling rabbit's response to chronic plumbism, in respect to the occurrence of elevated cerebrospinal pressure, is not similar to the human's response.

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Cerebrospinal Fluid Pressure in Weanling
Rabbits with Chronic Plumbism 1/

R. C. Hall, Jr., H. I. Frier, R. S. Bartlett and J. E. Rousseau, Jr. 2/

In mammals, cerebrospinal fluid is formed at the choroid plexuses and at the ependymal lining of the ventricles, flows through the ventricular system out into the subarachnoid spaces and in part returns to the blood via arachnoid villi (Davson, 1967, Rall, 1967, Hammerstad et al., 1969, Davson et al., 1970 and Milhorat, 1972). Cerebrospinal fluid provides buoyancy for the brain, regulates in part a constant ionic environment for the brain and provides for removal of chemical debris (Rall, 1967). Within a species, under specified conditions of measurement, the cerebrospinal fluid pressure is maintained within reasonably narrow limits (Davson, 1967). Either decreases or increases in cerebrospinal fluid pressure for the most part are indicative of defects in rates of formation or absorption of the fluid, of anatomical defects resulting in restriction or blockage of flow, or of physiological defects such as alterations in arterial or venous blood flow or pressure.

Elevated cerebrospinal fluid pressure has been reported in chronic plumbism of children (Follis, 1948, Weissberg et al., 1971, Bell and McCormick, 1972 and Milhorat 1972). This may be accompanied by papilledema as a result of the increased cerebrospinal fluid pressure and eventually by optic atrophy and blindness (Rodger and Sinclair, 1969 and Bell and McCormick, 1972). The actual incidence of elevated cerebrospinal fluid pressure in chronic plumbism of children is not known, possibly due to the general recommendation that lumbar puncture should be avoided except when necessary for differential diagnosis (Chisolm, 1967, American Academy of Pediatrics Subcommittee on Accidental Poisoning, 1969 and Bell and McCormick, 1972). In a summary of 425 children with plumbism, 59 initially exhibited encephalopathic signs indicative of increased intracranial pressure (Perlstein and Attala, 1966).

The most prominent pathological changes of the central nervous system caused by lead include cerebral edema associated with elevated cerebrospinal fluid pressure, endothelial cell proliferation and swelling accompanied by capillary and arteriole dilation, glial cell proliferation and focal necrosis and neuronal degeneration (Goyer and Rhyne, 1973). Investigators have not been in agreement as to the relative importance of these changes; however, Goyer and Rhyne stated that "the primary vascular change must account for the cerebral edema and increase in intracerebral pressure". In addition, in the case of animal models of plumbism, these researchers noted

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the paucity or even absence of reported clinical data on manifestations of central nervous system toxicity.

To ascertain possible effects of chronic plumbism on intracranial pressure, we measured cerebrospinal fluid pressure in weanling rabbits fed supplementary lead acetate intakes ranging from 3 mg of lead/kg body weight/day to 100 mg. In addition, we monitored the lead status of these animals by estimating the lead concentration in the whole blood and liver,

EXPERIMENTAL PROCEDURES

Animals. In two experiments 60 male weanling New Zealand rabbits, in sets of four at thirty-one days of age, were obtained (Hertzler Enterprises, Inc., Mansfield Center, Connecticut) numbered at random and each placed in an individual stainless steel cage, 51 cm wide x 56 cm long x 38 cm high, with a 1.25 cm mesh #12 wire floor, and fed according to the regimen in Appendix TABLE I. This consisted of a one week transition period in which the rabbits were fed 100 g of the basal ration, Appendix TABLE II, plus decreasing amounts of chopped alfalfa hay, a two week standardization period in which the rabbits were fed basal ration only, then either a ten week (first experiment) or twelve week (second experiment) comparison period in which the rabbits were fed the basal ration plus designated amounts of lead. Assignment to lead intake was at random in sets of four.

