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Magnesium and Methionine Deprivation Affect the Response of Rats to Boron Deprivation

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ABSTRACT

A series of nine experiments were done to obtain further evidence that boron might be involved in major mineral metabolism (Ca, P, and Mg), thus indicating that boron is an essential nutrient for animals. Eight factorially arranged experiments of 6–10 wk durations were done with weanling Sprague-Dawley male rats. One factorially arranged experiment was done with weanling spontaneously hypertensive rats. The variables in each experiment were dietary boron supplements of 0 and 3 μg/g, and dietary magnesium supplements of either 200 (Experiments 1–3) or 100 (Experiments 4–9) and 400 μg/g. In Experiments 7 and 9, a third variable was dietary manganese supplements of 25 and 50 μg/g. Methionine status was varied throughout the series of experiments by supplementing the casein-based diet with methionine and arginine. Findings were obtained indicating that the severity of magnesium deprivation and the methionine status of the rat strongly influence the extent and nature of the interaction between magnesium and boron, and the response to boron deprivation.

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When magnesium deprivation was severe enough to cause typical signs of deficiency, a significant interaction between boron and magnesium was found. Generally, the interaction was characterized by the deprivation of one of the elements making the deficiency signs of the other more marked. The interaction was most evident when the diet was not supplemented with methionine and especially when the diet contained luxuriant arginine. Signs of boron deprivation were also more marked and consistent when the diet contained marginal methionine and luxuriant arginine. Among the signs of boron deprivation exhibited by rats fed marginal methionine were depressed growth and bone magnesium concentration, and elevated spleen wt/body wt and kidney wt/ body wt ratios. Because the boron supplement of 3 μg/g did not make the dietary intake of this element unusual, it seems likely that the response of the rats to dietary boron in the present study were manifestations of physiological, not pharmacological, actions, and support the hypothesis that boron is an essential nutrient for the rat.

Index Entries: Boron; magnesium; methionine; arginine; sulfur amino acids; boron-magnesium interaction; trace element; calcium; minerals.

INTRODUCTION

Boron has been known to be essential for higher plants for over half a century, but only recently evidence has been obtained suggesting that boron is required by animals. Between 1939–1944, several attempts to induce a boron deficiency in rats were unsuccessful, although the diets fed apparently contained only 155–163 ng B/g *(1–3)*. In 1945, Skinner and McHargue (*4*) reported that supplemental dietary boron enhanced survival, maintenance of body fat, and elevated liver glycogen in potassium-deficient rats. Those findings were not confirmed by Follis (*5*), who fed a different diet with an unknown boron content and different levels of boron supplementation. After those reports, the study of boron as a possible essential nutrient for animals was neglected until 1981 when Hunt and Nielsen (*6*) reported that boron deprivation depressed growth and elevated plasma alkaline phosphatase activity in chicks fed inadequate cholecalciferol. Some subsequent experiments suggested that cholecalciferol deficiency enhanced the possible need for boron and that boron might interact, in some manner other than through a direct effect on cholecalciferol metabolism, with the metabolism of calcium, phosphorus, or magnesium (*7*). The relationship seemed strongest between boron and magnesium, because boron tended to normalize the abnormalities associated with magnesium deficiency in chicks. Boron did not consistently alleviate signs of calcium and phosphorus deficiency. Nonetheless, because the B:Mg ratio was quite low in both plasma and diet, boron apparently indirectly affected magnesium metabolism (*7*).

The response to magnesium deprivation by rats is quite different than by chicks. For example, in chicks magnesium deprivation induces hypocalcemia which is suggestive of hypoparathyroidism (*8*). In contrast, magnesium-deficient rats often develop hypercalcemia, hypophosphatemia, hypocalcuria, and hyperphosphaturia, which are suggestive of hyperparathyroidism (*8*).

The following experiments were done to ascertain whether boron affects the response of the rat to magnesium deprivation and vice versa. In these experiments, several other nutrients were inadvertently or intentionally varied to ascertain whether diet composition affected the response of rats to boron deprivation and/or the interaction between boron and magnesium. In one experiment, spontaneously hypertensive rats were used to ascertain whether dietary boron would alter the enhanced hypertension response caused by magnesium deprivation in these animals (*9*). The experiments were part of an overall plan to obtain further evidence that boron might be involved in major mineral metabolism (Ca, P, and Mg), thus indicating that boron is an essential nutrient for animals.

