quired for attainment of the steady state. We have failed, however, to detect any impairment of this response by prereserpinization. The Nefa concentration was found to increase continuously in response to DNP at a rate greater than that found in anesthetized control animals. The reserpinized animals differed only by an initial decrease in plasma Nefa concentration following DNP.

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Transaminase Inhibition by Decaborane* (32776)

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The use of the boron hydrides, pentaborane and decaborane, in recent years as high energy propellants has stimulated interest in the mechanism(s) by which this class of compounds expresses its toxic effects.

Boron hydrides are known to cause marked derangements in the biochemical and functional relationships of the central nervous system (1). While there is indirect evidence that many of the effects may be due to inhibition of aromatic amino acid decarboxylases (2), direct evidence has been obtained that 5HTP decarboxylase (EC 4.1.1.26) is inhibited by decaborane ($B_{01}H_{14}$) (3); these are all pyridoxal-requiring enzymes. For this reason, we chose to examine a number of pyridoxal enzymes, as well as several non-pyridoxal-requiring enzymes, to test the hypothesis that the toxic manifestations of boron hydrides are primarily due to their inhibition of pyridoxal enzymes.

We are reporting our initial results showing the effects of decaborane ($B_{10}H_{14}$) upon glutamic-oxalacetic transaminase (aspartate aminotransferase, EC 2.6:1.1) activity in the

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brain, liver, kidneys, serum, and heart of the rat.

Methods. Ten male Sprague-Dawley rats weighing 280–480 gm were injected intraperitoneally with 10 mg, and 10 with 20 mg, of decaborane per kg of body weight as a solution in corn oil. Nine control animals were injected with like quantities of corn oil. All the animals had free access to Purina laboratory chow and to water. It was noted, however, that those animals injected with decaborane seemed to abstain from eating. After 16 hours, the rats were anesthetized with methoxyflurane, and a sample of blood was aspirated by cardiac puncture into a heparinized syringe. The liver, kidneys, heart, and brain were quickly removed, frozen in liquid nitrogen, and stored for 1–3 days at −25°C until assayed. At the time of assay, the tissues were homogenized in 9 volumes of 0.25 *M* sucrose (4) with a Ten Broeck glass homogenizer. The homogenates were centrifuged at 700*g* for 15 min and at 20,000*g* for 30 min in a refrigerated Servall RC-2 centrifuge.

The resulting soluble fraction was diluted 1:100 and its aminotransferase activity determined in duplicate at 25°C by the method of Bergmeyer and Bernt (5). The aminotransferase activity of the precipitate material, which included the mitochondria, was not determined. The assay mixture initially contained 700 μmoles of *L*-aspartate, 0.6 μmoles of NADH, 25 μg of malic dehydrogenase (MDH), and 0.1 ml of diluted tissue supernatant. Ten min later the reaction was started by adding 20 μmoles of α-ketoglutarate; the final volume was 3 ml. Transamination was measured by the initial rate of decrease in optical density at 340 mμ as the MDH reductively converted to malic acid the oxalacetate formed from aspartate by transamination. When either MDH or α-ketoglutarate was omitted from the experimental assay mixture, there was no significant change in its absorbance at 340 mμ. The enzyme activity of the malic dehydrogenase preparation used in the aminotransferase assay was determined by the method of Bergmeyer and Bernt (6). The activity of the MDH preparation was not inhibited by the tissue extracts of the animals treated with decaborane. The L-aspartic acid, reduced nicotinamide adenine nucleotide, and α-ketoglutaric acid were reagent grade chemicals, and the malic dehydrogenase was obtained from Mann Research Laboratories. The absorbance measurements were made in a Beckman DU monochromator fitted with a Gilford model 220 absorbance indicator and recorder. The cuvette chamber was water-jacketed and the temperature was maintained at 25 ± 0.5°C with a constant temperature water bath.

Following the initial determination of enzyme activities, the supernatant extracts were dialyzed overnight against a 10 m*M* phosphate buffer (pH 7.6) and the enzyme activities again determined after the addition of 6 μg pyridoxal phosphate/ml of tissue supernatant.

Results and Discussion. Rats treated with only 10 mg/kg of decaborane showed a significant decrease in amino transferase activity in all assayed tissues except the brain (Table I). While the changes were most marked in the liver and serum, the kidney and heart also displayed significant losses in activity.

Treatment of the rats with 20 mg/kg of decaborane, a dosage that is less than the reported LD_{50} dose of 27 mg/kg (7), caused an even greater loss in enzyme activity in all tissues but the serum. This enzyme inhibition was most striking in the liver (97% inhibition at the 20 mg level) and serum (91% at the 20 mg dosage). The heart and kidney each displayed more moderate aminotransferase inhibitions, and the brain least of all the organs studied (25%). The relatively small loss of enzyme activity in the brain tissue is of particular interest since this is the organ usually incriminated for the toxic manifestations of boron hydrides (1–3). It would seem that although the dramatic CNS effects (lethargy, ataxia, convulsions) do suggest significant alterations in the function of various brain enzymes, the effects upon other organs may be more profound though less evident. The relative inhibition of enzyme activity in each of the various tissues follows a similar pattern in animals treated at the two dosage levels. This pattern of inhibition perhaps represents the

TABLE I. Decaborane Inhibition of Aspartate Aminotransferase Activity.[a]

