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Effect of Dietary Calcium and Phosphorus on Toxicity of Lead in the Rat: Rationale of Phosphate Therapy.*

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Since the introduction of calcium salts or calcium containing foods, by Aub and his associates,[1] in the treatment of lead poisoning, numerous papers have appeared in the literature advocating such therapy. The rationale of the procedure was based on the following facts: First, the solubility of lead phosphate was found to be analogous to that of calcium phosphate; hence Aub and his associates believed that lead might be deposited in the bones in the same manner that lime salts are and thus be removed from the circulation. Second, when diets low in calcium were fed to cats, the trabeculae of the bones of the animals were diminished in size and in number as compared to those of animals fed diets to which calcium had been added. This was interpreted to mean that the addition of calcium salts to diets *in general* results in an increased storage of lime salts in the trabeculae; and since lead was supposed to behave like calcium, its deposition in the bones could be hastened or increased by furthering the process of calcification through the administration of calcium. Third, the fact that lead colic may be alleviated, almost instantly, by the intravenous administration of calcium chloride was thought to add additional evidence that calcium "drives" lead into the bones. However, the later studies of Aub and coworkers[2] discredit such an assumption since the alleviation of pain is too rapid to be due to precipitation of lead in the osseous tissue. Aub and his coworkers are, therefore, now inclined to believe that the action of calcium in this instance is to relax the intestinal musculature.

That the administration of calcium salts does not always result in improved calcification unless the phosphorus in the diet is controlled, may be inferred from the experiments of McCollum, Ship-

* Aided by a grant from Mead Johnson and Company, Evansville, Indiana.

[1] Aub, J. C., Fairhall, L. T., Minot, A. S., and Reznikoff, P., *Medicine*, 1925, **4**, 1.

[2] Fitzhugh, G., Miller, M. L., Taylor, G. W., and Aub, J. C., *Am. J. Physiol.*, 1931, **97**, 142.

ley and Park[3] on the production of rickets in rats. When animals are reared on diets in which the calcium and phosphorus ratio is about 1:1, the bones appear normal, but when this ratio is increased to about 4:1 a most florid rickets develops. Apparently the excessive intake of calcium over phosphorus results in an increased excretion of the former through the intestines as the insoluble phosphate, so that the body is robbed of lime salts which are necessary for calcification. Similarly, if the phosphorus greatly exceeds the calcium intake, the excretion of the excess of phosphorus in the feces as the insoluble calcium salt leads to rickets or, more frequently, to osteoporosis—a condition produced by Aub and associates in their experiments with cats, since their low calcium diets were relatively high in phosphorus. These derangements in calcium metabolism may be averted or rectified by adding calcium to the low-calcium diet and by either adding phosphorus to or decreasing the calcium in the low-phosphorus ration, so that the calcium and phosphorus ratio approaches a more appropriate value. Later Shipley *et al.*,[4] and others[5] demonstrated that rickets may be produced not only by diets high in calcium and low in phosphorus but also by the addition of other cations equal in molality to calcium which are excreted as insoluble phosphates in the feces. Rickets has been produced in the rat by replacing the whole or part of the calcium in ricketogenic diets with strontium, magnesium, beryllium, thallium, iron or lead. For example, when part of the calcium of the Steenbock ricketogenic diet is removed so that the calcium-phosphorus ratio approaches one, rickets is usually absent. When, however, the removed calcium is replaced by an equimolar amount of lead so that the sum of the amounts of calcium and lead is equal to the total amount of calcium in the Steenbock diet, or about 4 times that of phosphorus, severe rickets develops. The rickets may be intensified by adding calcium to the lead-containing diet, or by merely adding lead to the usual Steenbock ricketogenic diet. Obviously, aside from the toxicity of the lead, the addition of calcium to lead diets which contain an inadequate amount of phosphorus does not lead to improved deposition of either calcium or lead phosphate in the bones but, on the contrary, it inhibits such a process. The deposition of calcium phosphate in the course of normal ossifica-

[3] McCollum, E. V., Simmonds, N., Parsons, H. T., Shipley, P. G., and Park, E. A., *J. Biol. Chem.*, 1921, **45**, 333; *Bull. Johns Hopkins Hosp.*, 1921, **32**, 363.

[4] Shipley, P. G., Park, E. A., McCollum, E. V., Simmonds, N., and Kinney, E. M., *Bull. Johns Hopkins Hosp.*, 1922, **33**, 216.