The basal ration met the NRC minimum daily essential nutrient requirement for the rabbit (National Research Council, 1966). The form of dietary lead fed was lead acetate, $Pb(Ac)_2 \cdot 3H_2O$, incorporated in basal ration pellets at concentrations of either 0.5 mg Pb/g (first experiment) or 10.0 mg Pb/g (second experiment). In the first experiment, 36 rabbits were fed Pb intakes of either 0, 3, 6 or 12 mg/kg body weight/day and in the second experiment, 24 rabbits, either 0, 25, 50 or 100 mg/kg body weight/day. Supplementary lead was fed at 11 a.m. After each rabbit had completely consumed its respective supplement, the daily basal ration allowance (minus the weight of the supplement) was fed.

For three weeks following arrival, all rabbits received, in their drinking water, 0.1% sulfaquinoxaline sodium (Sulfa-Nox Purina containing 3.44% sulfaquinoxaline) for prevention of intestinal coccidiosis (Siegmund, 1967), and 0.5% sulfamethazine (Sulfa-Purina containing 12.5% sodium sulfamethazine) to control liver coccidiosis (Jones, 1965). In an attempt to control enteritis, 50 mg of oxytetracycline hydrochloride (Puramycin Purina containing 25 mg oxytetracycline hydrochloride per ml) was added to each rabbit's water cup according to the schedule shown in Appendix TABLE I (Jones, 1965). Water was given ad libitum throughout the experiments. Feed hoppers and water cups were removed daily from the cages, washed and sterilized.

Observations and analysis. All feeds offered and refused were weighed daily to the nearest gram. The basal ration was analyzed for lead (Dalton, 1969) and found to contain 0.002 mg Pb/g. Rabbits were weighed upon arrival at the Animal Nutrition Research Barn and on the morning of the last day of each experimental week (Tuesday), as well as the morning of cerebrospinal fluid pressure measurement.

The mean and standard error for the minimum and maximum ambient temperatures during the comparison period were 20.0 ± 0.2 and $24.7 \pm 0.2C$ for the first experiment and 16.8 ± 0.2 and $19.8 \pm 0.2C$ for the second. Light intensity, limited to incandescent sources within the animal room, averaged 194 ± 13 lux (first experiment) and 215 ± 12 (second experiment).

During the course of both experiments, blood samples were obtained from the mar-

ginal ear vein (Hoppe et al., 1969) and two consecutive 24-hour urine collections were made and analyzed for biochemical constituents indicative of Pb-toxicity. The results of these analyses are reported elsewhere (Roscoe et al., 1972 and Roscoe, 1973).

On the 3rd morning of the 11th comparison period week (first experiment) and of the 13th comparison period week (second experiment) each rabbit was weighed and anesthetized with pentobarbital sodium solution (Fort Dodge M-122, Pentobarbital sodium, concentration 65 mg/ml) diluted 1:1 with sterile physiological saline. The anesthetic was given intraperitoneally at a dose of 0.8 ml/kg body weight. After muscular relaxation, the rabbit was clipped about the head, neck and shoulders and placed in sternal recumbency in a stereotaxic device (David Kopf #900 base and David Kopf rabbit adaptor) with the snout secured by a nose clamp and adjustable tooth bar and the head positioned and secured with two zygoma clamps. The zygoma clamps were at the same level as the diaphragm of a transducer (Statham P23AC, 0.8 mm³/100 mm Hg displacement) in order to establish a zero reference point. A sliding, measuring metric reservoir, containing sterile physiological saline, was connected to the side-arm of the pressure dome with polyethylene tubing (Clay Adams PE 190) whilst a modified 22 gauge, 2 inch spinal Quincke needle with 30 mm of polyethylene tubing (Clay Adams PE 50), for the cisterna magna puncture, was connected to a two-way stopcock (B-D MS02) attached to the second luer-lock fitting of the dome. A simulated pressure of 272 mm saline was applied to the transducer and allowed to drip through the filled dome and out the modified cisternal cannula. The neck, dorsal to the interparietal bone, was palpated to distinguish the point of entry for the cannula (Wells, 1964). The cannula placed at this point and at a 45° angle to the slope of the neck was thrust through the musculature. Once the cannula pierced the dura mater, which provides a more discernable resistance, the connection from the reservoir was closed allowing for communication between the rabbit and transducer diaphragm. Proper placement of the cisternal cannula was evidenced by a rapid negative movement of the recorder pen, respiratory fluctuation and a positive Queckenstadt test (Davson, 1967). A two and one-half minute recording was made on a physiological recorder (Grass Polygraph 7, Model 7A1ZP35). In the second experiment upon completion of the recording, the vertical distance from the midpoint of the zygoma clamps to the site of puncture was measured. This averaged 15 mm with a SD of 3.4.

