INEL BNCT Research Program September/October 1992

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ABSTRACT

This report presents summaries for two months of current research of the Idaho National Engineering Laboratory (INEL) Boron Neutron Capture Therapy (BNCT) Program. Information is presented on development and murine screening experiments of low-density lipoprotein, carboranyl alanine, and liposome boron containing compounds. Pituitary tumor cell culture studies are described. Drug stability, pharmacology and toxicity evaluation of borocaptate sodium (BSH) and boronophenylalanine (BPA) are described. Treatment protocol development via the large animal (canine) model studies and physiological response evaluation in rats are discussed. Supporting technology development and technical support activities for boron drug biochemistry and purity, analytical and measurement dosimetry, and noninvasive boron quantification activities are included for the current time period. Current publications for the two months are listed.

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ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of variance	INEL	Idaho National Engineering Laboratory
BBB	Blood Brain Barrier	IR	Infrared
BMRR	Brookhaven Medical Research Reactor	ISU	Idaho State University
BNL	Brookhaven National Laboratory	IV	Intravenous
BNCT	Boron Neutron Capture Therapy	LDL	Low density lipoproteins
BPA	Boronophenylalanine	LSD	Least significant differences
BSA	Bovine serum albumin	MR	Magnetic resonance
BSH	Borocaptate Sodium ($\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$)	MRI	Magnetic resonance imaging
CHN	Carbon nitrogen hydrogen	NMR	Nuclear magnetic resonance
CNS	Central nervous system	ORSU	Oregon State University
CRADA	Cooperative Research and Development Agreement	OSU	Ohio State University
CT	Computed tomography	PAL	Phenylalanine ammonia lyase
CTEM	Conventional transmission electron microscopy	SIMS	Secondary ion mass spectrometry
DSA	Dog serum albumin	T/B	Tumor boron/blood boron
EC	European Collaboration	TBAS	Tetra butyl ammonium sulfate
EIRMC	Eastern Idaho Regional Medical Center	TLD	Thermo luminescent dosimeter
FTIR	Fourier transform infrared spectroscopy	UCLA	University of California, Los Angeles
HPLC	High performance liquid chromatography	UCSF	University of California, San Francisco
HRP	Horseradish peroxide	UofR	University of Rochester
HSA	Human serum albumin	UofU	University of Utah
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy	V	Volt
		WSU	Washington State University

INEL BNCT RESEARCH PROGRAM SEPTEMBER/OCTOBER 1992

INTRODUCTION

This report contains an overview of progress for the Idaho National Engineering Laboratory (INEL) Boron Neutron Capture Therapy (BNCT) Research Program for September/October 1992, including information on the Research Programs, Technical Support Details, and Miscellaneous Project Information.

The following sections are the reports from the various investigators about their current research.

BORON DRUG DEVELOPMENT

Carboranyl Alanine and Low-Density Lipoprotein (LDL) Development and Evaluation

No progress reported.

Boronated Liposome Development and Evaluation

Liposome Encapsulation and Development: University of California, Los Angeles (UCLA) researchers have the first liposome biodistribution results obtained with the new tumor line, P1798 murine lymphosarcoma. The boron compounds encapsulated were $n\text{-B}_{20}\text{H}_{18}^{2-}$ and its reduced analog $\text{B}_{20}\text{H}_{19}^{3-}$. Although only blood and tumor boron analyses have been completed, the results obtained appear to be very similar to the behavior of these species with the EMT6 tumor line previously studied. The $\text{B}_{20}\text{H}_{19}^{3-}$ results are characterized by high initial tumor uptake followed by rapid clearance of boron from the tumor. The $n\text{-B}_{20}\text{H}_{18}^{2-}$ ion (injected dose 295 μg boron, 16 mg B/kg body weight) exhibits good initial tumor uptake of boron ($>20 \mu\text{g}$ B/g tumor) with reasonably good retention of boron in the tumor over the first 48 hours. The tumor boron concentration is 19.6 ppm after 24 hours with a tumor bo-

ron/blood boron (T/B) ratio of 4.2; at 48 hours these values are 15.6 ppm with T/B of 7.6.

