Effects of Boranes Upon Tissues of the Rat[1]
II. Tissue Amino Acid Content in Rats on a Normal Diet (35109)

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It has been demonstrated that exposure of animals to boranes (or boron hydrides) is followed by marked reductions in two enzymatic reactions dependent upon pyridoxal coenzyme, the decarboxylation of DOPA (dihydroxyphenylalanine) (1), and the transamination of aspartate (2, 3). The aromatic amino acid decarboxylase enzyme (EC 4.2.2.26), decarboxylates DOPA, 5-hydroxytryptophan, and *o*- and *m*-tyrosine, which are necessary for the synthesis of serotonin, epinephrine, and dopamine (4). The inhibition of this enzyme by boranes, and the depletion of brain serotonin and norepinephrine in exposed animals have been cited as evidence that the primary toxic effects of the boranes involve the depletion of biogenic amines of the central nervous system. However, monoamine oxidase inhibitors did not consistently ameliorate the reserpine-like effects of the boranes nor did they reduce the mortality rate in treated rats (5).

Our studies of aspartate aminotransferase (EC 2.6.1.1) showed this enzyme was markedly inhibited in animals injected with decaborane (2, 3). Moreover, the degree of enzyme inhibition was greater in the liver, kidney, and heart than in the brain, indicating that the effects of the boranes are much more generalized than had been previously appreciated. Furthermore, if the inhibition of these two pyridoxal enzymes were interpreted as indicative of a general predilection of boranes for enzymes requiring pyridoxal coenzyme, then the primary toxic effects of the boranes might be due to their widespread inhibition of this class of enzymes, which includes both the aminotransferases and the decarboxylases. Such a widespread effect should result in extensive alterations in amino acid and carbohydrate metabolism in the major organ systems.

This study was designed to assess the degree to which decaborane ($B_{10}H_{14}$) alters amino acid metabolic patterns. We measured the free amino acid content of the liver, heart, brain, kidney, and serum of adult rats 16 hr following the intraperitoneal injection of decaborane. Our observations demonstrate that boranes cause significant alterations in the amino acid pool size in all these tissues but the heart.

Methods. Five male Sprague-Dawley rats having free access to laboratory chow and water were injected intraperitoneally with 20 mg of decaborane/kg of body weight as a solution in corn oil. Five control animals were injected with a similar quantity of corn oil. After 16 hr, the rats were anesthetized with methoxyflurane, and a blood sample collected by cardiac puncture. The animals were then sacrificed and the brain, heart, kidneys, and liver quickly removed and frozen in liquid nitrogen until analysis. All further procedures were performed at 4°. An aliquot of tissue was dissolved in 0.2 *N* NaOH and the protein concentration determined by the biuret method. The tissues were homogenized in 9 vol of a buffered sucrose solution (6) with a mechanically driven Ten Broeck glass homogenizer. The homogenates were centrifuged in a refrigerated Sorvall RC-2B at 700*g* for 15 min and the resultant supernatant centrifuged at 20,000*g* for 30 min. An aliquot of the supernatant was deproteinized with sul-

[1] This study was supported in part by U. S. Public Health Service Grant AM-13135.

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fosalicylic acid and chromatographed on a Technicon amino acid analyzer using the citrate buffers described by Ellis and Prescott (7). The column was water jacketed and maintained at 60° with a Haake circulating water bath. Norleucine was used as an internal standard in each sample, and a commercially prepared amino acid standard (Technicon) was analzyed periodically. If separation between adjacent chromatographic peaks was not satisfactory to permit accurate calculation, the values were not included in the reported data. The data were analyzed statistically by means of the Student's *t* test.

Results. The liver, kidney, heart, brain, and serum from five normal rats and five decaborane-treated rats were used for this study. The free amino acid content of the tissues, expressed as millimoles per gram of tissue protein, are given in Table I.

With the exception of arginine, all the amino acids measured in this study were reduced in the kidney and brain of those rats treated with borane. Glycine was reduced to a greater extent in the brain than in the kidney, but for the remainder of the amino acids, the relative changes in each were approximately the same in these two tissues.

