Bull. Environm. Contam. Toxicol. 29, 550–553 (1982)

Blood Lead Levels After Chronic Feeding to Mice of Lead Acetate with Calcium Phytate in the Diet

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It has been found that the subacute toxicity of lead to mice was reduced by adding calcium phytate to the diet, and the action of this natural dietary ingredient may explain the protective effect of stock diets compared to purified diets, when fed concurrently with lead (WISE 1981). Chronic exposure of children may have extremely important effects on mental development (BRYCE-SMITH AND STEPHENS 1981) and therefore research on phytate-lead interaction has been extended to chronic lead administration. Phytic acid binds to many trace metals, including lead (CHERYAN 1980; MAGA 1982; WISE AND GILBURT 1981), and since lead absorption and metabolism depends on the availability of other trace metals (CALABRESE 1980) it is clear that the effects of adding phytate to the diet are not predictable from the evidence of a single experiment with toxic doses of lead. However, use of the same diet and form of phytate permits the influence of phytate on blood lead resulting from chronic lead administration to be compared directly with the results obtained in the earlier subacute experiment. This paper reports blood lead analyses of mice fed low dietary concentrations of lead with, or without calcium phytate.

METHOD

The experiment was similar to the previous one (WISE 1981), but in this case MF1 female mice were obtained commercially from OLAC 1976 Ltd, Bicester, U.K. and were a little heavier (average 23g). The basal diet was made to the same formula and both the lead acetate and calcium phytate were taken from the same bottles as previously. Briefly, the basal diet was made from casein, starch and maize oil, with vitamins, minerals and trace elements to conform to the NATIONAL RESEARCH COUNCIL (1978) recommendations. Eight diets were made containing either 0 or 20 g/kg calcium phytate with 0, 2, 10 or 50 mg/kg lead acetate. The lead acetate was ground to pass through a 53 um sieve and then incorporated into a pre-mix with starch before the diets were prepared. Since the diets were freshly prepared several times from different batches of ingredients, the basal intakes of lead varied during the study, although the contamination was the same for all groups at any time. Calcium phytate contributed only 0.017 mg lead/kg to the diet. Distilled water was provided ad libitum. Mice were distributed in eight groups of 10/group, such that the average weights within each group varied by only 0.6g. The mice were maintained on grids in filter boxes.

0007-4861/82/0029-0550 $01.00

After 3 months, 5 mice were taken at random from each cage and the remainder maintained on the diet for a further 3 months. The mice were killed by CO_2 inhalation and blood taken by cardiac puncture. Aliquots of 0.2 ml were diluted 1:5 with 0.125% Triton-X-100, sonicated and analysed for lead. Blood samples from animals fed diets containing no added lead acetate were pooled and used to make standard mixtures of lead in blood, which were used to construct a standard curve over the range of blood lead concentrations in the other groups. Lead analyses were performed in triplicate, using a Perkin-Elmer 460 flameless atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, USA). The absorption was recorded on a chart recorder, and the peak heights were estimated by using a Graphics Tablet coupled to an Apple II computer (Apple Computer Inc., Cupertino, USA). The baseline was constructed by placing the pen at each end of a segment containing 15 peaks. Triplicate measurements of peak heights were made, and in each case the baseline was separately constructed.