Liposomes containing $\text{Na}_2\text{B}_{10}\text{H}_6\text{NCO}$ have been sent to Washington State University (WSU) for screening with the P1798 tumor. Experiments at Vestar using the EMT6 tumor line are being scheduled for liposomes prepared with $\text{NaB}_{10}\text{H}_6\text{NH}_3$ (encapsulated), $\text{K}^+[\text{C}_2\text{B}_9\text{H}_{11}(\text{CH}_2)_{16}\text{CH}_3]^-$ (embedded in the lipid bilayer), $\text{Na}_3\text{B}_{20}\text{H}_{17}\text{NH}_3$ (double injection), and $\text{Na}[\text{Co}(\text{C}_2\text{B}_9\text{H}_{11}\text{SH})_2]$ (encapsulated).

Liposome biodistribution results have been obtained for encapsulated $\text{Na}_3\text{B}_{20}\text{H}_{17}\text{NH}_3$, encapsulated $\text{NaB}_{10}\text{H}_6\text{NH}_3$, and embedded $\text{KC}_2\text{B}_9\text{H}_{10}(n\text{-C}_{16}\text{H}_{33})$ in the P1798 murine lymphosarcoma tumor line. The $\text{Na}_3\text{B}_{20}\text{H}_{17}\text{NH}_3$ species has produced the most promising results in the EMT6 tumor line to date. P1798 lymphosarcoma results (injected dose = 222 μg boron) are similar to the EMT6 results obtained previously. The six hour tumor boron concentration was 24.5 μg boron/g tissue. The boron concentration increased over a period of approximately 12 hours and then began to decrease. The 48 hour tumor boron concentration was 20.1 μg boron/g tissue, and the T/B was 25. $\text{NaB}_{10}\text{H}_6\text{NH}_3$ (injected dose = 122 μg boron) was examined to determine if the retention of the reduced $\text{Na}_3\text{B}_{20}\text{H}_{17}\text{NH}_3$ species was due to the amine functionality. The six hour tumor boron concentration was 11.3 μg boron/g tissue. After 48 hours, the tumor boron concentration of 2.2 μg boron/g tissue. Therefore, the retention of tumor boron exhibited for $\text{Na}_3\text{B}_{20}\text{H}_{17}\text{NH}_3$ is probably not due to the amine moiety. The ability to be easily oxidized to a more reactive species, $\text{B}_{20}\text{H}_{17}\text{NH}_3^{1-}$, is one mechanism by which $\text{B}_{20}\text{H}_{17}\text{NH}_3^{3-}$ could be retained, and is supported by the cyclic voltammogram of $\text{Na}_3\text{B}_{20}\text{H}_{17}\text{NH}_3$. The $\text{KC}_2\text{B}_9\text{H}_{10}(n\text{-C}_{16}\text{H}_{33})$ species was the first attempt to increase the boron concentration of the liposomes by embedding a lipophilic boron compound into the bilayer membrane. This first experiment used liposomes encapsulating isotonic buffer (no boron encapsulated). Although it was attempted to dope the membrane with 3% added boron by weight, only

half of this was actually embedded, possibly due to some degree of water solubility of the *nido*-carborane. Therefore the injected dose of this species was low at 83 μg boron. However, the results are still quite promising. The six hour tumor boron concentration of 10.7 μg boron/g tissue is 80% retained over a 24 hour time period. When viewed as a function of the percent injected dose delivered and retained, these values are comparable to the best results obtained with water-soluble encapsulated boron species.

Liposomes encapsulating $\text{Na}[\text{Co}(\text{C}_2\text{B}_9\text{H}_{10}\text{SH})_2]$ were sent to WSU. However, this compound appears to be quite toxic to the mice with P1798 lymphosarcoma. Unencapsulated compound has been sent for *in vitro* toxicity screening. Liposomes doped with the hydrophobic species, $\text{KC}_2\text{B}_9\text{H}_{10}(n\text{-C}_{16}\text{H}_{33})$, but containing hyperosmolar buffer have been sent to WSU. This screening should provide information on the stability of liposomes with altered bilayer membranes encapsulating a solution which more closely resembles the liposomes containing the borane solutions.