MATERIALS AND METHODS

In all experiments, except Experiment 5, male weanling Sprague-Dawley rats (Harlan-Sprague Dawley, Indianapolis, IN) were weighed individually on arrival and housed three/all-plastic cage measuring 50 × 24 × 16 cm (*10*) and located inside a laminar flow rack (Lab Products, Carfield, NJ). In Experiment 5, spontaneously hypertensive male weanling rats (SHR) (Taconic Farms Laboratory Animals, Germantown, NY) were used. In all experiments, the rats were randomly assigned to groups with no significant differences in weight and in a fully crossed, two-factor (Experiments 1–6 and 8) or three-factor (Experiments 7 and 9) arrangement. Tables 1–5 indicate the number of rats/group, which ranged from 5–9, in each experiment. The variables in each experiment were dietary boron supplements of 0 and 3 μg/g, and dietary magnesium supplements of either 200 (Experiments 1–3) or 100 (Experiments 4–9) and 400 μg/g. The 100 μg/g supplements were added as part of the mineral mix. In Experiments 7 and 9 the third variable was dietary manganese supplements of 25 and 50 μg/g.

The basal diet (Tables 6–8) contained between 0.1–0.3 μg boron/g. The basal diet supplemented with 200 μg Mg/g analyzed about 240 μg Mg/g, and supplemented with 100 μg Mg/g analyzed about 120 μg Mg/g. The basal diet supplemented with 25 μg Mn/g analyzed about 25 μg Mn/g, and supplemented with 50 μg Mn/g analyzed about 54 μg Mn/g. Dietary boron, magnesium, and manganese content were determined by using the ashing and inductively coupled argon plasma atomic emission methods described subsequently. The basal diet was calculated to con-

Table 1
Effects of Dietary Boron and Magnesium (Mg Low = 200 μg/g) and Their Interaction[a] on Selected Parameters of Rats Fed Casein-Based Diets With No Supplemental Methionine and 50 μg Manganese/g (Experiments 1 and 2)

Treatment[a]		Final Wt,		KW/BW[b],		SW/BW[c],		Plasma Alkaline Phosphatase,		Bone Ca[d],	Bone Mg[d],
B	Mg	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 2	Exp. 2
μg/g	μg/g	g						units[e]		mg/g	mg/g
0	200	333 (7)[f]	287 (6)[f]	0.31	0.31	0.17	0.22	1.20	0.80	166	2. 99
0	400	369 (7)	281 (9)	0.29	0.30	0.17	0.24	1.36	0.82	166	3.71
3	200	357 (7)	301 (6)	0.30	0.31	0.19	0.23	1.48	0.78	160	2.92
3	400	390 (7)	301 (9)	0.29	0.30	0.18	0.23	1.64	0.80	170	3.88
				Analysis of Variance—*P* Values							
Boron		0.04	0.03	NS	NS	0.02	NS	0.005	NS	NS	NS
Magnesium		0.003	NS	0.01	NS	NS	NS	NS	NS	NS	0.0001
B × Mg		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
EMS[g]		738	369	0.0002	0.0001	0.0003	0.0005	0.05	0.01	358	0.07

[a]Amount of boron (H_3BO_3) and magnesium [$Mg(C_2H_3O_2)_2 \cdot 4H_2O$] supplemented to the diet.
[b]Kidney (one) weight/body wt × 100.
[c]Spleen wt/body weight × 100.
[d]Dry wt basis.
[e]Units are μmol *p*-nitrophenyl phosphate hydrolyzed/min/mL plasma × 10.
[f]Number of rats in each group that gave the means for each of the indices shown for the indicated experiment. Age: 10 wk in Experiment 1, 6 wk in Experiment 2.
[g]Error mean square.

Table 2
Effects of Dietary Boron and Magnesium (Mg Low = 200 μg/g) and Their Interaction on Selected Parameters of Rats Fed Casein-Based Diets Containing Supplemental Methionine (2.5 mg/g) and 50 μg Manganese/g (Experiment 3)

Treatment[a]							
B	Mg	Final Wt	KW/BW[b]	SW/BW[c]	Plasma Alkaline Phosphatase	Bone[d], Calcium	Bone[d], Magnesium
μg/g	μg/g	g			units[e]	mg/g	mg/g
0	200	257 (6)[f]	0.31	0.22	0.86	180	3.29
0	400	283 (9)	0.31	0.23	0.81	147	3.78
3	200	299 (6)	0.33	0.24	0.81	152	2.88
3	400	285 (9)	0.31	0.23	0.75	165	3.91
		Analysis of Variance—*P* Values					
Boron		0.01	0.02	NS	NS	NS	NS
Magnesium		NS	NS	NS	NS	NS	0.0001
B × Mg		0.02	NS	NS	NS	0.007	NS
EMS[g]		433	0.0002	0.0003	0.008	409	0.15

[a]Amount of boron (H_3BO_3) and magnesium [$Mg(C_2H_3O_2)_2 \cdot 4H_2O$] supplemented to the diet.
[b]Kidney (one) wt/body wt × 100.
[c]Spleen wt/body wt × 100.
[d]Dry wt basis.
[e]Units are μmol *p*-nitrophenyl phosphate hydrolyzed/min/mL plasma × 10.
[f]Number of rats in each group, age 6 wk, that gave the means for each of the indices shown for the indicated experiment.
[g]Error mean square.

