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Technical Progress Report

Abstract:

The synthesis and structural confirmation of the disulfide, $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ is reported as well as a new structural model for its mono-oxidized derivative, $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$, which showed useful tumor:blood concentration ratios in our rat model after three I.V. injections. The material, $B_{12}H_{11}(SH)_2^{-2}$, was isolated and identified from impure $Cs_2B_{12}H_{11}SH$. The former anion was shown to be responsible, in its oxidized form, for the excellent tumor uptake results shown by impure $B_{12}H_{11}SH^{-2}$ in the past. As a consequence of this work, a borane polymer was synthesized which has shown extremely encouraging tumor uptake properties. The synthesis of this polymer depended on our production and characterization of the first stable thiyl radical at room temperature, $B_{12}H_{11}\dot{S}^{-2}$. The retesting of certain phosphate boranes is reported. And a reagent, namely acetyl sulfenyl chloride, $ClSCOCH_3$, has been found which will produce $B_{12}H_{11}SH^{-2}$ in a laboratory synthesis.

The ability of sulfur derivatives of $B_{12}H_{11}SH^{-2}$ to bind to proteins was examined, as was the blood distribution of two of these interesting boranes. The amount of non-protein bound borane was related to the rate of blood clearance of the borane in rats. Finally, we continue to report our progress on the dog irradiation experiments.

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Technical Progress Report

Principal Investigator: William H. Sweet, M.D., D.Sc.
Current Term: 8/1/74-4/30/75: 8 hours/week
Remainder of Current Term 5/1/75-7/31/75: 8 hours/week

TECHNICAL PROGRESS REPORT

INTRODUCTION

The major effort of our research has been to develop a borane which will be selectively taken up by glioblastoma relative to normal brain and blood and therefore allow neutron-capture therapy. In addition to our chemical work, we have also been investigating the chronic effects of cerebral neutron capture irradiation on dogs under conditions at the nuclear reactor essentially the same as those for treatment of patients.

Of great interest to these laboratories has been the investigation of $B_{12}H_{11}SH^{-2}$ which had been reported by others to possess excellent tumor uptake properties(1). We found, however, that purified $Cs_2B_{12}H_{11}SH$ did not possess outstanding tumor uptake properties in rats or in man. Consequently, we followed the hypothesis that the excellent tumor uptake properties seen in earlier, and probably impure $B_{12}H_{11}SH^{-2}$, were due to another borane which was either produced as a side product in the bomb synthesis of $B_{12}H_{11}SH^{-2}$, or was an oxidized product of this anion. Two years ago we reported that the product of a sample of $Cs_2B_{12}H_{11}SH$ oxidized with H_2O_2 showed excellent tumor uptake properties. As this product was a mixture, we synthesized and crystallized a number of mixed disulfide and other oxidized derivatives of $B_{12}H_{11}SH^{-2}$ in order to isolate the active oxidized product of $B_{12}H_{11}SH^{-2}$. As indicated in last year's progress report, none of these isolated compounds showed excellent tumor uptake properties in rats after a single injection.

In this year's progress report, we describe three especially significant results with regard to development of a borane suitable for neutron-capture therapy. These are: 1. The characterization of crystallized, oxidized product

of $B_{12}H_{11}SH^{-2}$, namely $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$, which shows excellent tumor uptake and a tumor:blood boron concentration ratio greater than four after 96 hours following a triple injection of the compound in rats. 2. The species, $B_{12}H_{10}(SH)_2^{-2}$, (found in impure samples of $B_{12}H_{11}SH^{-2}$) when oxidized, showed excellent tumor uptake properties in rats after a single injection; its oxidized form, a borane polymer with -SOS- linkages, was largely responsible for the favorable biological results seen in oxidized impure $B_{12}H_{11}SH^{-2}$. 3. Consequently, a class of borane polymers derived from the thiyl radical $B_{12}H_{11}-\dot{S}^{-2}$ was developed, one of which showed outstanding tumor uptake properties. A polymer analogous to these was probably responsible for the excellent tumor uptake properties of unoxidized impure samples of $B_{12}H_{11}SH^{-2}$.

All the proposed areas of research described in our last year's A.E.C. report, and more, were investigated during the past year. These will now be described first briefly, and then in detail, under the following headings:

- I. Characterization of Oxidized Derivatives of $B_{12}H_{11}SH^{-2}$.
- II. The Thiyl Radical, $B_{12}H_{11}-\dot{S}^{-2}$, Produced from the Disulfide, $B_{12}H_{11}SSB_{12}H_{11}^{-4}$.
- III. The Borane, $B_{12}H_{10}(SH)_2^{-2}$, Found in Impure Samples of $B_{12}H_{11}SH^{-2}$.
- IV. Polymers from Borane Thiyl Radicals.
- V. A Side Product in the Synthesis of $B_{12}H_{11}SH^{-2}$ from H_2S and $B_{12}H_{12}^{-2}$.
- VI. Phosphate Derivatives including Rat Uptake Studies.
- VII. Laboratory Synthesis of $B_{12}H_{11}SH^{-2}$.
- VIII. Borane Binding to Proteins and Blood Distribution.
- IX. Biological Studies - Dog Irradiation Studies at M.I.T. Reactor.

I. Characterization of Oxidized Derivatives of $B_{12}H_{11}SH^{-2}$.

The synthesis and structural confirmation of the disulfide $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ was finally achieved by us. This species, which had been the one hypothesized to be responsible for good tumor uptake in impure samples of $B_{12}H_{11}SH^{-2}$, was tested in our animal tumor model and it failed to show favorable tumor binding properties.

The anion $B_{12}H_{11}SOH^{-2}$ reported last year by us, was subsequently shown to be the thiosulfinate ester $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$. This compound has shown excellent tumor uptake properties when administered in a multiple injection schedule.

II. The Thiyl Radical, $B_{12}H_{11}-\dot{S}^{-1}$, Produced from the Disulfide, $B_{12}H_{11}SSB_{12}H_{11}^{-4}$.

Under acidic conditions, the disulfide $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ produced the thiyl radical, $B_{12}H_{11}-\dot{S}^{-1}$. This is the first example of a thiyl radical which is stable at room temperature. Some kinetic measurements on the formation of the radical are described, as is its production from other boranes containing a sulfur-sulfur bond.

III. The Borane, $B_{12}H_{10}(SH)_2^{-2}$, Found in Impure Samples of $B_{12}H_{11}SH^{-2}$.

The dithiol $B_{12}H_{10}(SH)_2^{-2}$ was shown to be present in samples of $B_{12}H_{11}SH^{-2}$ previously thought to be pure. We have shown that this impurity, when oxidized, was responsible for the good tumor uptake observed by us two years ago.

IV. Polymers from Borane Thiyl Radicals.

A radical mechanism for the formation of the blue colored polymer (derived from $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$) described in last year's report is suggested. This polymer has been shown to be a radical. On the basis of this work we synthesized a different blue radical polymer, a base treated form of which was shown to give rise to good tumor uptake after a single injection, coupled with low toxicity.

V. A Side Product in the Synthesis of $B_{12}H_{11}SH^{-2}$ from H_2S and $B_{12}H_{12}^{-2}$.

A side product produced in the synthesis of $B_{12}H_{11}SH^{-2}$ by the reaction between H_2S and $B_{12}H_{12}^{-2}$ was crystallized by us. Since animal testing of this material revealed no favorable tumor binding properties, this work was terminated in order to pursue more promising lines of investigation.

VI. Phosphate Derivatives Including Rat Uptake Studies.

Two borane phosphates synthesized by Kaczmarczyk's group at Tufts University, and shown previously to have good tumor binding properties were re-investigated by us. These results were discouraging and we were not able to substantiate the favorable biological properties seen earlier.

