Interactive Effects of Dietary Silicon, Copper, and Zinc in the Rat

Royce J. Emerick and Henry Kayongo-Male

Departments of Chemistry and Biology, South Dakota State University, Brookings, SD, USA

A factorial rat experiment using two dietary concentrations each of copper, zinc, and silicon was conducted to identify areas in which interrelationships involving silicon may exist. The concentrations used were (mg/kg of diet): copper, 1 and 5; zinc, 2 and 12; and silicon, 5 and 270. An antagonism between silicon and zinc, whereby increases in dietary levels of either one resulted in a reduction in blood plasma concentrations of the other, was demonstrated. The depressing effect of silicon on plasma concentrations of zinc and on alkaline phosphatase occurred only in zinc-deficient rats. However, silicon had no effect on growth. Effects on aortic composition, interpreted as beneficial, accompanied increases in the silicon content of copper-deficient diets. Silicon-dependent increases in the chloroform-methanol extractable fraction of aorta closely approximated a similar response to copper. High dietary silicon increased aortic elastin in copper-deficient rats when dietary zinc was adequate. The aortic effects of silicon, while mimicking the gross effects of copper, occurred in the absence of any silicon-related changes in blood copper concentrations. Interrelationships of silicon with other elements, particularly copper and zinc, may warrant consideration in future nutritional and metabolic studies.

Keywords: Silicon; copper; zinc; trace elements

Introduction

A progression of research findings point to possible overlapping nutritional effects for silicon, copper, and zinc. The essential role of silicon has been reviewed by Carlisle.[1,2] Silicon is essential for the production of connective tissue with specific involvement in the biosynthesis of collagen. Stabilization of collagen and related proteins (e.g., vascular elastin) through cross-linking of polypeptide chains involves the copper-dependent enzyme, lysyl oxidase.[3] Aortic rupture, presumably due to defects in elastin, is a common consequence of copper deficiency in several species*[,4,5] Furthermore, a high ratio of zinc to copper may be a predisposing factor to coronary heart disease.[6]

An interaction between copper and molybdenum, in which effects of an excess of dietary molybdenum are countered by increases in dietary copper, is well-established, particularly in ruminants. Similarly, interactions between silicon and molybdenum† and silicon and aluminum[7] have been reported in chicks and rats, respectively.

The apparent overlapping nutritional and metabolic effects of silicon, copper, and/or zinc in the biosynthesis or structural integrity of connective tissues point to a potential triad of interrelationships for these elements. This study was conducted to identify areas in which these interrelationships may occur.

Materials and Methods

Animals and procedures

Male Sprague-Dawley albino rats (SASCO, Omaha, NE, USA) were used in a 2 × 2 × 2 factorial experi-

Received June 26, 1989; accepted for publication August 9, 1989. Published with approval of the Director of the South Dakota Agricultural Experiment Station as publication number 2386 of the journal series.

Address reprint requests to Dr. Royce J. Emerick, Olson Biochemistry Laboratories, Animal Science Bldg, South Dakota State University, Brookings, SD 57007, USA.

*O'Dell, B. L., Hardwick, B. C., Reynolds, G., Savage, J. E. (1961). Connective tissue defect in the chick resulting from copper deficiency. Proc. Soc. Exp. Biol. Med. **108,** 402 (abstr).

†Carlisle, E. M. (1979). A silicon-molybdenum interrelationship in vivo. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **38,** 553 (abstr).

ment involving two levels each of copper, zinc, and silicon. One hundred twenty rats with average weights of 64 ± 7 g were randomly allotted across the eight treatment groups of 15 rats each. Rats were housed individually in hanging stainless steel cages with wire-mesh floors in a room maintained at 23 to 25°C. A normal, 12-hour light-to-dark cycle was maintained. Diets representing the treatment variables and water treated by reverse osmosis followed by deionization were fed ad libitum.

