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Protective Action of Calcium Phytate Against Acute Lead Toxicity in Mice

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It is well recognised that diet affects lead absorption and toxicity (MOORE 1979) but, of all dietary components, only the part played by nutrients has been investigated at all thoroughly. MYLROIE et al. (1978) showed that a natural stock diet protected rats from lead toxicity, when compared to a semi-synthetic diet. That result remained unexplained by the authors, who were unable to reproduce the effect by adding extra nutrients to the semi-synthetic diet. Stock diets do vary greatly in their nutrient profile (WISE 1981), but what is more important here is the difference between them, as a group unrefined, and the refined human diet in industrial societies (WISE 1980). For example, their dietary fibre content may have considerable, and unforseen, effects that may complicate the interpretation of experimental results (WISE & GILBURT 1980). Two natural ingredients of unrefined diets have already been shown to protect animals against lead toxicity: pectin, a dietary fibre (ARCHIPOVA & ZORINA 1965) and tannic acid (PEASLEE & EINHELLIG 1977). Another substance that occurs to variable extents in stock diets is phytate (inositol hexaphosphate), which forms the store of phosphate in plants. WISE & GILBURT (1981) have shown that there is sufficient calcium in stock diets to precipitate all the phytate in vitro at intestinal pH, and that calcium phytate has a great affinity for lead. It is not possible to predict the solubility of lead in complex salt mixtures, because these exhibit very different solubilities when compared to single chemical compounds (SEIDELL 1953). For example, a mixture of magnesium sulphate and lead acetate that forms a precipitate of lead sulphate, does not do so in the presence of potassium citrate, all being present in the same molar concentrations as in the semi-synthetic diet that is used in this laboratory. Therefore, although the in vitro binding of lead to calcium phytate is encouraging, it is necessary to test its predicted effect in vivo.

This paper reports a study of lead toxicity in mice that were fed diets with, and without calcium phytate.

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METHOD

Forty female MF1 mice were distributed into 4 groups of equal average body weight (16g). They were kept on grids, and fed powdered semi-synthetic diet ad lib. The basal diet consisted of (g/kg): 200 casein, 50 maize oil (containing 0.05g dl- - tocopheryl acetate/kg diet), 35.4 mineral mix[1], 5 vitamin mix[2] and 5 choline chloride mix (134 + 366 maize starch). Maize starch was used as the energy source to make up the kilogram. To a second diet was added calcium phytate (Sigma Chemical Co., St. Louis, USA; Product No. P-5253) to make a final 20g/kg, which yields dietary calcium, phosphorus and phytate contents similar to the average stock diet (WISE & GILBURT 1981). To a third diet was added lead acetate (BDH Chemicals Ltd, Poole, England; Product No. 10142) to make a final 1g/kg. To a fourth diet was added calcium phytate and lead acetate. Feeding continued until it appeared that the toxic effects of lead might result in unscheduled fatalities (after 8 days). The mice were killed by carbon dioxide, blood samples were taken from the heart and collected in heparinized tubes, and both liver and kidneys were removed for lead analysis. Since little blood was collected, the blood from each of 2 animals was pooled and diluted 1:5 with 0.125% Triton-X-100, sonicated and analysed for lead by flameless atomic absorption spectrophotometry. Approximately 200 mg samples of liver and 1 kidney were separately wet-ashed with concentrated nitric acid, and analysed for lead. The data for body and organ weights were statistically analysed by t-tests after one-way analysis of variance, while organ lead concentrations were subjected to the Mann-Whitney U-test (Beyer, 1966).

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1. Mineral mix (g/kg): $K_3C_6H_5O_7.H_2O$ 290.1, $MgSO_4.7H_2O$ 139.2, NaCl 37.3, $CaCO_3$ 241.6, $CaH_4(PO_4)_2.H_2O$ 275.9, $Na_2SiO_3.5H_2O$ 5.3, trace mix 10.6.

Trace mix (g/kg): $C_6H_5O_7Fe.5H_2O$ 52.23, $MnCO_3$ 28.54, $ZnCO_3.2ZnO.3H_2O$ 5.79, Nickel sulphate (Ni= 20.7-21.9%) 3.71, $CuCO_3.Cu(OH)_2.H_2O$ 2.62, $SnCl_4.5H_2O$ 0.78, NaF 0.61, NH_4VO_3 0.30, $CrCl_3.6H_2O$ 0.26, $Na_2SeO_3.5H_2O$ 0.09, KI 0.05, $(NH_4)_6Mo_7O_{24}.4H_2O$ 0.02.

