Investigations of Chemical Basis of Zinc-Calcium-Phytate Interaction in Biological Systems.* (30176)

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The chemistry of phytic acid or inositol hexaphosphoric acid and its salts has long been of concern with respect to animal as well as to human nutrition(1,2,3). Phytin, the Ca-Mg salt of phytic acid, is present in appreciable amounts in the germinating seeds of grains. The phosphorus of insoluble phytates is relatively unavailable for absorption by most animals, with the exception of ruminants(4) and among non-ruminants the rat appears to have a high degree of ability to utilize phytic acid(5,6). Since phytin phosphorus comprises 60-80% of the total phosphorus in cereals(7) the problem of the unavailability of phosphorus and of the metals bound to phytate salts is a significant one, especially in those countries where cereals make up a large portion of the diet(8).

In recent years, attention has been given to the problem of Zn deficiency in animals fed diets in which the protein is of plant origin. It has been found(9,10,11) that an increase in the amount of Ca in the diets of swine aggravates parakeratosis, or Zn deficiency disease. It has also been shown biologically with chicks(12,13) that there is an interaction of Ca, Zn and phytate which brings about a decrease in Zn availability. The objective of this research was to investigate the chemical reactions involved which might explain the Zn-Ca effect displayed biologically in the presence of phytate.

Methods and materials. Various Ca phytate salts are formed at different pH levels (14,15). The tri-Ca salt is formed at pH 2.5 and the hexa-Ca salt at pH 6+(14). Since the pH of the intestinal tract ranges from acid to alkaline, experiments were conducted at both the 2.5 and 6+ levels. The solvent system for the tri-salt was diluted HCl (approximately 0.05 M) and for the hexa-salt, distilled water. Zinc and/or calcium salts dissolved in the appropriate solvents were added dropwise, with stirring, to the Na phytate dissolved in the corresponding solvent. In each experiment a trace of Zn^{65} was added to the Zn solutions to utilize the isotopic dilution technique for determining the percent Zn incorporated into the Zn phytate precipitate. The reaction volume was generally 18 ml. In Experiments 1, 2 and 3, the precipitate was centrifuged at 17,300 *g* for 25 minutes, the supernatant decanted, washed with 10 ml of the appropriate solvent, centrifuged, decanted and again washed with 10 ml of the appropriate solvent. Radioactivity was determined on 2 ml of the combined supernatant solutions in a Nuclear Chicago Gamma Scintillation Counter. In Experiments 4 and 5 washing of the precipitate was omitted. Precipitates analysed for zinc and calcium were brought into solution by ethylenediaminetetra-acetate and the concentration of the metals was determined with the Model 303 Perkin-Elmer atomic absorption spectrometer. Sodium phytate was analysed for sodium by flame photometry and for phosphorus by the molybdivanadate method(16). From the sodium and phosphorus analyses of the phytate used in this investigation the molecular composition was calculated to be $C_6H_6P_6O_{24}Na_{12} \cdot 38H_2O$, a value in agreement with that in the literature (15).

Results and discussion. Experiment 1 was conducted to ascertain (a) whether or not tri-zinc phytate salts are formed in diluted HCl (pH 2.5) similarly to the formation of tri-Ca phytate(14), and (b) the effect of Ca on Zn incorporation into phytate. The same information was sought for the hexa-salt environment in Experiment 2. Experi-

* Contribution for Animal Science Department, North Carolina Agri. Exp. Station, Raleigh. Published with approval of Director of Research as Paper No. 1915 of the Journal Series.

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TABLE I. Effect of Several Factors on Zinc Incorporation into Phytate.

Molarity of Zn†	Molarity of Ca‡	% Zn incorp.	M Zn/M phytate in ppt* Detn'd	M Zn/M phytate in ppt* Theoretical
	Tri-salt environment, Experiment 1§			
.015	—	89.9	2.7/1	3/1
"	.015	75.1	2.3/1	"
"	.03	62.0	1.9/1	"
.03	—	60.2	3.6/1	
"	.015	47.3	2.8/1	
"	.03	43.8	2.6/1	
	Hexa-salt environment, Experiment 2§			
.03	—	82.7	5.0/1	6/1
"	.03	50.6	3.0/1	"
"	.06	33.7	2.0/1	"
.06	—	41.2	5.0/1	6/1
"	.03	29.8	3.6/1	"
"	.06	21.9	2.6/1	"
	Dilution effect (tri-salt environment), Experiment 3			
.015	—	67.5		
.01	—	54.7		
.006	—	46.2		
.0043	—	39.3		

* The reaction was carried out at room temperature for one hr.
† $ZnSO_4 \cdot 7H_2O$.
‡ $CaCl_2$.
§ Molarity of sodium phytate in reaction volume was .005.

ment 3 was conducted to determine the effects of dilution on Zn incorporation. In this study the total amounts of the reactants were held constant but the solvent volume was varied. The various combinations of metals tested and the results are shown in Table 1.

As is shown in Table I, in the tri-environment (Exp. 1) when the Zn:phytate ratio in the reaction volume was varied from 3:1 to 6:1 the actual ratio obtained varied from 2.7:1 to 3.6:1. In the hexa-environment the corresponding analogous ratios were 5:1 *vs* 6:1. It was assumed from these results that the Zn:phytate stoichiometry in the 2 environments are similar to those of calcium(14). It can also be observed from Table I that in all cases the presence of calcium decreased the amount of zinc incorporation into the phytate. The results of Exp. 3 (Table I) show, as one might expect, that the greater the dilution of constant amounts of Zn and phytate the smaller the incorporation of zinc into phytate.

