Blood Selenium in Thai Children with Protein-Calorie Malnutrition[1] (34937)

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Selenium is essential for normal growth and development in a variety of animals and plants (1). Whether it is essential for man is not known. If it is essential for man, evidence for its deficiency might best be sought in disorders which imitate selenium deficiency disease in animals by involvement of systems vulnerable to selenium lack, *i.e.*, liver, muscle, and capillary vessels. Because of the involvement of these systems in protein-calorie malnutrition (PCM) in children and because protein is a well-established vehicle for selenium in various chemical forms, evidence for selenium deficiency was sought in children in Northern Thailand with various forms of protein-calorie malnutrition including marasmus and kwashiorkor. Indeed, Schwarz (2) and Majaj (3) have reported an effect of selenium in stimulating recovery of malnourished children from kwashiorkor. Burk *et al.* (4) found a significant reduction in the selenium content of the blood in Guatemalan children with PCM. This report deals with a similar study in both children and adults living in the vicinity of Chiang Mai, Thailand.

Materials and Methods. Clinical aspects. These studies were carried out at the Anemia and Malnutrition Research Center of Chiang Mai Medical College and St. Louis University School of Medicine located in Chiang Mai, Thailand. Patients reported in this study were admitted either to the malnutrition ward of the general pediatric service of the Chiang Mai General Hospital or the research ward of the Anemia and Malnutrition Research Center. The latter is a sealed unit equipped for metabolic studies and operating under a research protocol devoted to the study of hematopoietic responses of malnourished children to repletion with protein and other nutrients. All children were males except one control child. Post-kwashiorkor children reported in this study were those that made a good recovery over a period of 6–8 weeks. Treatment of these children consisted of correction of fluid and electrolyte imbalance by parenteral and oral fluids, administration of antibiotics known to be effective against organisms cultured from the individual child, parenteral and oral multivitamin and mineral therapy with high doses of vitamins A and D and an aggressive feeding program with milk and supplementary homogenized soft foods increasing from initial intakes of 2 g protein, 30 cal/kg, and reaching finally an intake (after about 10 days) of 6 g protein and 200 cal/kg. In 6 weeks, the average gain in weight was 2 kg with notable improvement in plasma proteins, hematocrit, activity, appearance, and sense of well being.

All of the malnourished children were studied within 14 days of admission. Treated children were studied after 42 days of admission and the other well-nourished children, admitted for indifferent complaints, such as congenital heart disease, pneumonia, and diptheria, were studied after recovery from the primary illness. One case of thalassemia major was studied. Blood samples were obtained from adults as outpatients. These included

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three healthy adult Thais and one adult American subject. All blood and plasma samples were frozen and brought to St. Louis University for selenium analysis.

Determination of selenium in blood (4, 5). Blood and plasma selenium was determined by the method of Watkinson (5) as modified by Burk *et al.* (4). Blood or plasma anticoagulated with sodium citrate was pipetted in amounts of 0.25 ml into a 100-ml Kjeldahl flask containing 1.75 ml deionized distilled water. After adding 5 ml digestion mixture (10 g sodium molybdate, 50 ml water, 150 ml conc sulfuric acid, 200 ml 70–72% perchloric acid), and two glass beads, the solution was boiled for about 5 min on an electric micro-Kjeldahl digestion rack until it became clear. Two milliliters conc hydrochloric acid were delivered to the flask and digestion continued 5 min more. The solution was then poured into a 125-ml glass beaker along with two rinses of 5 ml of water. One milliliter of 0.2 *M* EDTA ammonium salt was added, and the pH was brought to 1.0 with ammonium hydroxide using a pH meter. After standing overnight, a white mineral-EDTA precipitate was filtered off. After warming to 50°, the pH of the filtrate was meticulously readjusted to 1.0 with hydrochloric acid. The volume was then adjusted to 50 ml with 0.1 *N* hydrochloric acid and the temperature reestablished at 50°. A solution of 0.22%, 2,3 diaminonaphthalene (Aldrich Chemical Co. Inc.) which had been recrystallized from boiling water and extracted twice with 0.5 ml of cyclohexane/ml of solution was prepared and 1.0 ml added to the solution at 50°. The mixture was then incubated in a waterbath for 20 min at 50°. After cooling in tapwater, the incubation mixture was emptied into a 125-ml separatory funnel containing 10 ml cyclohexane. The funnel was shaken for 2 min to extract diaminonaphthalene-bound selenium into the organic phase. Three milliliters of the cyclohexane containing piazselenol was added to a cuvette in an Aminco-Bowman spectrophotofluorometer. The sample was activated at 376 mμ and the fluorescence read at 520 mμ.

Each blood or plasma sample was analyzed in duplicate. Selenium standards containing 1 ml of 0.1 μg Se/ml solution as sodium selenite (dried overnight at 102°) were run in triplicate against a water blank. Mean variance of replicate samples of blood or plasma was 2.5% with a standard deviation (SD) of 2.2%. The variance of duplicate blood analyses run at different times (five sets) was 7.5% with a standard deviation of 2.3%. Percentage recovery of selenium added to blood as compared with an aqueous selenium standard was 100 ± 5% (SD). Percentage recovery of selenium standard from the digestion was 70–90%, calculated by comparison with nondigested selenium standard. Using radioactive $^{75}SeO_3^=$ it has been demonstrated that of the 10–30% selenium lost during the digestion process, 5% is retained in the EDTA precipitate. The remainder cannot be detected by fluorometric assay, apparently due to oxidation of Se ^{IV} during digestion to Se ^{VI}, which does not react with diaminonaphthalene. Selenium content of plasma or blood was corrected for recoveries of selenium standards used in each individual assay. No correction was made for differences in recovery of selenium from blood, plasma, or water. Cell selenium content was computed from the difference between whole blood and blood plasma selenium content.