2. Vitamin mix (g/500g): thiamin hydrochloride 0.6, riboflavin 0.6, pyridoxine hydrochloride 0.7, nicotinamide 3.0, calcium pantothenate 1.6, folic acid 0.2, biotin 0.02, cyanocobalamin 0.001, retinyl palmitate (250000 iu/g) 1.6, cholecalciferol 0.0025, menadione sodium bisulphite 0.05, inositol 20, maize starch 471.595.

RESULTS

Table 1 shows that phytate did not affect weights of the whole animal, nor the liver and kidneys. Lead acetate added to the basal diet was extremely toxic, while in the phytate diet lead did not have a significant effect on weight gain.

TABLE 1. Weights of body and organs of mice fed lead acetate in diet with or without calcium phytate (± SE). Probabilities assessed by individual t-tests using the standard deviation obtained from analysis of variance: *** $P<0.001$, ** $P<0.01$, * $P<0.05$ for lead vs. no lead; ++ $P<0.01$, + $P<0.05$ for lead in basal diet vs. lead with phytate.

	Body Weight g	Liver Weight g	Kidneys' Weight (2) g
Basal diet	21.4 ± 1.2	1.37 ± 0.08	0.357 ± 0.02
+ lead	13.7 + 1.6***	0.88 + 0.13**	0.294 ± 0.03*
Phytate diet	20.9 ± 0.8	1.32 ± 0.07	0.355 ± 0.01
+ lead	19.5 + 1.2++	1.33 + 0.08++	0.404 ± 0.02++

Blood lead was higher in the former group (2.32 µg/ml) than in the mice given phytate (0.64 µg/ml). No statistical analysis was attempted because blood had been pooled, and in one case blood clotted, despite the heparin. Similar differences between organ lead concentrations were found, with 6.4 ± 1.4 (SE) µg/g and 1.2 ± 0.2 µg/g in livers, and 40.1 ± 9.7 µg/g and 3.9 ± 0.4 µg/g in kidneys. Differences due to the dietary phytate were highly significant ($P<0.001$) for both organs, when subjected to non-parametric statistics, despite the wide range of values found in the mice given lead in the basal diet (1.2 to 12.1 µg/g liver and 6.6 to 108 µg/g kidneys). The data were not skewed (medians were very close to the means). In the mice fed basal diet, more of the dietary lead was found in the kidneys (10.1 ± 2.3 µg) than in the liver (4.4 ± 0.7 µg), but in the mice given phytate the lead burden was equally shared (1.6 ± 0.2 µg/kidney and 1.5 ± 0.2 µg/liver). In the former group, part of the variation in organ lead concentrations was related to the size of organ; liver weights and lead concentrations were well correlated ($r = -0.76$), for kidneys less so ($r = -0.65$).

DISCUSSION

Calcium phytate did not obviously affect the nutritional status of the mice, and this is not surprising since it has been present for decades in natural stock diets. Under certain experimental circumstances, phytate can interfere with absorption of some essential metals (COSGROVE, 1966), and it is important to understand the factors determining whether

phytate will have deleterious nutritional consequences, or may only have possible protective effects against toxic metal absorption. The present research programme is attempting to answer this question; at the same time it is important to extend the experiments to chronic lead toxicity.

It was concluded from the present study that calcium phytate might be employed as a natural protective factor against lead toxicity. This contrasts with an unpublished study by Barltrop[3], in which addition of sodium phytate to the diet did not affect lead absorption. In that case, since the sodium salt was used, a large proportion of dietary calcium would have been precipitated, and the resultant low available calcium would have markedly increased the absorptive capacity of the intestine for lead (MOORE 1979), and hence cancelled out the effect of phytate.

Nutritionists have only considered the harmful aspects of phytate, but it has rarely been implicated as a direct cause of human trace element deficiencies, and only in non-industrialized societies, where unrefined cereal diets are eaten (BOROUMAND et al. 1979). Perhaps it may be found to have some value in polluted industrial countries, but it must be understood that sufficient extra calcium must be provided with phytate to enable it to interact beneficially with lead toxicity.

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