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Lead Toxicity and Nutritional Deficiencies

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Under appropriate conditions, deficiencies of certain minerals and vitamins as well as high intakes of dietary fat increase the toxicity of a given dose of lead in experimental animals. The severity of lead poisoning can also be increased by the consumption of either deficient or excessive levels of protein. Mineral deficiencies appear to have some of the most profound effects on lead toxicity, since the consequences of plumbism can be exaggerated by feeding diets low in calcium, phosphorus, iron, zinc, and, in some cases, copper. Evidence for an antagonism between lead and nutritional levels of selenium is inconclusive. Vitamin E deficiency and lead poisoning interact to produce an anemia in rats that is more severe than that caused by either treatment alone. Lead apparently exerts a pro-oxidant stress on the red cell, thereby causing its accelerated destruction. One of the biochemical mechanisms of lead poisoning may be the disruption of normal membrane architecture, thereby leading to peroxidative damage. Epidemiological surveys have suggested a negative correlation between the poor nutritional status of children with regard to calcium and the concentration of lead in blood. Other examples of potential interactions of mineral status and lead poisoning in humans include the hypothesized hazards of soft water to public health in areas with lead plumbing and the possible role of mineral deficiencies in the etiology of pica. Experimental studies have shown that in some situations combined nutritional deficiencies can have an additive effect in potentiating lead toxicity.

Introduction

Toxicologists are becoming increasingly aware of the fact that nutritional status has a profound effect on the response of an organism to a given dose of a poisonous substance. This is especially true in the case of lead poisoning, the severity of which is influenced by a wide variety of nutrients (*1–5*). The importance of dietary factors in determining the degree of lead poisoning is emphasized by the observation of Morrison et al. (*6*) that the dietary content of certain major minerals often had greater effects on the toxicity and tissue content of lead than did a doubling of the dietary lead content itself. Thus, one cannot talk about the relative toxicity of this or that level of lead exposure without knowing the nutritional status of the organism involved.

This review summarizes certain recent data concerning lead toxicity and nutritional deficiencies, with a discussion of new work documenting a relationship between vitamin E deficiency and lead poisoning. Also, a novel hypothesis implicating a possible pro-oxidant effect as a mechanism of lead toxicity is presented.

General Considerations

In the following sections are described several experiments in nutritional toxicology that have been carried out with laboratory animal models. Many of these studies are somewhat unrealistic in that severe nutritional deficiencies and/or very high doses of lead were used. Why scientists often resort to such drastic experimental conditions has been discussed elsewhere (*5*). Such experiments are extremely difficult to extrapolate to humans who are likely to be suffering from multiple marginal nutritional deficiencies and low level exposures to several heavy metals simultaneously. Nonetheless, research with animal models is useful because it allows one to establish specific effects of particular nutrients and toxicants. In this review, the relevance of the animal data to possible human health problems is pointed out wherever possible.

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Macronutrients

Protein

Several early experiments amply demonstrated that the toxicity of lead can be increased by feeding diets low in protein (*7, 8*). More recently, Der and associates (*9*) showed that rats fed low protein diets and poisoned with lead suffered marked retardation of growth and reproduction and increased incidence of infection. However, as pointed out by Mahaffey (*10*), inappropriate lead dosage may have confounded these studies. Barltrop and Khoo found that high as well as low protein diets elevated the lead content of certain tissues (*3*). The practical significance of these studies is that while protein deficiency may accentuate lead toxicity, protein excess may also, so the answer to lead exposure is not to consume high levels of dietary protein in the form of "health food" supplements or otherwise.

Fat

Barltrop discovered that lead absorption is dependent upon the quantity and kind of dietary fat (*4*). For example, increasing the corn oil in the diet from 5 to 40% resulted in 7- to 14-fold increases in the lead content of various tissues. Decreasing the dietary fat from 5 to 0% had no effect on lead absorption. In animals fed fats with different contents of various fatty acids, butterfat caused the greatest increases in lead absorption whereas fats containing large proportions of polyunsaturated fatty acids (rapeseed and sunflower oils) had little effect. No satisfactory explanation was available to account for these different effects. The practical consequences of increased butterfat consumption for individuals exposed to lead would seem to be clear, but Barltrop cautioned that "although the enhanced absorption associated with butterfat would seem to be of particular relevance to the normal human diet, further studies are required for a more detailed evaluation of the effects of individual fatty acids."

