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Influence of Dietary Protein Composition on Lead Absorption in Rats

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Nutritional factors such as dietary levels of calcium, iron zinc, fats, protein and vitamin D are known to affect the extent of lead uptake in a number of species and current knowledge about these interactive relationships has been reviewed (GOYER AND RHYNE 1973; NORDBERG 1978; MOORE 1979; CHISOLM 1980). Levels of dietary protein, in particular, are known to affect lead absorption; diets low in protein content generally enhance lead uptake (BAERNSTEIN AND GRAND 1942; BARLTROP AND KHOO 1975; MYLROIE et al. 1977).

As part of a general programmatic effort involving the development and definition of animal models of lead neurotoxicity, we observed that rats orally exposed to high doses of lead exhibited more severe toxic response when a purified diet containing casein was employed versus effects in animals maintained on regular rat chow. We have explored this observation further by assessing the relative tissue uptake of lead in young adult rats maintained on two different synthetic diets differing only in the type of protein source: casein or soy bean meal. Effects of lead as a function of these diets on the rat hematopoietic system were also determined. Data obtained using the two diets are described in this report.

MATERIALS AND METHODS

Dosing Protocol. Rats were obtained from our colony of Long-Evans strain animals derived from the Charles River Breeding Laboratory. The vivarium is maintained at 25°C. and a 12 h. light-dark cycle. A total of 23 animals were used. The rats were weaned at 25 days, housed individually in stainless steel cages and acclimated to the diets. Male rats from a given litter were randomly assigned to treatment or control groups: 4 controls, casein diet; 7 animals on casein + lead; 4 controls, soybean meal diet and 8 animals, soybean meal + lead. At 30 days of age, the two treatment groups received lead acetate by gastric intubation: 25 mg Pb/kg b.w., 6X weekly for 3 weeks; control animals received the same treatment with acetate as the sodium salt.

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Basal Diet. The casein-based diet was obtained from U.S. Biochemical Corp., Diet No. 12131 with the following composition (component and percent): casein, 20; sucrose, 68; cottonseed oil, 5; salt mixture, 3 and vitamin mix, 2.2. To this mixture was added Ca CO_3 to yield 0.9% Ca. The soybean diet was prepared in our laboratory with 20 percent soybean meal with all other proportions as for casein diet.

Tissue Lead Analyses. At 3 weeks, animals were anesthetized with pentobarbital (4.6 mg/100 g) and venous blood samples obtained from the inferior vena cava. Animals were sacrificed by perfusion to reduce tissue contamination by lead in retained blood. Aortic arch cannulation was achieved through the left ventricule and body perfusion obtained with 50 ml. of a solution of 2 % glutaraldehyde/1% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). Brain, liver, kidney, sciatic nerve, calvarium and femur were obtained from each animal for lead and morphological analysis. Blood lead determinations were carried out using atomic absorption spectrometry via the Delves Cup procedure (DELVES 1970) as modified by EDIGER AND COLEMAN 1972) and samples treated with Triton X-100 and ultrsonicated. Quantitation of lead was by the method of additions, using matrix-matched standards. Samples of brain, liver, kidney and sciatic nerve were wet ashed-2X- using ultra-pure nitric acid (Ultrex) and the residues taken up in dilute HC1. Femur and calvarium samples were cleaned of muscle and connective tissue using lead-free instruments followed by short soaking in dilute EDTA solution to remove surface lead. Further processing was the same as for soft tissue. Aliquots of analyte were analyzed by flameless atomic absorption spectrometry in a unit equipped for background compensation. Quantitation was by the method of additions with matrix matching.

Morphological Studies. Perfused animal tissues for histological study were stored in 10% formaldehyde. Tissues were washed with water, dehydrated and embedded in Paraplast. Eight-micron sections were stained with hematoxylin and eosin, Luxol Fast Blue-periodic acid schift or Nissl. For election microscopy, tissue was washed in phosphate buffer (0.1M, pH 7.4) and post fixed in 1% OsO_4 in phosphate buffer for 1 h. at room temperature. After buffer washing, samples were dehydrated in a graded ethanol series. Propylene oxide was used as a transitional fluid for infiltration with Araldite epoxy resin. Ultra thin sections were collected on copper athene grids and were post-stained with uranyl acetate and lead acetate. Micrography was done with a JEOL Model 100B electron microscope operated at 80 KV.

