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Comparative Effects of Feeding Lead Acetate and Phospholipid-bound Lead on Blood and Tissue Lead Concentrations in Young and Adult Rats

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The harmful effects of lead exposure have been recognized for many years. Most of the early evidence came from either industrial accidents or children ingesting paint containing lead chromate or lead carbonate. Recently, clinical and experimental evidence has been gathered which indicates that neuropsychological dysfunction occurs in children exposed to chronic low levels of lead (LANDRIGAN et al. 1975; DAVID 1974).

Differences in the bioavailability and toxicity of different chemical forms of heavy metals are well recognized, the most noteworthy example being the much greater hazard of methylmercury as compared with inorganic mercury (KURLAND et al. 1960). Data relating toxicity of lead to its different chemical forms are sparse. CREMER (1959) showed that tetraethyl lead is more toxic than lead acetate in rats. BARLTROP and MEEK (1975) described a rapid method for the determination of the relative absorption of dietary lead by rats. They found that basic lead carbonate had the highest absorption rate of all the inorganic forms of lead which were tested. The exact forms in which lead may exist in food are not known; possible forms are inorganic, protein-bound, and phospholipid-bound.

Phospholipids are known to bind heavy metals. Cadmium (MACPHERSON 1954) and lead (SCHOLFIELD and DUTTON 1955) have been used to complex and purify phospholipids. However, the toxicity and rate of absorption of these compounds have not been studied. The purpose of the present investigation was to compare the bioavailability of phospholipid-bound lead to that of lead acetate. Tissue lead concentrations and urinary excretion of delta-aminolevulinic acid (δ-ALA), a sensitive test for lead intoxication (DAVIS et al. 1968), were used as indicators of bioavailability.

MATERIALS AND METHODS

Phospholipid-bound lead was prepared as follows: 100 g of L-α-lecithin (Type II S from soybeans, commercial grade, Sigma Chemical Co.) was extracted with five successive 800-ml portions of absolute methanol in a 1-liter Erlenmeyer flask with stirring (magnet) for 30 min. The methanol fractions were combined and filtered. To the filtrate, a saturated methanol solution of lead acetate (Fisher Scientific Co.) was slowly added until no further precipitation occurred. The liquid phase was removed

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after centrifugation. The precipitate was washed six times with 250-ml portions of methanol by repeated suspension of the precipitate and centrifugation. After the final wash, the precipitate was filtered through a Buchner funnel and washed ten times with 50-ml volumes of methanol. The product was dried in a vacuum desiccator. The dried material weighed 38 g and contained 28% lead and 1.9% phosphorus. When this material was spotted on a thin layer chromatographic plate (Redi-coats, Supelco Inc.) and developed with a chloroform-methanol-water (65-25-4) solvent system, one major spot (R_f 0.52) was revealed after the plate was sprayed with a molybdenum reagent (Phospray, Supelco Inc.). No attempt was made to identify which phospholipid was bound to lead.

Male Sprague-Dawley rats were assigned to groups so that the average body weights for each group were similar. In the adult rat experiment, ten rats (295 to 390 g) were assigned to each of five groups; in the young rat experiment, eight rats (87 to 151 g) were assigned to each of five groups. The rats were housed individually in stainless steel wire-mesh cages. Basal diet (Table 1) was given to all groups. The diets contained either background lead levels (<0.03 ppm) or an added 300 ppm; lead diets modified by different forms of lead are outlined in Table 2. Food and deionized water were supplied ad libitum. The feeding period lasted 10 weeks. Several samples of the lead-fortified diets were analyzed and the lead levels were found to range from a minimum of 253 ppm to a maximum of 368 ppm.

Lead concentrations of various tissues were determined by using atomic absorption spectrophotometry (Perkin-Elmer Model 503 and HGA 2100 graphite furnace). All samples except blood were wet ashed with nitric acid and perchloric acid. Blood concentrations were determined by the method of FERNANDEZ (1975).

On the first day of the tenth week, each rat was transferred to a metabolic cage and urine samples were collected for determination of δ-ALA by the method of GRANICK et al. (1973).