Quarterman et al. also found that the degree of lead absorption varied with the type of dietary fat and showed that lecithin, mixed bile salts, and, to a lesser extent, choline stimulated lead uptake (*11*). They suggested that the stimulating effect of crude dietary fat on lead absorption may be due partly to the phospholipids contained therein and partly to the stimulation of bile flow which would add phospholipids and bile salts to the lumen contents. Rats with cannulated and exteriorized bile ducts did not absorb a significant portion of an oral dose of radiolead. Others have shown that the bioavailability of phospholipid bound lead is similar to that of lead acetate (*12*).

Miscellaneous Dietary Components

Barltrop (*4*) found no effect of low and high fiber diets (0 and 12% cellulose, respectively) on the absorption of lead by rats in short-term experiments but cellulose may not behave in the same way as fiber that occurs naturally in animal or human diets. Alginates are well known to decrease the absorption of strontium by children (*13*), but these materials either increased (*4*) or had only a small effect (*14*) on the absorption of lead by rats and had no effect (*15*) on the absorption of lead by humans. Pectin added at 1% of the diet elevated blood lead levels in 2-day experiments with rats (*4*), but calcium pectate given at 10% of the diet markedly lowered blood lead levels of rats after 2 weeks (*16*). Sodium phytate fed at 1% of the diet had no effect on lead absorption by rats (*4*). Dietary lactose is thought to stimulate the intestinal absorption of calcium and magnesium by infants (*17*), but Barltrop reported that addition of 10% lactose to their control diet had no effect on lead absorption in rats (*4*). Tannic acid had a protective effect against lead poisoning in mice (*18*), but the tannates in tea have also been shown to interfere with iron absorption (*19*). Iron status is important in determining an individual's ability to resist the toxic effects of lead (see next section on minerals).

Minerals

Calcium

The interaction of calcium and lead was pointed out in 1926 by Aub et al. (*20*), who noted that the "lead stream" follows the "calcium stream" and other early workers amply confirmed this relationship (*21, 22*). More recently, Six and Goyer demonstrated that feeding a low calcium diet markedly increased the susceptibility of rats to the effects of lead toxicity (*23*). The lead-poisoned rats on the low calcium diet suffered elevated body burdens of lead, more severe anemia, increased urinary excretion of δ-aminolevulinic acid, and a higher incidence of renal intranuclear inclusion bodies. A dose-response study showed that rats fed a high calcium diet developed renal inclusion bodies only when they were given 200 μg Pb/ml of drinking water, whereas rats fed a low calcium diet developed inclusions when given as little as 12 μg Pb/ml (*24*).

Although the metabolic interrelationship of lead and calcium has been the subject of numerous investigations, the mechanism by which low dietary calcium affects lead metabolism is still not completely understood (*25*). Barton et al. (*26*) demonstrated that different levels of intraluminal calcium

decreased the absorption of test doses of lead from ligated loops of small intestine in a dose-related manner. The mechanism of this effect presumably was a competition between lead and calcium for mucosal acceptor ligands. These results confirm those of Barltrop and Khoo, who found that increased levels of calcium in perfusate media decreased the transfer of lead across ligated gut loops (*27*). Meredith et al. showed that oral calcium administered immediately before lead was highly effective in decreasing lead absorption (*28*). However, Barton et al. observed that manipulation of dietary calcium had no significant effect on lead absorption (*26*). Rather, calcium-deprived rats had decreased excretion and thus increased body retention of lead. This observation agrees with the work of Quarterman and Morrison (*29*), who noted that the release of lead already incorporated into the skeleton as a result of previous lead dosing was inhibited by the subsequent feeding of diets low in calcium. The physiological mechanism whereby low calcium diets increase lead retention is not known, but Goyer speculated that the site of action may be the kidney (*25*).

The effect of dietary phosphate on lead metabolism and its interaction with calcium has been studied by several workers. Barltrop and Khoo showed that halving the recommended level of phosphate increased lead uptake by rats in short-term experiments but to a slightly lesser degree than halving the calcium (*27*). On the other hand, Quarterman and Morrison found that feeding 33 and 70% of the recommended levels of calcium and phosphate, respectively, caused similar increases in lead retention in long-term studies (*29*). Both groups agreed that feeding diets low in both calcium and phosphate resulted in roughly additive effects on lead uptake or retention. Quarterman and Morrison also demonstrated that diets low in phosphate reduced the inhibition by diets low in calcium of the release and excretion of lead already incorporated into the carcass. These workers found that dosing with vitamin D increased the absorption of lead by vitamin D-depleted rats and therefore suggested that the effects of calcium and phosphate on lead absorption could be partially accounted for by changes in the concentration of calcium binding protein in the intestinal mucosa. Barton et al. (*26*), however, suggested that lead absorption appeared to be mediated primarily by a second mucosal protein of higher molecular weight that was not dependent on vitamin D.