Compound Development: The most promising liposome results to date have been obtained with $\text{Na}_3\text{B}_{20}\text{H}_{17}\text{NH}_3$. This has been proposed to be due to the ability of this species to be oxidized *in vivo* to the more reactive, electrophilic $\text{NaB}_{20}\text{H}_{17}\text{NH}_3$, which could then react with intracellular proteins. Electrochemical studies have been initiated to examine the oxidation of $\text{B}_{20}\text{H}_{17}\text{NH}_3^{3-}$, $\text{B}_{20}\text{H}_{19}^{3-}$, and $\text{B}_{20}\text{H}_{17}\text{OH}^{4-}$. Preliminary experiments have examined the cyclic voltammetry of these species in acetonitrile (Et_4PF_6 supporting electrolyte) at a platinum electrode vs Ag/AgCl . All three species show irreversible oxidation waves. The peak potentials for $\text{B}_{20}\text{H}_{19}^{3-}$ and $\text{B}_{20}\text{H}_{17}\text{OH}^{4-}$ are 0.64 v and 0.55 v, respectively. Remarkably, the amine substituted ion is more than a half-volt easier to oxidize; showing an oxidation peak at -0.06 v.

Further electrochemistry experiments will be expanded to include other $\text{B}_{20}\text{H}_{18}^{4-}$ derivatives such as $\text{B}_{20}\text{H}_{17}\text{SH}^{4-}$ and $\text{B}_{20}\text{H}_{17}\text{CN}^{4-}$, and to examine the interconversion of $\text{Na}[\text{Co}(\text{C}_2\text{B}_9\text{H}_{11}\text{SH})_2]$ and $\text{Na}[\text{Co}(\text{C}_2\text{B}_9\text{H}_{11}\text{S})_2]$.

Pituitary Tumor Evaluation

AtT-20 cell viability studies have been carried out at the Oregon State University (ORSU) reactor with the new shielded system at five different power settings, 200, 300, 350, 450, and 600 kW to define the optimum radiation power/time. Cells were allowed to grow in culture for 13 days after irradiation and counted on an every three day basis to evaluate growth curves over time. The data suggest that *in vitro* AtT-20 cell irradiations for ten minutes at between 200 and 300 kW produce a minimum of cell death compared to non-irradiated controls, providing a level of irradiation for these cells that will best enable us to see a BNCT effect after exposure of the cells with the carborane-corticotrophin releasing hormone conjugate. Cell loading experiments with rodent pituitary tumor cells are planned to commence in the near future.

Measuring Dosimetry: A new bismuth sample holder has been designed for the ORSU Reactor that holds up to six cell irradiation vials. With this holder, anticipated gamma dose to the *in vitro* cell preparations will approach 2.0×10^{11} cGy gamma/ n_{th} /cm². The holder is a solid, milled bismuth box that will fit into the 12 x 4 inch thermal column port, inside of the already present bismuth and lead shielding. New, sterile, cell irradiation vials have also been obtained and tested for these *in vitro* experiments, each having a total volume capacity of 5 mL.

The new, smaller, 5 mL *in vitro* cell sample holders have been tested in the thermal column to assess possible complications during irradiation. Irradiations lasted for four minutes at full power (1 MW), using distilled water as the contained medium. This encapsulation test proved successful with no spills, leaks, or other complications. These smaller cell vessels provide additional bismuth shielding to further reduce the contaminating gamma dose to the cells, as described above.

The College of Oceanography at ORSU has purchased a new inductively coupled plasma (ICP)-mass spectrometer which will be available for the quantitation of intracellular boron from *in vitro* studies to reduce the analytical turn-around time. Anticipated levels of sensitivity of this unit for boron are in the part per billion range.

Previously, TLD-700s have provided false positive gamma level values, making interpretation of the dosimetry data very difficult. TLD-400s are currently being used to satisfactorily monitor contaminating gamma dose in the samples. The new bismuth sample holder has slots incorporated into its design for gamma ray (TLD) and neutron (gold foil) dosimetry.