Table 3
Effects of Dietary Boron and Magnesium (Mg Low = 100 μg/g) and Their Interaction on Selected Parameters of Rats Fed Casein-Based Diets With No Supplemental Methionine and 20 μg Manganese/g (Experiments 4 and 5)

Treatment[a]		Final Wt,		KW/BW[b],		SW/BW[c],		Plasma Alkaline Phosphatase,		Bone Ca[d]	Bone Mg[d]	Blood Pressure,
B	Mg	Exp. 4	Exp. 5	Exp. 4	Exp. 5	Exp. 4	Exp. 5	Exp. 4	Exp. 5	Exp. 4	Exp. 4	Exp. 5
μg/g	μg/g	g						units[e]		mg/g	mg/g	mm Hg
0	100	239 (6)[f]	237 (6)[f]	0.35	0.35	0.29	0.23	0.80	0.62	217	1.25	165
0	400	288 (6)	273 (6)	0.31	0.30	0.21	0.19	0.91	0.87	201	4.34	180
3	100	275 (6)	272 (5)	0.31	0.32	0.31	0.21	0.64	0.73	204	1.14	174
3	400	305 (6)	297 (5)	0.30	0.32	0.22	0.19	0.86	0.85	205	4.04	173
				Analysis of Variance—*P* Values								
Boron		0.02	0.006	0.03	NS	NS	0.06	0.05	NS	NS	0.02	NS
Magnesium		0.001	0.004	0.009	0.002	0.0001	0.0001	0.003	0.0001	NS	0.0001	NS
B × Mg		NS	NS	NS	0.003	NS	NS	NS	0.04	NS	NS	0.09
EMS[g]		680	488	0.0004	0.0004	0.0005	0.0001	0.01	0.005	244	0.04	101

[a]Amount of boron (H_3BO_3) and magnesium [$Mg(C_2H_3O_2)_2 \cdot 4H_2O$] supplemented to the diet.
[b]Kidney (one) wt/body wt × 100.
[c]Spleen wt/body weight × 100.
[d]Dry wt basis.
[e]Units are μmol *p*-nitrophenyl phosphate hydrolyzed/min/mL plasma × 10.
[f]Number of rats in each group that gave the means for each of the indices shown for the indicated experiment. Age: 7 wk in Experiment 4; age 10 wk in Experiment 5.
[g]Error mean square.

Table 4
Effects of Dietary Boron and Magnesium (Mg Low = 100 μg/g) and Their Interaction on Selected Parameters of Rats Fed Casein-Based Diets Containing Supplemental Methionine (2.5 mg/g) and 25 μg or 50 μg Manganese/g (Experiments 6 and 7)

Treatment[a]			Final Wt,		KW/BW[b],		SW/BW[c],		Plasma Alkaline Phosphatase,		BoneCa[d],	Bone Mg[d],
B	Mg	Mn	Exp. 6	Exp. 7	Exp. 6	Exp. 7	Exp. 6	Exp. 7	Exp. 6	Exp. 7	Exp. 6	Exp. 6
μg/g	μg/g	μg/g	g						units[e]		mg/g	mg/g
0	100	25	233 (6)[f]	231 (6)[f]	0.37	0.35	0.33	0.33	0.76	0.69	229	1.12
0	400	25	295 (6)	241 (6)	0.30	0.33	0.22	0.21	0.96	1.12	205	4.28
3	100	25	236 (6)	227 (6)	0.34	0.34	0.28	0.29	0.86	0.67	221	1.29
3	400	25	302 (6)	259 (6)	0.32	0.34	0.20	0.22	1.02	1.09	226	4.51
0	100	50	—	205 (6)	—	0.36	—	0.39	—	0.72	—	—
3	400	50	—	246 (6)	—	0.33	—	0.23	—	0.98	—	—
0	100	50	—	201 (6)	—	0.36	—	0.32	—	0.88	—	—
0	400	50	—	254 (6)	—	0.33	—	0.22	—	0.98	—	—
			Analysis of Variance—*P* Values									
Boron			NS	NS	NS	NS	0.006	0.001	NS	NS	NS	0.0006
Magnesium			0.0001	0.0001	0.0003	0.03	0.0001	0.0001	0.02	0.0001	0.02	0.0001
Manganese			—	0.009	—	NS	—	0.001	—	NS	—	—
B × Mg			NS	NS	0.02	NS	NS	0.003	NS	NS	0.0009	NS
B × Mn			—	NS	—	NS	—	NS	—	NS	—	—
Mg × Mn			—	0.01	—	NS	—	NS	—	0.002	—	—
B × Mg × Mn			—	NS	—	NS	—	NS	—	NS	—	—
EMS[g]			686	261	0.0006	0.0007	0.0005	0.0008	0.03	0.02	61	0.01