The metabolic interaction of lead and calcium has several practical ramifications. At one time, industrial hygienists hoped that increasing the dietary intake of calcium of workers in lead industries by providing them free milk might give partial protection against lead toxicity (*25*). But more recent work has shown that milk has no effect (*28, 30*) or may actually increase lead absorption (*31*). Possibly the high fat and protein content of milk might counteract the influence of calcium (*3*). Lead-calcium interactions are of interest also in that a significant negative correlation has been reported between dietary calcium intake and the concentration of lead in the blood of children (*32, 33*). Some nutrition surveys have indicated that certain population groups of children apt to be exposed to lead are also likely to be deficient in calcium (*2*). Whether the consequences of subclinical lead poisoning are greater in such individuals remains unanswered.

The lead-calcium relationship may also have implications with regard to the hypothesized public health hazards of drinking soft rather than hard water. The plumbosolvency of soft water is well established (*34*), and more recent work has shown that giving rats hard water or distilled water containing calcium at a concentration similar to that in hard water decreases the absorption of a concomitant oral dose of lead (*28*). Thus, people living in soft water areas appear to be in double jeopardy concerning lead poisoning, since such water not only dissolves more lead from lead plumbing but also fails to provide any protection against absorption of lead by the gastrointestinal tract. Water *per se* may also play an important role in lead absorption: Barltrop found that rats fed liquid diets containing lead in solution had four times as much lead in their blood as rats fed powdered diets containing lead in the solid form (*35*).

Another aspect of the influence of calcium on lead metabolism was reported by Jacobson and Snowdon (*36*), who showed that calcium-deficient monkeys failed to recognize the "presumed aversive effects" of ingesting lead-containing solutions. The animals continued to ingest relatively high amounts of lead until dietary calcium levels were restored to normal. These workers suggested that subclinical calcium deficiency may cause lead pica in children. Snowden later reported that deficiencies of zinc or magnesium also caused increased lead ingestion by rats (*37*), thus discounting a specific effect of calcium and suggesting rather generalized mineral deficiency as a possible factor in producing lead pica. However, in the latter study the control group was fed a crude laboratory chow type diet, whereas the deficient groups apparently were fed synthetic purified diets. Thus, there were several differences in the characteristics of the diets fed the control and deficient groups so that the differences in lead ingestion observed between these groups are not necessarily the result of changes in

only one dietary factor.

A tragic consequence of the similarity of lead and calcium metabolism is the recently reported case of severe lead poisoning in a film and television actress who took a "health food" supplement prepared from animal bone prescribed by her physician as a dietary calcium supplement (*38*). Unfortunately, the meal was derived from the bones of old horses and was contaminated with 60 to 190 ppm of lead. The patient diagnosed her condition herself after several physicians had been unable to do so, apparently because of their low "index of suspicion" regarding human lead intoxication.

Iron

As in the case of calcium deficiency, lead toxicity is accentuated in iron-deficient rats. Iron deficiency caused increased body retention of lead and increased urinary excretion of δ-aminolevulinic acid (*39*). Several hematopoietic effects were noted in the iron-deficient lead-poisoned rats such as depressed hematocrits, elevated reticulocyte counts and a more severe hypochromic, microcytic anemia. The mechanism by which iron deficiency potentiates lead toxicity is not clear, but there are several metabolic pathways in which an iron/lead interaction could occur. For example, lead is known to inhibit heme biosynthesis at many different steps (*40, 41*). Also, Kaplan et al. suggested that these two metals might compete directly for specific erythrocyte binding sites (*42*). Vanderkooi and Landesberg (*43*) found that cytochrome c isolated from liver mitochondria of lead intoxicated rats lacked iron. They suggested that "mistakes" may sometimes be made in lead poisoning such that this hemeprotein is synthesized without iron, perhaps in a way similar to the production of zinc protoporphyrin in either lead-poisoned or iron-deficient patients (*44*). Recently, Ragan has demonstrated a five-fold increase in the absorption of lead in rats when body-iron stores were reduced but before frank iron deficiency was manifested (*45*).