BORON LOCALIZATION SCREENING

Boronophenylalanine (BPA) Evaluation Studies

Plasma levels of free phenylalanine and tyrosine in mice have been shown in current literature to be reduced following injection of phenylalanine ammonia-lyase (PAL). WSU College of Pharmacy researchers hypothesized that tumor uptake of BPA can be enhanced by using PAL to reduce free phenylalanine and tyrosine in the plasma.

To investigate whether tumor levels of boron could be enhanced, an experiment was conducted using PAL to reduce plasma levels of free phenylalanine and tyrosine. To investigate whether tumor levels of boron increased, mice were injected intravenously into the dilated lateral tail vein with either 1 mg or 4 mg of BPA. One half of these mice were pretreated with PAL one hour prior to BPA injection. Tissue and blood samples were collected at 6 and 24 hours post BPA injection and sent to INEL for boron analysis. Blood samples were also sent to the Bioanalytical Center at WSU for amino acid analysis.

Table 1 lists tumor boron levels for the six hour time point. At 24 hours levels dropped sharply. Statistical analysis was done using analysis of variance (ANOVA), fisher least significant differences (LSD).

Amino acid analysis data indicate that PAL reduced plasma levels of both free phenylalanine and free tyrosine. Furthermore, BPA did not interfere with amino acid analysis.

Results indicate that PAL does enhance the uptake of BPA into tumor tissue. Experiments are underway to optimize PAL-modulated BPA uptake.

Liposome Evaluation Studies

The following compounds received from UCLA have been screened and analyzed for boron uptake using the BDF mouse bearing murine B16-BL6 melanoma:

$\text{Na}_2\text{B}_{10}\text{H}_{12}\text{NCO}$: Tumor boron levels peaked at 24 hours at 21.82 $\mu\text{g/g}$. Boron levels were maximum at six hours for blood at 52.68 $\mu\text{g/g}$. Maximum tumor:blood ratio was determined to be 1.82:1 at 72 hours.

$\text{Na}[\text{Co}(\text{C}_2\text{B}_9\text{H}_{10}\text{SH})_2]$: Tumor boron level was maximum at six hours at 18.17 $\mu\text{g/g}$ after pretreatment with buffer-containing liposomes. Blood boron levels were maximum at six hours with a concentration of 9.61 $\mu\text{g/g}$. Tumor-to-blood ratio was greatest at 24 hours (5.59:1). Figure 1 shows the boron level for both tumor and blood. At six hours blood and tissues were obtained from mice pretreated with buffer-containing liposomes (empty blood and empty tumor markers). The boron's half-life in the tumor appears to be 18 hours. Because of this time point and the tumor-boron levels

Table 1. Effect of PAL on tumor levels of boron following intravenous inoculation with 1 or 4 mg of BPA per mouse.

μg Boron/g of Tumor Tissue $\pm$ Standard Deviation		
	1 mg BPA	4 mg BPA
-PAL	1.2 $\pm$.42	4.64 $\pm$.84
+PAL	2.88 $\pm$.52	6.53 $\pm$.56

Figure 1. Boron levels in tumor and blood following intravenous injection of liposome encapsulated $\text{Na}[\text{Co}(\text{C}_2\text{B}_9\text{H}_{10}\text{SH})_2]$.

remaining above blood levels throughout the screening, a multiple injection group will be added in future screenings. It is the belief of the WSU BNCT researchers that a second injection at the 18 hour time point could increase tumor boron levels to therapeutic levels while still allowing the boron to clear from the blood.

The following compounds received from UCLA were screened and analyzed for boron uptake using the Balb/C mouse bearing P1798 lymphosarcoma:

$\text{Na}_2\text{B}_{20}\text{H}_{18}$: Tumor boron levels were maximized in mice pretreated with buffer-containing liposomes one hour prior to injection of liposome-encapsulated boron compound. At six hours, tumor boron levels averaged $22.71 \mu\text{g/g}$, and blood concentration was $27.01 \mu\text{g/g}$. Tumor-to-blood ratio peaked at 7.6:1 at 48 hours.