[a]Amount of boron (H_3BO_3) and magnesium [$Mg(C_2H_3O_2)_2 \cdot 4H_2O$] supplemented to the diet.
[b]Kidney (one) wt/body wt × 100.
[c]Spleen wt/body weight × 100.
[d]Dry wt basis.
[e]Units are μmol *p*-nitrophenyl phosphate hydrolyzed/min/mL plasma × 10.
[f]Number of rats in each group, age 7 wk, that gave the means for each of the indices shown for the indicated experiment.
[g]Error mean square.

Table 5
Effects of Dietary Boron and Magnesium (Mg Low = 100 μg/g) and Their Interaction on Selected Parameters of Rats Fed Casein-Based Diets With No Supplemental Methionine, Luxuriant Arginine (10 mg/g) and 25 μg or 50 μg Manganese/g (Experiments 8 and 9)

Treatment[a]			Final Wt,		KW/BW[b],		SW/BW[c],		Plasma Alkaline Phosphatase,		BoneCa[d],	Bone Mg[d],
B	Mg	Mn	Exp. 8	Exp. 9	Exp. 8	Exp. 9	Exp. 8	Exp. 9	Exp. 8	Exp. 9	Exp. 9	Exp. 9
μg/g	μg/g	μg/g	g						units[e]		mg/g	mg/g
0	100	25	244 (6)[f]	232 (6)[f]	0.33	0.39	0.36	0.46	0.57	0.55	183	0.72
0	400	25	302 (6)	260 (6)	0.30	0.33	0.20	0.27	0.88	1.22	187	4.06
3	100	25	287 (6)	238 (6)	0.30	0.36	0.28	0.39	0.78	0.65	199	0.86
3	400	25	293 (6)	304 (6)	0.29	0.31	0.21	0.28	0.79	0.89	182	3.93
0	100	50	—	201 (6)	—	0.39	—	0.38	—	0.80	212	0.94
0	400	50	—	230 (6)	—	0.33	—	0.26	—	1.45	168	4.01
3	100	50	—	233 (6)	—	0.35	—	0.30	—	0.90	257	2.11
3	400	50	—	241 (6)	—	0.33	—	0.26	—	1.17	183	4.24
Analysis of Variance—*P* Values												
Boron			0.004	0.0001	0.01	0.001	0.04	0.008	NS	0.03	0.0001	0.0001
Magnesium			0.0001	0.0001	0.005	0.0001	0.0001	0.0001	0.0006	0.0001	0.0001	0.0001
Manganese			—	0.0001	—	NS	—	0.0003	—	0.0001	0.0002	0.0001
B × Mg			0.0001	NS	0.09	0.01	0.008	0.003	0.001	0.0001	0.003	0.0001
B × Mn			—	NS	—	NS	—	NS	—	NS	0.005	0.0001
Mg × Mn			—	0.006	—	NS	—	0.02	—	NS	0.0001	0.0001
B × Mg × Mn			—	0.006	—	NS	—	NS	—	NS	NS	0.005
EMS[g]			175	300	0.0003	0.0005	0.001	0.002	0.009	0.02	182	0.03

[a]Amount of boron (H_3BO_3) and magnesium [$Mg(C_2H_3O_2)_2 \cdot 4H_2O$] supplemented to the diet.
[b]Kidney (one) wt/body wt × 100.
[c]Spleen wt/body wt × 100.
[d]Dry wt basis.
[e]Units are μmol *p*-nitrophenyl phosphate hydrolyzed/min/mL plasma × 10.
[f]Number of rats in each group, age 7 wk, that gave the means for each of the indices shown for the indicated experiment.
[g]Error mean square.