VII. Laboratory Synthesis of $B_{12}H_{11}SH^{-2}$.

Last year we suggested that a sulfonyl chloride, $ClSCOCH_3$, might be useful in a laboratory synthesis of $B_{12}H_{11}SH^{-2}$ from $B_{12}H_{12}^{-2}$. The production of $B_{12}H_{11}SH^{-2}$ and also of $B_{12}H_{10}(SH)_2^{-2}$ with this reagent has now been realized. Problems found in an attempt to develop a large scale laboratory synthesis of $B_{12}H_{11}SH^{-2}$ using the reagent $ClSCOCH_3$ are discussed.

VIII. Borane Binding to Proteins and Blood Distribution.

The ability of various boranes to bind covalently to proteins in aqueous media was examined. The only interactions observed appeared to be ionic. The boranes, $B_{12}H_{11}SH^{-2}$ and $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$, were shown to be distributed in significantly different ways which could be correlated with the rate of blood clearance of these compounds in rats.

IX. Biological Studies - Dog Irradiation Studies At M.I.T. Reactor.

Finally, we report on the surviving dogs which underwent slow neutron irradiation several years ago.

We will now describe the work accomplished in these nine areas in detail.

I. Characterization of Oxidized Derivatives of $B_{12}H_{11}SH^{-2}$

- 1) Product of the reaction in which 1 mol of $B_{12}H_{11}SH^{-2}$ reacts quantitatively with 1 mol of H_2O_2 .

In the last year's Technical Progress Report (A.E.C. Report No. C00-3334-3) for the period May 1, 1973 - April 20, 1974, we described the crystallized product of the above reaction as being the sulfenic acid $B_{12}H_{11}SOH^{-2}$, a conclusion based on reaction stoichiometry,

elemental analysis, and on a preliminary ^{11}B nuclear magnetic resonance spectrum, all of which appeared to indicate a mono substituted B_{12} cage.

Since that report we have carried out a more extensive boron-11 nmr study (Table I), including proton decoupled boron-11 resonance, of a number of our sulfur substituted borane derivatives including this compound. These results provide strong evidence for a coupled cage structure $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$. This thiosulfinate ester structure is also consistent with our elemental analysis

	C	H	O	N	B	S	H ₂ O
Calculated:	28.41	10.73	4.73	8.28	38.68	9.68	2.66
Found:	27.86	10.80	4.97	8.85	36.25	10.66	2.67
	28.23	10.63			37.57	10.26	

and reaction stoichiometry

The rate of elution of this compound on a gel filtration system developed by us for the estimation of molecular weight and anionic charge of borane anions is also consistent with two $B_{12}H_{12}^{-2}$ cages carrying a -4 charge.

The biological uptake of this compound is shown in Figure 1. It is encouraging that multiple injections in contrast to a single injection results in an increase of the tumor:blood boron concentration ratio after 96 hours.

Finally,

/the conclusion that H_2O_2 oxidation of $\text{B}_{12}\text{H}_{11}\text{SH}^{-2}$ produces linked cages has been extremely valuable in our understanding of the nature of the product produced in the H_2O_2 oxidation of $\text{B}_{12}\text{H}_{11}\text{SH}^{-2}$ contaminated with the di-mercaptan, $\text{B}_{12}\text{H}_{11}(\text{SH})_2^{-2}$.

2) Product of the reaction of $\text{B}_{12}\text{H}_{11}\text{SH}^{-2}$ and $\text{OIC}_6\text{H}_4\text{COOH}$.

When two mols of $\text{B}_{12}\text{H}_{11}\text{SH}^{-2}$ react with one mol of iodosobenzoate, a single product is formed.

When this reaction had been carried out initially (and described in our last year's report) we had observed the formation of several products. However, using pure $\text{B}_{12}\text{H}_{11}\text{SH}^{-2}$ (determined to be free of the $\text{B}_{12}\text{H}_{11}(\text{SH})_2^{-2}$), we observed only a single product which was isolated as crystals of the cesium salt. The reaction stoichiometry indicated that the product was, in fact, the disulfide $\text{B}_{12}\text{H}_{11}\text{S-SB}_{12}\text{H}_{11}^{-4}$, postulated by Soloway and Hatanaka to be responsible for the good tumor uptake properties seen in samples of impure $\text{B}_{12}\text{H}_{11}\text{SH}^{-2}$.

This was confirmed by the following elemental analysis ^{for} $\text{Cs}_4\text{B}_{12}\text{H}_{11}\text{SSB}_{12}\text{H}_{11} \cdot \text{H}_2\text{O}$

	Cs	B	H	S	H ₂ O
Calculated:	59.37	28.98	2.70	7.16	2.01
Found:	59.61	28.77	2.86	7.29	0.80

Further confirmation of the expected C_2 symmetry of this molecule was obtained by ^{11}B nmr which gave a spectrum similar to that of $\text{B}_{12}\text{H}_{11}\text{SH}^{-2}$. The presence of a disulfide group was positively demonstrated by a Raman absorption at 496 cm^{-1} which is quite characteristic of this bond.

The tendency of this disulfide ^{to fission} to free radicals was observed. The radicals formed are postulated to be either the thiyl radical $\text{B}_{12}\text{H}_{11}\text{-}\dot{\text{S}}^{-2}$, or thiol

radical $B_{12}H_{11}-\dot{S}-H^{-1}$. Because of the importance of such radicals in polymerization reactions which we have observed, the kinetics of formation of this radical was studied by us (see Part II).

(These studies, including the synthesis of the disulfide, have been submitted for publication).

II. The Thiyl Radical, $B_{12}H_{11}-\dot{S}^{-2}$, Produced From the Disulfide, $B_{12}H_{11}SSB_{12}H_{11}^{-4}$.

When the sodium salt of the disulfide, $B_{12}H_{11}SSB_{12}H_{11}^{-4}$, is added to DMF and trifluoroacetic acid ($5 \times 10^{-3}M$) at $25^{\circ}C$, the solution turns blue giving rise to a maximum absorbance of $0.465 \times 10^4 S_0$ (λ_{max} , 630 nm), where S_0 is the initial disulfide concentration (10^{-4} to $2 \times 10^{-3}M$). Initial $t_{1/2}$ readings of 343 sec at $4^{\circ}C$ and 190 sec at $25^{\circ}C$ were observed on a Bausch & Lomb spectronic 70. For times up to the initial $t_{1/2}$, the intensity of the blue color (B) at $4^{\circ}C$ for the various concentrations of the disulfide corresponds well to the following expression: $B^{3/2} = 3.270 \times 10^2 S_0^{3/2} t$, where again S_0 is the initial disulfide concentration and t , the time.

This equation takes into account the observation that different initial concentrations of the disulfide have the same initial $t_{1/2}$. Normally, this fact implies that the log of the observable (in this instance color absorbance) is linear with time. However, here the log function is not linear; only a $B^{3/2}$ plot is linear. (Figure 2 shows plots of the blue color raised to some power or as log B versus time for a run involving $2 \times 10^{-4}M$ $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ at $4^{\circ}C$).

Varying the concentration of trifluoroacetic acid, which is completely dissociated in DMF, at a constant disulfide concentration of $1.8 \times 10^{-4}M$ suggests an inverse relationship between the rate of blue formation and $[H^+]$ of the form, $dB/dt \propto 1/(1+k/[H^+])$ where k is a constant (see Table II). A similar blue color

was formed in trifluoroacetic acid solutions of acetonitrile, formic acid, acetone, and ethanol. Aqueous solutions of the disulfide (0.01 M) at pH 2 are yellow, but go reversibly to blue on heating to 90°C. An esr spectrum of the blue DMF solution taken with an Alfa X-Band Spectrometer at 100 KHz showed a resonance with a g factor of 2.019, similar to literature values for sulfur radicals, and a $w_{1/2}$ of 19.3 gauss. No fine structure was observed. The esr and blue color disappeared when excess sodium ethoxide was added to the disulfide-DMF-trifluoroacetic solution. Adding further trifluoroacetic acid returned the esr and blue color which proceeds to fade slowly ($t_{1/2}$, 8 days) on standing at 25°C (see Figure 3).