After measurement of each rabbit's CSF pressure, 30 ml of blood was withdrawn by heart puncture. To 10 ml, heparin was added to prevent clotting and whole blood lead determined (Dalton, 1969). To another 20 ml, citrate was added to prevent clotting, the sample centrifuged and plasma obtained for determination of vitamin A (Kimble, 1939) and of total tocopherols (Quaife and Harris, 1944). Additional anesthetic, if necessary, was administered and the rabbit decapitated.

The brain, left kidney and liver were removed and weighed. The liver was homogenized in a blender (Waring), refrigerated at -18C, and subsequently analyzed for dry matter, ash, lead (Dalton, 1969) and vitamin A (Bunnell et al., 1954). Other tissues were obtained for histopathological examination and these results are to be reported elsewhere (Kircher and Nielsen, 1972-73).

The data of the respective criteria from each experiment were subjected to analysis of variance and covariance, the latter where applicable, so as to isolate the variation due to Pb intakes, treatments, and to rabbits within treatments, (Snedecor and Cochran, 1967).

RESULTS

Feed consumed, growth and health. In the first experiment, feed consumed and growth were unaffected by level of supplemental Pb intake, TABLE 1. In the second experiment, both feed consumed and growth were less in those rabbits fed the 100 mg Pb intake, TABLE 2.

No outward signs of Pb toxicity, such as depression, walking in circles, ataxia, standing with head pressed against a firm object, crying out, muscular or eyelid twitching, staggering or convulsions, were observed in any rabbit of either experiment. In the first experiment, one rabbit fed no supplement Pb, 0 mg Pb intake, died the first week of the comparison period after exhibiting mucus diarrhea for two consecutive days. Based upon postmortem examination, death was attributed to focal ulcerative gastritis.

Cerebrospinal fluid pressure was unaffected by Pb intake, TABLES 1 and 2.

Whole blood lead, plasma vitamin A and plasma tocopherol concentrations.

In the first experiment, only plasma vitamin A concentrations were successfully determined and averaged 34, 32, 32 and 26 $\mu\text{g}/100\text{ ml}$ for the 0, 3, 6 and 12 mg Pb intake groups, SD=8. In the second experiment, whole blood lead increased with Pb intake and averaged 53, 121, 107 and 189 $\mu\text{g}/100\text{ ml}$ for the 0, 25, 50 and 100 mg Pb intake groups with the respective SD-s equal to 26, 30, 16 and 101. Averages for plasma vitamin A concentration, in $\mu\text{g}/100\text{ ml}$, were 50, 47, 50 and 33 with SD = 8 and for plasma tocopherol, in mg/100 ml, were 1.5, 0.9, 1.6 and 1.4 with SD = 0.5.

Brain, kidney and liver weights and liver lead and vitamin A concentrations.