The possible practical implications of iron deficiency in human lead poisoning have been commented upon several times (*2, 46*), for the children that are most apt to be exposed to lead are also likely to suffer from iron deficiency. In fact, one physician has stated (*47*) that he believes that "there are very few children with anemia due to lead intoxication alone" and that iron deficiency is a common complicating factor in pediatric lead poisoning. However, it must be pointed out that Angle et al. (*48*) found that administration of the usual dose of oral iron to children with mild iron deficiency appeared to increase lead absorption. Thus, additional investigation is needed before any large scale iron supplementation trials are begun in an attempt to minimize the incidence of lead poisoning in children. A role for iron deficiency in human pica has been proposed (*49*), but Snowdon found no support for this idea in experiments with iron-deficient rats (*37*).

Zinc and Copper

Cerklewski and Forbes (*50*) showed that increasing dietary zinc from 8 to 200 ppm in lead-poisoned rats decreased lead concentrations in tissues, urinary excretion of δ-aminolevulinic acid, and inhibition of δ-aminolevulinic acid dehydratase activity in the kidney. This antagonistic effect of zinc on lead was thought to be due to its interference in lead absorption since zinc did not affect urinary lead excretion and injected zinc had no effect on lead toxicity. These authors felt that the effect of zinc on lead absorption was not likely due to formation of a zinc-lead complex of low solubility. Rather, they hypothesized that zinc and lead competed for similar binding sites on a metallothionein-like protein in the intestine responsible for metal transport. Interactions between zinc and lead are possible beyond the level of the gastrointestinal tract, however, since zinc added *in vitro* or given *in vivo* has been shown to activate the enzyme δ-aminolevulinic acid dehydratase and to prevent the inhibition of this enzyme by lead (*51, 52*). In fact, concern was expressed that in workers exposed to both zinc and lead the zinc might activate the enzyme enough to mask the excessive lead burden of the body (*53*). Some researchers have concluded that so many metals affect δ-aminolevulinic acid dehydratase that its activity may be of doubtful value in screening large populations for increased lead absorption (*54*).

Cerklewski and Forbes (*55*) also found that supplementary dietary copper did not lessen the severity of lead poisoning but rather exaggerated it. Their evidence included increased renal lead concentrations and increased urinary excretion of δ-aminolevulinic acid. In contrast, Klauder and Petering (*56*) noted that many of the characteristics of the anemia due to lead poisoning are similar to those of the anemia due to copper deficiency and postulated that lead may induce copper deficiency and thus interfere with iron metabolism and utilization. The discrepancy in the results obtained by these two groups was thought to be due to differences in length of exposure to lead and to differences in the dietary regimens employed. More work is needed to establish the role of copper status in lead poisoning.

The possible significance of zinc and copper nu-

triture in human lead poisoning is still to be determined, but over two-thirds of the self-selected diets analyzed in a recent survey contained less than two-thirds of the recommended nutritional allowance for zinc and copper (*57*). Thomasino et al. (*58*) have suggested that zinc supplementation may be a useful adjunct to chelation therapy for lead toxicity.

Selenium

As pointed out by Ganther (*59*), Wagner found no evidence for selenium antagonizing lead in rats, either in chronic or acute toxicity experiments (*60*). Similarly, Stone and Soares reported that there was no significant interaction of lead and selenium in Japanese quail since selenium had no effect on the reduced red cell δ-aminolevulinic acid dehydratase caused by lead poisoning (*61*). Levander et al. found that excess dietary selenium partially protected vitamin E-deficient rats against lead poisoning, but the levels of selenium needed were toxic in themselves (*62*). Cerklewski and Forbes showed that selenium was mildly protective against the toxic effects of lead at low levels but exaggerated lead toxicity at excessive levels (*63*). Rastogi et al. observed that toxic levels of selenium counterbalanced toxic levels of lead in rats as judged by growth rate, food consumption, and δ-aminolevulinic acid dehydratase and P-450 enzymic activities (*64*). Levander and Argrett, however, noted that injecting rats with lead acetate had no effect on the short-term metabolism of an injected dose of sodium selenite (*65*). Bell et al. found that feeding 250 ppm lead had minor effects on chicks with some indication that lead aggravated selenium deficiency signs (*66*). Thus, although current data do not allow complete definition of the scope of the interaction of lead and selenium, present indications suggest that the nature of the interaction is not nearly as profound as those of mercury and selenium (*67*), cadmium and selenium (*68*), or arsenic and selenium (*69*).