The calcium effects obtained in these experiments were contrary to those postulated for explaining the biological effects(10-14). It occurred to us, from consideration of the fact that animal diets actually contain 30 to 100 ppm of Zn and as much as 1 to 2% Ca, that the Zn:Ca ratios studied in these experiments were too narrow. Accordingly, Exp. 4 was designed to test Zn:Ca ratios more comparable to those found in animal diets. The Zn:Ca ratios employed and the results are shown in Table II. The data show clearly that at wide Zn:Ca ratios, calcium does, in fact, increase markedly the amount of Zn incorporated into phytate, regardless of the type of environment. It seemed reasonable from the data obtained up to this point of the investigation that at wide Zn:Ca ratios, Ca enhanced the incorporation of Zn into phytate and that at narrow Zn:Ca ratios Ca decreased Zn incorporation into phytate. The next experiment, therefore, was designed to describe the overall response curves of varying ratios of Zn:Ca on incorporation of Zn into phytate in the two environments. In this experiment the phytate was held constant at 0.005 M and the calcium at 0.03 M with gradually increasing concentrations of Zn. The curves are shown in Fig. 1. In the

TABLE II. Effect of Wide Ratios of Zn/Ca on Zn Incorporation into Phytate (Exp 4).

Rep. No.	Molarity of Zn*	Molarity of Ca†	% Zn incorp.‡
	Tri-salt environment		
1	.00025	—	16.0
2	"	—	13.8
1	"	.03	98.8
2	"	"	99.0
1	.0005	—	51.1
2	"	—	39.9
1	"	.03	98.7
2	"	"	98.8
	Hexa-salt environment		
1	.00025	—	0 (− .1)
2	"	—	0 (−1.05)
1	"	.03	99.5
2	"	"	99.4
1	.0005	—	7.9
2	"	—	9.3
1	"	.03	99.4
2	"	"	99.4

* $ZnCl_2$.
† $CaCl_2$.
‡ Molarity of sodium phytate in reaction volume was .005. Reaction was carried out at room temperature for 16 hr.

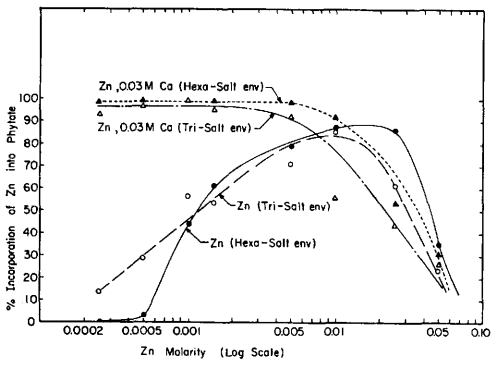

FIG. 1. Investigations of chemical basis of zinc-calcium-phytate interaction in biological systems.

tri-environment the Zn concentration at which the Ca enhancing effect reverses is about .0075 M Zn or a Zn:Ca ratio of 1:4, and in the hexa-environment it occurs at about .015 M Zn or a Zn:Ca ratio of 1:2. As shown in Fig. 1 and by Zn and Ca analyses of the precipitates, at Zn concentrations above 0.0075 M in the tri-salt environment and above 0.015 M in the hexa-salt environment, Ca decreased the incorporation of Zn into phytate. Presumably, at low Zn, high Ca concentrations, Ca increases the formation of insoluble phytate co-precipitating or adsorbing Zn. At high levels of Zn and Ca concentrations, Ca competes for positions on the phytate molecule, thereby reducing the amount of Zn precipitated.

The biological observations that an increase in the amount of Ca in diets of high phytate content aggravates a Zn deficiency in animals can be explained by the results of the present work. Since Zn is a trace element, it does not supply enough cations to form a significant precipitation of Zn phytate. However, Ca cations increase the total cationic environment sufficiently to initiate a co-precipitation with Zn to form insoluble phytates. Therefore, Zn in the presence of Ca would not be as available for absorption and Zn deficiency would be aggravated. On the other hand, when supplemental zinc is added to the diet, the concentration effect would at least partially overcome the Ca effect which has been observed biologically.

The total "cationic" concept developed in this study presumably would implicate any metal ion which forms an insoluble phytate complex. It is conceivable that under limiting dietary levels other trace elements could be affected similarly to Zn. Alternatively, metal ion excesses could affect the availability of limiting essential trace elements.

Summary. The effect of Ca on Zn incorporation into phytate in 2 solvent systems (pH 2.5 and pH 6+) was investigated in a series of 5 experiments. It was found that in both solvent systems at wide Zn:Ca ratios, Ca enhanced the incorporation or adsorption of Zn into phytate and at narrow Zn:Ca ratios, Ca decreased Zn incorporation into phytate. The findings offer a rational explanation of biological observations concerning Zn-Ca effects in the presence of phytate.

This work was done while one of the authors (C.A.B.) was a research participant in a National Science Foundation Undergraduate Science Education Program for the summer of 1964.

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Received January 13, 1965. P.S.E.B.M., 1965, v119.