$\text{Na}_3\text{B}_{20}\text{H}_{19}$: Tumor boron uptake was maximum at a concentration of $32.01 \mu\text{g/g}$ at the six hour time point. For blood, the maximum concentration $38.60 \mu\text{g/g}$ was also at the six hour time point. Tumor-to-blood ratio was greatest at 48 hours, with a value of 9.3:1.

$\text{NaB}_{10}\text{H}_9\text{NH}_3$: Tumor boron levels were maximum at six hours at a concentration of $11.32 \mu\text{g/g}$. At six hours blood concentration averaged $22.93 \mu\text{g/g}$. Tumor-to-blood ratio was greatest at 72 hours (2.25:1).

$\text{K}^+[\text{C}_2\text{B}_9\text{H}_{11}(\text{CH}_2)_{16}\text{CH}_3]^-$ and $\text{Na}_3\text{B}_{20}\text{H}_{17}\text{NH}_3$: Samples have been screened and sent to INEL for analysis. Results are pending.

Initial screenings using P1798 lymphosarcoma were done in Balb/C mice. Histopathological results indicate tumor involvement and damage in liver and spleen. Tumor in axillary nodes is also indicated. To circumvent these aforementioned observations, recent screenings have been conducted using Balb/C hybrid mice (CD_2F_1). Histological data are pending for P1798 lymphosarcoma injected intradermally and subcutaneously into CD_2F_1 mice. The following compounds have been screened using this model and results from INEL are pending.

$\text{NaCo}(\text{C}_2\text{B}_9\text{H}_{10}\text{SH})_2$: Samples have been screened and sent to INEL for analysis. Results are pending.

$\text{Na}_2\text{B}_{10}\text{H}_9\text{NCO}$: Samples have been screened and sent to INEL for analysis.

The following LDLs have been received from the University of California, San Francisco (UCSF) for the evaluation of boron uptake. Screenings for these LDLs used BDF mice bearing murine B16-BL6 melanoma.

LDL (Carbamyl compound): Boron uptake by tumor was maximum at six hours at a concentration of 5.36 $\mu\text{g/g}$. Liver levels were highest at six hours, averaging 28.09 $\mu\text{g/g}$. The greatest tumor-boron-blood ratio was 1.31:1 at 24 hours.

LDL (UCSF Fourth LDL): Boron uptake by the tumor was maximized at six hours at a concentration of 3.17 $\mu\text{g/mL}$. Boron levels peaked in the blood with a concentration of 3.22 $\mu\text{g/mL}$, also at six hours. The greatest tumor-to-blood ratio was found to be 1.58:1 at 72 hours.

LDL (UCSF #LW01-45): LW-I-45 was screened for boron uptake, and all tissue samples were sent to INEL for analysis. Results are pending.

DRUG STABILITY, PHARMACOLOGY AND TOXICITY

Toxicological Evaluation of Borocaptate Sodium (BSH)

The historical clinical experience has identified few adverse effects associated with intravenous (I.V.) infusion of BSH, but few patients have received BSH doses greater than 80 mg/kg. Animal data concerning BSH toxicity is also limited. However, the few available studies do suggest that high doses of BSH can cause hepatic necrosis, affect liver metabolism, induce erythrocyte aggregation and cause respiratory arrest, renal necrosis and skeletal muscle paralysis. I.V. administration of BSH is reported to kill at doses as low as 24 mg/kg (rabbit) or 25 mg/kg (dog), but there are also reports that animals tolerate i.v. infusion of 600 mg/kg BSH.

Idaho State University (ISU) researchers have been developing a tiered toxicity testing program for evaluation of new boron delivery agents. As part of this program, ISU research-