These data are consistent with the formation of either a thiyl radical, $B_{12}H_{11}\dot{S}^{-2}$, or thiol radical, $B_{12}H_{11}\dot{S}-H^{-1}$. Rapid exchange of protons in solution with the sulfur radical would interconvert these two forms and be consistent with the lack of hyperfine splitting in the esr. The stability of these sulfur radicals

is enhanced by the negative charges on the borane polyhedral cage. Associating the blue color $\tilde{B}$ with the thiyl radical and then by substituting stoichiometrically $2[S_0-S]$ for $\tilde{B}$ into the previous equations for $\tilde{B}$ and $d\tilde{B}/dt$, where S_0 is the initial and S the time dependent disulfide concentration suggests that the disulfide is protonating before fission and then undergoes a complex mechanism of fission. The fission reaction is reversible and goes essentially to completion throughout the disulfide concentration range investigated. The same blue color is formed, though less intensely when the following oxidized $B_{12}H_{11}SH^{-2}$ derivatives are heated to reflux in DMF and trifluoroacetic acid for 30 minutes:

Each of these are capable of forming the borane thiyl radical by the fission

of a sulfur-sulfur bond.

The formation and characterization of the thiyl radical, $B_{12}H_{11}-\dot{S}^{-2}$, is exciting in that it is the first reported stable thiyl radical at room temperature. Subsequently, this study paved the way for the understanding and synthesis of a class of blue radical polymers which show outstanding tumor uptake properties. (see Part IV).

See ~~COO-3334-20~~ for our communication on $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ and the Thiyl Radical, $B_{12}H_{11}-\dot{S}^{-2}$.

III. The Borane, $B_{12}H_{10}(SH)_2^{-2}$, Found in Impure Samples of $B_{12}H_{11}SH^{-2}$.

1) Characterization of an Oxidized Product Showing Excellent Tumor Uptake Properties.

In this section, we describe the identification of a side product formed during the synthesis of $B_{12}H_{11}SH^{-2}$ by the reaction of H_2S on $B_{12}H_{12}^{-2}$.

The oxidation by H_2O_2 of a supposedly pure sample of $Cs_2B_{12}H_{11}SH^{-2}$ which was crystalline and gave a single spot by TLC was observed to give rise to two products. This product mixture was found to have good tumor uptake properties (see A.E.C. Report 1974). It has since been shown that one of these oxidation products is $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$. A different sample of $Cs_2B_{12}H_{11}SH$ prepared more recently was found, however, to give rise to only a single product under conditions of oxidation identical to that under which the previously mentioned sample of $Cs_2B_{12}H_{11}SH$ gave two products. This single product is $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$. In attempts to determine the cause and nature of the other unidentified product we spent considerable effort varying reaction conditions, adding inorganic ions which could conceivably have been present in the original oxidation reaction, and using different samples of H_2O_2 , all with negative results. Finally, using a Sephadex column, we were able to show that a sample of $B_{12}H_{11}SH^{-2}$, which oxidized to give two products, contained

a borane impurity which was chromatographically similar to $B_{12}H_{11}SH^{-2}$ and which oxidized to give a material which was not $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$. Samples of $B_{12}H_{11}SH^{-2}$ which contained this impurity reacted with dithiobis-(2-nitrobenzoic acid) (DTNB, a thiol specific reagent) to give two chromatographically separable materials of Rf 0.37 and 0.16, whereas pure $B_{12}H_{11}SH^{-2}$ gave rise to only a single product with DTNB of Rf 0.37.

It was determined that a mixture of the DTNB derivatives could be separated by a solvent extraction procedure under conditions of controlled pH.

The DTNB derivatives of $B_{12}H_{11}SH^{-2}$, a mixed disulfide ($B_{12}H_{11}SSC_6H_3NO_2COOH^{-2}$; Rf 0.37), had been previously characterized by us (see Part 1). The other DTNB derivative (Rf 0.16) was identified as the bis disulfide $B_{12}H_{10}(SSC_6H_3NO_2COOH)^{-2}_2$ by elemental analysis.

Analysis for $[(CH_3)_4N]_2B_{12}H_{10}(SSC_6H_3NO_2COOH)_2$

	C	H	N	S	B
Calculated:	35.0	5.56	7.42	16.96	17.6
Found:	34.76	5.09	7.37	17.19	17.03

A uv maximum at 365 mμ is the same for $B_{12}H_{11}SSC_6H_3NO_2CO_2H^{-2}$ but with twice its extinction coefficient ($\epsilon = 9.36 \times 10^3 \text{ l m}^{-1} \text{ cm}^{-1}$). The ^{11}B nmr shows a singlet of area 2 at 7.0 ppm. and a doublet of area 8 at 15.8 ppm. ($J(B-H)$, 120 Hz; for B-H). Although the decoupled peak at 15.8 was narrowed to a $w_{1/2}$ of 43Hz (from 148Hz), no resolution of overlapping peaks was attained in spite of the asymmetry of the coupled doublet. This fact in addition with our inability to crystallize any sample of $B_{12}H_{10}(SSC_6H_3NO_2CO_2H)^{-2}_2$ suggests that it is a mixture of geometrical isomers, specifically 1-2, 1-7, and 1-12 disubstituted.

2) Regeneration of the Newly Discovered Borane - $B_{12}H_{10}(SH)_2^{-2}$.

As a final note to its identity, 1.0 g of $(Me_4N)_2B_{12}H_{10}(SSC_6H_3NO_2CO_2H)_2$ was converted to the Na^+ salt by ion exchange and reduced with 0.5 ml of mercaptoethanol at pH 11. After one day, the pH was adjusted to one and the solution was extracted with ethylacetate until the extracts were clear. Adding aqueous tetrabutylammonium chloride to a portion of the aqueous layer gave a precipitate $(Bu_4N)_2B_{12}H_{10}(SH)_2$, which, when dried, had an infrared spectrum containing pronounced peaks at 12.25μ and 10.3μ , which were observed to be weakly present in the original mixture. TLC of the solution indicated that its R_f was identical with that of $B_{12}H_{11}SH^{-2}$ and nitroprusside reagent indicated the presence of thiol groups.

3) Oxidation of a Sample Containing 50% $B_{12}H_{11}SH^{-2}$ and 50% $B_{12}H_{10}(SH)_2^{-2}$.

A sample shown to contain approximately 50% $Cs_2B_{12}H_{11}SH$ and 50% $Cs_2B_{12}H_{10}(SH)_2$ by TLC of their DTNB derivatives was oxidized and the mixture (TW001C) was given I.P. to rats: (A.E.C. Report NO. COO-3334-5). After 96 hours, the tumor contained $17.67 \mu g$ B/gm tumor and the tumor:blood boron concentration ratio was 5.8. When a sample containing only $Cs_2B_{12}H_{11}SH$ was given to rats, the tumor:blood boron concentration ratio did not exceed two after 96 hours. Thus $B_{12}H_{10}(SH)_2^{-2}$ must be present during the oxidation of $B_{12}H_{11}SH^{-2}$ or by itself in order for the product to show good tumor uptake properties by a single injection.

4) Structure of the Oxidized Product Responsible for the Excellent Tumor Uptake Properties.

As $B_{12}H_{10}(SH)_2^{-2}$ is disubstituted, its oxidation product is consequently a borane polymer with -SOS- linkages similar to that found in $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$. A limited amount $B_{12}H_{11}SH^{-2}$ during oxidation would act as chain terminators and restrict the length of the polymer.