The basal diet is shown in *Table 1*. Before use, the spray-dried egg albumin was denatured as described previously.[8] The basal diet contained copper, zinc, and silicon in concentrations considered to be suboptimum. These concentrations, based on analysis, were (mg/kg diet) copper, 1; zinc, 2; and silicon, 5. These low concentrations are designated as −Cu, −Zn and −Si. The higher treatment concentrations of each, designated as +Cu, +Zn, and +Si, were (mg/kg diet) copper, 5; zinc, 12; and silicon, 270. These were provided by respective additions of $CuSO_4 \cdot 5H_2O$, $ZnCl_2$, and tetraethylorthosilicate (J. T. Baker Chemical Co., Phillipsburg, NJ, USA). Dietary levels (mg/kg diet) of 5 copper and 12 zinc are considered to be adequate for normal growth.[9]

Tetraethylorthosilicate is readily hydrolyzed in the gastric stomach to monomeric silicic acid and ethanol. Thus, it is particularly suitable as a readily available source of silicon. It has been used routinely in our laboratory at a high level of intake (2% of the diet) for the experimental production of siliceous uroliths[10-12] and in studies concerning silicic acid-protein complex formation.[13]

The rats were weighed weekly during the 8-week experiment. Blood samples were obtained at termination by cardiac puncture following anesthetization with Halothane (Abbott Laboratories, Chicago, IL, USA). Blood was placed in heparinized tubes. Following the immediate determination of hemoglobin, as described below, and packed cell volume (Autocrit Centrifuge; Clay Adams, Inc., New York, NY, USA), plasma was separated and stored at −25°C until analyzed. While continuing under anesthetization, the rats were killed by decapitation and the liver, kidneys, tibia, and a sample of aorta were removed, weighed, and stored at −25°C until analyzed. The aorta sample represented approximately 3 cm of the descending thoracic aorta beginning immediately below the aortic arch.

Concentrations of calcium and magnesium in plasma, and copper and zinc in plasma and in nitric acid digests of liver and kidney were determined by atomic absorption spectrophotometry (Perkin-Elmer model 5000, Norwalk, CT, USA). Plasma inorganic phosphorus was determined using the Fiske and Subbarow phosphomolybdate method.[14] Silicon content of the basal diet was determined by graphite furnace atomic absorption spectrophotometry following fusion with sodium carbonate. Atomic absorption conditions were the same as those described later for plasma. Total hemoglobin was determined by the cyanomethemoglobin method and alkaline phosphatase by the hydrolysis of p-nitrophenyl phosphate (test kits, hemoglobin 525 and alkaline phosphatase 104; Sigma Chemical Co., St Louis, MO, USA). Ceruloplasmin activity was determined using o-dianisidine dihydrochloride substrate as described by others.[15]

Silicon in blood plasma was determined by graphite furnace atomic absorption (Perkin-Elmer model 5000 equipped with model 500 heated graphite atomizer and autosampler). An uncoated graphite tube was used. An equal volume of 2,000 mg nickel/L (from $Ni(NO_3)_2 \cdot 6H_2O$) was used for matrix modification and was delivered via the alternate volume feature of the autosampler. Instrument parameters were those recommended by the equipment manufacturer. Under these conditions, an absorbance approximating 0.14 was obtained for 20 μl of a standard containing 0.05 mg silicon/L.

The aortas were extracted for 20 minutes with chloroform/methanol (2:1 vol/vol), washed with ethanol and ether, and allowed to air dry. Aortic elastin was determined as the amount remaining insoluble following treatment with 0.1 N NaOH at 98°C for 1 hour.[16]

Tibia were extracted for 12 hours with ethanol followed by a 12-hour extraction with diethylether. After air-drying, they were ashed overnight at 500°C, and ash was calculated as percent of the dry, fat-free bone. Calcium and phosphorus were determined on the acid soluble bone ash by the methods described for plasma.

Statistical analyses were by analysis of variance using a model consisting of copper, zinc, and silicon effects and all two- and three-way interactions.[17] Significant differences between appropriate means were identified by the method of least significant difference protected by a significant F value.

Table 1 Basal diet composition

Ingredient	Amount (%)
Dextrose, anhydrous	69.0
Egg albumin, spray-dried	20.0
Corn oil	5.0
Salt mixture[a]	4.0
Vitamin mixture[b]	2.0

[a] The percent composition of salt mixture (salt mixture P-H with reduced amounts of $CuSO_4$ and $ZnCl_2$; ICN Nutritional Biochemicals, Cleveland, OH, USA) is as follows: dipotassium phosphate, 32.2; calcium carbonate, 30.0; sodium chloride, 16.7; magnesium sulfate heptahydrate, 10.2; manganese sulfate, 0.51; dibasic calcium phosphate dihydrate, 7.5; ferric citrate, 2.75; potassium iodide, 0.08; copper sulfate pentahydrate, 0.0098; zinc chloride, 0.0104; and cobalt chloride, 0.005.

