ment appear to be important factors in elevating blood pressure. Administration of $NaNO_3$ is also accompanied by an increase in thyroid to body weight ratio.

The technical assistance of Mr. Howard Clark is gratefully appreciated.

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Received September 9, 1963. P.S.E.B.M., 1964, v115.

Phytic Acid Studies.* (28922)

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Phytic acid has interested analytical chemists, biochemists, and nutritionists for several years. Brown *et al*(1) recently supported Neuberg's proposed structure and concluded that of the 18 acid hydrogens in the Neuberg model, only 12 were available for titration in aqueous solution.

Chicks fed purified diets containing sesame meal(2,3) or isolated soybean protein(4) as the sole source of protein developed zinc deficiency symptoms, although the diet contained more than the optimum level of zinc. Autoclaving the protein source or addition of EDTA (ethylenediamine tetraacetic acid) to the diet prevented the deficiency symptoms. O'Dell and Savage(5) observed that phytic acid formed complexes with isolated soybean protein and casein. When this complex was fed in the diet of chicks as a source of protein, zinc was found to be far less available for growth.

The work reported here was undertaken to estimate qualitatively the complex formation between metal ions and phytic acid using the "pH drop method"(6,7). The effect of feeding phytic acid on utilization of dietary zinc by chicks has been studied.

Methods. Phytic acid,§ 4.48×10^{-3} M,

* Arizona Agric. Exp. Station Journal Article 831.

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and 1.085×10^{-1} M sodium hydroxide solutions were used for titrations. A 25 ml sample of phytic acid with and without equimolar concentrations of Zn^{++}, Cu^{++}, Co^{++}, Mn^{++}, or Ca^{++} were titrated with sodium hydroxide. The metal ions were supplied as the sulfate salts of zinc, copper and the chlorides of cobalt, manganese and calcium. During titration, the solution was stirred continuously by a magnetic stirrer. Measurement of pH of the titration solution was made after each addition of 0.5 ml of base with a Beckmann (model N) pH meter equipped with an outer glass and saturated calomel electrode pair and standardized with Beckmann buffers. The pH values were plotted against the ratio of moles of NaOH added per mole of phytic acid.

In the study of the effect of sodium phytate on zinc utilization, 10 New Hampshire $\times$ Delaware chicks (5 males and 5 females) were used for each lot. Four replicate lots were fed on each of the dietary treatments. Zinc levels with and without added sodium phytate (0.25%) were fed for a 4-week experimental period. The basal diet employed isolated soybean protein as the amino acid source (Table I). Birds were housed in electrically-heated batteries with raised wire floors. Feed and distilled water were supplied *ad libitum*. The bird weights and feed consumption were measured at the end of 2 and 4 weeks. Mortalities were noted as they occurred. The isolated soybean protein basal diet employed contained no added zinc (Table I). Zinc was supplied in the form of $ZnSO_4 \cdot 7H_2O$ to obtain the respective dietary levels (Table II).

TABLE I. Composition of Basal Diet.

Ingredients	%
Isolated soybean protein (84% protein)	32.0
Cerelose	50.0
Soybean oil	2.7
d-Alpha-tocopheryl acetate	.3
Mineral Mix*—Zn deficient	8.0
Solka floc	3.0
Vitamin Mix†	4.0

* Supplied the following per kg of diet: 8.20 g $CaCO_3$, 42.1 g $CaHPO_4 \cdot 2H_2O$, 5.0 g NaCl, 320 mg $MnSO_4 \cdot H_2O$, 160 g ferric citrate, 16 mg $CuSO_4 \cdot 2H_2O$, 4 mg KI, 5 mg $CoCl_2 \cdot 6H_2O$, 5.72 g KCl, 5.70 g $MgSO_4 \cdot 7H_2O$, and 8 mg $NaMoO_4 \cdot 2H_2O$.

† Supplied the following per kg of diet: 22,000 I.U. Vit. A, 2,200 I.C.U. Vit. D_3, 1.25 mg ethoxyquin, 20 mg thiamine hydrochloride, 6 mg riboflavin, 15.2 mg d-calcium pantothenate, 4 mg pyridoxine hydrochloride, 20 mg para-aminobenzoic acid, 1,000 mg inositol, 100 mg niacin, 5.65 g DL-methionine, 2.5 g glycine, 3 μg Vit. B_{12}, 0.1 mg biotin, 1 mg folic acid, 1.5 mg menadione sodium bisulfite, and 1,100 mg choline chloride.