Example:

The possibility that all short anionic borane polymers might demonstrate good tumor binding properties in our animal tumor model has some support as indicated in work reported in the next section.

IV. Polymers from Borane Thiyl Radicals.

The excellent tumor uptake results of an anionic borane polymer containing $\text{O}=\text{S}-\text{S}-$ linkages, described in part II, encouraged us to examine the tumor uptake properties of other anionic borane polymers. In our report last year, we described the formation of a blue polymer made by concentrating an acidic solution of what is now known to be $\text{B}_{12}\text{H}_{11}\text{SOSB}_{12}\text{H}_{11}^{-4}$. A solution of this blue polymer was given I.P. (29.5 $\gamma\text{B/g}$ rat) to tumor bearing rats (Figure 4).* Although this result was encouraging it nevertheless fell short of the 5.8 ratio found for TW001C (see part III), a -SOS- linked borane polymer. The possibility that the polymer was restricted from leaving the intraperitoneal cavity initiated a further study of this compound using intravascular injection (I.V.). The material was shown to be fairly toxic relative to other boranes tested in this laboratory in a preliminary toxicity study (Table III). Even on injections as low as 10 $\gamma\text{B/g}$ rat, toxicity was evident. Consequently this polymer had to be discarded from further studies as the high toxicity would preclude clinical use. Other polymers investigated by us include a borane tetramer (given to us by Dr. R. Bechtold) and a high molecular weight purple borane polymer. The borane tetramer, actually shown by us to be composed of several different compounds was injected into tumor bearing rats and gave tumor:blood boron concentration ratios of 1.5 after 96 hours. The purple borane polymer ($[\text{B}_{10}\text{H}_8 \text{NNC}_6\text{H}_4\text{NN}]_n \text{B}_{10}\text{H}_9)^{-Y}$ synthesized by Tolpin et. al. (2) was shown to

(* NOTE: After 96 hours, the tumor:blood boron concentration ratio was 1.7)

be very high molecular weight by chromatography on Sephadex G-50. This material was toxic in rats at even a dose of 2 mg B/kg rat and was consequently not further studied.

The toxicity of these polymers relative to T0001 (part II) was postulated to be in part due to the chain length of these polymers. Consequently, we directed our efforts to producing other polymers with shorter chain lengths.

1) Postulated Mechanism for the Formation of the Blue Polymer Derived from $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$.

A blue color similar to that found in the blue polymer derived from $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ is formed when the sodium salt of disulfide, $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ (described in part I), is dissolved in dimethylformamide or formic acid as described in our communication entitled "Synthesis of Dithiobis-(undecahydro-dodecaborate(-2)) and of a Derived Thiyl Radical" (see C00-3334-10).

As described in the next section, this blue color was associated with the thiyl radical, $B_{12}H_{11}\dot{S}^{-2}$ or the thiol radical, $B_{12}H_{11}\dot{S}-H^{-1}$. This suggested that the blue polymer derived from $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ had a terminal thiyl radical which gave it similar visible spectral characteristics to $B_{12}H_{11}\dot{S}^{-2}$. An electron spin resonance spectra of this polymer in DMF- H_2O showed a g factor of 2.020 with a $w_{1/2}$ of 16.5 gauss confirming the presence of a thiyl radical. Analogous to the $B_{12}H_{11}\dot{S}^{-2}$ radical, the blue polymer in DMF lost its color with the addition of base, (sodium ethoxide), and regained it after the addition of excess trifluoroacetic acid. From this information we hypothesized the mechanism in Figure 4 for the formation of the blue polymer.

According to this mechanism, a sulfide linked borane polymer is produced with a terminal thiyl radical (See Figure 5- IIA). Under basic conditions, it converts to the disulfide form (See Figure 5-IIIB).

It was suggested that the toxicity of the blue polymer was due to the radical terminal group of the polymer. Therefore, we tested the base treated, disulfide form of this polymer, which is colorless, for toxicity. As shown in Table II the toxicity changed very little.

2) A Shorter Blue Polymer with Excellent Tumor Uptake and Low Toxicity.

The hypothesized mechanism of formation of the blue polymer (See Fig. 3-IIIA) from $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ suggested that a similarly sulfide linked polymer could be derived from the disulfide, $B_{12}H_{11}SSB_{12}H_{11}^{-4}$. In fact when 1 mmol of the disulfide in the hydrogen ion form was concentrated to a syrup at room temperature, a yellow color developed. When 20 microliters of this material was dissolved in 1 ml of DMF it turned blue indicating the presence of thiyl radical. This blue polymer is shorter than the one derived from $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ based on the observations in the following section.

3) Evidence Supporting the Shorter Chain Length of the Blue Polymer Derived from the Disulfide.

The blue polymers in aqueous solution seem to obey the following overall equilibrium in analogy to $B_{12}H_{11}SSB_{12}H_{11}^{-4}$.

In concentrated acidic disulfide solution, the syrupy polymer stays in the center according to LeChatelier's principle forming a protonated yellow species. Here the rate of radical recombination, $k_r[Pol-S]^2$, is much greater than the rate of radical fission, $k_f[Pol-S-S-Pol]$. [Note: α and β are kinetic parameters probably not simply related to the above expression in analogy with mechanistic

studies on $B_{12}H_{11}SSB_{12}H_{11}^{-4}$]. Upon several fold dilution, the disulfide dissociates forming the blue radical where now the rate of radical recombination is much less than the rate of radical fission. Finally, in basic solution the disulfide is deprotonated forcing the radical and the disulfide to the far left or unprotonated disulfide having no color.

When 20 microliters of the yellow syrup of the polymer derived from $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ is dissolved in 1 ml water, the solution is yellow blue indicating only partial dissociation to the radical state. However when 20 microliters of the yellow syrup of the polymer derived from $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ is dissolved in 1 ml water, the solution turns blue indicating complete dissociation to radicals. Thus, the equilibrium constant K , where $K = [Pol-S.]^2 / [Pol-\overset{H}{S}-\overset{H}{S}-Pol]$, is greater for the $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ derived polymer than for the $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ derived polymer. From thermodynamics, it is known that the free energy, ΔG , of a reaction is given by the following expression:

$$\Delta G = \Delta H - T\Delta S = -RT \ln K$$

Where ΔH is the change in enthalpy, T the temperature, ΔS is the change in entropy, R is the gas constant, and K the equilibrium constant. Therefore, the decrease in free energy for dissociation of the $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ derived polymer is greater than for the $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ derived polymer.

Suppose that the change of entropy was zero, then the change of enthalpy would be more negative for the dissociation of the $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ polymer than for the $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ polymer. If both polymers are simply chains of dinegatively charged cages, then the polymer having the greater length and subsequent negative charge $(-X)$ will release the most energy on dissociation into $(-\frac{X}{2})$ charged radicals according to the expression for the change in electrostatic potential which is proportional to $(-X/2)^2$. By this argument, the $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$

derived polymer is longer. On the other hand suppose that the enthalpy is zero, then the change of entropy must be more positive for dissociation of the $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ polymer. Using a kinetics argument here and assuming that the change of entropy in the forward direction to the transition state to be zero for both undissociated polymers, then the change of entropy for the overall reaction is only that entropy change from the thiyl radical polymer state to the recombination transition state. Now a longer chain thiyl radical would have a fewer percentage of states with the thiyl radical "sticking out", available for dimerization than a shorter chain polymer. Since the change in entropy here is proportional to the log of the number of states of the transition state divided by the square of the number of states of the thiyl radical polymer, the longer chain thiyl radical polymer will have the greater entropy change.