[b] Vitamin mixture composition (g/kg) triturated in dextrose (vitamin diet fortification mixture, ICN Nutritional Biochemicals) is as follows: vitamin A concentrate (500,000 IU/g), 1.8; vitamin D concentrate (850,000 IU/g), 0.125; α-tocopherol, 5.0; ascorbic acid, 45; inositol, 5.0; choline chloride, 75; menadione, 2.25; p-aminobenzoic acid, 5.0; niacin, 4.5; riboflavin, 1.0; pyridoxine hydrochloride, 1.0; thiamine hydrochloride, 1.0; calcium pantothenate, 3.0; biotin, 0.20; folic acid, 0.90; and vitamin B-12, 0.00135.

Results

Mean data are presented in *Tables 2* through *4* depending on whether the main treatment effects and interactions involved silicon and zinc (*Table 2*); silicon, copper, and zinc (*Table 3*); or copper and zinc (*Table 4*).

Effects and interactions of silicon and zinc

Blood plasma concentrations of zinc and silicon were each increased (Zn, $P < .01$; Si, $P < .05$) in response to dietary increases in the respective elements (*Table 2*). A zinc-silicon antagonism was evident in that lower ($P < .01$) plasma silicon concentrations accompanied the +Zn treatment with either level of dietary silicon. Conversely, lower ($P < .05$) plasma zinc concentrations were associated with the +Si treatment, but this occurred only when the silicon was fed in combination with the lower (−Zn) level of dietary zinc. The effect of the +Si treatment in lowering the zinc status of animals fed a zinc-deficient diet is additionally supported by lower ($P < .05$) plasma alkaline phosphatase activity in the −Zn+Si rats, alkaline phosphatase being a zinc-dependent enzyme.

Effects and interactions of silicon, copper, and zinc

Aorta mass (mg/cm length, wet basis) was increased by +Zn ($P < .01$) but not by +Cu (*Table 3*). Silicon tended to increase aorta mass in the −Cu but not in the +Cu treatment (Si × Cu interaction, $P < .05$).

The chloroform-methanol extractable fraction of aorta was increased by +Cu ($P < .01$). This fraction was also increased by +Si (Si effect, $P < .01$) but only in conjunction with the −Cu treatments (Si × Cu interaction, $P < .05$). These effects of +Cu or +Si in promoting increases in the chloroform-methanol extractable fraction of aorta were approximately equal but not additive. Aortic elastin was increased by +Si fed in combination with −Cu+Zn (silicon effect, $P < .05$), but the elastin-promoting effect of +Si was not evident in conjunction with the higher +Cu treatments or with the −Cu−Zn treatment (silicon × copper × zinc interaction, $P < .01$).

Effects and interactions of copper and zinc

Lower body weights ($P < .05$) were observed for rats in the −Zn treatments at all weighings (*Table 4*). At 4 and 8 weeks, rats fed the zinc-deficient (−Zn) diets weighed approximately 60% as much as those fed the zinc-adequate (+Zn) diets ($P < .01$). There was also a tendency for the higher level of copper (+Cu) to lower weight gains in the −Zn groups and to increase weight gains in the +Zn groups (copper × zinc interaction, $P < .05$).

Lower ($P < .01$) packed cell volume and hemoglobin values for the −Cu+Zn rats indicate that the lower level of dietary copper became limiting for hematopoiesis only when accompanied by the higher level of dietary zinc. Compared with the −Cu or −Zn treatments, blood plasma copper concentrations were increased ($P < .01$) by +Cu and were lowered ($P < .01$) by +Zn treatments. Also, the +Cu treatments promoted larger increases in plasma copper concentrations in zinc-deficient (−Zn) rats compared with those fed the +Zn diets (Cu × Zn interaction, $P < .01$). Ceruloplasmin activities were highly correlated ($r = .93$, $P < .01$) with plasma copper concentrations and showed the same treatment effects.

Treatment effects, albeit of doubtful physiologic importance, occurred in relation to blood plasma calcium, magnesium, and phosphorus concentrations. Blood plasma calcium concentrations were increased ($P < .01$) by +Zn, with the +Zn treatments averaging 103% of the −Zn treatments. Plasma magnesium was lowered ($P < .01$) by +Zn, but only in the −Cu treatments (Cu × Zn interaction, $P < .05$). In this instance, the plasma magnesium concentration of the +Zn−Cu treatment was 91% of the average for all other treatments involving combinations of −Zn or +Cu. Plasma phosphorus concentration for the +Cu+Zn treatment was 112% of the average of all other treatments involving combinations of −Cu or −Zn (Cu effect, $P < .05$; Zn effect, $P < .01$; Cu × Zn interaction, $P < .05$).