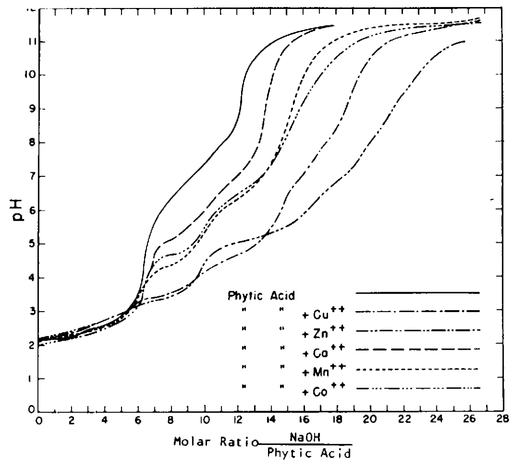

FIG. 1. Titration of 4.48×10^{-3} M phytic acid with NaOH in presence of equimolar concentrations of metal ions.

Results and discussion. The titration curve (Fig. 1) of phytic acid with NaOH indicated 2 inflection points corresponding to 8 and 12 moles of base per mole of acid. At the first inflection point, 8 acid hydrogen ions of phytic acid were completely neutralized (equation 1). The remaining 4 hydrogens were completely neutralized when 12 moles of base per mole of acid were added. This second inflection point is shown in equation 2.

The dissociation of 12 titratible hydrogens per mole of phytic acid in an aqueous solution may be represented as:

$$H_{12}phy \longrightarrow H_4phy^{-8} + 8H^+ \quad . \quad . \quad 1$$
$$H_4phy^{-8} \longrightarrow phy^{-12} + 4H^+ \quad . \quad . \quad 2$$
$$H_{12}phy \longrightarrow phy^{-12} + 12H^+$$

The presence of 12 dissociable hydrogens per mole of phytic acid greatly alters the shape of the titration curve for phytic acid in presence of equimolar concentrations of the metal ions tested. When the metal complexes were formed, the hydrogen ions were replaced, hence the acid-base titration curve was shifted to a lower pH range. The titra-

TABLE II. Effect of Phytic Acid on Utilization of Zinc by Chicks Fed a Zinc Deficient Diet (Table I).

% sodium phytate	Zinc in ppm	Avg wt at 4 wk (g)
.00	0	376.0[ab]
.00	10	431.0[c]
.00	50	464.0[d]
.25	0	357.0[a]
.25	10	393.0[b]
.25	50	466.0[d]

Means without a common letter are significant at the 0.05% level of probability.

tion curves (Fig. 1) show a significant pH drop when phytic acid was titrated in presence of equimolar concentrations of metal ions, the largest drop being in the case of zinc. The pH drop began only after 7-8 moles of base per mole of acid had been added. Therefore, only H_4phy^{-8} ions, which were formed at physiological pH ranges, would appear to be involved in the complex forming reaction. The magnitude of the observed pH drop is directly related to the metal $-H_4phy^{-8}$ complexing tendency and, therefore, served as a qualitative measure of the stability of the complex formed. Among the metals studied, zinc produced the largest pH drop and appeared to exhibit a greater tendency for complex formation with phytic acid. From these data we conclude that (1) at lower pH ranges, phytic acid showed a greater affinity for hydrogen than for the metal ions studied or the metal complexes formed were completely dissociated; (2) stability of the complexes formed between phytic acid and the metals studied was in the order $Zn^{++} > Cu^{++} > Co^{++} > Mn^{++} > Ca^{++}$. The higher stability of zinc was not surprising since similar findings have been reported(8).

The growth rate of chicks fed 10 ppm zinc with 0.25% sodium phytate was significantly lower than the growth of birds not fed the phytate. These data (Table II) indicate that the added sodium phytate inhibited growth presumably by formation of a complex with zinc, and thus reduced the absorption and utilization of the metal. The phytate anions (H_4phy^{-8}) formed near physiological pH ranges would appear to be involved in the complex forming reaction in the intestinal tract. At the 50 ppm zinc level, there was no significant difference in the growth rate due perhaps to the fact that the zinc requirement of chicks has been reported to be only 35 ppm(5).

Summary. Relative tendency of some metals to complex with phytic acid, and the influence of sodium phytate on the availability of zinc in chicks were studied. Of the metals studied, zinc appeared to form the most stable complex with phytic acid in the physiological pH ranges. Addition of sodium phytate (0.25%) to a low-zinc chick diet depressed growth rate presumably by binding zinc and rendering the element less available for growth. Therefore, the greater tendency exhibited by zinc to form a complex with phytic acid near physiological pH ranges would possibly explain the adverse effect of phytic acid on zinc metabolism.

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