Effects of Boranes Upon Tissues of the Rat. III. Tissue Amino Acids in Rats on a Pyridoxine-Deficient Diet[1] (35498)

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The present study was designed to study the effects of boranes upon the tissue amino acid content of various tissues to gain insight into the basic mechanisms of borane toxicity. Our earlier studies demonstrated marked inhibition of aspartate aminotransferase (AAT) activity in several organs of the rat following exposure to decaborane (1, 2). The inhibition of AAT was irreversible, reached its maximum extent shortly after the intraperitoneal injection of borane, and was most pronounced in the liver and kidneys. AAT activity assayed with endogenous pyridoxal was reduced in rats maintained on a pyridoxal-deficient diet; decaborane treatment of these pyridoxal-deficient animals did not cause a further reduction in AAT activity. Moreover, when these tissues were assayed with added pyridoxal phosphate, the AAT activity was higher than in the controls, indicating that AAT was inhibited by decaborane only when the enzyme contained the pyridoxal coenzyme. These findings, together with the report that boranes inhibit aromatic amino acid decarboxylase (3), led us to speculate that one of the primary toxic effects of boranes was their inhibition of pyridoxal enzyme systems by chemical reduction of the imine linkage between the apoenzyme and the pyridoxal group.

To assess the extent of borane inhibition of aminotransferase enzymes *in vivo* we studied the effects of decaborane upon tissue free amino acid content in normal rats (4). The alterations in amino acid content ranged from marked reductions in almost all the amino acids in the liver to slight elevations in several amino acids in the heart, the tissue least affected. These data indicated that many aminotransferase enzymes, and perhaps decarboxylase enzymes as well, were significantly inhibited as a result of the exposure to decaborane.

The current study was designed to extend these observations of the effects of boranes upon tissue amino acids to animals maintained on a pyridoxine-deficient diet. If boranes act primarily by inactivating pyridoxal enzymes, then one might expect that boranes administered to pyridoxine-deficient animals to only accentuate the alterations in amino acid metabolism already induced by the dietary deficiency. But if boranes cause significant inhibition of enzymes other than those catalyzed by pyridoxal, then one might expect to find an altered profile of the amino acid content of tissues from treated pyridoxal-deficient rats.

Methods. Fifteen male Sprague-Dawley rats were given free access to water and to a pyridoxine-deficient chow supplied by the California Biochemical Corporation. After 2 to 3 weeks, the rats developed the characteristic stigmata of pyridoxine deficiency; the borane study was begun 6 weeks after the rats were started on the diet. Five rats started at the same time on a Purina laboratory chow served as controls. Nine rats in the pyridoxine-deficient group were injected with decaborane, 20 mg/kg of body weight, as a solution in corn oil. The remaining six pyridoxine-deficient rats, and the five control rats, were injected with a similar amount of corn oil. Sixteen hr after injection, the animals were anesthetized with methoxyflurane; and the liver, kidneys, heart, and brain were removed quickly and frozen in liquid nitro-

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TABLE I. Tissue Content of Amino Acids and Derivatives (mean ± SEM).[i,j]