At the end of the tenth week, animals were killed by carbon dioxide asphyxiation. Blood and tissues were removed immediately and kept frozen until analysis.

The Student t-test was used to determine statistical significance.

RESULTS AND DISCUSSION

Both adult and young rats readily consumed the experimental diets and showed no apparent toxic symptoms such as failure to gain weight, skin lesions, etc., over the 10-week period. Averages of body weight gains, food intakes, and urinary δ-ALA

TABLE 1

Composition of Basal Diet

Constituent	%
Casein	20.00
Sucrose	30.37
Corn starch	31.19
Corn oil	5.00
Fiber	3.00
Calcium gluconate	5.32
Minerals[a]	2.92
Vitamins[b]	2.20
Total	100.00

[a]The mineral mixture provided per 100 g of diet: $NaHPO_4$, 2287 mg; $MgCO_3$, 128.2 mg; NaCl, 127.0 mg; KCl, 343 mg; $CuSO_4$, 2.0 mg; $MnSO_4$, 15.0 mg; $ZnCO_3$, 5.0 mg; $Na_2MoO_4.2H_2O$, 0.079 mg; KIO_3, 0.03 mg; and $FeSO_4$, 10.61 mg.

[b]Vitamins - Vitamin Diet Fortification Mixture, Nutritional Biochemicals, Cleveland, Ohio.

TABLE 2

Dietary Assignment

Group	Diet
A	Basal diet
B	Basal diet + phospholipid[a,b]
C	Basal diet + 300 ppm Pb as lead acetate
D	Basal diet + 300 ppm Pb as phospholipid-bound lead
E	Basal diet + 300 ppm Pb as lead acetate + phospholipid (added separately)

[a]Phospholipid and lead were added at the expense of corn starch.

[b]In diets B and E, soy lecithin (0.77 g/kg of diet) was added to provide an equivalent amount of phospholipid as diet D.

concentrations of adult and young rats are listed in Table 3. In the adult rat experiment, body weight gains in groups of rats given added lead in the diet were slightly lower than those fed control diets. However, these differences were not significant. As expected, urinary δ-ALA concentrations were elevated in rats that received lead. No significant differences were detected in δ-ALA values between rats fed lead acetate (Group C) and those fed phospholipid-bound lead (Group D). δ-ALA concentrations in rats fed lead acetate with phospholipid (soy lecithin) added separately (Group E) appeared to be higher than Group C ($P<0.05$). The control with added phospholipid (Group B) also appeared to be higher ($P<0.05$) than the control (Group A). In the experiment with young animals, the body weight gains of rats receiving lead in their diet were significantly lower than those of controls. The lower gains in body weight may indicate that young rats are more susceptible to lead intoxication.

No significant differences in lead concentrations were found in brain, liver, kidney, or femur of adult rats fed the different forms of lead (Table 4). However, in blood, the lead concentrations in Group E were significantly higher than those of Groups C and D ($P<0.01$). These higher values for lead in blood cannot be interpreted because the lead concentrations in other tissues did not show the same trend. No significant differences in lead concentrations were detected in liver, kidney, or blood among young rats fed the different forms of lead. Our data indicate that the bioavailability of lead acetate and phospholipid-bound lead were similar at the 300 ppm level of lead in the diet.

SUMMARY

Two different forms of lead, lead acetate and phospholipid-bound lead, were fed to young and adult male rats for 10 weeks at the 300 ppm dietary level. Based on the lead concentrations found in selected tissues, our results indicate that the bioavailability of phospholipid-bound lead is similar to that of lead acetate at the 300 ppm level. Young rats had higher concentrations of lead in tissues than did adult rats.

REFERENCES

BARLTROP, D., and F. MEEK: Postgrad. Med. J. 51, 805 (1975).

CREMER, J.E.: Brit. J. Ind. Med. 16, 191 (1959).

DAVID, O.J.: Environ. Health Perspect. 7, 17 (1974).

DAVIS, J.R., R.H. ABRAHAMS, W.I. FISHBEIN and E.A. FEBREGA: Arch. Environ. Health 17, 164 (1968).