Vitamin E

Vitamin E and Lead Toxicity

The first suggestion that vitamin E might have a protective effect against lead toxicity was made in 1954 by de Rosa (*70*), who found that vitamin E decreased the coproporphyrinuria and anemia in rabbits suffering from subacute lead poisoning. Some 20 years later, Bartlett et al. observed that vitamin E deficiency increased the anemia and basophilic stippling caused by lead in rabbits (*71*). Beginning in 1975, Levander and associates published a series of papers that related lead poisoning and vitamin E. The initial paper showed that lead poisoning caused a profound anemia, splenomegaly, and increased red blood cell mechanical fragility in vitamin E-deficient rats (*72*). Lead poisoning *per se* caused a lesser anemia and splenomegaly in vitamin E-supplemented rats but had no effect on red cell mechanical fragility. In nonpoisoned rats, vitamin E deficiency alone had none of these effects. A pronounced reticulocytosis in the lead-poisoned vitamin E-deficient rats indicated that the anemia in these animals was not due to impaired red cell production but rather was the result of increased red cell destruction. The increased mechanical fragility of red cells from poisoned deficient rats suggested that lead might be reacting with the red cell membrane thereby making it more "brittle" and less resistant to mechanical trauma. A reaction of lead with the red cell membrane was also suggested by the decreased osmotic and peroxidative fragilities observed in erythrocytes from poisoned deficient rats in the Fragiligraph apparatus (*73*), since lead would be expected to make the membrane "tougher" as well as more brittle and thereby more resistant to osmotic stress. Moreover, a reaction of lead with the red cell membrane might be expected to alter erythrocyte deformability and this might help explain the increased splenomegaly seen in poisoned deficient rats. Rat erythrocytes have a diameter of about 7.5 μm and must be able to stretch, bend, and squeeze through microcapillaries as narrow as 3 μm. The physical demands placed on the red cell are greatest in the spleen where not only are the capillaries the narrowest but the red cell is also subject to severe metabolic stress because of the low pH, low glucose concentration, low oxygen tension, and slow blood flow in this organ. Thus, when red cells lose their flexibility, they are trapped in the spleen, their survival in the circulation is shortened, and congestive splenomegaly results.

A convenient technique for estimating red cell deformability or flexibility in the so-called erythrocyte filtration test (*74, 75*). In our modification of this procedure (*76*), a 1% suspension of red cells in Tris-buffered saline is incubated under air. After various incubation periods, a 2-ml aliquot of suspension is drawn off, and the time required for this sample to pass through a polycarbonate filter with 3 μm pores under 10 cm water vacuum is determined. As the red cells lose their deformability, the time required for the cells to pass through the filter (filtration time) increases, and the cells lose their "filterability." Red cell filterability is considered by most hematologists to be a valid index of erythrocyte deformality and therefore a useful guide for

Table 1. Effect of vitamin E and lead on erythrocyte filterability.[a]

Diet	Lead in water, ppm	Filtration time (seconds) after incubation for 0 hr	2 hr	4 hr	6 hr
Vitamin E-supplemented	0	12 ± 1[b]	14 ± 0[b]	20 ± 2[b]	40 ± 6[b]
Vitamin E-supplemented	250	13 ± 1[b]	15 ± 1[b]	20 ± 2[b]	32 ± 10[b]
Vitamin E-deficient	0	12 ± 0[b]	15 ± 1[b]	22 ± 1[b]	229 ± 96[c]
Vitamin E-deficient	250	17 ± 1[c]	56 ± 11[c]	144 ± 53[c]	453 ± 89[d]

[a] Data from Levander et al. (*76*); mean values of four rats ± SE; means in the same column with different superscript letters differ significantly at the $p < 0.05$ level (Duncan's multiple range test); vitamin E added at 100 ppm as *dl*-α-tocopheryl acetate; lead added as lead acetate.

predicting the ability of red cells to survive *in vivo*.