While the changes in amino acid content in the liver generally were similar to those in the kidney and brain, there are several notable differences. Ornithine, which was reduced to 30% of the control value in the kidney, was not significantly lower (78%) in the liver of the decaborane-treated animals. Histidine, on the other hand, was reduced to a greater extent (25%) in the liver than in the kidney (50%) or brain (40%). The most striking differences, however, were observed in the levels of glycine. Glycine, which was lowered moderately in the kidney and brain, was increased by 150% in the liver of the decaborane-treated rats. This increase in liver glycine may reflect inhibition of the glycogenic pathways of glycine metabolism, while the decreases of this amino acid in the brain and kidney may be the result of a lowered rate of synthesis of the amino acid in these tissues. There were no significant changes in the levels of the amino acids in the heart of treated animals.

TABLE I. Tissue Amino Acid Content in Decaborane Treated Rats.[a]

	Kidney (mean ± SEM)		Liver		Brain		Heart		Serum	
	Control	Decaborane	Control	Decaborane	Control	Decaborane	Control	Decaborane	Control	Decaborane
Aspartic acid	70.1 ± 5.0	35.6 ± 3.4[f]	10.5 ± 1.5	7.2 ± 0.7	169.8 ± 12.5	69.4 ± 8.8[f]	11.7 ± 0.7	15.0 ± 2.4	0.076 ± 0.005	0.050 ± 0.011
Threonine	45.5 ± 4.8	20.5 ± 1.4[f]	6.1 ± 0.7	2.8 ± 0.6[b]	17.5 ± 2.4	8.4 ± 1.0[c]	6.9 ± 0.7	9.0 ± 1.9	0.420 ± 0.047	0.303 ± 0.031
Serine	71.7 ± 7.0	34.7 ± 2.6[f]	8.3 ± 1.1	5.0 ± 0.7[c]	27.3 ± 3.6	13.8 ± 1.9[c]	8.5 ± 1.0	6.3 ± 1.5	0.382 ± 0.005	0.213 ± 0.013[f]
Glutamic acid	94.0 ± 6.2	64.8 ± 3.9[c]	31.9 ± 1.7	18.2 ± 3.1[c]	166.6 ± 21.4	112.3 ± 13.0	72.5 ± 5.6	63.9 ± 9.9	0.323 ± 0.040	0.270 ± 0.040
Glycine	113.7 ± 11.1	91.6 ± 7.3	31.0 ± 4.8	76.9 ± 11.5[c]	41.5 ± 4.4	25.2 ± 3.6[c]	7.4 ± 0.8	16.9 ± 4.6	0.392 ± 0.012	1.373 ± 0.256[c]
Alanine	101.2 ± 6.8	39.7 ± 2.3[f]	14.3 ± 2.4[a]	12.6 ± 1.3[f]	32.1 ± 3.6	12.6 ± 1.3[f]	17.6 ± 1.7	23.0 ± 5.1	0.688 ± 0.063	0.557 ± 0.053
Valine	40.4 ± 2.6	18.0 ± 1.5[f]	5.5 ± 0.8	2.6 ± 0.2[a]	4.2 ± 0.4	1.9 ± 0.3[a]	2.3 ± 0.2	2.8 ± 0.5	0.273 ± 0.030	0.281 ± 0.030
Methionine	14.8 ± 1.1	7.5 ± 0.7[f]	2.6 ± 0.4	1.1 ± 0.03[a]	2.9 ± 0.4	1.3 ± 0.2[a]	1.1 ± 0.1	0.9 ± 0.3	0.070 ± 0.002	0.065 ± 0.004
Isoleucine	22.2 ± 1.8	9.3 ± 0.8[f]	3.2 ± 0.4	—[g]		1.3 ± 0.2	1.6 ± 0.2	1.7 ± 0.4	0.113 ± 0.015	0.143 ± 0.015
Leucine	47.1 ± 3.6	21.2 ± 1.9[f]	7.3 ± 1.0	3.1 ± 0.3[a]	7.5 ± 0.9	2.9 ± 0.4[a]	3.1 ± 0.4	3.7 ± 0.7	0.219 ± 0.023	0.225 ± 0.022
Tyrosine	16.8 ± 1.4	9.1 ± 0.6[f]	3.1 ± 0.4	1.3 ± 0.2[a]	3.5 ± 0.4	1.8 ± 0.3[d]	1.6 ± 0.1	2.0 ± 0.4	0.098 ± 0.003	0.104 ± 0.010
Ornithine	35.4 ± 4.1	10.6 ± 1.1[f]	8.9 ± 1.2	6.9 ± 1.4					0.106 ± 0.002	0.152 ± 0.036
Lysine	57.1 ± 4.5	22.2 ± 2.5[f]	12.1 ± 1.7	6.1 ± 0.4[a]	12.8 ± 1.4	4.9 ± 1.1[c]	8.0 ± 0.4	8.7 ± 1.9	0.535 ± 0.005	0.339 ± 0.038[f]
Histidine	15.8 ± 1.2	7.7 ± 0.8[f]	7.3 ± 0.6	1.8 ± 0.3[f]	5.7 ± 0.6	2.0 ± 0.5[c]	2.6 ± 0.2	2.4 ± 0.4	0.096 ± 0.016	0.062 ± 0.010[d]
Arginine	10.0 ± 1.4	9.7 ± 1.0	0	0	14.4 ± 2.0	7.8 ± 1.2[c]	5.0 ± 0.2	5.1 ± 0.9	0.235 ± 0.049	0.152 ± 0.009
Phenylalanine	15.5 ± 2.1	10.3 ± 1.1[c]	3.6 ± 0.3	4.3 ± 0.6	5.4 ± 0.8	2.1 ± 0.3[c]	1.7 ± 0.05	2.1 ± 0.3	0.079 ± 0.002	0.087 ± 0.004
Taurine	87.4 ± 6.2	61.0 ± 4.3[c]	23.4 ± 7.9	12.8 ± 2.3	86.0 ± 13.3	64.5 ± 9.0			0.221 ± 0.024	0.381 ± 0.105