Hematology. Hemoglobin and hematocrit values were determined using standard clinical methods. Erythrocyte protoporphyrin (EP) levels were obtained using a Searle Buchler hematofluorometer.

RESULTS AND DISCUSSION

Animals receiving either of the two basal diets with or without added lead showed no significant differences in weight gain or food and water consumption within the 3-week exposure period. Weight gain was 3-4 g/day, daily food intake was 10-12 g and daily water consumption ca. 20 ml.

Whole blood and other tissue lead values for the 4 study groups are set forth in Table 1. Control animals maintained on either casein or soybean meal diets showed very similar blood lead values, ca. 10 μg/dL, which were consistent with levels seen in all of our other rat studies. With lead treatment, however, blood lead concentrations were significantly greater (p= .001) in the casein diet group than were those from animals fed the soybean meal, 280 vs. 110 μg/dL respectively. Similarly, statistically significant increases were also seen for brain, liver, kidney, sciatic nerve,

TABLE 1. Tissue Lead Levels[a] in Exposed Rats on Different Protein Diets.

Tissue	Control/Casein n=4	Control/Soybean n=4	Lead/Casein n=7	Lead/Soybean n=8
Blood	10.0(2.5)	10.0(0.0)	280.0(26)*	110.0(10.6)
Brain	N.D.	0.1(0.0)	1.5(.15)*	0.7(.04)
Kidney	0.6(0.0)*	0.2(.03)	21.9(1.6)*	10.9(.74)
Liver	0.2(0.0)	0.2(.03)	6.4(.79)*	2.9(.14)
Sc.Nerve	N.D.	N.D.	4.0(.87)*	2.7(.42)
Femur	6.3(.85)*	0.1(.03)	1659.0(128)*	680.0(31)
Calvarium	4.7(.65)*	0.5(.07)	853.0(60)*	254.0(15)

[a]Mean (+/- s.e.); blood, μg/dL
all others, μg/g

N.D. Not Detected

*p= .001, casein vs. soybean meal, treated or controls

femur and calvarium lead levels (p= .001) for the casein-fed rats. The enhanced lead uptake with casein feeding was also seen in

control rat kidney, femur and calvarium (p= .001) where the lead source was the trace amount in food and water.

In Table 2 are summarized the hematological effects of lead exposure in these animals. Hematocrit and hemoglobin values were significantly lowered (p= .001) with casein-fed and lead-exposed rats relative to exposed animals eating soybean diet. Erythrocyte protoporphyrin (EP) values for the two exposure groups were not statistically different from each other.

Morphological studies revealed no alterations in liver, brain or sciatic nerve tissue, while lead-containing nuclear inclusion bodies were observed in the proximal convoluted tubule of the kidney in both exposure groups.

TABLE 2. Hematological Effects[a] in Lead-Exposed[b] Rats on Different Protein Diets

	Control/ Casein(4)	Control/ Soybean(4)	Lead + Casein(7)	Lead + Soybean(8)
EP(ug/dL)	17.0(3.5)	16.0(2.4)	60.0(4.9)	47.0(1.8)
Hematocrit(%)	40.0(3.5)	44.0(2.3)	25.0(1.2)*	36.0(1.0)
Hemoglobin(g/dL)	12.2(0.3)	12.0(0.4)	7.4(0.4)*	10.8(0.2)

( ) Number of animals (males)

[a] Mean (+/- s.e.)