[5] Park, E. A., *Physiol. Rev.*, 1923, **3**, 129.

tion, or the deposition of other insoluble phosphates such as strontium or lead, can occur only when the phosphorus intake is adequate for their deposition and for the excretion of the excess cations as the insoluble salts in the feces.

On theoretical grounds alone, it would seem logical that if the aim of therapy in lead poisoning is to deposit or excrete the lead in an insoluble and hence in an innocuous form, *i. e.*, as the phosphate, an abundance of phosphorus or foods containing phosphorus should be supplied. Certainly, the introduction of large amounts of calcium without phosphorus into the animal organisms merely diverts the available phosphorus to rid the body of the excess calcium as the phosphate and thus interferes with the formation of such a compound with lead. The correctness of such an assumption was tested experimentally in rats.

Thirty-two rats, 30-40 days old, averaging 65 gm. in weight, the offspring of healthy stock, were divided into 8 groups of 4 each. They were fed 1.5 gm. % of $2PbCO_3$-$Pb(OH)_2$ in the Steenbock-Bills stock diet[6] with and without the additions of either $CaCO_3$, Na_2HPO_4 or 3 $MgCO_3.Mg(OH)_2+3H_2O$. Four of the groups received the respective diets without vitamin D while the remainder received the corresponding diets and vitamin D in the form of viosterol 1-D in levels of 1% of the diet. The compositions of the diets are given in Table I. The animals were allowed food and water

TABLE I.
Composition of Diets Used.

Diet No. 1	Diet No. 2	Diet No. 3	Diet No. 4
Stock + 1.5 gm. $2PbCO_3.Pb(OH)_2$	Diet No. 1+1.5 gm. $CaCO_3$	Diet No. 1+0.9 gm. $3MgCO_3Mg(OH)_2+3H_2O$	Diet No. 1+2.75 gm. Na_2HPO_4 (Anhyd.)
" + Viosterol	" + Viosterol	" + Viosterol	" + Viosterol

The stock diet is that described by Bills[6] and contains approximately 0.475 gm. calcium and 0.515 gm. phosphorus %. The viosterol was diluted with olive oil to make 1D, or equal to cod liver oil in antirachitic potency. The added calcium, magnesium and phosphorus are, approximately, in equimolar amounts.

ad libitum, and their weights and changes in health and behavior were noted at frequent intervals. The effect of the variations in the calcium and phosphorus of the diets on the toxicity of lead as indicated by weight and longevity of the animals is shown in Figs. 1, 2, and 3. It is seen that among the groups *not* receiving vitamin D, the weight and longevity were the poorest in the following order:

[6] Bills, C. E., Honeywell, E. M., Wirick, A. M., and Nussmeier, M., *J. Biol. Chem.*, 1931, **90**, 619.

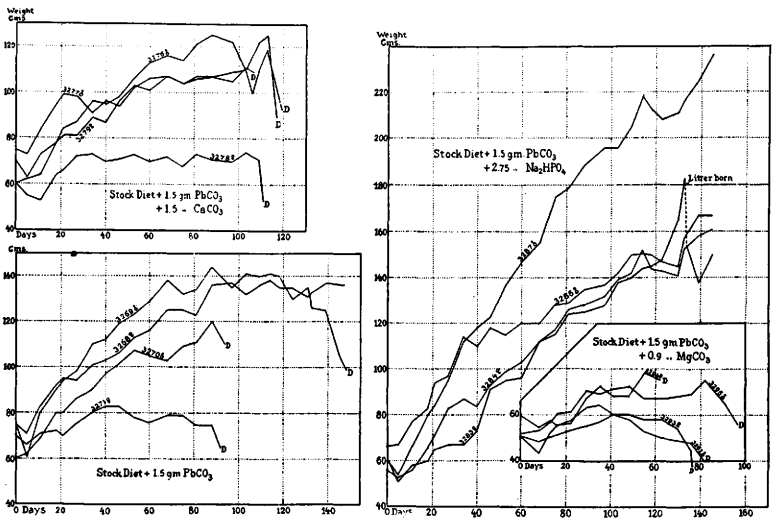

FIG. 1.

Growth curves of rats fed the stock diet and lead carbonate, with and without additions of calcium, magnesium and phosphate. The formulae for the carbonate of lead and magnesium are abbreviated in Figs. 1 and 2. The complete formulae are given in the text.