Table 6
Composition of Basal Rat Diet for Boron Studies[a]

Ingredient	Amount, g/kg dry diet
Casein, high protein[b]	160.00
Ground corn, acid washed[c]	712.50
Corn oil[c]	75.00
Arginine, free base[d]	5.00
Mineral mix[e]	24.96
dl-α-tocopherol[c]	0.20
Choline chloride[f]	0.75
Vitamin mix[g]	4.55
$CaHPO_4$[h]	17.00
Iron sponge[i]	0.04
	1000.00

[a]Diet contained between 0.1–0.3 mg of boron/kg on an air-dried basis. The diet was calculated to contain 5.8 mg methionine and 1.7 mg cystine/g.

[b]Teklad Division, Harlan Industries, Madison, WI.

[c]NBCo Biochemicals, Division of ICN Biomedicals, Inc., Cleveland, OH. *See* reference *11* for the procedure for acid washing corn.

[d]Sigma Chemical Co., St. Louis, MO.

[e]See Table 7.

[f]Grand Island Biological Co., Grand Island, NY. The choline chloride was dissolved in 1.5 mL of H_2O.

[g]See Table 8.

[h]"Reagent" grade, J. T. Baker Chemical Co., Phillipsburg, N.J.

[i]"Puratronic" grade, Johnson Matthey Chemicals from Aesar, Seabrook, NH. The iron sponge was dissolved in "Instra-Analyzed" grade HCl (J.T. Baker Chemical Co., Phillipsburg, NJ) before mixing into diet.

tain 5.8 mg methionine and 1.7 mg cystine/g. In Experiments 3, 6, and 7, the basal diet was supplemented with 2.5 mg methionine/g. In Experiments 8 and 9, the basal diet was supplemented with 5 mg arginine/g. Thus, the total amount of arginine added to the diet was 10 mg/g in Experiments 8 and 9. Titles and headings of Tables 1–5 show the diet combination of magnesium, methionine, arginine, and manganese that was fed to the rats in each experiment. Reasons for these different combinations are given in the Results section.

The rats were given de-ionized water (Super Q System, Millipore Corp., Bedford, MA) in plastic cups. Fresh food in plastic cups was provided each day. The diets were mixed three d before the start of each experiment and about once every two wk thereafter. They were stored at −16°C in tightly capped plastic containers. Plastic equipment and cleaning procedures have been described (*10–12*). Absorbent paper under the cages caught droppings and was changed daily. Room temperature was maintained at 23 °C. Room lighting was controlled automatically to pro-

Table 7
Composition of Mineral Mix for Basal Diet Used in Boron Studies

Ingredient	Amount, g/kg diet
NaCl[a]	2.0000
$Mg(C_2H_3O_2) \cdot 4H_2O$[a]	0.8750
$Mn(C_2H_3O_2) \cdot 4H_2O$[b]	0.1125
$CuSO_4 \cdot 5H_2O$[c]	0.0300
KI[d]	0.0004
$Zn(C_2H_3O_2) \cdot 2H_2O$[a]	0.0500
Na_2SeO_3[e]	0.0003
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$[c]	0.0040
$Cr(C_2H_3O_2)_3 \cdot H_2O$[b]	0.0020
NH_4VO_3[c]	0.0003
$NiCl_2$[d]	0.0020
$Na_2HAsO_4 \cdot 7H_2O$[a]	0.0050
KCl[a]	3.5000
$Na_2SiO_3 \cdot 9H_2O$[a]	0.0500
NaF[d]	0.0020
Ground corn, acid washed[f]	18.3265
	24.9600

[a]"Reagent" grade, J. T. Baker Chemical Co., Phillipsburg, NJ.

[b]"Certified" grade, Fisher Scientific, Co., Fair Lawn, NJ.

[c]"Specpure" grade, Johnson Matthey Chemicals from Aesar, Seabrook, NH.

[d]"Ultrapure" grade, Alfa Inorganics/Ventron, Beverly, MA.

[e]Alfa Inorganics/Ventron, Beverly, MA.

[f]NBCo Biochemicals, Division of ICN Biomedicals, Inc., Cleveland, OH. *See* reference *11* for the procedure for acid washing corn.

vide 12 h each of light and darkness. Animals were weighed and provided clean cages weekly.