Liver and kidney copper concentrations were increased ($P < .01$) by +Cu and lowered ($P < .01$) by +Zn. Zinc concentration in kidneys was increased

Table 2 Dependent variable mean values showing silicon effects and/or silicon × zinc interaction[a,b]

Item	Treatment				SEM[c]
	−Zn		+Zn		
	−Si	+Si	−Si	+Si	
Blood plasma composition					
Zinc[d,e] (mg/L)	0.70*	0.53†	1.39‡	1.34‡	0.05
Alkaline phosphatase[d,e,f] (U/dl)	3.96*	3.15†	4.89‡	4.88‡	0.20
Silicon[d,e] (mg/L)	0.69*†	0.83*	0.51‡	0.59†‡	0.06

[a] Mean values within a row not sharing a common superscript symbol differ by the method of least significant differences protected by a significant F value, $P < .05$.
[b] Statistical effects are by analysis of variance.
[c] Standard error of the mean calculated from the error mean square.
[d] Zinc effect, $P < .01$.
[e] Silicon effect, $P < .05$.
[f] Silicon × zinc interaction, $P < .05$.

Table 3 Dependent variable mean values showing silicon, copper, or zinc effects and/or interactions[a,b]

Item	Treatment								SEM[c]
	−Cu				+Cu				
	−Zn		+Zn		−Zn		+Zn		
	−Si	+Si	−Si	+Si	−Si	+Si	−Si	+Si	
Aorta mass, wet basis[d,e] (mg/cm)	8.1*†	9.0†‡	9.7‡§	10.6§	8.3*†	7.8*	10.3§	10.3§	0.38
Aorta composition (percent)									
Chloroform-methanol extractables, wet basis[e,f,g]	68.5*	71.5†‡	69.2*†	72.7‡	71.8‡	71.9‡	72.6‡	72.8‡	0.9
Elastin, dry, fat-free basis[d,f,h,i,j]	21.3*	21.8*	23.8*	30.9†	32.4†‡	36.2‡	37.4‡	36.3‡	1.5

[a] Mean values within a row not sharing a common superscript symbol differ by the method of least significant differences protected by a significant F value, $P < .05$.
[b] Statistical effects are by analysis of variance.
[c] Standard error of the mean calculated from the error mean square.
[d] Zinc effect, $P < .01$.
[e] Silicon × copper interaction, $P < .05$.
[f] Copper effect, $P < .01$.
[g] Silicon effect, $P < .01$.
[h] Silicon effect, $P < .05$.
[i] Silicon × copper × zinc interaction, $P < .01$.
[j] Air-dried following extraction with chloroform-methanol.

Table 4 Dependent variable mean values showing copper or zinc effects and/or copper × zinc interaction[a,b]

Item	Treatment				SEM[c]
	−Cu		+Cu		
	−Zn	+Zn	−Zn	+Zn	
Body weight (g)					
Initial	64.6	65.6	64.2	64.6	1.4
4 wk[d,e]	142.5*	220.2†	129.7*	227.1†	5.0
8 wk[d,e]	185.4*	294.3†	167.0*	315.4†	8.1
Blood parameters					
Packed cell volume[d,f,g] (%)	44.7*	40.1†	44.7*	44.9*	0.5
Hemoglobin[d,f,g] (g/dl)	14.2*	12.3†	14.0*	14.3*	0.2
Blood plasma parameters					
Copper[d,f,g] (mg/L)	0.29*	0.08†	0.80‡	0.36*	0.04
Ceruloplasmin[d,f,g] (U/L)	19*	2†	70‡	23*	4.3
Calcium[d] mg/L	107.8*†	110.5‡	106.0*	109.3†‡	0.9
Magnesium[d,e] (mg/L)	23.4*	21.5†	23.3*	22.9*	0.4
Phosphorus[d,e,h] (mg/L)	69.9*	70.4*	69.5*	78.0†	1.6
Tissue parameters (mg/kg dry wt)					
Liver copper[d,f]	8.8*	3.9†	14.4‡	10.0*	0.5
Kidneys copper[d,f]	17*	14*	27†	21‡	1.2
Liver zinc[e,f]	74*	69†	77*‡	79‡	1.3
Kidneys zinc[d]	75*	81†	76*	82†	0.8
Bone ash[d,i] (%)	59.2*	60.2†	59.1*	60.9‡	0.25

[a] Mean values within a row not sharing a common superscript symbol differ by the method of least significant differences protected by a significant F value, $P < .05$.
[b] Statistical effects are by analysis of variance.
[c] Standard error of the mean calculated from the error mean square.
[d] Zinc effect, $P < .01$.
[e] Copper × zinc interaction, $P < .05$.
[f] Copper effect, $P < .01$.
[g] Copper × zinc interaction, $P < .01$.
[h] Copper effect, $P < .05$.
[i] Dry, fat-free basis.