This greater change in free energy for dissociation of the $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ derived polymer is also observed when it takes several hours for the blue color to fade in the $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ derived polymer in DMF after adding base. In the $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ derived polymer, the blue color fades under similar conditions in several minutes. The rate of disappearance of the blue color is dependent here only on the rate of recombination of thiyl radicals since the base restricts any disulfide from dissociating. And the rate of recombination depends on the charges of the polymers and on the number of conformations possible.

We have been developing a gel filtration system to determine the molecular weights and the corresponding length of these polymers; however this system cannot as yet resolve boranes with greater than a (-6) charge and thus could not determine the blue polymers' molecular weights.

4) Biological Testing of $B_{12}H_{11}S^{-2}$ Radical Derived Polymers.

The four polymers, 1) Blue $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ derived polymer; 2) Colorless base-treated $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ derived polymer; 3) Blue $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ derived polymer; and 4) Colorless base-treated $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ derived polymer were tested for toxicity in young male Wistar rats in the following manner. Each polymer was given to three pairs of animals. One pair received 10γB/g, a second pair, 25γB/g and a third pair, 50γB/g, by I.V. injection of an approximately isotonic solution of the borane polymer at pH 7. The resultant death rates are given in Table III. As a result of this toxicity test, the most non-toxic polymer, i.e. the colorless base-treated $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ derived polymer, was administered to tumor bearing rats at a dose of 50γB/g. The rats were sacrificed at intervals and the tissue distribution of boron at these times determined. (See Table IV which summarizes these results.)

It can be seen that significant tumor binding does take place. This observation combined with its apparent low toxicity makes this compound an extremely promising one.

V. A Side Product in the Synthesis of $B_{12}H_{11}SH^{-2}$ from H_2S and $B_{12}H_{12}^{-2}$.

In last year's progress report we described the isolation of the pyridine salt, a major side product from the H_2S - $B_{12}H_{12}^{-2}$ synthesis of $B_{12}H_{11}SH^{-2}$. Fractional recrystallization yielded light green crystals which were found by TLC to be composed of two boranes with an overall boron to sulfur ratio of about 36:1 (via chemical analysis). When this material was converted to the sodium form and given to rats (50 mg B/Kg rat), the resulting tumor:blood boron concentration ratios were poor. After oxidation with H_2O_2 , the material showed little improvement in this biological property. As encouraging uptake results were obtained at this time with polymers, we decided not to pursue the characterization and testing of this material.

VI. Phosphate Derivatives Including Rat Studies.

As stated in our last proposal we were interested in further investigating some of the phosphate borane derivatives which had been synthesized by Dr. Alexander Kaczmarczyk at Tufts University. Some of these boranes had, in our rat tumor model, demonstrated good tumor binding capacity with high tumor: blood and tumor:brain boron concentration ratios.

Of those phosphate derivatives which had shown evidence of good tumor binding abilities in our laboratories, we selected two that were most easily synthesizable. These were the $B_{12}H_{12}^{-2}$ derivative $B_{12}H_{10}(OPO(OH)OPO(OH)_2)_2^{-2}$ which is derived from the available dihydroxy compound $B_{12}H_{10}(OH)_2^{-2}$ and the $B_{20}H_{18}^{-2}$ derivative $B_{20}H_{16}OPOH^{-2}$ which is derived from the equally available $B_{20}H_{17}OH^{-3}$. For the purpose of synthesizing these compounds we employed Dr. R. Bechtold, a recent graduate student of Dr. Kaczmarczyk, who had had personal experience in the synthesis of the $B_{12}H_{10}(OPO(OH)OPO(OH)_2)_2^{-2}$ and who had acquired considerable experience in such phosphate derivative synthesis during her doctorate work.

The reaction sequences for each of these phosphate derivatives are as follows:

and

Despite the fact that these synthetic routes had been worked out in Dr. Kaczmarczyk's group, considerable difficulty was experienced by Dr. Bechtold in repeating these reactions, particularly the phosphorization steps, during which several products were produced. The materials which we tested in rats proved to be quite toxic and although they did demonstrate tumor:blood boron ratios great than 1, the amounts in the tumor were small (See Table V).

One of our conclusions (see parts III and IV of this report) is that anionic borane polymers tend, as a class, to possess tumor binding capabilities. The formation of such polymers by a reaction between hydroxylated $\text{B}_{12}\text{H}_{12}^{-2}$ cages and phosphoryl chloride appeared to us to be quite possible, even probable:

Also we have obtained evidence in these laboratories that the $\text{B}_{12}\text{H}_{12}^{-2}$ cage is attacked by chloro-phosphorus reagents (specifically $\text{Cl}_2\text{P}(\text{O})\text{C}_6\text{H}_4\text{NO}_2$); thus it is feasible that the direct coupling by phosphorous of $\text{B}_{12}\text{H}_{12}^{-2}$ or $\text{B}_{20}\text{H}_{18}^{-2}$ cages could have occurred under the reaction conditions carried out by Dr. Bechtold leading to the formation of the polymers.

In order to test the possibility that such hypothetical polymers could have been carried along in the subsequent workup of the phosphorylation reactions, we conducted some preliminary dialysis experiments. Although this work cannot be regarded as being conclusive, the slow rates of dialysis observed of the phosphorylated boranes relative to monomeric boranes tends to support this hypothesis. A further point of evidence is that the borane phosphates possess a low to zero R_f in our anion exchange TLC systems. This is a characteristic we associate with multiple linked borane cages.

VII. Laboratory Synthesis of $B_{12}H_{11}SH^{-2}$.

1) Background.

In last year's progress report, we described our initial attempt to find an electrophilic reagent which would allow the synthesis of $B_{12}H_{11}SH^{-2}$ in the laboratory.

Our approach was to synthesize a sulfide derivative of $B_{12}H_{12}^{2-}$, i.e. $B_{12}H_{11}SR^{-2}$, which could be cleaved to $B_{12}H_{11}SH^{-2}$ under appropriate conditions. Several potential reagents which we investigated last year failed to give a cleavable sulfide derivative. A reagent which had properties that might overcome previous difficulties was acetyl sulfenyl chloride, $ClSCOCH_3$, which we proposed to investigate last year. We now report that the combination of this reagent with $B_{12}H_{12}^{2-}$ does indeed produce $B_{12}H_{12}SH^{-2}$ -- in a single step reaction! However, at this writing we have not achieved a large scale isolation of pure $B_{12}H_{11}SH^{-2}$ by this reaction.

2) The Reaction Conditions for Production of $B_{12}H_{11}SH^{-2}$ Using $ClSCOCH_3$

Initial reaction trials with $ClSCOCH_3$ and $Na_2B_{12}H_{12} \cdot 2H_2O$ in acetonitrile gave $B_{12}H_{11}SH^{-2}$ and $B_{12}H_{10}(SH)_2^{-2}$ as the major products. These

were both initially identified by TLC after conversion to the mixed disulfide derivative using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). Evidently ClSCoCH_3 was acting as a thiolating reagent by the following scheme:

Reaction trials consisted of adding several microliters of ClSCOCH_3 to 0.0357 mmol of $\text{Na}_2\text{B}_{12}\text{H}_{12} \cdot 2\text{H}_2\text{O}$ in 0.4 ml acetonitrile plus five to twenty microliters water. Changing an acid ($\text{CF}_3\text{CO}_2\text{H}$) or base (pyridine) for the added water did not alter the reaction course. Neither did the use of diglyme instead of acetonitrile. If water was not added to the acetonitrile, a TLC baseline material appeared which was converted to $\text{B}_{12}\text{H}_{11}\text{SH}^{-2}$ (by TLC) after the addition of water. This baseline material was probably the very hydrolytic thioester $\text{B}_{12}\text{H}_{11}\text{SCOCH}_3^{-2}$.

