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Effect of Boron on Vitamin D Deficient Rats

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ABSTRACT

The effects of different levels of dietary boron were determined in vitamin D deficient rats. Vitamin D deficient diets containing either 0.158 ppm or 2.72 ppm of boron were fed to rats for 11 w, and calcium, magnesium, and phosphorus apparent absorption and balance were measured in the twelfth week. Higher apparent absorption and balance values for calcium and phosphorus were observed in the rats with higher dietary boron, but very few differences were seen in body wt, organ wt, and bone parameters. Balance measurements represented the present status of the rats after 12 w on the diets, but other measurements represented an accumulation over the lifetime of the rat, including a suckling period with ample vitamin D and boron. The data demonstrated that when rats are vitamin D deficient, as indicated by hypocalcemia, the level of boron in the diet affects mineral balance.

Index Entries: Boron; vitamin D; calcium; magnesium; phosphorus; apparent absorption and balance; hypocalcemia.

INTRODUCTION

Boron is known to be an essential element in plant nutrition, but at this time, it is not recognized as an essential nutrient for animals. Improved growth and reduced plasma alkaline phosphatase activity after

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supplemental boron was added to a vitamin D deficient diet for day-old chicks renewed speculation, not active since the 1940s, that boron may be a potential ultratrace element for animals (*1,2*). These studies by Nielson and Hunt on chicks have also shown that diets low in boron but adequate in all other nutrients did not result in any deficiency signs. However, if the diet is also low in other minerals, such as, magnesium or potassium, or low in vitamin D, deficiency signs occur, indicating an interaction between boron and other nutrients. Initially, it was suspected that boron's interaction with vitamin D deficiency was caused by a role of boron in the metabolism of vitamin D. Later studies have found evidence for other modes of interaction (*3*). Since the knowledge of possible roles of vitamin D has increased in the last few years, boron and vitamin D may interact in one or more of several biological functions.

Although studies in rats have demonstrated effects of boron when rats are deficient in both calcium and magnesium (*4*), effects of boron in rats deficient only in vitamin D are not known. As a result, the present study focuses on the use of vitamin D deficient, hypocalcemic rats fed lower and higher dietary boron levels.

MATERIALS AND METHODS

Weanling Harlan Sprague-Dawley rats were obtained at 21 d of age. The rats were fed a corn meal based diet supplemented with an AIN 76A-vitamin mix without vitamin D, and a mineral mix with or without supplemental boron. The formulation of the mineral mix essentially followed that of Nielson and Hunt (*5*), except for the addition of a higher level of potassium that may have been low in their previous formulation. Composition of the diets is shown in Table 1. Corn meal was stripped of minerals by washing three times with 2*N* hydrochloric acid and rinsing with ultrapure water (*6*). Diets were analyzed for boron content by Curtis Hunt of the USDA, ARS Human Nutrition Research Center, Grand Forks, ND. The rats were fed the diets *ad libitum* for 12 w, and had free access to ultrapure water. A total of 19 rats were used in the study, 10 consuming the diet deficient in both boron and vitamin D, and nine consuming the diet deficient in vitamin D with supplemental boron. Plasma levels of calcium and magnesium were monitored in three different rats/treatment groups at 3-wk intervals (*3, 6, and 9*) during the study. Plasma phosphorus, as well as calcium and magnesium levels, were measured in seven rats on lower dietary boron and six rats on higher boron at the end of the study (12th week). All rats had blood samples taken twice, with blood taken three times from a few rats. Apparent balance of these same minerals was assessed in 13 rats for a 4-d period prior to sacrifice. Blood was obtained by cardiac puncture of rats under ether anesthesia, and rats were sacrificed by exsanguination.