In the first experiment, TABLE 3, organ weights were unaffected and the liver lead concentration of the rabbits fed the highest Pb intake was three times that for the rabbits fed no added lead. In the second experiment, TABLE 4, those animals fed the 100 mg Pb intake had smaller organ weights than the other three groups; however, when expressed on a per unit body weight basis, only liver was found to be affected by Pb intake. The lead concentration in the liver increased in both experiments with increasing Pb intake. In the liver of those rabbits fed the 100 mg Pb intake, Pb concentration averaged twelve times that determined in the liver of the rabbits fed no supplemental lead. The concentration of vitamin A in the liver was not significantly affected by lead intake. Vitamin A, expressed as the total amount in the liver or as the amount per unit of body weight, tended to be less in the rabbits fed supplemental Pb and this was most pronounced in those fed the 100 mg Pb intake.

TABLE 1. Effect of lead intake upon feed consumed, growth and cerebrospinal fluid pressure of weanling rabbits in Experiment I.

Criteria	Pb intake, mg/kg body wt/day				SD per rabbit
	0	3	6	12	
Duration of comparison period (wk)	10	10	10	10	----
Animals (no)	8	9	9	9	----
Feed, (kg)					
Offered	7.73	7.88	8.35	8.32	0.94
Consumed					
Actual	7.68	7.82	8.30	8.28	0.93
Adjusted ^{a/}	8.00	7.82	8.08	8.18	0.42
Body weight (kg)					
Initial	1.29	1.38	1.44	1.40	0.23
Gain	1.99	2.03	2.10	2.16	0.21
Terminal cerebrospinal fluid pressure (mm saline)	44	48	44	44	26

^{a/} Adjusted by covariance for body weight the day prior to the beginning of the comparison period.

TABLE 2. Effect of lead intake upon feed consumed, growth and cerebrospinal fluid pressure of weanling rabbits in Experiment II.

Criteria	Pb intake, mg/kg body wt/day				SD per rabbit
	0	25	50	100	
Duration of comparison period (wk)	12	12	12	12	----
Animals (no)	6	6	6	6	----
Feed (kg)					
Offered	10.18	10.48	10.20	9.07	1.19
Consumed	10.18	10.48	10.20	8.76	1.27
Body weight (kg)					
Initial	1.42	1.46	1.44	1.40	0.18
Gain	2.33	2.37	2.27	2.02	0.36
Terminal cerebrospinal fluid pressure (mm saline)	73	61	58	64	16

TABLE 3. Effect of lead intake upon organ weights and lead concentration of the liver of weanling rabbits in Experiment I.

Criteria	Pb intake, mg/kg body wt/day				SD per rabbit
	0	3	6	12	
Brain					
Total wt (g)	9.4	9.2	9.3	9.3	0.5
Weight per unit body wt (mg/g)	3.0	2.8	2.7	2.7	0.3
Left kidney					
Total wt (g)	8.4	8.6	9.0	9.3	1.4
Weight per unit body wt (mg/g)	2.6	2.6	2.6	2.7	0.3
Liver					
Total wt (g)	93	109	106	109	24
Weight per unit body wt (mg/g)	29	33	31	32	8
Dry matter (g/100g)	27.32	27.49	27.24	27.27	0.89
Ash (g/100g)	1.15	1.11	1.16	1.14	0.10
Lead content of liver					
Fresh (ug/100g)	107(31) ^{a/}	225(70)	254(58)	368(180)	----
Dry (ug/100g)	392(113)	818(259)	932(226)	1365(669)	----
Ash (mg/100g)	9(3)	20(6)	22(6)	32(17)	----

^{a/} Standard deviation per rabbit given in parentheses.

TABLE 4. Effect of lead intake upon organ weights and lead and vitamin

A concentrations of the liver of weanling rabbits in Experiment 11.

