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EVALUATION OF BORON COMPOUNDS FOR USE IN NEUTRON CAPTURE THERAPY OF BRAIN TUMORS. I. ANIMAL INVESTIGATIONS[1]

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The neutron capture irradiation of intracranial neoplasms using nonradioactive boron^{10} was first proposed by Sweet and Javid (1952). For this form of therapy to be successful, it is essential that there be a high differential concentration of boron between the tumor and the adjacent normal brain. Under local bombardment with thermal neutrons of the area containing residual tumor, the neoplastic tissue would be selectively destroyed by the following nuclear reaction: boron^{10} + neutron^{1}→(boron^{11})→ lithium^{7} + alpha particle + 2.4 MEV. The alpha particle and lithium atom emitted travel a maximum of nine microns in tissue, thereby releasing this destructive energy only in the immediate vicinity of the cell containing the original disrupted atom of boron^{10}.

The feasibility of this form of therapy is based upon a difference in permeability between normal and neoplastic tissues. Fortunately, there is a breakdown of the normal blood-brain barrier (BBB) in brain tumors (Moore, 1947) and consequently many substances which are restricted in their passage into the brain enter the tumor readily (Selverstone *et al.*, 1949; Sweet, 1951).

Of 125 boron compounds screened in mice (Soloway, 1958; Soloway *et al.*, 1960; Soloway and Gordon, 1960; Thiry, 1958), fifteen have given higher glioma:brain boron ratios than were observed with borate (Locksley and Sweet, 1954). In the present study, ten of these compounds were compared with each other and with boric acid to ascertain which compounds deserved additional study on the basis of tumor:brain ratios and toxicity in mice. Of the five more promising compounds, two have been tested in cats to determine toxic manifestations and tissue concentrations. This was done prior to their evaluation in terminal patients (Sweet *et al.*, unpublished).

METHODS. In other studies (Soloway *et al.*, 1960), it has been shown that C3H mice bearing subcutaneously transplanted gliomas[2] provide a useful means of assaying the tumor:brain ratios of various boron compounds. The present variety of glioma, an ependymoma, was used throughout the investigations. The methods used for transplanting the tumor and determining the boron content in tissues have been described by Soloway *et al.* (1960). The animals were injected intravenously or intraperitoneally usually under ether anesthesia with doses of from 70 to 300 mg of boron per kilogram of body weight (mg B/kg). They were sacrificed by ether inhalation after periods from 15 minutes to 3 hours and various tissues were weighed and analyzed for boron content.

In general, the toxicity of the compound which proved most favorable was then evaluated by intravenous injection of the aqueous solutions into the tail veins of white Swiss albino mice. Survivors were followed for 72 hours. The solutions were prepared in a concentrated form and at a pH range of 7.35 to 7.40, whenever possible, to minimize volume and pH as factors in the toxicity determinations. However, many compounds were soluble in high concentration only in an alkaline medium.

The more promising compounds were tested more thoroughly by intravenous and intracarotid injection in cats anesthetized with pentobarbital. The latter route was included in the event that intracarotid administration of boron compounds

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[2] Dr. D. M. Perese of the Department of Neurosurgery at the Roswell Park Memorial Institute in Buffalo, New York, very generously supplied us with the original subcutaneously grown ependymoma.

becomes the preferable method of injection for neutron capture therapy. Continuous electroencephalographic and electrocardiographic tracings were made and vital signs followed closely. Urinary excretion of several compounds or their metabolites was measured by collecting urine with indwelling catheters and analyzing aliquots for boron content. At intervals of from 15 minutes to several days after injection, serial blood samples were taken and boron analyses were performed on them as well as on other tissues of each animal. Vital organs were then examined for histologic changes.

RESULTS AND DISCUSSION. *Mouse studies.* Tumor:brain boron ratios in mice were approximately the same whether injected intravenously or intraperitoneally. Consequently, in table 1, the average boron ratio listed for the ten compounds at the times specified includes mice injected by either of these routes of administration. At each time interval 3 to 10 mice were used and from these data, standard deviations of each group were calculated to ascertain the variation in this study. Though the deviations were large, it was apparent that the ratios for all of these compounds were appreciably greater than was observed in this laboratory with the borate ion (Locksley and Sweet, 1954) and confirmed in this present study. This is especially true at times greater than 1 hour after injection, since with borate at such times a ratio of only one was observed.

Since all of the compounds described here appeared to be quite promising from the standpoint of ratio, it was essential to compare them with regard to toxicity. The LD50 values of each of these ten compounds as well as of boric acid are listed in table 2. Of those screened the following five, based on boron content, were the most satisfactory from toxicity considerations: 1) *m*-Boronosuccinanilic acid. 2) 3-Amino-4-carboxybenzeneboronic acid. 3) 2-Acetamidobenzene-1, 4-diboronic acid. 4) *o*-(2-Carboxy-2-acetamidoethyl)-benzeneboronic acid. 5) Sodium perhydrodecaborate. Of these five, *m*-boronosuccinanilic acid, 3-amino-4-carboxybenzeneboronic and more recently sodium perhydrodecaborate have been investigated more extensively in larger animals and finally in terminal glioma patients (Sweet *et al.*, unpublished). In view of its encouragingly low toxicity based on boron content, sodium perhydrodecaborate would appear to be the compound of choice. Doses of 50 mg B/kg have been administered to man with no untoward effects. Surprisingly enough, it is a boron hydride.

