# Complexation of Phytate with Proteins and Cations in Corn Germ and Oilseed Meals

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The extent of phytate–protein interaction in aqueous extracts of a high lysine and a commercial hybrid corn germ, soybean flakes, and sesame meal was investigated. The phytate in corn germ is relatively more water soluble than is the protein; in soybean flakes it is proportionally soluble and in sesame phytate is markedly less soluble than total protein. As shown by gel electrophoresis at pH 9 or greater, several proteins in an aqueous extract of soybean flakes complex with phytate whereas those of corn germ do not. Gel filtration on Sephadex G-50 at pH 4.4 also clearly separates phytate from corn germ proteins. The amino acid compositions of the crude extracts do not explain the difference in phytate binding inasmuch as there was no correlation with the concentrations of the basic amino acids. Dilute HCl (0.3 M) extracted phytate and counterions from a sesame meal residue and these precipitated when neutralized with NaOH. The insoluble phytate had a composition ($Na_2Mg_5$ phytate) which suggests that phytate exists in sesame seed, and perhaps most seeds, as a magnesium phytate, probably $K_2Mg_5$ phytate, and not as phytin ($Ca_5Mg$ phytate).

The phytate ion complexes with metallic ions and in many cases forms insoluble compounds. For example, complexes with iron, zinc, and calcium are highly insoluble and a combination of calcium and zinc forms an even less soluble complex (Oberleas et al., 1966). Phytate also complexes with proteins making them less soluble (Smith and Rackis, 1957; Courtois and Barré, 1953; Bourdillon, 1951). There is evidence that phytate–protein complexes are less subject to proteolytic digestion than the same protein alone (Barré, 1956). It has been shown that calcium ions interact with protein and phytate to further decrease their solubility (Wolf and Briggs, 1959; Saio et al., 1967).

Phytate clearly decreases the biological availability of zinc, presumably by formation of a stable insoluble complex which does not release zinc to absorption sites in the intestinal mucosa (O'Dell et al., 1972b). Conceivably indigestible phytate–protein complexes could make zinc and other nutrients even less biologically available. Because of this possibility and the fact that little is known about the chemical nature of phytate as it exists in plant seeds, this study was initiated to investigate the stability of the phytate–protein and phytate–metal complexes that can be extracted from corn germ, soybean meal, and sesame meal.

## MATERIALS AND METHODS

**Source of Samples.** The high lysine (0.44% lysine) and commercial hybrid corn (*Zea mays*) samples analyzed were produced by the Pioneer Hi-Bred Corn, Co., Johnston, Iowa. The phytate and mineral compositions of these samples have been reported (de Boland et al., 1975; O'Dell et al., 1972b). The germ, obtained by dry milling in a pilot plant, was extracted six times by refluxing with petroleum ether to remove lipid. The soybean flakes, processed by the U.S. Department of Agriculture Northern Regional Laboratory, Peoria, Ill., were prepared by hexane extraction of dehulled raw soybeans. Defatted sesame meal was prepared from a commercial full-fat meal by extraction with petroleum ether as described above.

**Preparation of Water-Soluble Protein Fractions.** A 20-g sample was extracted with 200 ml of distilled water for 3 h at 25 °C, centrifuged at 4000g, and finally filtered. The residue was reextracted in the same manner. It was then washed three times with water and lyophilized. An aliquot of the aqueous extract (pH 6.0–6.5) was adjusted to the pH of maximum precipitation (corn germ, pH 4.8; soybean flakes, pH 4.5; sesame, pH 4.3) with dilute HCl and centrifuged at 5000g. The supernate was dialyzed twice against 10 vol of water and lyophilized; the insoluble fraction was washed three times with water and lyophilized.

**Salt and Sodium Hydroxide Extraction of Sesame Meal.** Following water extraction of 100 g of sesame meal, the residue was dried and then extracted three times with 500 ml of 0.85 M NaCl for 24 h at 4 °C. After water washing and drying, the residue was extracted three times with 250 ml of 0.05 M NaOH for 24 h. The final residue was washed with water and dried.

**Acid Extraction of Sesame Residue after NaOH Treatment.** One gram of dry residue from above was extracted with 20 ml of 0.3 M HCl for 18 h at 25 °C. After centrifugation at 4000g, the supernate was adjusted to pH 7.2 with NaOH whereupon a white precipitate formed. The precipitate was collected by centrifugation, washed three times with water, and lyophilized prior to analysis.