In order to separately collect the feces and urine, rats were housed in stainless steel metabolic cages. Dry fecal samples were charred on a

Table 1
Composition of Rat Diet for Boron Studies

Ingredient	Amount
	g/kg dry diet
casein, high protein[a]	160.00
ground corn, acid washed[a]	708.00
corn oil[b]	75.00
methionine, L.[c]	3.00
mineral mix[d]	42.00
choline bitartrate[c]	2.00
AIN-76A vitamin mix[a]	10.00

[a]United States Biochemical Corporation, Cleveland, OH.
[b]Mazola corn oil
[c]Sigma Chemical, St. Louis, MO.
[d]Mineral mix contained (in g/kg of diet): sodium chloride, 2.0; magnesium acetate, 3.5; manganese acetate, 0.1125; copper sulfate, 0.03; potassium iodide, 0.0004; zinc acetate, 0.05; sodium selenite, 0.0003; ammonium molybdate, 0.004; chromic chloride, 0.002; ammonium vanadate, 0.0003; nickel chloride, 0.002; sodium arsenate, 0.005; potassium chloride, 3.5; potassium acetate, 4.53; sodium metasilicate, 0.05; potassium fluoride, 0.0025; ferrous sulfate, 0.2; calcium phosphate (dibasic), 17; acid washed ground corn, 11.011. This mineral mix yielded a dietary Ca, Mg, and P content of 0.5, 0.04 and 0.39%, respectively. The boron supplemented diet contained 0.017 g boric acid/kg diet, to provide 3 mg B/g diet.

hotplate after addition of 1 mL concentrated nitric acid and then ashed at 500°C overnight. The samples were then redissolved in a hydrochloric acid solution (1 part concentrated HCl:9 parts ultra pure water), and diluted in 0.5% lanthanum oxide for calcium and magnesium analyses. Diet and tissue samples were treated in a similar manner. Aliquots of urine were boiled down to dryness, charred and ashed, and then redissolved in the abovementioned hydrochloric acid solution to original vol. Plasma samples were directly diluted in 0.1% lanthanum oxide. A Perkin-Elmer 3030B flame atomic absorption spectrometer was used to analyze samples for calcium and magnesium content, whereas phosphorus content was estimated, using a spectophotometric method developed by Chen et al. (7). Apparent balance for calcium, magnesium, or phosphorus was estimated by subtracting the fecal and urinary mineral content from the level of intake [I-(F + U)]. Apparent absorption (%) was estimated by subtracting fecal content from intake and dividing by the intake [(I-F/I) × 100].

At the time of sacrifice, the liver, both kidneys, spleen, brain cortex, cerebellum, and brain stem were removed. These tissues were analyzed for calcium, magnesium, and phosphorus content, as described above.

The right anatomical femur was taken for bone analyses. After

placing the bones in a pressure cooker for 15 min at 10 psi, the bones were cleaned of tissue. The femurs were placed in ultrapure water and hydrated under vacuum for 1 h and the density was calculated after obtaining the wt of the hydrated bone out of water and the buoyancy (wt of bone immersed in water) using a density measurement apparatus (Mettler, Hightstown, NJ). Dry fat-free wt was obtained after extraction of the femurs in a Soxhlet extraction device for 48 h with ethanol, followed by 48 h with diethyl ether. The ash and the calcium, magnesium, and phosphorus content of the ash were obtained as described previously. The data were statistically analyzed using the General Linear Models and Univariate procedures of SAS®.

RESULTS

Body Weight Gain, Tissue Weights, Calcium, Magnesium and Phosphorus Content, and Bone Parameters

Weanling rats 21 d old, were fed vitamin D-deficient diets containing either 0.158 ppm of boron or 2.72 ppm of boron (unsupplemented, supplemented, respectively) for 12 w. No significant differences in body wt gain or in food intake occurred over the time span of the study, and the final wts (mean ±SEM) were 364.9 ± 24.8 g and 356.5 ±42.2 g for the lower boron and the higher boron diets, respectively. Weights of organs/body wt also were not different. Rats on the unsupplemented diet had higher total calcium ($p < 0.007$) and higher mg calcium/g ($p < 0.012$) in the brain cortex and higher total phosphorus ($p < 0.028$) and higher mg phosphorus/g ($p < 0.044$) in the cerebellum than the boron supplemented group (Table 2). This same group also had higher total phosphorus ($p < 0.046$) and higher mg phosphorus/g in liver ($p < 0.035$). No significant differences in magnesium content were found for any of the tissues measured. In addition, all bone parameters measured, except one, that included length, density, dry fat—free bone wt, ash wt, % ash, total mg calcium, magnesium, and phosphorus, and mg/g bone calcium, magnesium, and phosphorus, showed no differences (Table 3). The only exception was a significantly higher mg/g bone magnesium in rats on the boron-supplemented diet.