The rats were fed their respective diets for ten wk in Experiments 1 and 5, six wk in Experiments 2 and 3, and seven wk in Experiments 4 and 6–9. Then they were fasted overnight, weighed, and decapitated subsequent to ether anesthesia and cardiac exsanguination with a heparin-coated syringe and needle. Over 60 different physiological and biochemical parameters were examined using various organs and blood of the rats. Parameters selected for presentation were those representative of the changes caused by boron, magnesium, and their interaction. The kidney wt/body wt (KW/BW) and spleen wt/body wt (SW/BW) ratios were determined by removing one kidney and the spleen, blotting with absorbent tissue, and weighing immediately. Plasma alkaline phosphatase was determined by using *p*-nitrophenyl phosphate as substrate (Phosphatase Test Kit, Sigma Chemical Co., St. Louis, MO). Bone calcium and magnesium were determined as follows. Immediately after decapitation,

Table 8
Composition of Vitamin Mix for Basal Diet Used in Boron Studies

Ingredient	Amount, g/kg diet
Retinyl palmitate (250,000 IU/g)[a]	0.0320
Menaquinone[b]	0.0010
Thiamine · HCl[b]	0.0100
Pyridoxine · HCl[b]	0.0150
Niacin[b]	0.0300
D-pantothenic acid, calcium salt[a]	0.0480
Vitamin B_{12} (0.1% triturate)[a]	0.0200
Folic acid[b]	0.0020
Biotin[b]	0.0010
Riboflavin[b]	0.0270
Inositol[b]	0.0500
Para-aminobenzoic acid[b]	0.0050
Cholecalciferol (400,000 IU/g)[a]	0.0038
Dextrose[a]	4.3052
	4.5500

[a]NBCo Biochemicals, Division of ICN Biomedicals, Inc., Cleveland, OH.
[b]Grand Island Biological Co., Grand Island, NY.

the right thigh was removed and frozen until the femur could be excised from the flesh and cleaned with cheesecloth. After cleaning, the bones were dried at 85°C for four h in a vacuum oven. The dried bones were weighed in 30 mL teflon vials. After 5 mL of concentrated ultrapure nitric acid (G. Frederick Smith Chemical Co., Columbus, OH) were added, the vials were tightly capped and allowed to sit at room temperature for 24 h. Then the vials were placed uncapped on hot plates at low heat and allowed to evaporate to dryness. An additional 3 mL of HNO_3 were added to the vial and the above procedure repeated. Finally, 1 mL of concn HNO_3 and 3 mL of 30% H_2O_2 (J.T. Baker Chemical Co., Phillipsburg, NJ) were added before the vial was heated to dryness. Then 2 mL of 8*N* HNO_3 were added to the vial which was heated to redissolve the sample. All samples were diluted to 10 mL with distilled, de-ionized water. The samples were analyzed using an inductively coupled argon plasma atomic emission spectrometer (ICP 6500, Perkin-Elmer Corp., Norwalk, CT). The spectrometer was operated under the following conditions: Perkin-Elmer RF generator, 27.12 MHz; incident power, 1250 W; reflected power, <5W; plasma argon flow, 12 L/min; nebulizer argon flow, 1 L/min; auxiliary argon flow, 0.7 L/min; peristaltic pump supplied cross flow nebulizer, flow rate 2 mL/min; and vertical observation zone, 15 mm above load coil.

To ascertain the adequacy of the analytical methods used, citrus leaves (SRM #1572, NBS, Dept. of Commerce, Gaithersburg, MD) were subjected to the same procedures as those used for bones; the methods

produced values within 98% of the certified values for calcium and magnesium.

In Experiment 5, the systolic blood pressure of the SHR rats was measured with a tail cuff and a pressure transducer linked via an amplifier to a physiograph (Narco Bio-Systems, Inc., Houston, TX). This method was described by Wu et al. (*13*). Data were statistically analyzed using analysis of variance methodology (*14*). Differences between values were considered significant when $P < 0.05$.

RESULTS

In the first three experiments (Tables 1 and 2) the low magnesium treatment of 200 μg/g of diet did not have a marked effect. Rats fed the low-magnesium diet in Experiment 1 exhibited a slightly depressed growth; in Experiments 2 and 3, they exhibited a slightly depressed concentration of magnesium in the bone. In Experiments 1–3, boron deprivation depressed growth. No other consistent effect of boron was found with the other indices shown. Experiments 1 and 2 did not produce any marked significant findings indicating an interaction between boron and magnesium. However in Experiment 3, where methionine was added to the diet because we became cognizant of the possibility that the basal diet might be low in methionine, evidence for an interaction between magnesium and boron was obtained. Boron-deprived rats fed the low magnesium diet exhibited depressed growth and an elevated bone calcium concentration; these changes did not occur in boron-supplemented rats fed the low magnesium diet.