A decreased filterability of red cells from vitamin E-deficient rats was observed after various periods of incubation *in vitro* and lead poisoning accentuated this effect (Table 1). The fact that only minor differences in erythrocyte filterability among the different treatment groups were observed in cells before *in vitro* incubation suggested that lead bound to the cell membrane was not in itself responsible for the different filtration characteristics. Rather, the decreases in filterability of red cells from vitamin E-deficient poisoned or nonpoisoned groups seemed to be related to increases in red cell lipid peroxidation and could be prevented by feeding synthetic antioxidants. Also, the decreases in red cell filterability could be partially prevented by adding tocopherol-rich plasma to the incubation medium (*77*), whereas *in vitro* addition of prooxidants such as hydrogen peroxide or dialuric acid greatly accelerated the decline in filterability (*78*). The decreased filterability of red cells from vitamin E-deficient poisoned or nonpoisoned rats was shown to be related to striking changes in the morphology of these cells from the normal discocytic shape to the highly abnormal stomatospherocytic shape (*79*). These morphological changes fully account for the changes in filterability, for spherocytes are hard, rigid bodies that cannot squeeze through narrow passageways, whereas discocytes with their high surface to volume ratio are quite able to do so. The decreased filterability of blood samples from vitamin E-deficient poisoned or nonpoisoned rats was shown to be due primarily to a decreased filterability of the old red cells which are known to be more spherical and less deformable than young red cells (*80*).

Since humans are likely to be deficient in several nutrients simultaneously, we investigated the possible additive effects of calcium and vitamin E deficiency on lead-poisoned rats. The theoretical rationale for assuming that certain multiple nutritional deficiencies may have additive effects in potentiating heavy metal toxicity has been published elsewhere (*81*). A deficiency of calcium was studied because of its presumed occurrence in the human population and because of its known potentiation of lead toxicity (see discussion about calcium under section on minerals above). For this experiment, weanling male Fischer 344 rats were fed one of four experimental diets which contained either 0.5 or 0.37% calcium, with or without supplemental vitamin E (Table 2). These amounts of calcium represent 100 and 75%, respectively, of the National Research Council (NRC) recommended nutrient level for growth in rats (*83*). Each dietary group was

Table 2. Composition of diets adequate or low in calcium.

Ingredient	Adequate Ca (0.5%) diet, %	Low Ca (0.375%) diet, %
Casein[a]	16.000	16.000
Sucrose	75.258	75.565
Stripped lard[b]	5.000	5.000
Vitamin mix[c]	1.000	1.000
Low-Ca salts[d]	1.184	1.184
$CaHPO_4$	1.072	1.072
$CaCO_3$	0.454	0.147
DL-methionine	0.012	0.012
Vitamin E[e]	0.020	0.020

[a] Vitamin-free test casein, lot #610833, Teklad Test Diets, Madison, WI 53713.

[b] Tocopherol-stripped lard, Teklad Test Diets, Madison, WI 53713.

[c] Vitamin E-deficient vitamin mix as described in Levander et al. (*82*).

[d] The salt mix provided, per kg diet: Na_2HPO_4, 1.544 g; KCl, 5.606 g; KH_2PO_4, 2.300 g; $MgSO_4$, 1.980 g; $MnSO_4 \cdot H_2O$, 0.151 g; $ZnCO_3$, 0.044 g; $CuSO_4$, 0.012 g; $FeC_6H_5O_7 \cdot 5H_2O$, 0.205 g; KIO_3, 0.000185 g; Na_2SeO_3, 0.000219 g. The casein used in this experiment (lot #610833) also contributed (by analysis) when added at a level of 16% of the diet the following minerals, per kg diet: Ca, 0.0159 g; P, 1.2144 g; Zn, 0.007 g; Cu, 0.002 g; I, 0.00004 g; Fe, 0.0009 g; Mn, 0.050 g. The salt mix, casein, $CaHPO_4$, and $CaCO_3$ provided in the calcium-adequate diet 100% of the National Research Council (NRC) recommended nutrient requirement level of the rat (*83*) for the following minerals: Ca, P, Na, Cu, I, Fe, Mg, Mn, K, Cl, Zn, and Se were added at 3.6, 1.0, 0.030, and 0.0001 g/kg diet, respectively, levels which are somewhat higher than the NRC recommendations.

[e] *dl*-α-Tocopheryl acetate, powder (500 I. U. vitamin E/g), Grand Island Biological Co., Grand Island, N. Y. 14072. This was omitted in the vitamin E-deficient diets.