[b] 25 mgPb/kg, 6X weekly, 3 weeks

* Significant at p=.001 as compared to soybean diet group

The use of casein as protein source in the diet of lead-exposed animals clearly enhances both lead assimilation and hematological effects relative to rats ingesting a soybean diet, the difference being 2-3 fold across various tissues in the case of lead concentrations. The sensitivity of diet protein as a factor is seen from the fact that the very small amounts of lead taken in by control animals are lodged in tissues at significantly different amounts in those cases where lead is less mobile.

The difference in the extent of lead's effect on hemoglobin and hematocrit follows from the enhanced lead assimilation in rats

fed the casein diet. A non-significant trend to increased EP levels in the casein + lead group is also seen and it is probable that with longer periods of exposure this hematological end point would be increasingly affected. EP is only increased in those red cells formed during lead exposure (EPA 1977) leading to a lag in the time for such cells to be in large enough proportion to significantly affect the total EP content of blood. The lifetime of the rodent erythrocyte is ca. 50 days (BURWELL et al. 1953) contrasted to a lead treatment interval of 21 days in this study.

The major morphological change in the tissues of the animals in both lead exposure groups is the presence of lead-containing nuclear inclusion bodies in the proximal convoluted tubule cell observed by light and electron microscopy. These inclusion bodies appear to be a commonly observed feature in renal tubule and other cells from various species and are proposed to serve a protective role against lead cytotoxicity (GOYER AND RHYNE 1973). It was not possible to make any quantitative distinction as to the density of tubule cell inclusions between the two diet groups.

Based on available experimental data in the literature, it is probably not surprising that casein in the diet of lead-exposed animals promotes both enhanced lead retention in various tissues and greater toxicity nor that soybean meal would be less effective in this regard.

MYLROIE et al. (1978) found that a casein-based synthetic diet was associated with greater tissue lead content in lead-dosed rats when compared to animals maintained on ordinary laboratory rat chow. While these workers found that the difference could not be ascribed to mineral, carbohydrate or fat composition they did not address the influence of dietary protein differences. KELLO AND KOSTIAL (1973) reported the enhanced absorption of lead in rats fed cows milk, powdered milk or milk additives as part of the diet and attributed this to a reduced iron content in such diet mixtures compared to the regular rat diet used. KAO AND FORBES (1973) demonstrated greater hematological effects of lead in rats fed a casein-containing diet vs. those maintained on ordinary rat chow. In this connection, several laboratories (LEE et al. 1980; MYKKANN AND WASSERMAN 1980) have demonstrated that calcium absorption in rats or chicks is enhanced by casein in the diet when compared to other forms of protein including egg albumin or soybean protein.

A second factor is the possible suppressing effect of soybean meal protein on lead absorption which is suggested by available data. FORBES AND YAFE (1960) and MOMCILOVIC et al. (1976) demonstrated that zinc absorption in animals is less with a soybean-based diet than with casein or milk-based diets. Since VOHRA et al. (1965) have reported that phytate present in soybean meal will strongly bind such divalent cations as copper (II), zinc (II) and calcium (II), it is likely that lead-phytate interactions in the GI tract of our animals follow a similar course.

Our results as well as the data of MYLROIE et al. (1978) and KELLO AND KOSTIAL (1973) clearly indicate that dietary composition employed by various investigators in the evolution of animal models of lead toxicity that involve oral exposure is of prime importance. It is also likely that any lack of consistency in dose-internal burden or dose-effect relationships for lead among various studies relate in no small measure to the absence of standardized diets.

Our results also support the concern of MYROLIE et al. (1978) regarding the use of oral-exposure animal data in supporting conclusions about human lead exposure. These workers noted that data for helping establish an acceptable level of lead in current commercial paints were obtained from animal studies using a standard rat chow as basal diet, a diet shown by these workers to promote much less lead assimilation than that prepared using casein. KOSTIAL AND KELLO (1979), furthermore, found that lead bioavailability in the rat using various "human" diets greatly exceeds that using ordinary rat chow, varying from 3-20% intestinal absorption and being in all cases greater than that for rat chow, less than 1%. The former values approximate the reported percentages for lead absorption in man (KEHOE 1961; RABINOWITZ et al. 1974).

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