**Electrophoresis.** A discontinuous polyacrylamide gel electrophoresis system was used (Buchler Instruments, Fort Lee, N.J.). The procedure was that described by the manufacturer. The system used a 7.5% acrylamide gel, a Tris-glycine buffer (pH 8.9), and had a running pH of 9.3. The general procedure was similar to that described by Gabriel (1971).

The protein sample was dissolved in buffer and diluted 1:2 with 60% sucrose. An aliquot containing 100–150 μg of protein was placed directly on the stacking gel. To detect protein the gels were stained for 60 min in 7% acetic acid containing 0.1% amido schwarz and destained by diffusion in 7% acetic acid. For phytate detection a similar gel was placed in a $FeCl_3$ solution (0.074 M $FeCl_3$ in 0.15 M HCl) and heated at 100 °C for 20 min. After cooling a white precipitate appeared on an orange background indicating the presence of ferric phytate.

**Analytical Methods.** Nitrogen was determined by the AOAC microkjeldahl method. Phytate was determined as previously described (de Boland et al., 1975) and involved precipitation with ferric ion in acid solution (Early and Deturk, 1944) followed by determination of phosphorus after wet ashing.

For amino acid analyses samples were hydrolyzed with constant boiling HCl. A sample of protein (ca. 10 mg) was placed in a tube with 6 ml of the HCl; the tube was flushed

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Table I. Water Solubility of Solids, Nitrogen, and Phytate in Corn Germ, Soybean Flakes, and Sesame Meal

| Sample | Recovd solids, g | Nitrogen content, %, D.M. | Phytate P content, %, D.M. |
|---|---|---|---|
| Corn germ, commercial | 100.0 | 3.05 | 1.35 |
| Aq ext. | 20.3 | 6.53 (44%)[a] | 6.15 (93%)[a] |
| Residue | 62.6 | | 0.08 (4%) |
| H₂O insol., pH 4.8 | 7.5 | 10.85 (27%) | 0.36 (2%) |
| H₂O sol., pH 4.8 (dial.) | 3.8 | 4.88 (6%) | 6.78 (19%) |
| Corn germ, high lysine | 100.0 | 2.80 | 1.03 |
| Aq ext. | 18.5 | 6.12 (40%) | 6.27 (89%) |
| Residue | 63.6 | 2.00 (46%) | 0.07 (4%) |
| H₂O insol., pH 4.8 | 6.8 | 7.86 (19%) | 0.30 (3%) |
| H₂O sol., pH 4.8 (dial.) | 4.4 | 3.48 (5%) | 6.54 (22%) |
| Soybean flakes | 100.0 | 7.95 | 0.43 |
| Aq ext. | 38.0 | 12.4 (59%) | 0.68 (60%) |
| Residue | 60.0 | 4.19 (32%) | 0.14 (20%) |
| H₂O insol., pH 4.5 | 19.6 | 14.5 (36%) | 0.66 (30%) |
| H₂O sol., pH 4.5 (dial.) | 10.8 | 2.72 (3.7%) | 0.35 (9%) |
| Sesame meal | 100.0 | 6.0 | 1.44 |
| Aq ext. | 7.9 | 8.8 (11.5%) | 0.35 (2%) |
| NaCl (5%) ext. | 9.5 | 14.2 (22%) | 1.13 (7%) |
| NaOH (0.2%) ext. | 12.0 | 14.2 (28%) | 0.08 (<1%) |
| Residue | 23.0 | | 2.73 (44%) |

[a] The values in parentheses are percentages of the original component recovered.

with nitrogen and capped. It was then heated at 108 °C for 24 h, and then repeatedly evaporated to dryness under vacuum to remove HCl. Analyses were performed on a Beckman amino acid analyzer using one column packed with UR-30 (for acidic and neutral) and one with PA-35 (for basic amino acids) resin (Benson et al., 1967).

Mg and Ca were determined by atomic absorption spectrophotometry and Na and K by flame emission spectrophotometry (O'Dell et al., 1972a).