Criteria	Pb intake, mg/kg body wt/day				SD per rabbit
	0	25	50	100	
Brain					
Total wt (g)	10.1	10.1	9.9	9.3	0.6
Weight per unit body wt (mg/g)	2.8	2.7	2.8	2.9	0.3
Left kidney					
Total wt (g)	10.2	11.0	10.6	9.4	1.6
Weight per unit body wt (mg/g)	2.8	2.9	3.0	2.9	0.4
Liver					
Total wt (g)	118	128	129	99	21
Weight per unit body wt (mg/g)	33	35	36	31	6
Dry matter (g/100g)	26.93	25.98	26.06	26.66	0.98
Ash (g/100g)	1.16	1.11	1.14	1.23	0.13
Lead content of liver					
Fresh ($\mu\text{g}/100\text{g}$)	78(33) ^{a/}	468(72)	610(187)	935(297)	----
Dry ($\mu\text{g}/100\text{g}$)	288(120)	1802(294)	2340(726)	3475(1110)	----
Ash (mg/100g)	6(2)	42(7)	53(17)	76(19)	----
Vitamin A content of liver					
Concentration ($\mu\text{g}/100\text{g}$)					
Actual	1228	1015	980	1080	330
LOG ₁₀	3.08	3.00	2.98	3.00	0.12
Total (μg)					
Actual	1442	1297	1245	1028	332
LOG ₁₀	3.14	3.10	3.09	2.98	0.12
Per unit body weight ($\mu\text{g}/\text{kg}$)					
Actual	394	347	348	314	75
LOG ₁₀	2.59	2.53	2.54	2.48	0.10

^{a/} Standard deviation per rabbit given in parentheses.

DISCUSSION

In these two experiments, in which weanling rabbits doubled their weight and at the highest Pb intake, had whole blood Pb concentrations three times and liver Pb concentrations twelve times those observed in rabbits fed no supplemental Pb, cerebrospinal fluid pressure was unaffected by Pb intake. It was, therefore, tentatively concluded that the weanling rabbit's response to Pb intake, in respect to the occurrence of elevated cerebrospinal fluid pressure, was not similar to the human's response to Pb intake.

In contrast to the lack of response of cerebrospinal fluid pressure to dietary Pb, other criteria indicative of lead toxicity were affected, especially in those rabbits fed dietary intakes of Pb \geq 25 mg/kg body weight/day. These included red blood cell stippling and elevated red blood cell protoporphyrin concentrations as indicated by a positive fluorescent erythrocyte test, and elevated concentrations and daily urinary output of delta-aminolevulinic acid and are reported elsewhere (Roscoe, 1973). In a subsequent experiment (Bartlett, 1974), rabbits fed a 25 mg Pb intake exhibited lower erythrocyte delta-aminolevulinic acid dehydratase enzymatic activities and greater urinary concentrations and daily output of delta-aminolevulinic acid and phorphobilinogen than did rabbits fed no supplemental Pb. Therefore, with respect to these criteria, the weanling rabbit's response to toxic intakes of Pb was found to be essentially similar to humans (Vallee and Ulmer, 1972).

To date, attempts to reproduce in laboratory animals the vascular changes and edema of human lead encephalopathy have not been very successful except in suckling rats by administration of lead to the dam (Clasen et al., 1974). Had such a procedure been employed in the present experiment instead of using the post-weaning period, the results with respect to cerebrospinal fluid pressure, may have been different. However, little if anything is known about cerebrospinal fluid dynamics in lead toxicity (Goyer and Rhyne, 1973) and in addition, man and four-footed animals differ in cerebrospinal fluid dynamics (Cutler et al., 1968). Lastly, in another toxicity, hypervitaminosis A, cerebrospinal fluid pressure decreased in four-footed animals (Eaton, 1969) but increased in man (Feldman and Schlezinger, 1970).

The effects of dietary Pb on total liver vitamin A contents, especially in those rabbits fed the 100 mg Pb intake, may appear to contradict the negative findings of the effect of Pb on liver vitamin A recently reported for the rat (Phillips et al., 1971). However, the 100 mg Pb intake rabbits of the present study consumed less basal ration, TABLE 2, which contained the vitamin A than did the rabbits fed no supplemental Pb. Thus less vitamin A was consumed by the former group. Therefore, it was not possible to ascertain the effect of Pb on liver vitamin A because of this difference in feed intake.