ers have been characterizing some of the toxic effects of BSH (200-750 mg/kg) delivered by i.v. infusion to Long-Evans or Sprague-Dawley rats. Lethality following acute i.v. administration of drug can be influenced by dose-rate of administration, physiological state of the animal, vehicle and osmolarity of the infusion solution, and volume-rate of administration. These studies suggest that over the range of parameters examined, neither the dose-rate of administration nor volume-rate of administration influence the dose of BSH that elicits death. Whether BSH is dissolved in distilled water or phosphate buffered saline also seems to make little difference, and BSH-induced changes in systemic osmolarity do not seem to explain the observed lethality. The physiological state of the animal does seem to dramatically influence BSH toxicity. Anesthetized rats instrumented for measurement of cardiovascular and pulmonary function are much more sensitive to the toxic effects of BSH than are anesthetized, non-instrumented rats or conscious, non-instrumented rats. In non-instrumented rats, i.v. infusion of BSH doses greater than 550 mg/kg usually resulted in death and infusion of doses greater than 375 mg/kg occasionally resulted in the death of the animal. Animals infused with equal volumes of osmotically equivalent vehicle never died during the seven day observation period. At the higher BSH doses, death often occurred within six hours of infusion. This rapid onset of death suggested a drug-induced impairment of either the cardiovascular or pulmonary systems and prompted investigations of cardiovascular and pulmonary function. Pulmonary parameters (rate, flow and tidal volume) were little effected by BSH until just prior to death. Respiratory depression did not appear to be a primary cause of BSH-induced death, since artificial respiration was not an effective antidote. Measurement of insufflation pressure was impaired by the ability of BSH to reverse the effects of competitive neuromuscular junction blockade. Given the structural differences between BSH and neuromuscular junction antagonists, this effect was most unexpected, but suggests a possible BSH effect on skeletal muscle and may shed light on literature reports of BSH induced limb paralysis.

Cardiovascular parameters were rapidly and substantially altered by BSH administration. Blood pressure, heart rate, cardiac contractility and cardiac output initially increased, but then gradually declined until death intervened. Total

peripheral resistance began a slow but marked increase shortly after the BSH infusion was initiated. After infusion of 250-300 mg/kg, significant ventricular arrhythmias were noted and continued infusion exacerbated these arrhythmias. The worsening of the arrhythmias correlated with the decline in cardiac output and the precipitous fall in cardiac stroke output seemed to be immediately preceded by ventricular fibrillation.

Intravenous administration of BSH doses of 375-550 mg/kg were often associated with delayed death, the animals not succumbing until 1-4 days postinfusion. This delayed time-to-death seems to argue against cardiovascular failure or respiratory depression as a direct cause of death. Animals appeared to be edematous, had low urine output, and at autopsy, displayed enlarged kidneys. Renal failure secondary to cardiogenic shock was suspected, but anti-shock therapy failed to ameliorate the BSH-induced lethality. Preliminary histopathology studies on rats surviving i.v. infusion of 375-550 mg/kg BSH do suggest renal necrosis, and indicate a need to evaluate the possibility of BSH-induced changes in renal function.

TREATMENT PROTOCOL DEVELOPMENT

Large Animal Model Studies

Pharmacokinetics: No new dogs were infused during this time period.

Normal Tissue Tolerance (Neutron Irradiation): All the remaining dogs that have been irradiated are clinically normal. (Concentrations reported below are in $\mu\text{g } ^{10}\text{B/g}$).

Three dogs, all at 25 $\mu\text{g/g}$ and 19 Gy, had their six month post-irradiation checkups in the first part of October.

Two dogs from the 100 $\mu\text{g/g}$ and 27 Gy and two dogs from the 50 $\mu\text{g/g}$ and 27 Gy had their 12 month post-irradiation checkups. At this time these dogs were euthanized and necropsied.

One dog from the 25 $\mu\text{g/g}$ and 27 Gy had to be euthanized at nine months. This dog had acute onset of central nervous system signs, lateral recumbency, left face paralysis and lethargy. The other three dogs from the group are not showing any clinical signs and no abnormalities are evident on their 12 month post-irradiation scans and blood work. All four of these dogs showed changes in their computed tomography (CT) and magnetic resonance (MR) scans at six months, but no clinical signs. Only one of these dogs will be euthanized later this month. The other two will be saved for continued observation.

Three dogs, all at 100 $\mu\text{g/g}$ and 35 Gy, had normal 12 month post-irradiation scans and will be necropsied.

Spontaneously-Occurring Brain Tumor Dogs: "Shana" Hergenrader (#35447-168), an eleven year old spayed female Golden Retriever, was presented to WSU on October 30 for right facial paralysis and sudden onset of seizures. Shana will be evaluated, and if there is a mass, she will undergo brain surgery.