In Experiment 4 (Table 3), the low magnesium treatment was reduced from 200 μg/g diet to 100 μg/g diet in an attempt to create a marked magnesium deficiency that, we hoped, would enhance changes caused by boron deprivation and an interaction between boron and magnesium. The success of using 100 μg Mg/g of diet resulted in the use of that level of supplementation for the low-magnesium treatment in all subsequent experiments. In both Experiments 4 and 5 (Table 3), the manganese content of the diet was inadvertently reduced to 25 μg/g. It will be shown subsequently that this dietary change probably was of little consequence to the major objectives of this study. Methionine was not supplemented to the diet because we hoped to get growth differences, similar to those in Experiments 1 and 2, in the animals fed adequate magnesium. In both Experiments 4 and 5, magnesium deprivation significantly affected all parameters except bone calcium in Experiment 4 and blood pressure of the SHR rats in Experiment 5. The effects of magnesium deprivation were modified in several instances by dietary boron. For example, a marked elevation of KW/BW was seen in boron-deprived rats fed the magnesium-deficient diet; the elevation was slight to nonexistent in boron-supplemented rats. The growth depression caused by magnesium

deprivation tended to be more marked in the boron-deprived than boron-supplemented rats. Magnesium deprivation also tended to increase the concentration of calcium in the bone (Experiment 4) and decrease blood pressure (Experiment 5) in boron-deprived, but not in boron-supplemented, rats.

In Experiments 6 and 7 (Table 4) methionine was added to the diet, and in Experiment 7, manganese was fed either at the 25 or 50 μg/g diet level. These changes were made to ascertain whether the low dietary manganese or methionine in Experiments 4 and 5 were affecting the boron and magnesium findings.

The addition of methionine to the diet eliminated the effect of boron on growth. Moreover, magnesium-deprivation depressed growth to the same extent in both the boron-deprived and supplemented rats.

In Experiment 7, the growth depression caused by magnesium deprivation was more marked in rats fed adequate-manganese than low-manganese diets. In contrast with the growth findings, the depression in plasma alkaline phosphatase by magnesium deprivation was more marked in the rats fed low-manganese than adequate-manganese diets. Although manganese did interact with magnesium to affect growth and plasma alkaline phosphatase, manganese did not affect the response to dietary boron or to an interaction between boron and magnesium which were our primary concerns.

In contrast with growth, methionine supplementation apparently enhanced the changes in SW/BW and bone magnesium in response to dietary boron. The SW/BW was higher in boron-deprived than boron-supplemented rats; the effect was more marked in magnesium-deprived than magnesium-supplemented rats. In Experiment 6, bone magnesium concentrations were higher in boron-supplemented than boron-deprived rats.

Nonetheless, when we compared the methionine-supplemented to the nonsupplemented findings, our overall impression was that the interaction between magnesium and boron was more evident when dietary methionine was marginal. Thus, in Experiments 8 and 9, arginine was supplemented to the diet with the idea that it would enhance the need for methionine and create a relative methionine deficiency (*15*), and in this state, rats would show marked changes caused by an interaction between boron and magnesium. This manipulation was successful because, as seen in Table 5, all indices shown were affected by an interaction between boron and magnesium. In the growth data of Experiment 9, the interaction was affected by dietary manganese. In most cases, the significant interaction between boron and magnesium could be characterized by magnesium deprivation more adversely affecting the boron-deprived than boron-supplemented rats. For example, the elevation in KW/BW and SW/BW, and the depression in plasma alkaline phosphatase caused by magnesium deprivation was more marked in boron-deprived than boron-supplemented rats. This was true with both manganese-

adequate and manganese-low diets. One notable exception was that the elevation in bone calcium concentration was more marked in the boron-supplemented than boron-deprived rats.

An unexpected finding in Experiment 9 was that the growth response of rats to dietary manganese indicated that a low dietary level (25 μg/g) was more beneficial than the NRC requirement for this element (55 μg/g) with an arginine-supplemented, casein-based diet. Regardless of dietary magnesium or boron, growth was better in the rats fed low manganese than "adequate" manganese diets.