Table 3. Effect of calcium and vitamin E deficiencies on lead-poisoned and nonpoisoned rats.[a]

Dietary supplement		Lead in water, ppm	Weight gain, g	Spleen weight, % of body wt	Hematocrit value, % by volume
Calcium, %	Vitamin E, ppm				
0.5	100	0	235 ± 5[bc]	0.19 ± 0.01[b]	45 ± 1[b]
0.5	100	250	177 ± 4[d]	0.25 ± 0.01[b]	41 ± 1[c]
0.5	0	0	236 ± 2[bc]	0.20 ± 0.01[b]	45 ± 0[b]
0.5	0	250	177 ± 4[d]	0.24 ± 0.02[b]	40 ± 1[c]
0.375	100	0	244 ± 7[b]	0.18 ± 0.01[b]	45 ± 1[b]
0.375	100	250	141 ± 4[e]	0.34 ± 0.03[b]	38 ± 1[c]
0.375	0	0	237 ± 6[bc]	0.20 ± 0.01[b]	44 ± 1[b]
0.375	0	250	133 ± 6[e]	0.82 ± 0.31[c]	34 ± 2[d]

[a] Mean values of four rats ± SE; means in the same column with different superscript letters differ significantly at the $p < 0.05$ level (*Duncan's multiple range test*).

Table 4. Effect of calcium and vitamin E deficiencies on the filterability of erythrocytes from lead-poisoned and nonpoisoned rats.[a]

Dietary supplement		Lead in water, ppm	Filtration time (sec) after incubation for varying times				
Calcium, %	Vitamin E, ppm		0 hr	1 hr	2 hr	4 hr	6 hr
0.5	100	0	13 ± 1[b]	16 ± 1[b]	19 ± 1[b]	26 ± 3[b]	32 ± 3[b]
0.5	100	250	12 ± 0[b]	14 ± 0[b]	18 ± 1[b]	23 ± 2[b]	29 ± 2[b]
0.5	0	0	12 ± 1[b]	17 ± 1[b]	24 ± 3[b]	47 ± 9[b]	> 600[c]
0.5	0	250	13 ± 1[b]	16 ± 1[b]	18 ± 1[b]	29 ± 3[b]	495 ± 62[c]
0.375	100	0	13 ± 1[b]	16 ± 0[b]	19 ± 1[b]	30 ± 1[b]	43 ± 12[b]
0.375	100	250	15 ± 2[b]	18 ± 2[b]	18 ± 1[b]	24 ± 1[b]	35 ± 7[b]
0.375	0	0	12 ± 1[b]	14 ± 1[b]	18 ± 1[b]	34 ± 6[b]	559 ± 42[c]
0.375	0	250	37 ± 20[b]	193 ± 136[b]	222 ± 128[c]	292 ± 111[c]	> 600[c]

[a] Mean values of four rats ± SE; means in the same column with different superscript letters differ significantly at the $p < 0.05$ level (Duncan's multiple range test).

divided into two subgroups; one received distilled water and the other received water containing 250 ppm lead as lead acetate. Table 3 shows that the growth inhibition, splenomegaly, and depressed hematocrits caused by lead poisoning all tended to be exaggerated by feeding the diet low in calcium and simultaneous vitamin E deficiency further accentuated this trend. Likewise, lead poisoning accelerated the decline in the filterability of red cells from vitamin E-deficient rats at the low level of calcium intake (Table 4). Unfortunately, in this experiment lead poisoning had no effect on the filterability of red cells from rats fed the vitamin E-deficient calcium-adequate diet. Refractoriness of certain groups of vitamin E-deficient experimental animals to the effects of lead toxicity has been noted previously (*84*). It can be overcome by increasing the severity of the lead poisoning by increasing the dose of lead administered to the animal (*84*) or, as in this case, by intensifying the effect of a given dose of lead by producing an additional nutritional deficit (calcium deficiency) in the experimental animal. At any rate, the experiment described above verified the concept that discrete multiple nutritional deficiencies can have additive effects in potentiating the toxicity of certain heavy metals such as lead.

In attempting to relate these *in vitro* decreases in red cell filterability to *in vivo* increases in spleen size it was puzzling to note impaired red cell filterability and no splenomegaly in our nonpoisoned vitamin E-deficient rats. Increased red cell turnover is observed in vitamin E deficiency (*85*), but the extent of hemolysis is generally slight and splenomegaly is not usually observed. Apparently, both stresses—i.e., deficiency and toxicity—are required to precipitate the massive red cell destruction and accompanying splenomegaly observed in lead-poisoned vitamin E-deficient rats. How does lead cause this increased destruction of red cells? First of all, lead could react with the red cell membrane and disrupt the normal arrangement of lipids in bilayers thereby rendering polyunsaturated fatty acid residues more susceptible to peroxidative damage. Or lead could catalyze the lipid peroxidation of polyunsaturated lipids (*86*) or act to destroy tocopherol directly (*87*). Whatever the precise mechanism of any pro-oxidant effect of lead, the fact that vitamin E was much more effective than selenium in preventing the lead-induced changes in red cells (*62*) suggests that the site of action of lead

is in a hydrophobic area of the cell membrane (where vitamin E is apt to localize) rather than in a hydrophilic region of the cell sap (where selenium in the form of the soluble cytoplasmic enzyme glutathione peroxidase is likely to be found).