	Kidney			Liver			Heart			Brain		
	Control	PDD[k]	PDD + DB	Control	PDD	PDD + DB	Control	PDD	PDD + DB	Control	PDD	PDD + DB
Aspartic acid	69.9 ±3.2	43.4[a] ±2.6	48.5[a] ±3.0	8.1 ±0.5	6.5 ±0.5	8.1 ±0.6	18.9 ±1.6	14.3 ±2.0	60.5[be] ±8.3	118.4 ±11.2	123.0 ±13.2	156.6 ±13.1
Threonine	49.4 2.6	34.3[b] 2.2	30.2[a] 1.9	5.4 0.9	8.4 1.1	7.1 0.6	9.5 0.4	9.4 1.1	19.3[dg] 2.7	13.3 1.6	15.6 0.8	24.4[ae] 1.4
Serine	76.5 3.5	55.9[a] 2.2	40.7[ae] 1.9	8.0 1.4	13.1 2.0	7.7[f] 0.6	10.0 0.5	11.0 1.0	21.8[cg] 3.0	26.1 2.1	26.5 1.3	26.7 1.1
Glutamic acid	129.4 6.9	147.3 10.1	83.3[ae] 4.1	35.2 2.4	46.1[d] 3.8	20.1[ae] 2.0	76.6 7.0	78.8 6.7	76.4 6.0	174.0 16.0	214.8 19.7	217.0 19.3
Proline	46.0 2.9	32.0[b] 2.3	24.9[ah] 2.1	3.5 0.4	1.8[b] 0.3	2.1[b] 0.2	3.0 0.2	2.5 0.3	2.8 0.4	4.0 0.4	3.7 0.2	4.7[f] 0.2
Leucine	51.9 3.1	32.7[a] 1.5	27.9[a] 1.7	8.0 1.0	5.4[c] 0.3	5.6[d] 0.6	5.3 0.3	3.8[c] 0.4	7.2[f] 0.7	4.8 0.7	4.2 0.2	6.2[g] 0.5
Isoleucine	24.4 1.7	15.1[b] 1.0	12.8[a] 1.0	3.2 0.2	2.3[b] 0.2	2.1[c] 0.3	3.0 0.1	2.3[b] 0.2	3.5[f] 0.3	2.3 0.3	1.8 0.1	2.7[e] 0.1
Methionine	17.0 0.9	11.3[a] 0.5	8.4[ae] 0.5	3.2 0.2	1.9[a] 0.1	1.5[a] 0.2	1.2 0.1	0.6 0.4	1.3 0.2	2.0 0.3	1.6 0.1	1.6 0.2
Cystine	8.6 0.4	8.9 1.1	3.4[ae] 0.5	0.4 0.1	0.4 0.1	0.4 0.1	2.4 1.0	0.5 0.3	1.1 0.4	0.8 0.1	0.5 0.1	0.5 0.1
Valine	46.7 2.5	31.2[a] 2.1	26.9[a] 1.8	12.9 1.7	8.2 1.5	5.8[a] 0.5	5.9 0.8	4.1 0.4	6.6[f] 0.6	4.7 0.4	5.8 0.7	7.5[b] 0.5
Alanine	93.0 5.3	65.4[a] 2.4	45.4[ae] 2.2	36.6 3.3	36.2 1.6	13.0[ae] 1.2	37.3 2.0	28.9[d] 2.6	22.7[a] 1.7	29.6 1.1	29.3 1.1	24.8[dh] 1.3
Glycine	117.4 3.5	105.0[b] 1.7	87.5[af] 3.7	36.9 2.3	36.5 2.7	60.8[dh] 7.3	13.8 0.9	12.6 0.6	30.4[ae] 2.1	35.9 1.4	33.7 1.4	47.2[bh] 2.3
Tyrosine	13.6 0.6	11.8 1.8	6.8[bh] 1.4	3.3 0.14	2.4[b] 0.15	3.0 0.4	7.7 1.8	1.5[b] 0.4	4.4[h] 0.9	2.4 0.3	2.2 0.08	3.8[df] 0.4
Phenylalanine	29.6 2.0	17.3[a] 1.7	11.7[af] 0.7	7.0 1.1	4.7 0.6	4.0[e] 0.6	8.6 0.8	1.5[a] 0.4	5.0 1.6	3.7 0.4	3.0 0.2	3.7 0.5

TABLE I (continued)