TABLE 1

Tumor/brain boron ratios in mice at various intervals after administration of boron compounds*

Compound	15†	30	60	120	180
p-Borono-phenylalanine‡	6.1 ± 1.3	8.3 ± 2.1	6.9 ± 1.1	6.5 ± 2.1	—
2-Acetamidobenzene-1,4-diboronic acid	8.2 ± 2.4	9.1 ± 2.0	6.5 ± 0.9	5.8 ± 2.4	5.2 ± 3.2
m-Ureidobenzeneboronic acid	6.0 ± 2.2	11.0 ± 2.5	7.2 ± 0.6	3.7 ± 2.3	3.5 ± 2.7
m-Boronosuccinanilic acid	6.8 ± 2.3	6.4 ± 2.2	6.4 ± 2.5	4.9 ± 1.2	3.3 ± 1.2
m-Carboxybenzeneboronic acid	5.7 ± 1.7	7.8 ± 1.2	7.3 ± 1.3	4.4 ± 1.0	5.2 ± 0.6
p-Carboxybenzeneboronic acid	4.6 ± 1.7	7.0 ± 1.5	7.3 ± 1.7	3.9 ± 0.2	4.0 ± 2.3
2-Nitrobenzene-1,4-diboronic acid	4.2 ± 2.8	4.9 ± 1.1	5.4 ± 3.5	4.4 ± 1.0	4.4 ± 2.2
o-(2-Carboxy-2-acetamidoethyl)-benzeneboronic acid‡	4.8 ± 2.4	7.7 ± 2.6	6.5 ± 2.6	3.4 ± 1.3	2.8 ± 0.6
3-Amino-4-carboxybenzene-boronic acid	6.9 ± 1.8	7.2 ± 1.5	8.5 ± 2.2	6.7 ± 1.6	7.0 ± 0.8
Sodium perhydrodecaborate§	3.9 ± 1.2	5.4 ± 1.1	7.2 ± 2.2	5.7 ± 1.5	7.3 ± 2.1

* Ratios are recorded with the standard deviations; the mice received doses of 140 to 300 mg B/kg.

† Time of sacrifice in minutes after injection.

‡ Dr. H. R. Snyder, Professor of Chemistry at the University of Illinois, kindly supplied these compounds.

§ Dr. M. F. Hawthorne of the Redstone Arsenal Division of the Rohm and Haas Company kindly furnished triethylammonium perhydrodecaborate and the procedure for preparing the sodium salt.

TABLE 2

Mouse toxicity study

Compound	No. Mice*	pH	LD50†			Toxic Signs of Near Lethal Dose
			g/kg	*mg B/kg*	*mmol/kg*	
Boric acid	12	6.9	2.11	375	34.6	Seizures, respiratory depression, ataxia, diarrhea
	34	7.4	2.42	430	39.7	
	21	8.8	1.52	270	25.0	
	16	9.4	1.24	220	20.3	
3-Amino-4-carboxybenzeneboronic acid	25	8.1	3.29	200	18.5	Seizures, respiratory depression
	16	9.5	2.06	125	11.5	
p-Boronophenylalanine	11	10.0	1.52	80	7.4	Seizures, respiratory depression
m-Ureidobenzeneboronic acid	12	10.0	1.02	100	4.6	Seizures, respiratory depression
p-Carboxybenzeneboronic acid	25	9.4	1.74	115	10.6	Seizures, diarrhea, respiratory depression
m-Carboxybenzeneboronic acid	20	7.4	2.56	170	15.7	None
2-Nitrobenzene-1,4-diboronic acid	34	9.3	1.68	175	8.1	Seizures, respiratory depression
m-Boronosuccinanilic acid	48	7.4	4.09	190	17.5	None
2-Acetamidobenzene-1,4-diboronic acid	19	10.6	2.54	250	11.5	Seizures, respiratory depression
o-(2-Carboxy-2-acetamidoethyl)-benzeneboronic acid	14	7.5	5.72	250	23.1	None
Sodium perhydrodecaborate	11	7.2	1.04	685	6.3	Seizures

* The numbers of mice refer to those used in the LD50 range and not to the total number of mice required for the toxicity study.

† The values are accurate to within ± 25 mg B/kg.

The literature is replete with information concerning the high toxicity of such materials (Hill *et al.*, 1958; Walton *et al.*, 1955; Lowe and Freiman, 1957), but the high chemical stability of the decaborate ion, $B_{10}H_{10}^{=}$ (Hawthorne, unpublished) is possibly responsible for its biological inactivity.