Another possible mechanism by which lead could increase the splenic destruction of red cells is by marking them as abnormal. Red cells coated with polyvalent metals suffer increased susceptibility to splenic sequestration (*88*) and lead is known to render certain bacilli more susceptible to phagocytosis (*89*), apparently as a result of forming metalloprotein complexes on the cell surface. Splenectomy ameliorates the anemia induced by lead poisoning, presumably because this organ is no longer present to remove defective erythrocytes (*90*). Any effect of lead causing the red cell to be recognizable as unfit by the spleen might be independent of the vitamin E status of the animal and hence could account for the mild splenomegaly seen even in lead-poisoned vitamin E-supplemented rats. Of course, the red cells in deficient-poisoned animals would be under twin stresses since not only would the red cells be recognized by the spleen as abnormal due to lead exposure, but these cells would also be much more susceptible to splenic destruction due both to antioxidant lack and any pro-oxidant effect of lead (*91*).

Vitamin E and the Mechanism of Lead Poisoning

Many early investigations into the biochemical mechanism of lead poisoning focused on the reaction of lead with biological membranes (*92, 93*). More recently, however, there has been a shift in emphasis so that the primary thrust of research has been to study the effects of lead on soluble enzymes such as the δ-aminolevulinic acid dehydratase of red cells (*94, 95*). While there is no question that lead can inhibit several steps in the heme biosynthetic pathway (*40, 41*), lead can also react directly with the red cell membrane to produce changes in erythrocyte fragility (*96*) and this latter effect of lead should not be ignored. Our data relating lead poisoning and vitamin E deficiency present additional evidence that, at least under some conditions, lead can have profound effects on erythrocyte membrane stability and integrity. The most reasonable explanation for our results is that lead exerts a pro-oxidant effect in red blood cells. The observation that children living near lead smelters have an increased incidence of Heinz bodies (*97*), an indicator of oxidative stress to red cells, supports this hypothesis. Also, Kao and Forbes found that lead-exposed red blood cells are more susceptible to hemolysis by phenylhydrazine than are normal cells (*98*). Moreover, Sifri and Hoekstra have reported that administration of lead increases the overall extent of *in vivo* lipid peroxidation as judged by ethane evolution (*99*), so lead may have pro-oxidant effects in a variety of tissues other than red cells. For example, intravenous lead acetate injection increases oxygen toxicity in rats (*100*) apparently by activation of the intrinsic coagulation pathway, resulting in consumptive coagulopathy and disseminated intravascular coagulation (*101*). Whether any pro-oxidant effect of lead could be involved in lead-induced encephalopathy is not known at this time, but the focal cerebral hemorrhages observed in lead-treated rats are more probably related to damage to the vascular system of the brain than to depressions in the activity of δ-aminolevulinic acid dehydratase in the brain (*102*). Degeneration of cerebellar capillaries is a feature of vitamin E deficiency in chicks (*103*), and failure of cerebellar cell multiplication has been reported in studies of lead-induced encephalopathy in the developing rat (*104*). Vitamin E also protects against the cerebellar degeneration (*105*) and *in vivo* (*106, 107*) and *in vitro* (*108*) neurotoxicity caused by methylmercury. It has been recently suggested that the neurotoxicity of methylmercury may result partially from free radicals formed by its breakdown (*109*). Copper induces a peroxidative hemolysis in certain patients with Wilson's disease (*110*), iron causes a hemolytic anemia in premature infants low in vitamin E (*111*), and silver precipitates lesions of selenium-vitamin E deficiency in pigs fed diets otherwise adequate in these nutrients (*112*). Thus, pro-oxidant effects may be an important aspect of the mechanism by which some metals exert their toxic effects. Hopefully, study of the interaction of heavy metals with membranes will prove to be a fruitful field of investigation in the future.

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