Plasma Calcium, Magnesium, and Phosphorus Values

Plasma calcium levels were measured periodically during the study, not only to assess a possible boron and vitamin D deficiency interaction, but also to assess the state of vitamin D deficiency. Average plasma calcium levels decreased over time for rats in both treatment groups, demonstrating the development of vitamin D deficiency as body stores

Table 2
Tissue Levels of Calcium, Magnesium, and Phosphorus ± SEM

Mineral	Treatment Group	Brain Cortex total mg	Brain Cortex mg/g	Brain Stem total mg	Brain Stem mg/g	Brain Cerebellum total mg	Brain Cerebellum mg/g	Kidney total mg	Kidney mg/g	Liver total mg	Liver mg/g	Spleen total mg	Spleen mg/g
Ca	+B	0.053[a]±	0.043[a]±	0.038±	0.157±	0.033±	0.145±	0.132±	0.056±	0.280±	0.031±	0.023±	0.033±
		0.002	0.007	0.026	0.097	0.007	0.009	0.009	0.003	0.025	0.002	0.001	0.0008
	-B	0.082±	0.065±	0.011±	0.056±	0.130±	0.406±	0.143±	0.057±	0.310±	0.033±	0.027±	0.037±
		0.008	0.0004	0.0008	0.004	0.062	0.245	0.008	0.003	0.017	0.002	0.002	0.002
Mg	+B	0.180±	0.144±	0.031±	0.154±	0.037±	0.162±	0.424±	0.179±	1.91±	0.208±	0.137±	0.189±
		0.007	0.002	0.005	0.003	0.003	0.009	0.025	0.003	0.18	0.009	0.007	0.007
	-B	0.189±	0.150±	0.029±	0.147±	0.038±	0.153±	0.438±	0.177±	1.96±	0.213±	0.140±	0.193±
		0.007	0.002	0.003	0.003	0.003	0.007	0.017	0.004	0.034	0.005	0.004	0.003
P	+B	3.30±	2.63±	0.583±	3.08±	0.645[b]±	2.78[b]±	5.81±	2.49±	23.63[b]±	2.64[b]±	2.39±	3.31±
		0.44	0.32	0.038	0.28	0.063	0.25	0.56	0.22	2.16	0.23	0.32	0.12
	-B	3.76±	2.97±	0.649±	3.40±	0.873±	3.48±	6.71±	2.70±	29.81±	3.21±	2.48±	3.25±
		0.19	0.10	0.082	0.12	0.063	0.19	0.36	0.21	1.74	0.10	0.08	0.17

[a]Significant differences with $p < 0.02$.
[b]Significant differences with $p < 0.05$.

Table 3
Bone Parameters ± SEM

Treatment Group	Femur Density g/ml	Femur Length cm	Dry fat-free bone weight g	Ash weight g	% Ash	Total bone Calcium mg	Bone Calcium mg/g	Total Bone Magnesium mg	Bone Magnesium mg/g	Total Bone Phosphorus mg	Bone Phosphorus mg/g
+B	1.4506±	3.37±	0.4010±	0.2633±	65.69±	98.92±	246.33±	2.27±	5.65*±	49.0±	122.24±
	0.0081	0.045	0.0170	0.0109	0.30	5.49	6.31	0.08	0.09	2.20	2.20
-B	1.4629±	3.38±	0.4126±	0.2699±	65.38±	101.57±	246.02±	2.21±	5.35±	50.92±	123.44±
	0.0048	0.019	0.0074	0.0056	0.32	2.51	2.33	0.05	0.06	0.87	0.78

*Bone magnesium (mg/g) was significant between treatment groups at $p < 0.02$.

were depleted (Table 4), but were unaffected by boron level in the diet. Magnesium was unaffected by boron or vitamin D status, remaining unchanged through the course of the study. At the end of the study, plasma phosphorus levels were also unaffected by the level of boron in the diet (Table 4).