($P < .01$) by +Zn. In liver, the zinc concentration was increased ($P < .01$) by +Cu, but only in the presence of +Zn (copper × zinc interaction, $P < .05$).

Bone ash, calculated as a percentage of the dry, fat-free bone, was increased by +Zn ($P < .01$). The values averaged 59.2% for −Zn and 60.6% for +Zn treatments. Percentage calcium (36.1 ± 0.15) and phosphorus (17.6 ± 0.06) in bone ash showed no treatment effects ($P > .05$).

Discussion

An antagonism between silicon and zinc, whereby increases in dietary levels of either one resulted in a reduction in blood plasma concentrations of the other, was demonstrated in this experiment. In addition, effects on aortic composition, interpreted as beneficial, are attributed to increases in the silicon content of copper-deficient diets.

Silicon-zinc antagonism

Rats fed 270 mg silicon and 2 mg zinc/kg diet had blood plasma zinc concentrations that were 76% and alkaline phosphatase activities that were 80% of the corresponding values for those fed the same level of zinc with no added silicon. Silicon had no similar zinc-depressing effect in rats fed 12 mg zinc per/kg diet. The higher dietary silicon level clearly caused lower plasma zinc concentrations only in zinc-deficient rats. Whether higher levels of dietary silicon may extend this effect in rats fed nutritionally adequate levels of zinc was not determined.

Unlike the effect of silicon in lowering blood plasma zinc concentrations only in the low-zinc treatments, a zinc-induced lowering of blood plasma silicon occurred with both levels of dietary silicon. Rats fed the higher level of zinc, with or without added silicon, had plasma silicon concentrations that averaged 72% of the corresponding values for those fed the low-zinc diets.

The silicon-zinc antagonism described here, in which increases in the dietary concentration of one resulted in lower blood plasma concentrations of the other, may be similar to the antagonism reported by Carlisle† to occur between silicon and molybdenum. However, this is the first time that a silicon-zinc antagonism in animals has been demonstrated, and it cannot be concluded at this time whether it is due to reactions occurring intestinally or parenterally.

The lower plasma silicon concentrations of rats fed the higher zinc level may be partly related to differences in body size; those fed 2 mg zinc/kg of diet weighed only approximately 60% as much as those fed 12 mg zinc/kg of diet. However, the effect of silicon on plasma zinc concentration and alkaline phosphatase activity cannot be explained in this manner since an increase in dietary silicon did not influence body weights.

The 270 mg silicon/kg of diet, representing the highest silicon level in this study, is less than the 500 mg silicate silicon/kg of diet used by Schwarz and Milne[18] to obtain a growth response in rats. However, it approximates the 250 mg silicon/kg of diet used by Carlisle[1] to demonstrate a silicon-responsive increase in the rate of bone mineralization in rats. No effect of silicon on percentage of bone ash or on calcium to phosphorus ratio in the bone was observed in the current study.

Aortic effects of silicon

Aortic effects of dietary silicon were manifested only in groups fed the lower concentration of copper. Rats fed the low-copper diets (1 mg copper/kg of diet) with either concentration of zinc had lower percentages of aortic chloroform-methanol extractables and elastin. Silicon-dependent increases in the chloroform-methanol extractable fraction of aorta closely approximated an increase in this fraction that was associated with an increase in dietary copper.

Higher aortic elastin values in response to an increase in dietary silicon occurred only in the low-copper, high-zinc group, these being the only rats showing analytic signs of copper deficiency. These deficiency signs included lower packed cell volume, hemoglobin, plasma copper, and plasma ceruloplasmin. While mimicking the gross effects of copper, the effect of silicon on aortic elastin content did not involve any changes in blood copper concentrations. Further, the elastin-promoting effect of silicon also differed from that of copper in that the silicon effect was manifested only in the presence of an adequate level of dietary zinc while the response to copper occurred independent of dietary zinc levels.

Previous studies on essential functions of silicon have emphasized the effects of silicon on collagen formation in the growth of bone and cartilage.[1,2] The aortic effects of silicon, documented herein, have not been previously demonstrated. These observations of the effects of added dietary silicon, made without extraordinary measures to limit silicon in the basal diet or the surrounding environment, suggest that interrelationships of silicon with other elements may warrant consideration in nutritional and metabolic studies, particularly those involving copper or zinc.