	Kidney			Liver			Heart			Brain		
	Control	PDD	PDD + DB	Control	PDD	PDD + DB	Control	PDD	PDD + DB	Control	PDD	PDD + DB
Ornithine	25.8	19.4[b]	18.2[a]	8.5	6.4	8.1	2.4	2.1	4.2[dg]	1.8	1.9	3.4
	1.6	0.4	0.9	0.8	1.0	0.7	0.4	0.3	0.6	0.1	0.2	0.7
Lysine	36.5	22.8[a]	19.7[ah]	10.8	5.7[a]	5.3[a]	11.1	7.3[b]	8.3	5.4	4.0[d]	5.2[g]
	1.9	1.5	0.9	0.9	0.5	0.6	1.0	0.4	0.8	0.5	0.3	0.3
Tryptophan	3.7	2.0[a]	2.1[a]							0.79	1.57[b]	1.48[b]
	0.2	0.1	0.1							0.12	0.20	0.12
Histidine	15.4	11.7[b]	8.6[af]	1.1	1.2	0.5[g]	3.5	3.5	4.9	3.4	2.9	3.8
	0.7	0.8	0.5	0.4	0.2	0.1	0.2	0.1	0.7	0.6	0.3	0.3
Arginine	12.1	6.1[a]	4.1[a]				7.1	4.8[c]	3.1[af]	9.6	7.7	9.3
	0.6	0.7	0.7				0.8	0.2	0.4	1.5	0.4	0.8
Taurine	65.7	53.3	20.4[ae]	23.2	7.3[b]	4.5[a]	201.0	155.0	256.0	78.9	89.5	74.0
	5.3	4.6	2.5	3.7	2.3	0.5	23.9	51.0	27.0	5.0	4.3	5.6
Urea	2.9	4.7	5.1	0.50	0.95	2.3[bf]	1.3	2.1[d]	5.9[bf]	1.6	1.8	5.5[ae]
	0.5	0.8	1.0	0.04	0.19	0.3	0.1	0.2	0.8	0.2	0.3	0.6
Citrulline	2.9	1.6	1.5[b]	<0.1	0.34[a]	1.7[ae]	0.31	1.17	2.45[dh]	0.38	0.21	1.06[dg]
	0.5	0.3	0.2		0.06	0.3	0.17	0.30	0.30	0.05	0.13	0.20

[i] Expressed as mmoles/g of tissue protein.

[j] p Values comparing rats on pyridoxine deficient diet, untreated and treated, with controls: [a] $<.001$; [b] $<.01$; [c] $<.02$; [d] $<.05$. p values comparing treated pyridoxal deficient rats to untreated pyridoxal deficient rats: [e] $<.001$; [f] $<.01$; [g] $<.02$; [h] $<.05$.

[k] Abbreviations: PDD, pyridoxine-deficient diet; PDD + DB, pyridoxine-deficient diet plus decaborane.

gen until analysis. All further procedures were performed at 4°. An aliquot of each tissue was dissolved in 0.2 *N* NaOH and the protein concentration determined by the biuret method. An aliquot of each tissue was homogenized in a buffered sucrose solution (5) and centrifuged at 20,000*g* for 30 min. The tissue supernatant was deproteinized with sulfosalicylic acid and 1 aliquot of each tissue preparation chromatographed on a Technicon amino acid analyzer using citrate buffers (6). The column was water jacketed and maintained at 60°. Norleucine was used as an internal standard in each sample, and a commercially prepared amino acid standard was analyzed periodically. The data were analyzed statistically by means of Student's *t* test.

Results and Discussion. The mean concentrations of the tissue amino acids, and of taurine, urea, and citrulline, are given in Table I in values of millimoles per gram of tissue protein. Asparagine and glutamine were not separated by our system.

Pyridoxine-deficient rats. Tissue levels of most amino acids were reduced in the kidney of those rats maintained on the pyridoxine-deficient diet. The changes in the amino acids of the liver were basically similar to those in the kidney, except that the reductions were not so extensive. The increase in glutamic acid was significant ($p<.05$) in the liver; and serine and threonine were also slightly elevated. The most striking changes in amino acid levels in the heart were the marked reductions in tyrosine and phenylalanine. The amino acids of the brain were affected less than those of the other tissues.

Although the changes in free amino acid content we observed in these pyridoxal-deficient rats were largely consistent with published values, there are some disparities. While Swendseid *et al.* (7) found that pyridoxal deficiency caused significant changes in the glycine and alanine content of the liver, we observed no change; the alterations we found in hepatic serine and methionine were contrary to those observed by the same group. Taurine, which we found to be reduced to 30% of normal in the liver, has been reported to be unchanged in this tissue in pyridoxal deficiency (8). The decrease in taurine was even more pronounced in the decaborane-treated animals, where the taurine content of the kidney was also reduced to about 30% of the control value. Since the enzyme [cysteinesulfinate carboxylyase (EC 4.1.1.29)] responsible for the synthesis of taurine is pyridoxal dependent, the reduction in taurine content in pyridoxal deficiency presumably is due to a lessened activity of this enzyme (9).