RESULTS

**Extraction of Phytate.** The distribution of solids, phytate, and nitrogen in the aqueous fractions of corn germ, soybean flakes, and sesame meal is shown in Table I. Since nearly all of the phytate in the corn kernel exists in the germ (O'Dell et al., 1972a), this fraction was chosen to study the interaction of phytate and corn protein. Approximately 90% of the phytate in corn germ is extractable by water, whereas only 40% of the nitrogen is extracted. Hence, the aqueous extract is enriched in phytate. When the extract was adjusted to the point of maximum insolubility (pH 4.8), a large proportion of the protein came out of solution but little phytate was associated with it. The soluble fraction contained the major proportion of recovered phytate, but approximately 75% was lost during the washing and dialysis procedure. Even though the supernatant fraction from isoelectric precipitation contained 20% of the original phytate after dialysis, one should not conclude that it is bound to protein. Later dialysis experiments showed that phytate dialyzes through cellophane membranes extremely slowly.

Water extracted approximately 60% of both the nitrogen and phytate found in soybean flakes. Consequently the concentration of phytate in the soluble fraction was only slightly higher than that of the original flakes.
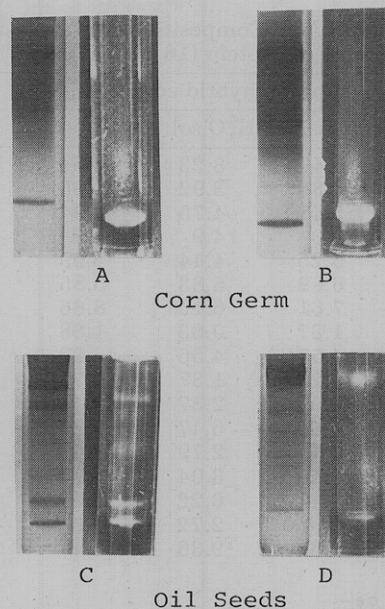


Corn Germ

Oil Seeds

Figure 1. Acrylamide disc gel electrophoresis of aqueous extracts of corn germ from a commercial hybrid (A) and a high lysine (B) corn, soybean flakes (C), and sesame meal (D). The gels were run in duplicate; one gel (left) was stained for protein with amido schwarz and one (right) for phytate with ferric chloride. The dark bands on the left indicate protein and the light bands on the right indicate phytate. Coincidence of bands suggests protein–phytate interaction.

Adjustment of the extract to pH 4.5 precipitated most of the nitrogen and phytate and the phytate was firmly bound to the protein.

In contrast to the phytate in corn germ and soybean flakes, that in sesame meal was not extractable by water. Subsequent extraction with 0.85 M NaCl and finally with 0.05 M NaOH removed most of the protein but relatively little of the phytate. Clearly, the phytate in sesame is not readily soluble in neutral or alkaline aqueous solutions. Since a large proportion of the protein is extracted by these procedures, it is not likely that phytate is bound to protein in the native state.

**Phytate–Protein Interaction.** At first, dialysis was used as a measure of phytate–protein interaction but this proved to be entirely unsatisfactory because phytate dialyzes extremely slowly from cellophane tubing. Several days were required to reach equilibrium with even dilute solutions of pure sodium phytate. The next approach was to use electrophoresis at a high pH, a condition that eliminates protonation of all except the basic amino acid residues. Equal aliquots of a fraction were placed on each of two gels and run simultaneously in the system which provided a running pH of 9.3. One gel was stained for protein and the other for phytate. Photographs of gel pairs are shown in Figure 1. In this electrophoretic system phytate moved at the buffer front which is delineated by the dark band in the gels on the left. When free phytate was present, a white band appeared at the front as shown by the gels on the right.