In assessing the toxicity of these compounds, it became apparent that the volume of the injected solution was not a major factor provided it did not exceed such large volumes as 2.5 to 3 ml per mouse. Interestingly enough, such volumes were well tolerated when injected intravenously over a 10- to 20-second period. The pH of the material was of greater concern since even small volumes (less than 0.4 ml) of alkaline solutions produced respiratory depression, seizures, and often death. To determine the effect of pH upon the toxicity of these boron compounds, we measured the LD50 for boric acid solutions at varying hydrogen ion concentrations (table 2). As might be anticipated, the lowest toxicity was attained at the physiological pH, increasing on either side of this value. By raising the pH to

9.4, the toxicity of the boric acid solution approximately doubled. Similar findings of the effect of pH have been reported recently by White (1960) with regard to the toxicity of nitrogen mustards.

TABLE 3

Average boron concentrations in cat tissues in mg B/kg*

Boric Acid

	mg B/kg		mg B/kg
Optic chiasm	375–415	Kidney	550–620
Sciatic nerve	360–400	Heart	475–550
Cerebellum	320–410	Skull	400–520
Cerebrum-cortex	300–390	Liver	450
White matter	300–330	Muscle	415–465
Brain stem	280–340	Scalp	360
Spinal cord	230–270	Fat	60–80
Pituitary	200–260	Blood	430–500
Feces (rectum)	340		

Cat studies. Preliminary to the injection of *m*-boronosuccinanilic acid and 3-amino-4-carboxybenzeneboronic acid in man, their toxic effects as well as lethal doses of boric acid were determined in cats. A comparison study of the toxicity of these aromatic boronic acids to boric acid would provide some intimation as to what might be an initial safe dose of these compounds in man. Much is known regarding the pharmacology of boric acid in man (Pfeiffer *et al.*, 1945; Goodbloom, 1953; Locksley and Farr, 1955; McNally and Rust, 1928; Watson, 1945; Connelly *et al.*, 1958) and this is the reason for its use as a reference standard.

Boric acid in intravenous doses of 600 mg B/kg produced generalized seizures, severe diarrhea, and ataxia in 2 cats. Occurrence of diarrhea after parenteral administration suggested the possibility that the intestinal wall was actively excreting the substance. Fecal specimens were taken from the large intestine and showed high concentrations of boron, corroborating

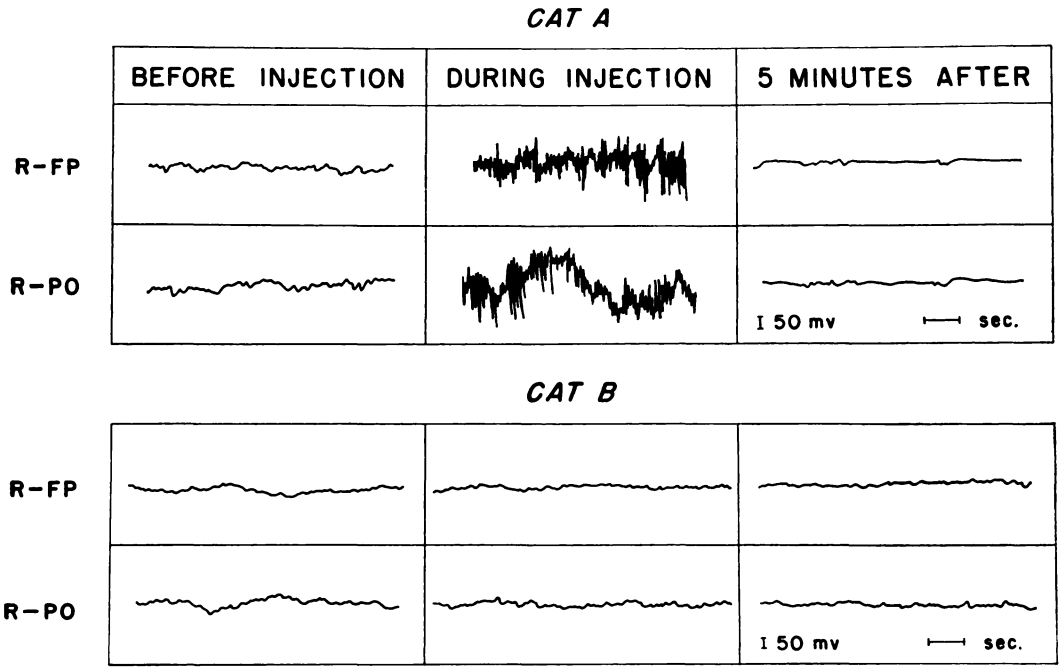

FIG. 1. Electroencephalographic tracings.
Leads: R-FP = fronto-parietal; R-PO = right parieto-occipital.
Cat A received 3-amino-4-carboxybenzeneboronic acid, 40 mg B/kg, by injection into the right common carotid artery after ligation of the facial portion of the external carotid artery. The pH of the solution was 8.1 and it contained 3 mg B/ml. A generalized seizure and respiratory arrest ensued.
Cat B received *m*-boronosuccinanilic acid, 40 mg B/kg. by the same route. This solution contained 5 mg B/ml and the pH was 7.4. It was well tolerated. The animal was sacrificed 110 hours later, and there was no sign of toxicity.

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