Treatment (mg/kg)	Liver			Brain			Heart			Kidney			Serum × 10^{-3}		
	No. of tissues	Mean	SE	No. of tissues	Mean	SE	No. of tissues	Mean	SE	No. of tissues	Mean	SE	No. of tissues	Mean	SE
Control	8	73.46	5.51	9	39.40	2.46	9	87.12	12.44	9	58.21	3.28	8	79.24	3.55
Decaborane treated															
10	10	24.92	3.59	10	36.68	1.25[b]	10	54.36	2.86[c]	2	34.60	1.50[d]	10	5.73	1.18[e]
20	10	2.27	.45	10	29.58	2.21[d]	10	21.7	2.33	10	14.82	1.75	9	6.81	1.27
20 (dialyzed)	10	1.57	.58	10	11.82	1.24	10	7.62	1.38	10	7.32	.96			

[a] Expressed as International Units (μM NAD oxidized/min)/gm of tissue except for the dialyzed samples, in which there is no correction for dilution of the enzyme during dialysis. Probability levels comparing the 10 and 20 mg/kg treated series with the controls: [b] $<.2$; [c] $<.02$; [d] $<.01$; all others $<.001$. Probability levels comparing the 10 and 20 mg/kg treated series: [e] $p<.6$; [b] $p<.02$; all others $<.001$. Probability levels were not determined for the dialyzed samples.

schema of exposure of the various organs to the toxic boron hydride as it enters the portal circulation from the peritoneum and passes into the larger systemic circulation.

It should be noted that the highly reactive nature of the boron hydrides makes it presently impossible to define the exact chemical composition of the boron compounds responsible for these effects. Their efficacy as reductants for high energy fuels, the extreme rapidity with which their boron–boron bonds undergo alcohololysis (8), and the velocity of the spontaneous conversion of boron hydrides to borates when incubated in rabbit plasma *in vitro* (9) all give testimony to the difficulties encountered in defining the exact chemical nature of the culpable boron compound. We therefore use the term "boron hydride" advisedly and perhaps with some degree of freedom.

To determine whether the loss in enzyme activity represented a deficiency of endogenous pyridoxal cofactor, we added supplemental pyridoxal phosphate, 6 μg/ml, to the tissue extracts. Measurement of the aminotransferase activity showed no recovery of activity. This did not rule out the possibility that the inhibition of the enzyme might be due to competitive inhibition by some pyridoxal analogue formed as a result of the action of the boron hydride. To remove any such inhibitor, the tissue extracts were dialyzed overnight against 200 volumes of 10 mM phosphate buffer at pH 7.6. This procedure should also remove any free residual borane derivatives or boron hydride. Pyridoxal phosphate (6 μg/ml) was added to the extracts following the dialysis; re-assay demonstrated no recovery of aminotransferase activity by any of the tissue extracts. The apparent loss of activity in the dialyzed extracts (Table I) is due to dilution of the enzyme by the buffer solution. Determination of the specific activity of the aminotrasferase showed no net change in the activity of these samples with dialysis. These experiments indicate that the loss in activity may be due in some way to an irreversible change in the apoenzyme.

The nature of this change in the apoenzyme is not clear, but possible causes include inhibition of synthesis of the enzyme by the tissues, irreversible binding of boron products

to the enzyme with consequent inactivation, or irreversible covalent changes in the apoenzyme or coenzyme–apoenzyme complex.

Our results to date do not allow us to determine which, if any, of these factors is operative here. The changes in dietary habits of the animals treated with decaborane are unlikely to have been a significant factor in the marked loss in enzyme activity since it has been shown that the activity of this enzyme is unchanged in the tissues of rats fasted overnight (10). Studies are in progress to determine if boron is bound by the aminotransferase apoenzyme or if the structural charactersitics of the enzyme are altered by boron hydrides.

These and other data from our laboratory suggest that boranes have a predilection for pyridoxal enzymes, and this hypothesis is supported by additional studies in this series indicating that lactic dehydrogenase (EC 1.1.1.2.7) activity is not altered significantly by decaborane (W. N. Scott, J. H. Landez, and H. D. Cole, in preparation). If substantiated by further studies, our findings may result in two advances: (a) an underlying hypothesis for the mechanism of the toxicity of boron hydrides; and (b) a simple and effective technique for inhibiting pyridoxal-catalyzed enzyme systems *in vivo*.

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Zinc Metabolism in Schistosomes (32777)

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The relationship of zinc metabolism to schistosomiasis is of particular interest due to recent observations by Prasad *et al.* of a dwarfism syndrome in Egyptian males associated with low serum zinc levels (1–3). Growth failure, hepatosplenomegaly and hypogonadism manifested by absence of pubic, axillary, and facial hair; small penis, and atrophic testes were consistent findings in these patients. Both hookworm and *Schistosome haematobium* infections were observed in many patients and all had hypochromic anemia. The diet of the patients was high in cereals, low in animal protein and green vegetables. Nutrition was proposed as one factor in the deficiency, and blood loss due to parasites as another. Since schistosomiasis is present in a very high percentage of the population in the Nile delta, it was hypothesized that there might be a relationship between zinc deficiency and schistosomiasis.

Two basic experiments were set up: (i) to determine the ^{65}Zn uptake by schistosome worms and eggs in infected hamsters, (ii) to

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