3. Report on the Scale-Up of the Synthesis of $B_{12}H_{11}SH^{-2}$ by $ClSCOCH_3$

a) Production of ClSCOCH_3

The synthesis of ClSClOCH_3 by William Bonner (J. Am. Chem. Soc., 1950) should be used. Several other literature methods were not reproducible. Starting reagents must be doubly distilled before use in order to approach the literature yield of ClSClOCH_3 .

b) Isolation of the DTNB Derivatives of Products from $B_{12}H_{12}^{-2}$ and $ClSCOCH_3$.

Tetramethylammonium bis-(3-carboxy-4-nitrophenyldithio)decahydro-dodecaborate(-2), $[(CH_3)_4N]_2 B_{12}H_{10}(SSC_6H_3NO_2CO_2H)_2^{-2}$. The reaction was run by adding to 40 ml CH_3CN and 1 ml H_2O 0.808g (3.58 mmol) of $Na_2B_{12}H_{12} \cdot 2H_2O$. To this was added slowly 1 ml of $ClSCOCH_3$ in increments of 50 μ l. After 30 minutes, the solvents were rotary evaporated and 20 ml of 1N NaOH was added to dissolve the residue. The solution was then acidified with concentrated HCl to pH 1 and after cooling in ice and filtering, 1.5 g of

5,5'-dithiobis-(2-nitrobenzoic acid), $[\text{SC}_6\text{H}_3(\text{NO}_2)\text{CO}_2\text{H}]_2$, was added. The pH was adjusted to 11 with 6N NaOH to give a dark red solution. After 24 hours, the pH was readjusted to one and the solution cooled. The unreacted $[\text{SC}_6\text{H}_3(\text{NO}_2)\text{CO}_2\text{H}]_2$ precipitated and after filtration the pH was adjusted to 2.6. To remove some byproduct $\text{HSC}_6\text{H}_3(\text{NO}_2)\text{CO}_2\text{H}$, the solution was continuously extracted with ether for 12 hours, readjusting the pH periodically. Adjusting the pH to 1.6, continuous ether extraction selectively removed $\text{H}_2\text{B}_{12}\text{H}_{10}(\text{SSC}_6\text{H}_3\text{NO}_2\text{CO}_2\text{H})_2$ according to TLC (Rf, 0.16). (The aqueous layer was set aside for the isolation of $\text{B}_{12}\text{H}_{11}\text{SSC}_6\text{H}_3\text{NO}_2\text{CO}_2\text{H}^{-2}$). After extracting the ether once with one volume of HCl (1N), one volume of petroleum ether was added and the borane extracted into water. The aqueous layer was reduced to a volume of 10 ml and aqueous excess $\text{Me}_4\text{N}^+\text{Cl}^-$ was added to precipitate a yellow salt. After filtering and then drying in vacuo, the yellow product weighed 0.50g (16%). The product was identical by TLC, and i.r. to $((\text{CH}_3)_4\text{N})_2\text{B}_{12}\text{H}_{10}(\text{SSC}_6\text{H}_3\text{NO}_2\text{CO}_2\text{H})_2$ described in part III.

Tetramethylammonium (3-carboxy-4-nitrophenyldithio) undecahydrododecaborate(-2) hydrate, $[(\text{CH}_3)_4\text{N}]_2\text{B}_{12}\text{H}_{11}\text{SSC}_6\text{H}_3\text{NO}_2\text{CO}_2\text{H} \cdot 2/3\text{H}_2\text{O}$. The aqueous layer set aside in the isolation of $[(\text{CH}_3)_4\text{N}]_2\text{B}_{12}\text{H}_{10}(\text{SSC}_6\text{H}_3\text{NO}_2\text{CO}_2\text{H})_2$ is adjusted to pH=1.75 and extracted twice with one volume of ethylacetate. After adjusting the pH to 0.0, the aqueous layer is extracted three times with ethylacetate. After combining the last three extractions, one volume of petroleum ether is added and the solution extracted with water. The aqueous solution is reduced in volume to 5 ml, and excess tetramethylammonium chloride is added. The solution is filtered and the precipitate dried to yield 0.65g (35%) of a yellow salt (Rf, 0.35). The borane can be recrystallized from water by adding base until the salt just dissolves and then slowly cooling the solution. The product was identical by TLC and i.r. to $[(\text{CH}_3)_4\text{N}]_2\text{B}_{12}\text{H}_{11}\text{SSC}_6\text{H}_3\text{NO}_2\text{CO}_2\text{H}$ described in last year's progress report.

Attempts to reduce $B_{12}H_{11}SSC_6H_5NO_2CO_2H^{-2}$ at pH 8 with a ten fold excess of mercaptoethanol or thioglycolic acid produced $B_{12}H_{11}SH^{-2}$ by TLC. The mercaptan can be removed after reduction by extraction with ethylacetate at pH 2. The remaining product, by TLC, is 90%+ $B_{12}H_{11}SH^{-2}$ and several percent baseline. Attempts to crystallize out $B_{12}H_{11}SH^{-2}$ without co-crystallizing the baseline material have failed with all the cations available to us. Thus although we have the $B_{12}H_{11}SH^{-2}$ isolated in a pure derivative form, we are presently unable to complete the synthesis.

VIII. Borane Binding to Proteins and Blood Distribution.

1) Protein Binding Studies

This experiment was carried out in order to determine if any of the $B_{12}H_{11}SH^{-2}$ derivatives would bind sufficiently tightly to proteins such that they would not be removed from the protein solution by exhaustive dialysis. If this occurred, it could have implications with regard to the tumor binding properties that these compounds show.

In the first series of tests, solutions of six different boranes were added to buffered $2 \times 10^{-5}M$ human gamma globulin at pH 7.0 and 8.5. The final borane concentrations were $2 \times 10^{-3}M$. As this generally produced cloudy solutions or outright precipitate, the experiment was modified and BSA was used as the protein. To 3 ml of buffered BSA (pH 7.0 and 8.5) aqueous solutions of the borane derivatives, which previously had been found to be dialyzable, were added so that the final solution was $2 \times 10^{-2}M$ in BSA. After 14 days of exhaustive dialysis, the non-dialyzable material was analyzed for protein and boron. In no instances were significant amounts of boron found in the dialysis bags thus tending to rule out the possibility of any covalent binding between the borane and BSA.(Table VI).

2) Dog Blood Experiment

This experiment was a preliminary investigation designed to reveal differences in the behavior of $B_{12}H_{11}SH^{-2}$ and $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ in blood in order to further our understanding of the differences observed in the behavior of these compounds with regard to tumor binding capacity and blood clearance rates (see Figure 6).

The behavior of these compounds in blood is of interest because the transport of a compound to the tumor is via the blood. Any mechanism of entry into the tumor must necessarily involve the compound as it occurs in the blood, and the rate of kidney excretion of a compound will depend on its rate of transport across the glomeruli. This in turn will depend on the proportion of compound which is neither protein nor cell-bound. To obtain initial information pertaining to these questions, experiments were carried out in which the boranes were gently mixed with anti-coagulated (citrate-dextrose) whole dog blood after which the plasma and cells were separated. Part of the plasma was ultra-filtered to obtain a protein free fraction. Boron analysis of the plasma and the protein free ultrafiltrate together with the blood hematocrit and original whole blood boron concentration permitted the boron distribution between the cells and plasma and the protein and non-protein boron distribution within the plasma to be determined. These results for the anions $B_{12}H_{11}SH^{-2}$ and $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$ are strikingly different (see Table VII). Approximately 14% of $B_{12}H_{11}SH^{-2}$ in the blood is in association with the cellular components, and of the remainder in the blood approximately 22% is non-protein bound. In the case of $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$, however, all that present in the blood is associated with plasma proteins (within experimental error). These plasma distribution data are consistent with the relative rates of blood clearance of these two anions as discussed above and studies of this type appear to hold potential for predicting the blood clearance behaviors of such compounds.