Hemoglobin was assayed by the cyanmethemoglobin method (6). Hematocrit was obtained from each blood sample by capillary tube centrifugation. Total plasma protein was determined by the biuret method (7) adapted for Beckman-Spinco microequipment.

Results and Discussion. The results are presented in Tables I and II. As shown in Table I, the plasma of malnourished children contains significantly less selenium than that of well-nourished children including those recovered from kwashiorkor. Subjects with protein-calorie malnutrition had been treated with the standard regimen which included high protein diet, vitamins, and minerals, but no added selenium.

Red blood cells appear to concentrate selenium as indicated by the increased concentration in red blood cells compared with plasma. The idea that the majority of red cell

TABLE I. Blood Selenium in Thai Infants.

Subject clinical diagnosis	Age (years)	Hemato-crit	Plasma protein (g/100 ml blood)	Hemoglobin (g/100 ml blood)	Selenium content: Blood (ng/ml blood)	Plasma (ng/ml plasma)	Cells (ng/ml cells)	Plasma Se / Plasma prot (μg/g)	Cell Se / Hemoglobin (μg/g)
Malnourished									
1 Kwashiorkor	4	16	4.0	4.8	92	16	493	0.40	1.65
2 Marasmus	3	27	4.3	10.1	116	43	317	1.00	0.84
3 Kwashiorkor	4	24	4.3	7.2	75	38	192	0.88	0.64
4 Marasmus	3	28	4.9	8.3	84	39	200	0.80	0.76
5 Marasmus	2	16	4.7	4.9	61	47	131	1.00	0.43
6 Marasmus	3	24	4.3	7.9	67	47	129	1.09	0.39
7 Marasmus	1	24	6.1	7.8	41	32	71	0.82	0.22
8 Kwashiorkor	2	32	4.2	9.4	168	52	116	1.24	1.41
Mean	2.7	24	4.6	7.6	88	39	206	0.87	0.79
SD	1.0	6	0.7	1.9	38	10	138	0.28	0.50
Well-nourished									
1 Congen. heart dis.	1	33	5.6	9.2	163	74	342	1.32	1.23
2 Pneumonia	1	28	7.1	8.3	84	54	161	0.76	0.54
3 Pneumonia	1	32	6.8	8.5	160	132	219	1.94	0.82
4 Diphtheria	3	42	6.5	12.2	133	75	212	1.15	0.73
5 Post-kwashiorkor	2	39	7.0	12.9	—	91	—	1.42	—
6 Post-kwashiorkor	1	33	5.5	11.2	74	63	97	1.15	0.29
7 Post-kwashiorkor	3	36	5.7	11.4	—	89	—	1.56	—
8 Post-marasmus	2	32	5.7	11.2	95	84	119	1.47	0.34
9 Diphtheria	4	37	5.1	11.2	130	82	214	1.61	0.70
Mean	2	35	6.1	10.7	120	83	195	1.38	0.66
SD	1.1	4	0.7	1.6	36	22	82	0.34	0.32

TABLE II. Blood Selenium in Normal Thai Youth and Adults and a Child with Thalassemia Major.

	Subject clinical diagnosis	Age (years)	Hematocrit	Plasma protein (g/100 ml blood)	Hemoglobin (g/100 ml blood)	Selenium content				
						Blood (ng/ml blood)	Plasma (ng/ml plasma)	Cells (ng/ml cells)	Plasma Se	Cell Se
									Plasma prot (μg/g)	Hemoglobin (μg/g)
1	Thai	39	41	6.1	13.7	147	125	178	2.05	0.53
2	Thai	12	38	7.4	11.3	202	106	358	1.43	1.20
3	Thai	22	48	6.8	16.0	144	81	213	1.19	0.64
4	American (R.J.L.)	29	46	7.7	16.4	250	131	390	1.70	0.85
	Mean		43	7.0	14.4	186	111	285	1.59	0.80
5	Thalassemia major	2	5	5.5	1.5	91	68	520	1.24	1.73

selenium is associtaed with hemoglobin gains support from the findings (a) that there is an increase in selenium concentration of red cells from the child with thalassemia major (Table II), whose hemoglobin is an abnormal mixture and (b) that the mean red blood cell selenium per gram hemoglobin in both malnourished and well-nourished groups (Table I) is similar. Unlike red cell selenium, plasma selenium per gram of plasma protein, was less in the malnourished group indicating the level of plasma selenium, if anything, is not a simple function of total plasma protein. These data suggest that when selenium is limiting, the red blood cell and perhaps other tissues may compete successfully with plasma for the limited supply.

The data obtained from Thai children corroborate, in large measure, the previous work of Burk *et al.* (4) at the Institute of Nutrition of Central America and Panama (INCAP) in Guatemala. The mean plasma selenium in malnourished children with PCM at INCAP observed by Burk was 70 ± 31 (SD) ng/ml. In our studies, the mean plasma selenium for malnourished children was significantly lower 39 ± 10 (SD) ng/ml. The probability of this difference arising by chance is less than 0.05. Also the response to therapy in the children in our study was somewhat more rapid than that observed in the INCAP study. The values for selenium in blood and plasma of control children were lower than those in adults as shown in Table II suggesting that selenium stores increase with maturity possibly in proportion to plasma proteins and hemoglobin. Whether or not the findings relate in any way to the pathogenesis of protein-calorie malnutrition cannot be determined at this time.

Summary. Protein-calorie malnutrition in Thai infants and young children is associated with significant reduction in plasma selenium without change in red cell selenium. Plasma selenium was restored to normal levels by treating these children with therapeutic diets containing protein, vitamins, and minerals but no added selenium.

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