TABLE 3

Body Weight Gains, Food Intakes, and Urinary δ-ALA Concentrations of Adult and Young Rats Fed Different Forms of Lead

Group	Diet[a]	Initial Body Weight, g	Body Weight Gain, g[b]	Food Intake, g/day/rat	Urinary δ-ALA, μg/24 hr[b]
			Adults rats		
A	Basal	343.5	127.0±6.3	22.27	11.86±3.2
B	Basal + PL	343.5	135.2±6.1	23.08	42.21±9.5[c]
C	Basal + $PbAc_2$	343.5	119.5±5.1	22.01	101.4±17.4
D	Basal + PL-Pb	343.5	115.2±6.7	21.75	134.6±20.8
E	Basal + $PbAc_2$ + PL	343.5	123.8±8.6	23.65	197.8±31.8[d]
			Young rats		
A	Basal	131.6	300.4±17.2	18.25	60.56±5.7
B	Basal + PL	132.2	296.9±13.2	19.26	64.94±3.3
C	Basal + $PbAc_2$	132.2	230.6±12.2[e]	17.20	279.4±18.1
D	Basal + PL-Pb	132.2	245.5±15.8[f]	16.61	303.6±29.3
E	Basal + $PbAc_2$ + PL	132.2	259.6±10.0[f]	17.64	346.4±42.0

[a]PL, phospholipid; PL-Pb, phospholipid-bound lead.

[b]Values are means ± SE of 10 adult rats or 8 young rats.

[c]Significantly higher than Group A ($P<0.05$).

[d]Significantly higher than Group C ($P<0.05$).

[e]Significantly lower than Groups A and B ($P<0.01$).

[f]Significantly lower than Groups A and B ($P<0.05$).

TABLE 4

Concentrations of Lead in Blood and Tissues of Adult and Young Rats Fed Different Forms of Lead[a]

Group	Diet[b]	Brain, µg/g wet tissue	Liver, µg/g wet tissue	Kidney, µg/g wet tissue	Femur, µg/g wet tissue	Blood, µg/100 ml
			Adult rats			
A	Basal	0.18±0.04	0.22±0.02	0.77±0.21	0.41±0.09	3.33±0.16
B	Basal + PL	0.16±0.06	0.24±0.06	1.18±0.23	0.22±0.06	3.15±0.46
C	Basal + $PbAc_2$	0.96±0.15	1.51±0.06	19.07±0.84	85.11±5.55	36.05±2.84
D	Basal + PL-Pb	1.44±0.34	1.77±0.12	18.48±1.32	80.58±5.14	39.50±1.82
E	Basal + $PbAc_2$ + PL	1.43±0.25	1.89±0.16	18.87±2.51	87.35±7.24	53.70±3.34[c]
			Young rats			
A	Basal	-	0.07±0.01	0.34±0.05	-	1.61±0.38
B	Basal + PL	-	0.08±0.03	0.36±0.06	-	4.84±0.97
C	Basal + $PbAc_2$	-	3.08±0.24	25.67±2.45	-	72.50±7.73
D	Basal + PL-Pb	-	3.98±0.34	24.96±2.23	-	78.75±5.61
E	Basal + $PbAc_2$ + PL	-	3.60±0.22	24.36±1.49	-	74.19±6.49

[a]Values are means ± SE of 10 adult rats or 8 young rats.

[b]PL, phospholipid; PL-Pb, phospholipid-bound lead.

[c]Significantly higher than Groups C and D ($P<0.01$).

FERNANDEZ, F.T.: Clin. Chem. 21, 558 (1975).

GRANICK, J.L., S. SASSA, S. GRANICK, R.D. LEVERE and A. KAPPAS: Biochem. Med. 8, 149 (1973).

KURLAND, L.T., S.H. FARGO and H. SIEDLER: World Neurol. 1, 379 (1960).

LANDRIGAN, P.J., R.W. BALOH, W.F. BARTHEL, R.H. WHITWORTH, N.W. STAEHLING and B.F. ROSENBLUM: Lancet i, 708 (1975).

MACPHERSON, L.B.: Nature 173, 1193 (1954).

SCHOLFIELD, C.R., and J.H. DUTTON: J. Biol. Chem. 214, 633 (1955).