Apparent Calcium, Magnesium, and Phosphorus Balance

After 11 w on the diets, the rats were placed in stainless steel metabolic cages for measurement of apparent balances conducted during the twelfth and final wk of the study. The group fed an unsupplemented diet had a significantly lower calcium ($p < 0.025$) and phosphorus ($p < 0.02$) apparent balance than the group fed a boron supplemented diet (Table 5). These differences basically resulted from lower apparent calcium ($p < 0.025$) and phosphorus ($p < 0.011$) absorption, with fecal phosphorus greater ($p < 0.05$) in the group on the unsupplemented diet (Table 5). The rats on a boron supplemented diet showed a relatively larger variation in apparent mineral balance, compared with the animals on the unsupplemented diet (Fig. 1). One of the rats with supplemental boron did not respond, as indicated by lower calcium, magnesium, and phosphorus apparent balances, compared with the rats without supplemental boron. When statistical analyses were run without this rat, apparent calcium ($p < 0.002$) and phosphorus ($p < 0.001$) balance became highly significant. In addition, magnesium apparent balance of the two groups that previously was not significantly different also became highly significant ($p < 0.002$).

DISCUSSION

In the last few years, the knowledge of the possible biological roles of vitamin D has increased as studies have shown that a number of types of tissues have vitamin D receptors (*8*). For example, evidence suggests that vitamin D may play a role in immune function, and it is known to affect insulin secretion. Interactions with other nutrients in these functions is likely. When diets that are low in boron and deficient in vitamin D are fed to chicks, deficiency signs representing a boron and vitamin D interaction occurred (*1*). Hunt (*3*) has reported that vitamin D-deficient chicks have elevated levels of plasma glucose, β-hydroxybutyrate, pyruvate, triglycerides, alkaline phosphatase, triiodothyronine (T_3), and lower levels of plasma ionized calcium. Supplementation with boron reduces these metabolic parameters and elevates the plasma ionized calcium in vitamin D deficient chicks. After the discovery of a boron-vitamin D interaction, initially, boron was thought to possibly affect the metabolism of vitamin D. Boron supplementation in the absence of any other nutrient deficiencies has been shown to have no effect on calcium, magne-

Table 4
Total Plasma Calcium, Magnesium, and Phosphorus (±SEM)
as mg/100mL at 3, 6, 9, and 12 Weeks of The Study*

		Weeks of Study			
Mineral	Treatment Group	3	6	9	12
Ca	+B	11.03±0.39	9.07±0.53	8.26±0.51	6.48±0.58
	-B	10.77±0.18	9.60±0.27	7.03±0.80	7.08±0.69
Mg	+B	1.60±0.03	1.75±0.03	1.60±0.10	1.64±0.05
	-B	1.57±0.04	1.52±0.09	1.53±0.08	1.63±0.06
P	+B	-	-	-	10.90±0.47
	-B	-	-	-	9.4±0.76

*3 rats per treatment/time were used except for 12 w when 6 rats were used from the +B group and 7 rats from the −B group.