RESULTS

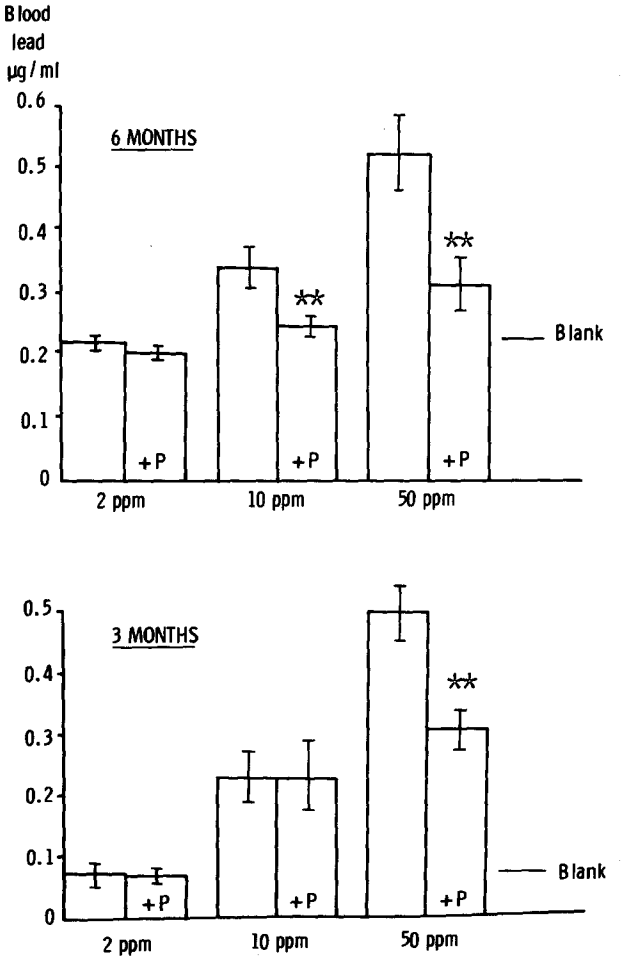
There were no significant effects of either calcium phytate or lead acetate on body weight during the study. After 3 months feeding, the average weight was 35 g, and this increased slightly to 37 g after a further 3 months. Figure 1 shows the results of blood lead analyses. After both 3 and 6 months feeding, there was no greater blood lead concentration in the 2 mg/kg group than in those given no lead acetate, although over the last 3 months both blood lead concentrations tripled. After feeding 10 mg/kg, a significant difference due to phytate appeared only in the 6 month group, however, at 50 mg/kg, there were significant effects of phytate after both 3 and 6 months feeding.

DISCUSSION

When 1000 mg/kg lead acetate had been fed to mice, the toxicity was readily apparent in animals given no phytate supplement (WISE 1981). Blood lead had been 2.3 ug/ml compared to 0.6 ug/ml in the phytate-supplemented group. The present results showed that blood lead was less influenced by calcium phytate when dietary lead concentrations were low. Furthermore, with 10 and 50 mg/kg lead acetate plus calcium phytate in the diet, blood lead tended to rise to a maximum between 0.25 and 0.30 ug/ml and remained relatively constant over the last 3 months, however in the absence of phytate, blood lead was higher and rose slightly, even during the last trimester. Blood lead is related in a curvilinear manner to dietary lead (DEPARTMENT OF HEALTH AND SOCIAL SECURITY 1980), which suggests that the lead available for absorption in the intestine is not linearly related to blood lead. Therefore phytate might have a proportionately greater effect on lead solubility in the intestine than would be reflected in the resultant blood lead.

Since calcium phytate removes even traces of lead from in vitro solution, the lack of effect, at the lowest concentration of dietary lead on blood levels, contrasts significantly with predictions that might have been derived from simple in vitro experiments (WISE AND GILBURT 1981). There is no evidence regarding the forms of phytate in the intestine (CHERYAN 1980), and it is possible that other forms exist in equilibrium with calcium phytate, however, even if all the phytate were to be present as a suspension

Figure 1. Blood lead concentration (ug/ml ± SD) of mice fed lead acetate with, or without 20g/kg calcium phytate for 3 or 6 months. Blank readings obtained from blood of mice fed diets containing no lead acetate. Probabilities assessed by t-tests.
** $P < 0.01$



of its calcium salt, unpublished in vitro experiments have provided some information that might explain the present observations. When calcium phytate has been co-precipitated with traces of lead in this laboratory, most of the lead is located on the surface of particles and is not firmly bound within them. Unidentified substances in rat intestinal contents were found to desorb some lead from the precipitate. Possibly the extent to which calcium phytate may influence lead absorption depends on the presence of some factor(s) in the intestinal lumen able to desorb lead, and which may be able to desorb a greater quantity from calcium phytate at lower lead concentrations. Similar mechanisms have been proposed for cadmium, copper, and zinc, to explain how they are absorbed from diets containing calcium phytate (WISE AND GILBURT 1982).

Much further work is required to elucidate the mechanisms of these interactions, which are likely to be important for all but the most refined human diets that are devoid of phytate. It is also worth noting that absorption studies using in vitro intestinal preparations without the modifying effects of phytate might be missing a very important factor involved in the control of lead availability from intestinal contents.

It is clearly impossible to predict from this experiment the effect of phytate in childrens' diets on blood lead levels, but there was a trend suggesting that the lower the dietary lead, the less phytate would influence blood lead, and therefore the potential usefulness of phytate against chronic lead accumulation is probably less than for acute toxicity.

ACKNOWLEDGEMENTS

The author would like to thank D. Holt for the lead analyses, and D.J. Gilburt for technical assistance.

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Accepted September 16, 1982