Essentially all of the phytate of the water extract of corn germ (A and B) moved at the buffer front unassociated with protein. The water-soluble corn germ protein which precipitated at pH 4.8 had a major band (gel not shown) near the top of the gel which was associated with a small phytate band. However, most of the phytate moved at the front. The water-soluble fraction extracted from soybean flakes showed three major and several minor protein bands on the electrophoretic gels. There were at least four

Table II. Amino Acid Composition of Water-Soluble Proteins in Corn Germ, Soybean Flakes, and Sesame Meal (Values as Percent of Protein (16 g of N))

| | Comm. hybrid corn germ | | | High lysine corn germ | | | Soybean flakes | | Sesame meal | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total | H₂O sol. | Iso. ppt | Total | H₂O sol. | Iso. ppt | H₂O sol. | Iso. ppt | H₂O sol. | Iso. ppt |
| Asp | 9.40 | 8.23 | 7.54 | 10.8 | 11.4 | 8.83 | 4.90 | 3.85 | 8.06 | 8.84 |
| Thr | 4.36 | 3.92 | 3.85 | 4.17 | 4.51 | 4.69 | 4.39 | 3.74 | 3.68 | 3.79 |
| Ser | 5.41 | 4.75 | 4.94 | 3.94 | 5.41 | 5.14 | 6.45 | 6.16 | 4.77 | 5.16 |
| Glu | 18.0 | 14.9 | 13.3 | 13.5 | 15.4 | 15.1 | 23.6 | 24.2 | 20.1 | 21.0 |
| Pro | 5.25 | 4.44 | 4.09 | 6.80 | 5.74 | 3.65 | 5.55 | 5.94 | 3.60 | 4.68 |
| Gly | 6.09 | 5.83 | 5.85 | 5.65 | 6.94 | 5.94 | 4.77 | 4.62 | 5.86 | 5.49 |
| Ala | 7.61 | 6.10 | 8.66 | 6.00 | 7.05 | 6.98 | 5.03 | 4.84 | 7.39 | 7.07 |
| Cys | 1.37 | 0.83 | 1.88 | 1.66 | 1.98 | 1.47 | 0.41 | 0.48 | 2.02 | |
| Val | 4.57 | 4.56 | 6.09 | 4.11 | 4.51 | 6.10 | 4.77 | 4.73 | 4.84 | 4.02 |
| Met | 0.26 | 1.37 | 1.75 | 0.80 | 0.95 | 1.06 | | 0.32 | 2.22 | |
| Ile | 2.99 | 2.57 | 3.48 | 2.51 | 2.80 | 4.04 | 4.77 | 4.95 | 3.66 | 3.85 |
| Leu | 8.45 | 5.47 | 7.89 | 5.88 | 5.48 | 8.40 | 9.16 | 9.35 | 7.21 | 7.14 |
| Tyr | 3.41 | 2.79 | 3.62 | 2.97 | 3.13 | 3.84 | 4.13 | 4.18 | 4.91 | 3.72 |
| Phe | 4.10 | 3.04 | 4.90 | 3.83 | 3.02 | 5.37 | 6.58 | 6.82 | 5.68 | 5.54 |
| Lys | 5.41 | 6.22 | 9.57 | 7.82 | 8.95 | 11.4 | 6.97 | 7.15 | 3.89 | 3.65 |
| His | 2.94 | 2.72 | 3.65 | 5.14 | 4.29 | 4.45 | 2.71 | 1.32 | 2.93 | 3.04 |
| Arg | 8.61 | 9.36 | 12.4 | 12.0 | 12.2 | 12.8 | 7.22 | 7.04 | 15.3 | 16.5 . |
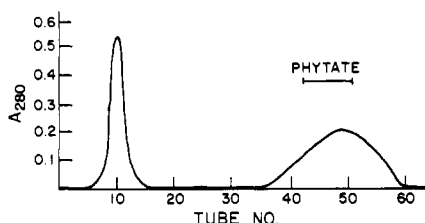


Figure 2. Sephadex G-50 chromatogram of a dialyzed and lyophilized aqueous extract of high lysine corn germ. Sample (100 mg) was dissolved in 3 ml of 0.025 M acetate buffer (pH 4.4), clarified by centrifugation, placed on a column, and developed at a flow rate of 120 ml/h. Aliquots of 4 ml were collected per tube and the absorbance measured at 280 nm. The phytate content of each tube was measured by ferric iron precipitation and phosphorus determination. Phytate was detected in tubes indicated by a horizontal line.
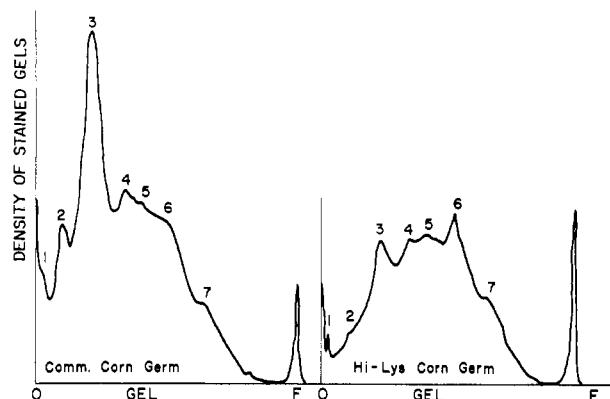