Table 5
Apparent Calcium, Magnesium, and Phosphorus Balance ±SEM

Mineral	Treatment Group	Urine mg/day	Feces mg/day	Mineral[a] Intake mg/day	Apparent Absorption %	Apparent Balance mg/day	Total Diet Intake g/day
Ca	+B	1.01±0.41	54.2±3.8	103.8±5.1	47.0±4.7[b]	49.6±7.1[b]	20.8±1.0
	-B	1.42±0.29	62.0±2.8	95.3±3.4	35.0±1.1	35.0±2.2	19.1±0.7
Mg	+B	1.50±0.10	3.41±0.31	8.3±0.4	59.0±4.7	3.40±0.54	-
	-B	1.58±0.30	3.77±0.11	7.6±0.3	50.1±1.9	2.28±0.19	-
P	+B	19.9±1.1	33.9±2.4[b]	83.1±4.0	58.7±3.8[c]	29.3±5.5[c]	-
	-B	22.0±1.2	40.3±1.8	76.2±2.7	47.2±1.2	12.9±2.1	-

[a]The diets contained upon analysis 5.0, 0.4, and 4.0 mg of calcium, magnesium, and phosphorus, respectively per g of diet.
[b]Significant differences with $p < 0.05$.
[c]Significant differences with $p < 0.02$.

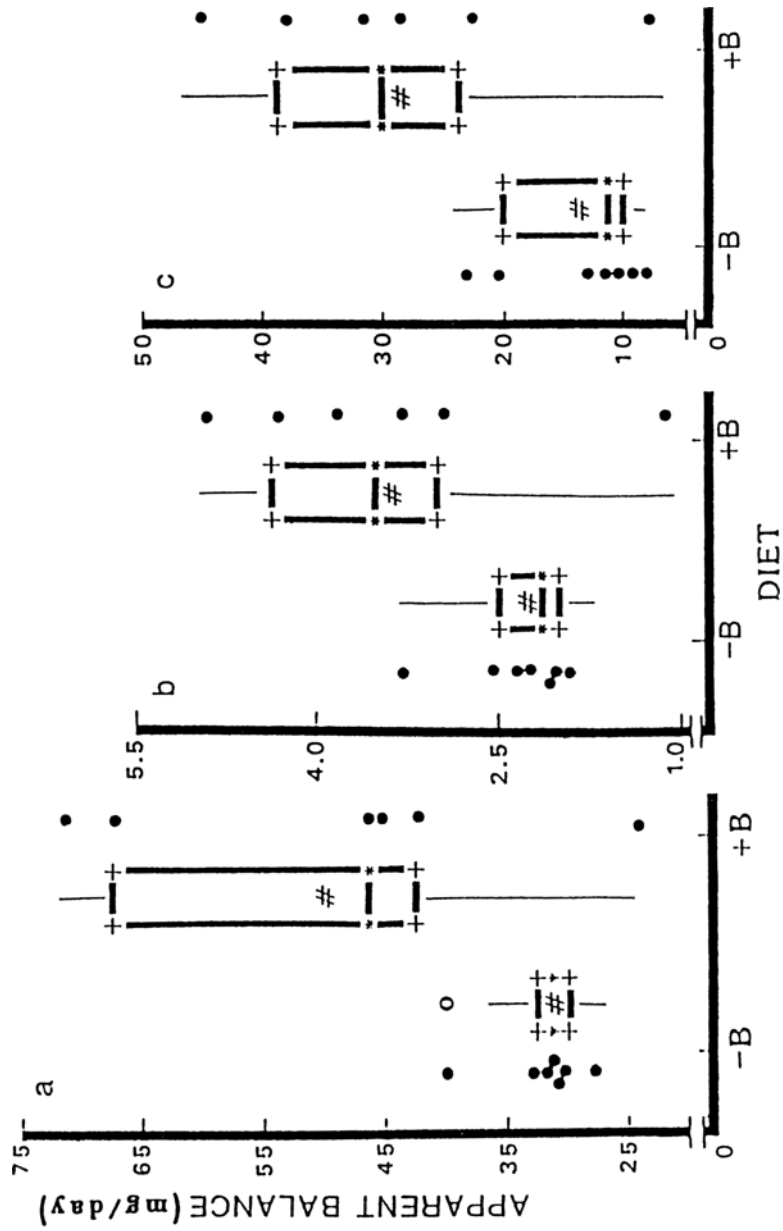

Fig. 1. Box plots and individual data points (●) of apparent calcium (a), magnesium (b), and phosphorus (c) balance in vitamin D deficient rats with (+B) or without (−B) supplemental boron. Univariate procedure of SAS® was used to distribute the data by quartiles and indicate the mean (#), median (central horizontal line), and the 25th and 75th percentiles (designated by the lower and upper horizontal lines, respectively). The zero above the extending "whiskers" of the box plot in (a) indicates that the data point is outside of the 1.5 interquartile range, which is equal to the distance between the 25th and 75th percentiles.

sium, or phosphorus balances in growing male rats (*9*). In addition, no effect on serum levels of vitamin D metabolites was seen.