IX. Biological Studies - Dog Irradiation Studies at M.I.T. Reactor

The limiting factor in determining the radiation dose in man in the proposed boron-slow neutron capture therapy is, we think, the dose just tolerated by the blood vessels in the normal brain. They must not develop a radiation fibrosis and a consequent ischemic necrosis of the normal tissue. This dose has never been determined. We hope to approximate it for man by a series of irradiations on the normal canine brain. The configuration of the irradiation portal at the M.I.T. Reactor operating room has been selected which will be approximately that for the human radiations. This will keep the ratios of fast neutrons, slow neutrons and gamma rays the same in the dogs as in the patients - obviously an important factor in increasing the validity of extrapolating from dog to man.

Seven dogs were irradiated at the M.I.T. nuclear reactor during September 1972 (Series I, 4 dogs) and January 1973 (Series II, 3 dogs) simulating conditions of patients undergoing neutron capture therapy.

The specific details of the irradiations are given in the 1973 report (COO-3334-2) report, and hence will not be described at length here. In brief, however, each dog was given an infusion of 95.0 - 97.2% ^{10}B enriched $\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$ from Shionogi Research Laboratory, Osaka, Japan, supplied by Dr. Hiroshi Hatanaka who collaborated with us on these dog studies. Approximately 22 hours before irradiation two dogs (R-dogs #1 and 4) in Series I received 48 mg B/Kg while the other two dogs (R-dogs #2 and 3) received 24 mg B/Kg. The first dog (R-dog #5) in Series II received no boron before irradiation (to test the effects of radiation without boron) while R-dog #6 received 28 mg B/Kg and R-dog #7 received 20 mg B/Kg. Dogs in Series II were irradiated approximately eight hours after the boron infusion.

The dogs were taken to the Therapy Room at the M.I.T. reactor, where craniectomies were performed following which the dog was placed appropriately and precisely at the radiation portal and the exposed brain irradiated for a specific number of seconds. For details on exact technical procedure and dosimetry for each dog, see the 1973 report (COO-3334-2).

Specific details of the dogs in Series I (R-dogs #1, 2, 3, and 4) are given in last year's report (COO-3334-3) and will not be described in detail here. They were sacrificed approximately one year after irradiation. Head autopsies were performed and gross autopsy descriptions were written up. Dr. Edward P. Richardson, Jr., neuropathologist at the Massachusetts General Hospital, dissected these brains after proper fixation and selected the appropriate areas for study. Histological slides were made from both the normal and irradiated sides of the brain. Dr. Tavcor, a neuropathologist and assistant to Dr. Richardson, examined these slides in detail. He could find no damage to the brains.

We planned to keep the dogs in Series II (R-dog #5, received irradiation but no boron and R-dogs #6 and 7, received both boron and irradiation) alive for two years. They were checked periodically, especially for adverse neurological signs. The dose data are given in the following table:

DOSE DATA FOR INCIDENT TISSUE LAYER
OF TUMOR-FREE DOG BRAINS IRRADIATED
WITH THE THERAPY BEAM OF THE M.I.T. REACTOR

Dog #	Total Dose to Boron-free tissue (rads)	Dose due to $^{10}\text{B}(n,\alpha)^7\text{Li}$ (rads)	Total dose to tissues containing ^{10}B (rads)
R-Dog #6	1444	2600	4044
R-Dog #7	1495	1095	2590

R-dogs #5 and 7 were kept alive and were without any adverse neurological signs for approximately 25 months, at which time they were brought from the kennel into the Animal Farm at the Massachusetts General Hospital. Physical examinations were performed by Dr. Teodoro Dagi, one of our neurosurgeons. Both dogs were alert and responded well to their environment, and grossly seemed neurologically normal. Dr. Dagi performed head autopsies on both dogs and no abnormalities were observed. The brains were removed and placed in formalin, and are now waiting to be sectioned so that the radiated areas and corresponding normal areas can be studied in detail histologically.

R-dog #6 was well and active until about 17 months following irradiation. At that time he began slowing down but not alarmingly until a few days later when he had a grand mal seizure. The kennel notified us of this development and we had him transported by car to the Animal Farm at the Massachusetts General Hospital. He had two mild seizures on the trip to the hospital, and he then became stuporous. He was given a neurological examination by Dr. Quindlen, a neurosurgeon, who found the following:

- No placing reflex and decreased hopping reflex.
- Almost no spontaneous movements.
- Respiration regular, but shallow, 20/min.
- Pupils equal and reactive to light, bilaterally.
- Cannot tell extent of lateral gaze, decreased left corneal reflex.
- No spontaneous movements of tongue.
- Will not open jaw, when mouth opened, tongue was flaccid bilaterally.
- Moves all four limbs to pain, left less than right.
- Possibly mild paresis on left side.
- Will not right himself, but remains motionless on side.

Dr. Quindlen's clinical impression was:

- ?? Increased intracranial pressure or post-ictal state.
- ?? Lesion in right hemisphere.
- ?? Internal hydrocephalus.

Dr. Quindlen then sacrificed the dog. The following are the autopsy notes of the head:

A midline incision was made with scalpel down back of head. Scalp was dissected away from skull. There was almost no temporalis muscle on right side (the side of original craniotomy performed for the radiation). A cut was made in spine between C3 and C4. Skin and muscle were cut all around the neck. The dog was then decapitated with bone cutters. Temporalis

muscle was removed from left side; such muscle was nearly non-existent on right side (site of operation).

Skull was removed. The brain did not show marked swelling or flattening of gyri (Figure 7).

Left cerebrum was firm, and right was somewhat softer.

Right side of brain at site of radiation showed a lesion on right posterior parietal region which was covered with a yellow plaque*, an extradural collection of grumous material which may have been an old epidural hematoma, measuring 1.5 cm in diameter. This material was thin and saucer-like and probably not a space-occupying lesion.

Brain was removed and placed in 10% formalin.

Dura was left intact on brain.

Anterior lobe of pituitary was removed and placed in 10% formalin.

The posterior lobe of pituitary is still attached to stalk of brain.

*NOTE: Was this yellow due to a powder left when Topazone aerosol powder, which is furazolidone with inert dispersing agent and propellants, was sprayed on wound at time of craniotomy? Some antibiotics like penicillin can cause seizures, when topically applied to brain.

Dr. Richardson examined the histological slides in great detail and compared those of this R-dog #6 with the slides of R-dogs #1-4 which showed no radiation effects. In regard to R-dog #6 he concluded that in the right cerebral hemisphere in region exposed to irradiation there was destruction of cortex and underlying white matter with gliosis and a macrophage reaction. A few small blood vessels appear necrotic with perivascular hemorrhage. Radiation change is a possibility in this case although most of the damage could be explicable by operative trauma.

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Figure 1

Concentration of Boron in Tumor Bearing Rats after Injections of $\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SO}_3\text{B}_{12}\text{H}_{11}$ BORON CONCENTRATION ($\mu\text{g B/g}$)

KEY:

① = Tumor } 38 $\mu\text{g B/g I.P.}$
 □ = Blood } single inj.^a

③ = Tumor } 53 $\mu\text{g B/g I.V.}$
 □ = Blood } triple inj.^b

② = Tumor } 46 $\mu\text{g B/g I.V.}$
 □ = Blood } double inj.

a. I.V. injection of 46 $\mu\text{g B/g}$ gives similar values of 6 hrs. and 72 hrs.

b. Dosage divided equally among injections, 24 hrs. apart.

Figure 2

Plots of the blue color (B) produced by $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ ($2 \times 10^{-4}M$) in trifluoroacetic acid ($5 \times 10^{-3}M$) and dimethylformamide. The units of absorbance depend on the particular function of B used.

Figure 3.