Figure 3. Chromoscans of electrophoretic gels showing relative densities of corn germ protein bands stained with amido schwarz. Conditions are the same as in Figure 1. The origin is indicated by O and buffer front by F. Gels were loaded with 100–150 μg of protein in the aqueous extract of corn germ.

phytate bands associated with protein and one at the buffer front.

The electrophoretic results show that, while water-soluble proteins in soybean flakes complex strongly with phytate even at alkaline pH, those in corn germ do not. Only after isoelectric precipitation of corn germ protein is a small quantity of phytate complexed. As pointed out earlier (de Boland et al., 1975) the phytate associated with soybean protein after isoelectric precipitation is also strongly bound. Although only a small proportion of the phytate in sesame meal is extracted with water, that portion is associated with protein during electrophoresis.

To confirm that the water-soluble corn germ proteins do not bind phytate, even under acidic conditions, a lyophilized water extract of high lysine corn germ dissolved in acetate buffer (pH 4.4) was chromatographed on Sephadex G-50. Absorbance at 280 nm was used to monitor protein in the filtrate. However, there was low molecular material present which also absorbed at this wavelength. A chromatogram is shown in Figure 2. The first peak appeared at the void volume and the second appeared at the salt volume. The nature of the compound(s) giving rise to the second absorption peak is unknown, but it is not protein. Pure phytate appears at the same filtration volume in the presence or absence of corn germ proteins so that there is no evidence of phytate interaction with the water-soluble corn germ proteins.

**Nature of Water-Soluble Proteins.** To clearly distinguish the electrophoretic patterns of the water-soluble proteins extracted from the two corn germ samples the

band densities were recorded by use of a scanning densitometer. Chromoscans of stained gels are shown in Figure 3. Seven bands could be detected visually as well as by the peaks in the scan patterns. Bands 2 and 3 of the high lysine corn germ were markedly lower than those in the commercial hybrid whereas bands 5 and 6 were more prominent.

In an attempt to explain the differences in phytate binding by the water-soluble proteins from oilseeds and corn germ, they were analyzed for amino acids. The results are presented in Table II. Analyses of the total corn germ fractions showed that the high lysine corn germ was truly higher in lysine than the commercial hybrid (7.8 vs. 5.4%). Furthermore, it was higher in histidine and arginine. These differences were reflected in the water-soluble proteins, including the isoelectric insoluble (Iso. ppt) fraction. The higher concentration of basic amino acids in the high lysine corn may well account for the differences in electrophoretic patterns of the two corn germs. One might expect that the high lysine corn germ proteins with their higher content of basic amino acids would bind phytate more readily, but there was little or no detectable difference in binding.

The water-soluble proteins in soybean flakes have relatively low concentrations of the basic amino acids and a high concentration of glutamic acid. Nevertheless, several of the constituent proteins bind phytate strongly. Soybean protein is relatively high in lysine and this residue

Table III. Analysis of the Phytate Complex Extracted from Sesame Meal Residue by HCl

| Constituent | % | Molar ratios |
|---|---|---|
| Phytate P | 16.2 | 6.0 |
| Phytate | (57.7)[a] | 1.0 |
| Mg | 10.2 | 4.8 |
| Ca | 0.72 | 0.20 |
| Na | 2.1 | 1.05 |
| K | 0.34 | 0.10 |
| Zn | 0.24 | 0.04 |

[a] Calculation based on the assumption that phytate contains 28.2% phosphorus.

may be critical in phytate binding. Sesame protein is rich in arginine but low in lysine. It does not bind phytate strongly.