The results from the present study have demonstrated that a relatively low boron diet (0.158 ppm B), in combination with a vitamin D deficient status in rats, produced a lower calcium and phosphorus apparent balance, as compared with vitamin D deficient rats with supplemental boron (2.72 ppm B). This mainly reflected a lower absorption of these minerals from their diet. The rats had been on vitamin D deficient diets for about 12 w, and all but two were vitamin D deficient as determined by low total plasma calcium levels (*10*).

A higher degree of variability was observed within the boron supplemented group for calcium magnesium, and phosphorus apparent balance, compared with a more narrow range in the group with no supplemental boron. This was possibly on account of variable boron requirements of individual rats in a vitamin D deficient state. Since one rat on the boron supplemented diet had apparent balance values lower than the rats in the unsupplemented group, the data were also statistically analyzed with data from this rat omitted. This resulted in greater significance levels for calcium and phosphorus apparent balance and absorption values. In addition, magnesium apparent balance and absorption for the two groups, that previously was not significantly different, became highly significant.

The increase in brain cortex calcium and the decrease in cerebellum phosphorus observed with a boron deficiency in vitamin D deficient rats provides a direct measure of changes occurring in brain tissue as a consequence of boron deficiency. Penland (*11,12*) has reported changes in electroencephalogram readings in human subjects consuming diets low in boron and magnesium. These effects of low boron intake on brain minerals (in rats) and brain electroencephalograms (in humans) occurred in conjunction with a vitamin D or a magnesium deficiency, respectively. This suggests that brain function could be altered by low dietary boron levels in combination with other nutrient deficiencies.

Since supplemental boron did not result in greater body wt, organ wt, organ mineral content, bone wt, density, and so on, it is possible that other nutrient interactions were occurring. Hunt and Nielson (*13*) have shown that the negative effects of low magnesium dietary levels on bone occurred when low dietary magnesium was combined with low boron and a vitamin D deficient diet. Therefore, bone and other parameters may not show evidence of boron deficiency interactions unless several nutrients are deficient in the diet.

The discrepancy between the bone and tissue mineral data and the balance data may be related in part to the timing of the measurements and the relative degree of vitamin D and/or boron depletion. The mineral content of the bone and tissues examined in this study reflect mineral retention over the lifespan of the rat (114 d), including a suckling period with adequate vitamin D and, presumably, ample boron in the diet. The

apparent balance measurements represented the state of the animal over the last four days prior to sacrifice, following twelve w on a vitamin D deficient diet with or without supplemental boron. Although rats in the present study were hypocalcemic, and most of them exhibited the frank hypocalcemia of 7–8 mg/dL previously reported by Halloran (*14*), even lower values of plasma calcium have been reported, suggesting that a greater depletion of vitamin D stores may be possible (*15*). This suggests that the rats in this study were still in the process of becoming vitamin D depleted. Day-old chicks, in contrast to weanling rats, apparently have relatively low vitamin D stores, since a vitamin D and boron interaction without other nutrient deficiencies has been previously demonstrated (*1*).

Underwood and DeLuca (*16*) have demonstrated that normal bone formation occurred in rats in a vitamin D deficient state as long as calcium and phosphorus were continuously infused into the rats. This indicated that vitamin D was needed for absorption of these minerals, but was not directly involved in bone formation. In addition, Nielsen (*17*) has shown in a study involving men and postmenopausal women that serum calcitonin was higher during an initial boron depletion period than during the subsequent boron repletion. It seems likely that until calcium and phosphorus absorption have been appreciably decreased for some time, protective hormonal effects may delay any detrimental effects on bone formation.