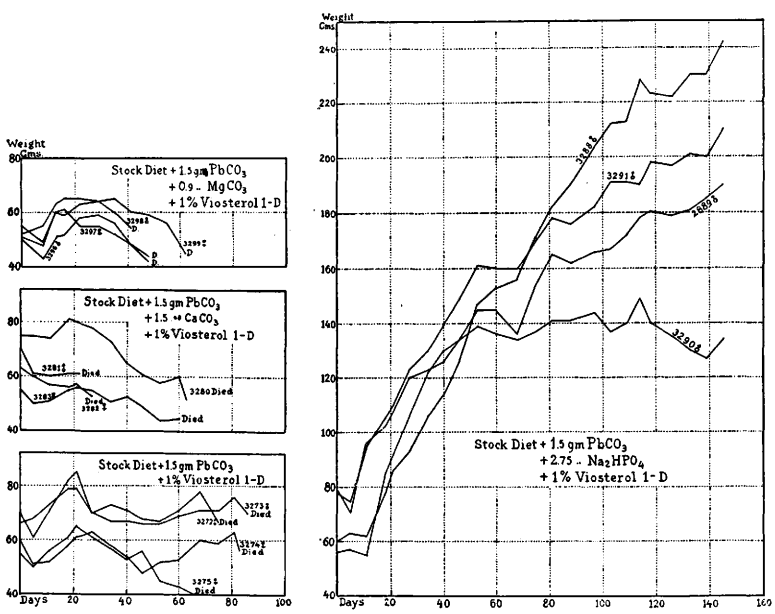

FIG. 2.

Same as Fig. 1, except that 1% of viosterol 1D was added to the corresponding diets.

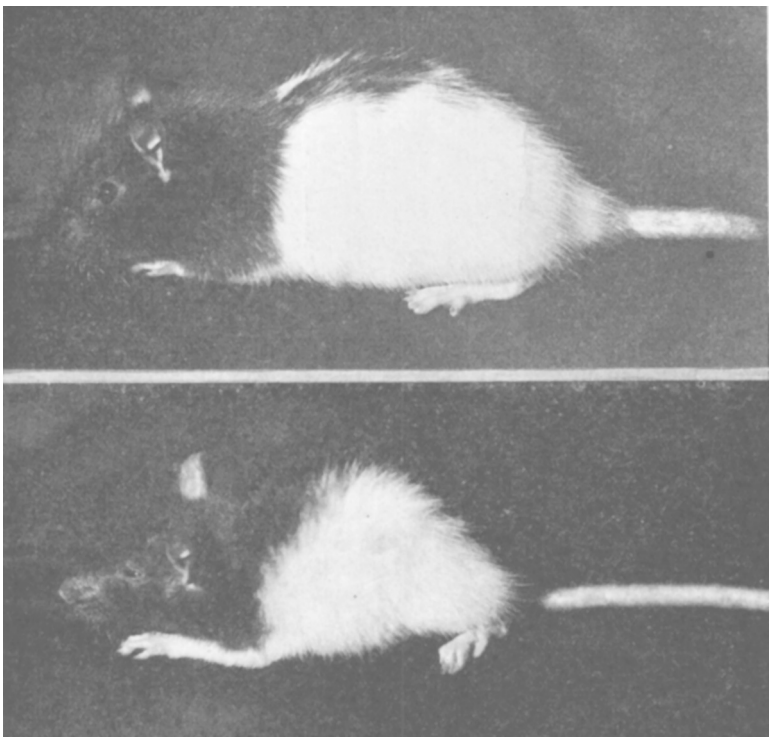

FIG. 3.
Appearance of rats poisoned with lead carbonate. *Upper:* Female, 117 days on lead and phosphate diet. Weight, 142 grams. Still living. Had litter at 133 days. *Lower:* Female, litter mate, 117 days on lead and calcium diet. Weight, 43 grams. Died on 118th day.

$MgCO_3$, > $CaCO_3$, > $PbCO_3$ (Without additions). Of the animals receiving $PbCO_3$ alone, one is still alive but is failing very rapidly. Those receiving Na_2HPO_4 gain weight steadily and appear normal outwardly. One female of this group had a litter of young after being on the diet for 133 days, but she killed her offspring soon after delivery.

With the exception of those in the Na_2HPO_4 group, which are alive and doing well, the animals receiving vitamin D all died sooner than their corresponding mates not receiving the vitamin. The interpretation of these results may be that vitamin D diverted *calcium* phosphate into the bones and allowed lead, not combined with phosphate, to circulate freely in the body fluids; whereas in the Na_2HPO_4 group the phosphate was adequate for both deposition and excretion of the cations (Ca^{++} and Pb^{++}) as the insoluble phosphate and the animals, therefore, seem well. In the latter case vita-

min D may have been helpful in depositing lead along with the calcium in the bones.