The time decay of the blue color (B) produced from $B_{12}H_{11}SSB_{12}H_{11}^{-4}$ ($1 \times 10^{-4}M$) in trifluoroacetic acid ($5 \times 10^{-3}M$) and dimethylformamide.

Figure 4.

Tumor and blood boron concentrations in rat as a function of time after an I.P. injection (29.5 γB/g rat) of the blue polymer derived from $B_{12}H_{11}SO_3B_{12}H_{11}^{-4}$

FIGURE 5

IIIA

IIIB

NOTE: III, IIIA, IIIB refer to compounds in Section III.

Figure 6

Tumor and blood boron concentrations in rat as a function of time. For rat: $\square$ -blood, ∇

$\bullet$ -tumor. For man: $\circ$ -blood, $+$ -tumor.

Figure 7

Brain of R-Dog #6

Table I

 ^{11}B nmr Data of a Number of Sulfur Boranes

Compound	Chemical Shifts (ppm) ^a		Relative Intensities
	BS	BH-(J(B-H)H _z)	
$\text{B}_{12}\text{H}_{11}\overset{\text{O}}{\parallel}\text{SSB}_{12}\text{H}_{11}^{-4}$	4.9, 8.1	16.9(131)	1:1:22
$\text{B}_{12}\text{H}_{11}\text{SSB}_{12}\text{H}_{11}^{-4}$	6.2	15.3(134), 16.8(128), 19.9(140)	2:10:10:2
$\text{B}_{12}\text{H}_{11}\text{SSCH}_2\text{CH}_2\text{OH}^{-2}$	7.1	16.6(125) ^b	1:11
$\text{B}_{12}\text{H}_{11}\text{SO}_3\text{H}^{-2}$	7.1	17.2(124) ^b	1:11
$\text{B}_{12}\text{H}_{11}\text{SSCH}_2\text{CO}_2\text{H}^{-2}$	7.2	16.6(119) ^b	1:11
$\text{B}_{12}\text{H}_{11}\text{SSC}_6\text{H}_3(\text{NO}_2)(\text{CO}_2\text{H})^{-2}$	7.2	16.6(120) ^b	1:11
$\text{B}_{12}\text{H}_{11}\text{SC}_6\text{H}_3\text{NO}_2^{-2}$	9.5	16.6(122) ^b	1:11
$\text{B}_{12}\text{H}_{11}\text{SH}^{-2}$	10.4	14.8(125), 17.0(112), 20.5(135)	1:5:5:1
$\text{B}_{12}\text{H}_{11}\text{SSO}_2\text{C}_6\text{H}_4\text{CH}_3^{-2}$	11.0	16.8(131) ^b	1:11

a Chemical shift relative to $\text{Et}_2\text{O} \cdot \text{BF}_3$

b Hydrogen decoupled peak asymmetric, with shoulder on high-field side. This appears to be due to the unresolved boron resonance para to the sulfur bonded atom.

NOTE: All chemical shifts of the sulfur bonded boron atoms are downfield, not exceeding 11.0 ppm.

Table II

The initial half life periods of the fission of
 $2 \times 10^{-4} \text{M Na}_4\text{B}_{12}\text{H}_{11}\text{SSB}_{12}\text{H}_{11}$ in DMF as a function
of the initial hydrogen concentration at 4°C .

$[\text{H}^+]$	2×10^{-4}	4×10^{-4}	1×10^{-3}	2×10^{-3}	5×10^{-3}
$t_{\frac{1}{2}} \text{ (sec)}$	> 3600	522 ± 15	410 ± 10	352 ± 10	343 ± 10

Table III

Toxicity of Borane Polymers in Rats

Compound	I.P. or I.V.	Dose γB/gm	Number of rats injected	Number Alive Same day	Number Alive 4 days
Blue Polymer from $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$	I.V.	10 25 50	3 2 1	2 2 0	2 1 0
Colorless Polymer from SOS (base-treated blue SOS polymer) ^a	I.V.	10 25 50	2 2 2	2 2 1	2 1 0
Blue Polymer from $B_{12}H_{11}SSB_{12}H_{11}^{-4}$	I.V.	10 25 50	2 3 1	2 2 0	2 2 0
Colorless polymer from SS (base-treated blue SS polymer) ^b	I.V.	10 25 50	2 2 2	2 2 2	2 2 2

a. SOS represents $B_{12}H_{11}SOSB_{12}H_{11}^{-4}$

b. SS represents $B_{12}H_{11}SSB_{12}H_{11}^{-4}$

Table IV

Boron Distribution in Rats After a Single
I.V. Injection of the Base Treated Polymer
Which Was Derived from $B_{12}H_{11}SSB_{12}H_{11}$

Time	Blood*	Brain*	Muscle*	Tumor*
6 hrs.	40.8	1.52	3.81	21.42
	36.6	1.06	3.73	17.56
	31.2	1.02	5.52	19.62
	Average 36.20	1.20	5.35	19.53
24 hrs.	7.00	0.41	2.54	11.67
	12.79	0.43	2.24	20.59
	16.20	0.58	2.10	18.51
	Average 11.99	0.62	2.29	16.92
48 hrs.	4.40	0.62	2.29	9.10
	8.50	0.40	1.87	11.87
	11.05		0.47	7.07
	Average 4.65	0.51	1.54	9.34
96 hrs.	1.45	0.20	0.46	9.39
	1.33	0.15	0.33	5.32
	4.13	0.40	1.07	14.36
	Average 2.30	0.25	0.62	9.69

* Concentration in $\mu\text{g B/g}$.

Table V

Boron Distribution of the Borane Phosphates

Time (hrs)	Tumor γ B/g	Blood γB/ml	Brain γ B/g	Muscle γ B/g	<u>Tumor</u> <u>Blood</u>	<u>Tumor</u> <u>Brain</u>
24	9.39	5.82	2.58	1.96	1.6	3.6
48	4.05	2.15	0.18	0.86	1.9	22
96	3.09	2.72	0.11	0.88	1.1	28

NOTE: At each time the results are the average of 4 animals.
3.79 γB/g administered by single I.V. Injection.

22	5.39	2.85	0.31	1.55	1.9	17
96	5.68	2.64	0.28	2.28	2.2	20
120	4.62	1.62	0.50	1.90	2.8	9

NOTE: At each time the results are the average of 3 animals.
Because of toxicity, the compound was administered by 5 daily
I.P. injections, total 24 γB/g.

Table VI

The Boron/Protein Concentration Ratio of Solutions
of Bovine Serum Albumin to Which Excess Borane
Had Been Previously Added, Followed by Exhaustive Dialysis.

Compound	Final Boron/Protein Concentration Ratio	
	pH 7	pH 8.5
$B_{12}H_{11}SOSB_{12}H_{11}-4$	0.026	0.017
$B_{12}H_{11}SSO_2C_6H_{11}CH_3-2$	0.014	0.010
$B_{12}H_{11}SSC_6H_3NO_2COOH-2$	0.005	0.004
$B_{12}H_{11}SSCH_2CHOH-2$	0.017	0.015
$B_{12}H_{11}SSCH_2COOH-2$	0.007	0.007
$B_{12}H_{11}SH-2$	0.013	----

Table VII

Distribution of Two Borane Anions,
 $B_{12}H_{11}SH^{-2}$ and $B_{12}H_{11}SO_3B_{12}H_{11}^{-4}$, in 1 ml Dog Blood

Compound	Total B (μg B)	Total B in Cells*	Boron in Plasma*		
			Total (μg B)	Protein Bound	Non-Protein Bound
$B_{12}H_{11}SH^{-2}$	50 γ	0 γ	43 γ	78%	22%
$B_{12}H_{11}SO_3B_{12}H_{11}^{-4}$	50 γ	0 γ	51 γ	98%	2%

* B analysis on duplicates accurate to 10%

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