Supplementation of diets of postmenopausal women on low boron diets (0.25 mg boron/d) with 3.0 mg of boron/d resulted in improvements in calcium and magnesium retention, and elevation of serum concentrations of 17β-estradiol and testosterone (*18*). This effect was greater in the women on low magnesium diets. The diets were adequate in vitamin D, but the postmenopausal women may have somewhat impaired vitamin D metabolism, as a result of lower estrogen levels in serum, that may have had an impact on the study.

The results from the present study demonstrated that supplemental boron improved calcium and phosphorus apparent absorption and balance in rats that were vitamin D deficient. These data verified an interaction between vitamin D deficiency and boron in the absence of other nutrient deficiencies. Furthermore, the data also suggested that a boron and vitamin D interaction in the absence of other nutrient deficiencies could only be seen when rats were vitamin D depleted. Depletion of the vitamin D stores in rats, as indicated by low plasma calcium levels, is a relatively lengthy process that other researchers may not have followed. Overall, the study has implications for a means of improving mineral absorption and balance in people with marginal vitamin D intakes and possibly low boron intakes.

Future studies in rats should involve measurements of body wt, tissue, and bone parameters only on rats that have been hypocalcemic for several weeks. Apparent balance and absorption measurements should

also show less variation and greater significance if performed at this same time. This protocol would elucidate a vitamin D and boron interaction in rats if all other nutrients in the diet are adequate.

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REFERENCES

1. C. D. Hunt and F. H. Nielsen, in *Trace Element Metabolism in Man and Animals*, 4th Intl. J. M. Howell, J. M. Gawthorne, and C. L. White, ed. Austral. Acad. Sci. Canberra, Australia, 597 (1981).
2. F. H. Nielson, *Annual Review of Nutrition*, vol. 4, W. J. Darby, ed. Annual Reviews Inc., Palo Alto,CA, 21 (1984).
3. C. D. Hunt, *FASEB J.* **3,** A760 (1989).
4. B. Brossart and F. H. Nielson, *ND Acad. Sci.* **40,** 128 (1986).
5. F. H. Nielsen, T. R. Shuler, T. J. Zimmerman, and E. O. Uthus, *Biol. Trace Elem. Res.* **17,** 91 (1988).
6. F. H. Nielsen, D. R. Myron, S. H. Givand, and D. A. Ollerich, *J. Nutr.* **105,** 1607 (1975).
7. P. S. Chen, Jr., T. Y. Toribara, and H. Warner, *Anal. Chem.* **28,** 1756 (1956).
8. J. W. Pike, *Nutr. Rev.* **43,** 161 (1985).
9. Personal communication with R. Brommage, Nestle Co., Switzerland.
10. J. L. Underwood, M. E. Phelps, and H. F. DeLuca, *Proc. Natl. Acad. Sci. USA* **81,** 2352 (1984).
11. J. G. Penland, *FASEB J.* **4,** A1045 (1990).
12. J. G. Penland, *FASEB J.* **3,** A1242 (1989).
13. C. D. Hunt and F. H. Nielsen, In: *6th Intl Symposium on Trace Elements in Man and Animals*, L. S. Hurley, C. L. Keen, B. Lonnerdal, and R. B. Rucker, ed. Plenum Publishing, NY, NY, 275 (1988).
14. B. P. Halloran, *Nutr. Rep. Intl.* **31,** 1149 (1985).
15. M. Keenan, M. Hegsted, and R. Mohan, *FASEB J.* **2,** A1098 (1988).
16. J. L. Underwood and H. F. DeLuca, *Am. J. Physiol.* **246,** E493 (1984).
17. F. H. Nielsen, *FASEB J.* **3,** A760 (1989).
18. F. H. Nielsen, C. D. Hunt, L. M. Mullen, and J. R. Hunt, *FASEB J.* **1,** 394 (1987).