DISCUSSION

The findings of the present study indicate that the severity of the magnesium deprivation and the methionine status of the rat strongly influence the extent and nature of the interaction between magnesium and boron and the response to boron deprivation. Dietary boron did not consistently or markedly affect the response of rats fed a marginally deficient magnesium diet (200 μg/g) which was manifested mainly by a reduction in bone magnesium concentration (Experiments 1–3). In other words, dietary boron deprivation did not induce signs of magnesium deficiency in rats fed marginal magnesium. On the other hand, when magnesium deprivation was severe enough to cause the typical signs of deficiency, including depressed growth, plasma alkaline phosphatase activity and bone magnesium concentration, and elevated SW/BW and KW/BW, dietary boron did affect the response of the rats. The influence was most evident when the casein-based diet was not supplemented with methionine, and especially when the diet contained luxuriant arginine. Generally, the result of the interactions between boron and magnesium was that the deprivation of one of the elements made the deficiency signs of the other more marked. For example, the signs of boron deprivation were more consistent and more marked when the diet was low in magnesium and was not supplemented with methionine or contained luxuriant arginine; among the signs for which this generally held true was depressed growth and elevated KW/BW and SW/BW.

The finding that the interaction between boron and magnesium, and the response of rats to boron deprivation, were more marked and consistent when the diet contained luxuriant arginine suggests that boron is physiologically important when enhanced in vivo demand for methionine cannot be met by the diet. Casein-based diets are known to be marginal in methionine content and to lack taurine (*16*). In the present experiments, the addition of arginine to the basal diet (5 mg/g), or to the arginine-luxuriant diets (10 mg/g), probably made the diets methionine-deficient because of the increased requirement for methionine in the metabolism of arginine through the pathway that utilizes methionine-methyl to form creatinine (*15*). Magnesium deprivation probably

enhanced the need for methionine because it induces elevated levels of taurine in muscle and urine (*17*); taurine is formed from methionine via cysteine (*18*).

Although the findings from the present study can lead to several hypotheses as to a possible role for boron in vivo, currently we are intrigued with the possibility that boron is important at the cellular membrane level. The reasons for this are:

1) Boron deprivation signs are most noticeable when taurine metabolism probably has been altered, and taurine might have a role in membrane stabilization (*17*).
2) Boron deprivation exacerbates the signs of magnesium deficiency, and some of these signs (e.g., elevated spleen size as the result of abnormal erythrocytes) may be the result of abnormal membrane structure (*19*).
3) Boron is suspected of regulating such plant hormones as auxin, gibberellic acid, and cytokinin (*20,21*), through an effect on a second messenger, such as cyclic AMP, at the cell membrane level (*20,22*).
4) Palytoxin, an extremely poisonous animal toxin from coral, raises the permeability of excitable and nonexcitable membranes of animals. The binding of palytoxin to membranes is potentiated by borate (*23*).
5) Aplasmomycin, a novel ionophoric macrolide antibiotic, is a boron-containing compound (*24*).
6) Several recent reviews have presented evidence consistent with the view that boron in plants is directly associated with membranes and is involved in maintaining their functional efficiency (*25–27*). In other words, many symptoms of boron deficiency in plants are secondary effects caused by changes in membrane permeability.
7) The major part of the boron of mung bean seedlings was found localized in the membranes (*28*).

In ultratrace element research, signs indicating essentiality of several elements apparently were misinterpreted and probably were manifestations of pharmacologic, not physiologic actions (*29*). Thus, it is reasonable to ask whether boron is acting through physiological or pharmacological mechanisms in the present study. High dietary amounts are usually required for an element to act pharmacologically. In the present study, a dietary boron supplement of 3 μg/g seems high but is actually relatively low in comparison to the boron contents of some foods. A recent extensive study of the mineral content of over 200 Finnish foods included boron (*30–32*). The average boron content (μg/g dry wt) in different food groups was: cereals, 0.92; meat, 0.16; fish, 0.36; dairy products, 1.1; vegetables, 13; and others, 2.6. Foods that contained the highest levels of boron (μg/g wet weight) included soy meal, 28; prune, 27; raisin,

25; almond, 23; rose hips, 19; peanut, 18; hazel nut, 16; date, 9.2; and honey, 7.2. Wines contained up to 8.5 μg B/g. The high level of boron in nuts, fruit kernels, fruits, and vegetables was also reported elsewhere (*33–36*).

Obviously, foods of plant origin are rich sources of boron. Meat or fish apparently are poor sources of boron. The preceding indicates that the daily intake of boron by humans and animals can vary widely depending upon the proportions of various food groups in the diet. Nonetheless, a reported average daily intake for Finnish people was reported to be 1.7 mg boron. If one assumes an intake of about 1 kg of food (dry wt) daily, the boron concentration of the diet is in the same range as that fed to the rats in the present study. Thus, because the boron supplementation did not make dietary intake unusual, it seems probable that actions of boron were manifestations of physiological actions, and support the hypothesis that boron is an essential nutrient for the rat.

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