[a] Expressed as mmoles/g of tissue protein.
[b] *p* values comparing decaborane-treated tissues to control: [c] $<.05$; [d] $<.02$; [e] $<.01$; [f] $<.001$.
[g] Two peaks present in liver extracts of treated rats.

Many of the alterations in the serum amino acid levels parallel the changes observed in the liver. For example, the increases in serum glycine and phenylalanine are of the same degree as those in the liver. However, a number of amino acids, including the essential amino acids, valine, methionine, and leucine, as well as tyrosine, ornithine, and taurine, were increased in the serum although they were reduced in the liver.

Discussion. It has been proposed that pyridoxal phosphate is required for the transport of amino acids (8, 9). Inactivation of membrane pyridoxal groups should therefore be followed by a reduction in the rate of accumulation of amino acids from the intestine, with a fall in the tissue and serum levels. If the inhibition of absorption were responsible for the marked reduction in serum amino acid levels in these animals however, the effect should be more pronounced among the essential amino acids than among those the rat is capable of synthesizing. Table I shows, however, that the essential amino acids were altered in the serum no more than were those amino acids considered nonessential for the rat. The blockade of intestinal absorption therefore does not seem a likely mechanism for the changes we found in the serum amino acids. This same reasoning cannot, however, be extended to the changes in the amino acid content in the individual organs. The possibility exists that the alterations in amino acid content of the various tissues may be due, at least in part, to inhibition of the transport of the amino acids from the circulating plasma into the tissue cells. The differences in the degree of alteration of amino acids levels in the various tissues might be due then to the differing degree of exposure of the tissues to borane as it is absorbed by the portal circulation (2), or even to differences in the composition (and susceptibility to inhibition by borane) of the plasma membrane in the different tissues.