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Appendix Table I. Feeding Regimen

Period	Exp. wk.	Basal ration	Water	Medication ^{a/}	
				Sulfa	Antibiotic
Transition	1	100 g plus decreasing amts. of hay ^{b/}	Ad Libitum	All days	Last 7 days, 2 ml/rabbit
Standardizing	2	6.0% ^{c/}		7-days	First 3-days, 2 ml/rabbit
	3	5.8%		7-days	Last day, 3 ml/rabbit
	4	5.6%		-	All days, 3 ml/rabbit
	5	5.4%		-	First 2-days, 3 ml/rabbit
	6	5.2%		-	-
	7	5.0%		-	-
Comparison	8	4.8%		-	-
	9	4.6%		-	-
	10	4.4%		-	-
	11	4.2%		-	-
	12	4.0%		-	-
	13	3.9%		-	-
	14	3.8%		-	-
	15	3.7%		-	-
	16	3.6%		-	-

^{a/} Sulfaquinoxaline sodium (Sulfa-Nox Purina containing 3.44% sulfaquinoxaline sodium) was added to the drinking water to provide a concentration of 0.1%; sulfamethazine sodium (Sulfa-Purina containing 12.5% sodium sulfamethazine) was added to the drinking water to provide a concentration of 0.5%; and the

antibiotic, oxytetracycline (Pura-mycin Purina containing 25 mg oxytetracycline hydrochloride per ml), was added in the volumes indicated directly into each rabbit's water cup.

b/ Five g of chopped alfalfa hay the first day and reduced 1 g daily until the 6th day when no hay was fed.

c/ The average daily feed allowance in grams was based upon the rabbit's anticipated body weight in grams times the percent times 10^{-2} .

Anticipated body weight was equal to $W_1 + \frac{W_1 - W_2}{4}$ where W_1 = current body weight and W_2 = body weight two weeks (14 days) previous.

Appendix Table II. Basal Ration.

Ingredients ^{1/}	kg/100 kg
Barley, grain, all analyses US, (40) ^{5/} (Ground barley)	20.540,462
Oats, grain, all analyses US, (40) ^{5/} (Ground oats)	22.500
Flax, seed, solv-extd, grnd, (52) (LOM solvent process)	6.750
Soybean, seed, wo hulls, solv-extd, grnd, mx 3 fbr, (52) (SOM 50%, solvent process)	6.750
Beet, sugar, pulp, extd-res, dehy, (10) ^{5/} (Dried beet pulp)	10.000
Wheat, bran, dry-mil, (40) (Wheat bran)	22.500
Cane, sugar, molasses, mech-expr, mn 48 invert sugar (40) (Cane molasses)	9.000
Limestone, grnd, mn 32 Ca, (60) (Calcium carbonate)	0.750
Animal, bone steamed dehy grnd, (60) (Steamed bone meal)	0.250
Iodized salt	0.900
Vitamiu A supplement ^{2/}	0.001,538
Yeast, irradiated, dehy, ^{3/} (52)	0.008
Vitamin E supplement ^{4/}	0.050,000
Total	100.000,000

^{1/} Nomenclature from NAS-NRC Publ. 1232. 1964.

^{2/} Hoffmann - La Roche type 325-40 vitamin A acetate beadlets, 325,000 USP units per gram, contributes 1500 µg of retinol equivalent per kg of ration.

^{3/} Standard Brands type 36-F, 36,000 I.U. vitamin D per gram, contributes 2880 I.U. vitamin D per kg of ration.

4/ Hoffmann - La Roche vitamin E acetate beadlets, 500 I.U. d, 1- α -tocopheryl acetate per gram, contributes 250 I.U. of vitamin E per kg of ration.

5/ Ingredients finely ground.