**Cationic Environment of Phytate in Sesame Meal.** As mentioned above, relatively little of the phytate in sesame meal is extracted by water, 0.85 M NaCl, or 0.05 M NaOH when these solvents are used in succession. However, these solvents extract most of the protein leaving a residue enriched in phytate. Extraction of the residue with 0.3 M HCl gave a clear solution which had a high concentration of phytate. When adjusted to pH 7.2 with NaOH a white precipitate formed. After washing and lyophilizing the product was analyzed for the constituents listed in Table III. As shown magnesium was the major cation in the complex and accounted for at least 80% of the counterions. Sodium accounted for 10% but this ion no doubt arose from the isolation procedure. Calcium made up a negligible portion of the complex so that the precipitate did not resemble phytin. It seems probable that phytate in sesame seed exists as a potassium magnesium salt which is not extracted by neutral or basic solvents.

DISCUSSION

This study confirms earlier reports (Smith and Rackis, 1957; Courtois and Barre, 1953; Bourdillon, 1951) that phytate interacts with some proteins to form insoluble products or complexes. These complexes are not easily dissociated by electrophoresis at a high pH. Several soybean albumins form strong associations with phytate while those of corn germ do not. The reasons for this difference are not obvious from the amino acid composition of the mixture of proteins extracted by water.

Barre and van Huot (1965a) observed that the different basic amino acids of serum albumin are more or less accessible to the anionic charge of phosphorus compounds. In the reaction of phytate with serum albumin the order of reactivity was lysyl, histidyl, and finally arginyl residues. The authors suggested that the arginyl residues must be occluded in the interior of the molecule and thus less reactive than the other basic residues which appear to be on the surface. These authors also investigated the affinity of phytate for the basic amino acid residues in ovalbumin (Barre and van Huot, 1965b). They suggested that, in the latter case, phytate reacted first with all of the arginyl, then with lysyl, and finally with histidyl residues. It appears that lysine may be important in the complexation of oilseed proteins because lysine is low in sesame and relatively high in soybean proteins. The latter proteins bind phytate more readily than those of sesame. Although corn germ albumins are rich in lysine and arginine they do not bind phytate and there was no difference between high lysine and commercial corn germ.

Water extracts essentially all of the phytate found in corn germ (de Boland et al., 1975), a large proportion of that in raw soybean flakes, but relatively little of that in sesame meal. Although phytate cannot be readily separated from the aqueous extract of corn germ by dialysis, it is not complexed with protein as shown by electrophoresis at high pH and by gel filtration at a low pH. The concentrations of total lysine, arginine, and histidine in the proteins extracted from the three sources do not differ significantly. The results suggest that the arginine and possibly the lysine residues of the corn germ proteins are not accessible for reaction with phytate.

Sesame seeds contain among the highest levels of phytate found in nature (de Boland et al., 1975). As it exists in sesame, phytate is not readily extracted by the usual aqueous solvents at neutral or alkaline pH. It is extracted from the residue by dilute HCl along with sufficient $Mg^{2+}$ to account for a large proportion of the required counterions. At neutrality the magnesium salt was insoluble. This property accounts for the failure of phytate to be extracted from sesame meal and suggests that phytate exists in the sesame largely complexed with magnesium. Clearly it does not exist there as phytin, $Ca_5Mg$ phytate. In fact, most seeds do not contain sufficient calcium to account for this stoichiometry (O'Dell et al., 1972a).

From the results presented here it is clear that one cannot make a general statement as to the chemical environment or native state of phytate in plant seeds. In some cases phytate may be complexe' with protein and in others with magnesium to form an insoluble compound. Obviously it may be complexed with both divalent cations and with proteins. It is known that calcium has a marked effect on the solubility of soybean protein–phytate complexes (Saio et al., 1967).

From the series of studies on phytate (de Boland et al., 1975; O'Dell et al., 1972a,b) completed in this laboratory some generalizations can be made. Essentially all of the phytate in corn seed exists in the germ portion. It can be readily extracted from corn germ with water along with the albumins. Although these proteins are relatively rich in arginine and lysine they do not bind phytate. The extractability of phytate is not significantly affected by autoclaving for 30 min, and there is relatively little destruction of phytate in natural products by this treatment (de Boland et al., 1975). All of the "phytate" in mature seeds, as determined by ferric iron precipitation, is inositol hexaphosphate (de Boland et al., 1975). The phytate in sesame meal is not strongly complexed to protein but is not readily extracted by neutral aqueous solvents because it exists there as an insoluble magnesium complex.