Klevay[19] summarizes numerous similarities between animals deficient in copper and people with ischemic heart disease, and has previously offered epidemiologic data to support the hypothesis that a high zinc to copper ratio may be an important contributor to cardiovascular disease.[6] Our data show that silicon is antagonistic toward zinc and ameliorates some of the aortic effects of copper deficiency. These findings, combined with the hypothesis proposed by Schwarz[20] that silicon associated with dietary fiber sources may be active in preventing atherosclerosis, provide a basis for including dietary silicon in future studies of the epidemiology of cardiovascular disease.

Acknowledgments

The authors gratefully acknowledge the technical assistance of Renata Wnuk.

References

1 Carlisle, E. M. (1986). Silicon as an essential trace element in animal nutrition. *Ciba Found. Symp.* **121,** 123–139

2 Carlisle, E. M. (1988). Silicon as a trace nutrient. *Sci. Total Environ.* **73,** 95–106

3 Hill, C. H., Starcher, B., Kim, C. (1967). Role of copper in the formation of elastin. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **26,** 129–133

4 Shields, G. S., Coulson, W. F., Kimball, D. A., Carnes, W. H., Cartwright, G. E., Wintrobe, M. M. (1962). Studies on copper metabolism. XXXII. Cardiovascular lesions in copper-deficient swine. *Am. J. Pathol.* **41,** 603–621

5 Guenthner, E., Carlson, C. W., Emerick, R. J. (1978). Copper salts for growth stimulation and alleviation of aortic rupture losses in turkeys. *Poult. Sci.* **57,** 1313–1324

6 Klevay, L. M. (1980). Interactions of copper and zinc in cardiovascular disease. *Ann. NY Acad. Sci.* **355,** 140–151

7 Carlisle, E. M., Curran, M. J. (1988). A silicon and aluminum interaction in the rat. In *Trace Elements in Man and Animals 6,* Proceedings of the Sixth International Symposium on Trace Elements in Man and Animals (L. S. Hurley, C. L. Keen, B. Lonnerdal, R. B. Rucker, eds), p. 279, Plenum Press, New York

8 Emerick, R. J., Lu, D. (1987). A possible synergism of dietary phosphate and urine acidifying salts in preventing silica urolithiasis in a rat model. *J. Nutr.* **117,** 1603–1608

9 National Research Council (1978). *Nutrient Requirements of Laboratory Animals,* 3rd Ed., National Academy of Science, Washington, DC

10 Emerick, R. J. (1984). Chloride and phosphate as impediments to silica urinary calculi in rats fed tetraethylorthosilicate. *J. Nutr.* **114,** 733–738

11 Schreier, C. J., Emerick, R. J. (1986). Diet calcium, phosphorus and acidifying and alkalizing salts as factors influencing silica urolithiasis in rats fed tetraethylorthosilicate. *J. Nutr.* **116,** 823–830

12 Emerick, R. J. (1986). Animal age and duration of exposure to a urolithic diet as factors influencing silica urolithiasis incidence in an animal model. *Nutr. Rep. Int.* **34,** 907–913

13 Emerick, R. J. (1987). Phosphate inhibition of protein-polysilicic acid complex formation as a factor in preventing silica urolithiasis. *J. Nutr.* **117,** 1924–1928

14 Oser, B. L. (1965). *Hawk's Physiological Chemistry,* 14th Ed., McGraw-Hill, New York

15 Lehmann, H. P., Schosinsky, K. H., Beeler, M. F. (1974). Standardization of serum ceruloplasmin concentrations in international enzyme units with o-dianisidine dihydrochloride as substrate. *Clin. Chem.* **20,** 1564–1567

16 Starcher, B., Hill, C. H., Matrone, G. (1964). Importance of dietary copper in the formation of aortic elastin. *J. Nutr.* **82,** 318–322

17 SAS. (1985). *SAS/STAT Guide for Personal Computers, Version 6 Ed.* SAS Institute Inc., Gary, IN

18 Schwarz, K., Milne, D. B. (1972). Growth-promoting effects of silicon in rats. *Nature* **239,** 333–334

19 Klevay, L. M. (1987). Ischemic heart disease, a major factor to becoming old. *Clin. Geriatr. Med.* **3,** 361–372

20 Schwarz, K. (1977). Silicon, fibre and atherosclerosis. *Lancet* **1,** 454–457