Magnesium was used in the experiments for two reasons: (1) $MgSO_4$ is frequently advocated as a remedy in lead encephalitis. (2) If phosphorus should be a determining factor in the inactivation of lead by forming an insoluble lead compound, it was anticipated that magnesium would increase the toxicity of lead because of the summation of total cations, and also because it was previously shown by Shelling, Kramer and Orent[7] and by Shipley and Holt[8] that magnesium hinders calcification, probably by increasing the solubility of insoluble phosphate compounds (Ca^{++}, Pb^{++}). The results of the experiment with magnesium are in accord with this view and indicate the danger of using soluble magnesium salts in the treatment of lead poisoning.

A few animals, not included in the above groups, were fed the stock diet and lead carbonate until they showed evidence of toxicity, as indicated by diminution of activity and loss in weight. They were then placed on the same diets to which, in one group $CaCO_3$ and in another group Na_2HPO_4, were added. Those receiving the Na_2HPO_4 improved rapidly while those getting $CaCO_3$ fared very poorly. The animals in the Na_2HPO_4 group, after being on the diet for 20 days and showing marked improvement, were suddenly changed to the high calcium diet. They lost weight very rapidly, became less active and on the 6th day one developed paralysis of the hind legs and died in convulsions during the following day. Apparently the sudden ingestion of an excess of calcium liberated the stored lead into the circulation, causing the toxic manifestations.

The application of these experimental results to the prophylaxis of lead poisoning in human beings, especially those poisoned by the gastro-intestinal tract, seems obvious. Fortunately, most diets of children and adults contain adequate amounts of phosphorus to take care of calcification and inactivation of the heavy cation lead which requires but a small amount of $PO^{=}_4$ to form an insoluble compound. In advocating calcium additions to the diets in the treatment of lead poisoning Aub and his associates advise milk, which contains, besides calcium, an abundance of phosphorus; but others, not realizing the phosphorus factor in this process, prescribe large amounts of calcium salts other than the phosphate. It would also seem that not only is phosphorus important in

[7] Shelling, D. H., Kramer, B., and Orent, E. R., *J. Biol. Chem.*, 1928, **77**, 157; *Bull. Johns Hopkins Hosp.*, 1927, **41**, 426.

[8] Shipley, P. G., and Holt, L. E., Jr., *Bull. Johns Hopkins Hosp.*, 1927, **41**, 437.

depositing lead in the bones but also in attempting to delead at a future date. This may be accomplished by giving a diet low in calcium and relatively high in phosphorus so that the cations removed from the bones are excreted as the insoluble phosphates. The metabolism data of Aub and his associates indicate that, aside from its acidity, H_3PO_4 was the most effective substance in deleading patients suffering from chronic lead poisoning.

More extensive experiments on the effect of dietary calcium and phosphorus in lead and thallium poisoning in rats, induced by oral and subcutaneous administration, are now in progress. The results of these experiments and also of those obtained with phosphate therapy in human cases of plumbism will be reported later.

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Relation of Pressure to Rate and Quality of Milk Secreted.*

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Recent work[1, 2] has shown that milk secretion takes place in the interim between milkings, and that practically all milk drawn at a milking is present in the udder at the time of milking. It is evident, therefore, that pressure must develop in the duct system as the secretion accumulates. The purpose of this work is to establish the amount of pressure developed by the accumulating secretion; the effect of such pressure upon the rate of secretion; and the maximum pressure against which milk will be secreted.

Neusch,[3] Isaachsen,[4] and Tgetgel[5] measured the pressure in the udder at milking time and reported wide differences due no doubt to a difference in distention of the gland, and as they measured the

* The data used in this paper are taken mainly from a thesis presented by T. V. Rigor in partial fulfillment for the Ph.D. degree.

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1 Petersen, W. E., Palmer, L. S., and Eckles, C. H., *Am. J. Physiol.*, 1929, **90**, 573.

2 Swett, W. W., *J. Dairy Sci.*, 1927, **10**, 1.

3 Neusch, J., *Uber das Sog. Aufzieben der Milch*, 1910, Paul Parey, Berlin.

4 Isaachsen, H., *Proc. World's Dairy Congress*, 1923, **2**, 1018.

5 Tgetgel, B., *Schweizer Arch. f. Tierheil.*, 1926, **68**, 335, 369.