Pyridoxine deficient rats treated with decaborane. Treatment of the pyridoxine-deficient rats with decaborane generally caused changes in amino acid content that could be considered an accentuation of a trend already established by the pyridoxine-deficient state. In the kidney, every amino acid but two was lower in the pyridoxine-depleted animal than in the control, and these levels were further reduced following decaborane treatment. The only exceptions were glutamic acid and cystine.

The liver content of threonine, serine, and glutamic acid, all slightly increased by the pyridoxine-deficient diet, were reduced following treatment with decaborane. Alanine and glycine, both unchanged in the pyridoxal-deficient rats, showed marked changes in the liver of the borane-treated rat, with alanine reduced to less than half the control level and glycine almost doubled. Taurine, markedly reduced in the pyridoxal-deficient rats, was lowered even further in the borane-treated animals.

The alterations in the amino acids of the brain were less extensive than in any other tissue, paralleling the earlier observations that decaborane inhibits AAT activity less in the brain than in the heart, liver, or kidneys of the normal rat (1). Aspartic acid, unchanged by pyridoxal deficiency, was increased approximately fourfold in the heart of the decaborane-treated animals.

As a result of our earlier studies we had suggested that the toxic effects of the boranes might be due primarily to their chemical reduction *in vivo* of the imine (Schiff base) formed by pyridoxal coenzyme with a lysine residue in the apoenzyme of aminotransferases and decarboxylases. This reaction, which has been used in structural studies of purified aminotransferase enzymes, causes

the pyridoxal to form an acid-stable secondary amine with the apoenzyme, with consequent inactivation of the enzyme. Extensive inhibition of pyridoxal enzymes by such a mechanism *in vivo* could be expected to result in serious derangements in amino acid metabolism. Moreover, then one should expect the metabolic alterations in decaborane toxicity to resemble those of pyridoxal deficiency; the effects of decaborane in the pyridoxal-deficient animal might then appear as an accentuation or exaggeration of the alterations already induced by the pyridoxine-deficient diet.

Although most of the changes in amino acid content in the borane-treated animals are an apparent extension of alterations already occurring as a result of the dietary deficiency, there are a number of striking reverses in the pattern when the boranes are administered to the pyridoxal-deficient rats. Glutamic acid was increased, though not always to a significant extent, in the kidney, liver, and brain of the pyridoxal-deficient rat. In both the kidney and liver, glutamic acid, instead of being increased further by decaborane, was decreased to a level about 40% lower than the control. These reductions in glutamic acid are approximately the same as those in the liver and kidney of the normal rat following decaborane (4).

Aspartic acid, which tended ($p<0.2$) to be lower in the heart of the pyridoxal-deficient rat, was increased threefold following decaborane administration. This increment is significantly greater than the 30% rise caused by decaborane in the normal rat (4).

Although most of the changes in amino acid content are compatible with the hypothesis that the primary effect of boranes is upon pyridoxal enzyme systems, the exceptions we have observed indicate that other enzyme systems are also significantly altered. Considering the apparent lack of specificity of the highly reactive boranes, this is not surprising. Our earlier studies, in fact, did show that lactic dehydrogenase is appreciably inhibited in some tissues of animals exposed to boranes (2). However, the data seem to support the thesis that pyridoxal enzyme systems are quite susceptible *in vivo* to the effects of boranes, and that these effects are not limited to the central nervous system.

Summary. Dietary pyridoxal deficiency causes extensive changes, mostly reductions, in the amino acid content of the liver and kidneys, and to a lesser extent, the brain and heart. Treatment of pyridoxal-deficient rats with intraperitoneal decaborane (20 mg/kg) tended to accentuate the changes induced by pyridoxal deficiency. The data support the suggestion that boron hydrides (borane) are effective inhibitors of pyridoxal-dependent enzyme systems *in vivo*. The data indicate that other enzyme systems are also inhibited by boron hydrides.

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