In most cases, the tissue level of a given amino acid is subject to the rates of several metabolic pathways, at least two of which, transamination and decarboxylation, are dependent upon pyridoxal phosphate. For this reason, the exact mechanism for the changes in tissue content of any given amino acid may not be readily deduced. One exception, however, should be taurine, whose synthesis is wholly dependent upon the pyridoxal enzyme cysteinesulphinate carboxylase (EC 4.1.1.29), and which should be reduced in pyridoxal-deficient states. However, it has been reported that even though pyridoxal-deficient rats cease excreting taurine in their urine, the tissue levels of this amino acid are unchanged (10). In our present study many of the changes in amino acid content resemble those observed in pyridoxal deficiency, but tissue taurine levels were decreased while the serum levels were slightly increased.

We have suggested that boranes have a predilection for pyridoxal enzymes in the exposed animal. Because pyridoxal enzymes are so important for the metabolism of amino acids, any condition causing a reduction in the activities of pyridoxal enzymes would necessarily be followed by extensive changes in the tissue amino acids. Therefore, one could consider that the boranes exert their effects by a form of pyridoxal-deficiency, but one that is due to an acute chemical-mediated loss of pyridoxal, rather than to chronic dietary deficiency of the coenzyme. However, the changes in amino acid content we found in the decaborane-treated rats were not the same in every instance as those reported for rats maintained on a pyridoxine-deficient diet (11). For example, serum aspartic acid is reported to increase by twofold or more in pyridoxal deficiency, while in these studies borane caused a reduction by one-third. Isoleucine, which is decreased in pyridoxal deficiency, was increased by 25% following decaborane. The changes in taurine levels are contrary to those reported for pyridoxal deficiency.

The mechanism by which boranes exert these effects may be due to their electron deficiency and their reducing capacity. The reducing properties of diborane (B_2H_6) have been used to reduce the double bond in the Schiff base formed by pyridoxal phosphate with the apoenzyme of muscle phosphorylase (12) and of aspartate aminotransferase (13). Diborane has also been used to reduce selectively the C-terminal carboxyl groups of pep-

tides and proteins (14). These reductive effects of boranes upon proteins *in vitro* surely have their parallel *in vivo*. It is probably just this property that Merritt was counteracting when he observed that the continuous infusion of methylene blue into decaborane-treated rabbits increased the survival time and prevented sedation (15). This mechanism would also account for the marked reduction in aspartate aminotransferase (AAT) we have observed in the tissues of rats treated with decaborane (2, 3). If one makes the analogy between the effects of diborane upon AAT *in vitro* and the effects *in vivo* in these tissues, and speculates that this inhibition is the result of the reduction of the unstable double bond linking the pyridoxal group with the apoenzyme; then one would expect a reduced effect of decaborane upon the AAT enzyme in the pyridoxal-deficient rat. Our earlier studies indeed showed that AAT, when assayed with endogenous pyridoxal, was not reduced by decaborane. When AAT was assayed with exogenous pyridoxal the activity was greater in pyridoxal-deficient rats than in animals maintained on a normal diet prior to the decaborane treatment.

The present study demonstrates that under our experimental conditions the effects of decaborane upon amino acid pool size, like those upon AAT (3), were greatest in the liver. However, the magnitude of the changes in amino acid content in the other tissues does not parallel the degree of AAT inhibition in those tissues. This may be due to the fact that other amino acid transferase enzymes may not have the same susceptibility to reduction by borane, perhaps as a result of differing affinities for pyridoxal coenzyme. Moreover, it must be noted that measurement of tissue content or pool size of a given amino acid does not necessarily reflect the turnover rate of the amino acid within the tissue.

Summary. Our data demonstrate that the administration of borane is followed by extensive changes in tissue amino acid levels. These changes are probably due to the extensive inhibition of aminotransferase and other pyridoxal-dependent enzymes. One possible mechanism for such an effect is the chemical reduction, and consequent inactivation, of the active holoenzyme by borane.

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Received May 6, 1970. P.S.E.B.M., 1970, Vol. 135.