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# Effect of Dietary Zinc and Copper Interrelationships on Blood Parameters of the Rat

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The effects of varying both dietary zinc and copper on blood chemistry in male rats fed a semipurified diet were studied. Hematocrit and hemoglobin were directly related to serum zinc and serum copper levels. Serum glutamate oxaloacetate transaminase (SGOT) values were directly correlated with serum zinc values, and serum uric acid levels were directly related to serum copper levels. Serum cholesterol was inversely related to dietary copper as well as to serum copper values. Since there was no significant relationship of dietary zinc or serum zinc with serum cholesterol it was not surprising to find a direct relationship between serum cholesterol and the ratio of serum zinc/serum copper.

Because of our finding that cadmium, lead, and certain chemicals can affect zinc and copper metabolism in rats (Klauder and Petering, 1976; Klauder, 1975; Book et al., 1973; Murthy et al., 1972, 1976; Petering et al., 1967, 1971; Rice et al., 1974) we have been interested in establishing dose–response relationships to zinc and copper nutriture when these are varied within the suboptimal and optimal dietary ranges. This is important since an animal model for evaluating the toxicity of some environmental chemicals depends on controlling dietary intake of protective nutrients, in this case the essential trace metals. We have recently reported the nutritional interaction of varying zinc and copper as this is reflected in the serum and tissue levels of these two essential elements (Murthy et al., 1974), and we now wish to present evidence of interaction of these elements on several blood parameters which are frequently assessed in studying biological effects of environmental toxicants.

MATERIALS AND METHODS

The details pertaining to materials and design of the experiment were described in detail in a previous communication (Murthy et al., 1974). Twenty groups of three weanling Carworth strain male rats were fed a semipurified diet low in zinc and copper. Dietary zinc (zinc acetate) and copper (copper sulfate) were given in distilled deionized water at the following concentrations ($\mu$g/ml): 2.5, 5.0, 10.0, 20.0, and 40.0; and 0.25, 0.50, 1.00, and 2.00, respectively. A 5 × 4 factorial design for the 20 groups of animals was used. Food and water were available ad libitum. The experiment lasted for 60 days. Hematocrit, hemoglobin, white blood cell count, lymphocytes, and granulocytes were determined prior to kill or at necropsy by the routine clinical laboratory procedures. At necropsy the animals were anesthetized with phenobarbital sodium,

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blood was drawn by cardiac puncture, sera were separated, and the serum profile was obtained using an Auto Technicon sequential multi-analyzer (SMA 12) in which calcium, inorganic phosphorus, glucose, total protein, albumin, alkaline phosphatase, serum glutamate oxaloacetate transaminase (SGOT), uric acid, and cholesterol values were determined. For purposes of obtaining correlation between blood and/or serum profiles, serum copper and serum zinc values which were published earlier are referred to.

**Statistical Calculations.** The statistical technique of analysis of variance was used to investigate the effects of alterations in dietary zinc and copper and their interaction on one another (Snedecor and Cochran, 1967). Subsequent comparisons testing individual effects (where appropriate) were carried out and then significance judged by Tukey's paired comparison test (Winer, 1962). Associations between the following measurements were investigated by calculating their correlation coefficients: hematocrit and hemoglobin; hematocrit and serum zinc; hematocrit and serum copper; hemoglobin and serum copper; hemoglobin and serum zinc; cholesterol and serum copper; cholesterol and serum zinc; cholesterol and the ratio of serum zinc to serum copper; cholesterol and the ratio of dietary zinc to dietary copper; SGOT and serum zinc; and uric acid and serum copper. (The values of serum zinc and copper were taken from the previously published paper; Murthy et al. (1974).)

RESULTS

**Blood and Serum Parameters Showing No Variation Due to Dietary Zinc or Copper.** Analyses of variance of the data for white blood cell count, lymphocytes, granulocytes, calcium, inorganic phosphorus, glucose, total protein, albumin, and serum alkaline phosphatase relating to the dietary levels of zinc and copper were done and found to be in the normal range for rats without any variation due to dietary zinc and copper.

**Parameters Showing Variation Due to Alteration in the Levels of Dietary Zinc and/or Copper.** *He-*