"Bozo" Doyle (#35447-167), a five year old castrated male Springer cross, was presented to WSU on October 19 for sudden onset of ataxia and circling, head tilt, and right eye blindness. Bozo will be evaluated, and if there is a mass, he will have surgery and possibly irradiation, if the mass enhances.

"Tank" Galitelo (#35447-166), a seven year old male Bullmastiff, was presented to WSU on September 7 for loss of balance, incoordination, tremors, and seizures. Tank's CT and MR scans revealed an unenhancing mass in the right temporal lobe. Tank was infused with 50 $\mu\text{g/g}$ of boron and underwent brain surgery later that day. Tank recovered and went home with owners.

"Dudley" Fiset (#35447-151), a seven year old castrated male Golden Retriever, is doing great.

"Brandy" Hoff (#35447-94), a seven year old female Golden Retriever, is also doing great. Brandy will be returning to WSU for her three year post-irradiation checkup by the end of October. If there are no changes, Brandy will be coming back to WSU every year instead of every six months.

Physiological Response Evaluation and Interdiction

Gamma Only Irradiation Studies: The effects of 60 Gy Co-60 irradiation using horseradish peroxidase (HRP) studies will be completed in the near future. University of Rochester (UofR) has serial MR studies to compare with the HRP demonstrating the severe injury to the microcirculation of the brain disrupting the blood brain barrier (BBB). This injury begins at two weeks and progresses to 12 weeks, and by the next sample time point of six months Severe injury has occurred. Although animals have been kept living longer, this is unnecessary since the six month endpoint very dramatically shows severe vascular injury. Thus, BNCT tumor treated animals (six month survivors with fractionated BNCT) are available for study and sacrifice. These plans will proceed next month.

Therapeutic Ratio in RAT 9L Gliosarcoma: BNCT vs. Photons: All tissue has been processed and analyzed for BBB leakage. The only remaining analysis is vascular density. All completed analyses have been previously reported.

9L Gliosarcoma: This study involved the implantation of 10^4 tumor cells into the left striatum using the procedure described by Coderre, et. al., (1992). The animals were treated with HRP and perfused 14 days post-implantation.

Data acquisition is virtually complete. Two brains remain to be analyzed. Analysis is currently underway for the completed brains. A photographic record of these brains will be completed within three weeks.

BNCT Cellular and Histopathology: Following UofR's national presentations, most recently in Columbus, OH at the Fifth International Symposium on Neutron Capture Therapy, UofR is becoming the reference center for cellular and histopathology studies. There has been a sharp increase in requests for the processing of specimens from BNCT irradiated cats, dogs and human patients. Requirements will be drafted for the national repository for the examination of the pathologic material, particularly brains, of animals and humans exposed to BNCT.

In addition to rats from Brookhaven National Laboratory (BNL), the following tissues have been received:

Dog brains from WSU that include controls as well as BNCT irradiated animals for processing and assessment, particularly utilizing UofR whole brain histopathologic techniques and electron microscopy for analysis of vascular injury.

Ten rat brains from OSU with melanoma implants which are believed to be cured at six months to a year post-BNCT-irradiation. Sectioning of the brains for evaluation is underway.

UofR has received inquiries about human specimens from Boston but has not received any to date.

The Petten BNCT Project has also requested a cooperative link to do pathological studies.

UofR collaborated with Jeff Coderre on a strategy to escalate the boron serum level and the neutron exposure for planned rat irradiations.

Future Planned Studies: Jeff Coderre visited UofR to initiate writing a series of papers under collaborative authorship. The following is a concise summary of the proposed publications:

"The Favorable Therapeutic Ratio of BNCT in Brain Tumors," will illustrate the ability to eradicate the rat 9L gliosarcoma in half of the animals without any severe injury to brain, unlike control animals treated with 250 kV irradiation who survive but with the price of severe brain damage to achieve tumor ablation. This paper will be submitted to *Science* or *Nature*, two of the more prestigious journals, since this is a major achievement and needs this kind of documentation.

"Truly Selective Effect of BNCT in Brain Tumors." Due to the large amount of material available, a separate paper will be submitted, probably to the *International Journal of Radiation Oncology Biology Physics*, to alert the radiation oncology community. The rat 9L gliosarcoma mimics the human glioblastoma radioresistance, and to be able to eradicate this tumor will virtually no effect on all the brain is worth documenting in much detail.

"Early and Persistent Disruptions of Blood Brain Barrier after Irradiation," will be based on pathologic review of HRP injections for 60 Gy animals with early time points of two weeks to six months.

UofR will also be doing some parallel studies in other normal tissues. The BNCT animals all have cataracts as compared to the 250 kV irradiation, which is also worth documenting.

Studies will be directed to look at the lung damage after BNCT. UofR has the capability of detecting any level of alteration with modern molecular biology probes and techniques. UofR has estimated that the negative systemic effect of BNCT would be in the lung, the major dose limiting tissue outside of the brain, for head irradiation.

SUPPORTING TECHNOLOGY DEVELOPMENT

Task 1: Biochemistry of BSH and Its Oxidation Products

No activity to report.

Task 2: Noninvasive Boron Quantification

The research of the nuclear magnetic resonance (NMR) study on the interaction between albumin and BSH has been completed at the University of Utah (UofU). Conclusions that have been arrived at are:

The ^{11}B line width, defined as $W^{b,c}$, contributed from the spin-spin relaxation rate of bound BSH and the exchange rate between bound and unbound BSH during the titration of serum albumin to $W^{b,c}$, was obtained by subtracting the ^{11}B line width of free BSH (W^u) from the observed line width of BSH (W^o) in albumin solution ($W^{b,c} = W^o - W^u$). The line widths $W^{b,c}$ of BSH during the titration of Human Serum Albumin (HSA) are listed in Table 2 and compared at two temperatures, 295°K and 310°K. The line width decreases as the temperature increases indicating that the bound and unbound BSH are under fast exchange.

The observed chemical shifts of the BSH remain the same for various temperatures (295 to 310°K) and varying concentrations (1 to 5%) of Bovine Serum Albumin (BSA), HSA and Dog Serum Albumin (DSA) as shown in Table 3. The data are the means analyzed statistically for each nucleus, and the errors are the standard deviation. The small change is considered the experimental error. This observation indicates that there is no covalent binding and no formation of disulfide bridge involved in this interaction. The observed coupling constant $^1J_{11\text{B-1H}}$ is 126 Hz, and did not change with temperature variation and albumin titration, which is consistent with the above conclusion.

The binding fraction, the number of binding sites of BSH on three types of serum albumin and the binding constraints of the interaction have also been calculated using the equations reported last time. The calculation procedures are listed below.

Table 2. The line width $W^{b,c}$ (Hz) of BSH vs temperature change.

	B2 1% HSA	B2 2% HSA	B2 3% HSA	B3 1% HSA	B3 2% HSA	B3 3% HSA
295°K	19.83 ± 1.77	46.03 ± 2.82	61.33 ± 3.42	15.57 ± 0.55	37.97 ± 2.37	56.92 ± 2.77
310°K	4.33 ± 3.22	16.33 ± 1.59	26.33 ± 7.35	1.60 ± 3.61	14.50 ± 2.51	20.47 ± 2.75

Table 3. The chemical shift of BSH.

B1	B2	B3	B4
-28.41 ± 0.08	-32.80 ± 0.04	034.92 ± 0.03	-38.50 ± 0.00

*Saturated H_3BO_3 = 0 as external reference.

The viscosity effect on the longitudinal relaxation rate R_1 was first corrected using the equation:

$$F_t = B \frac{\eta}{\eta^*} \implies \frac{\eta^*}{\eta} = \frac{F_t^*}{F_t} = f^v \implies R_1^*$$

where F_t is the flow time measured from the Ostwald viscometer, B is the viscometer constant, f is the density of the solution, f^v is the correction coefficient, and $*$ denotes water solution. The corrected longitudinal relaxation rates are plotted in Figure 2, indicated by the points. A saturation function $Y = Y_0 - ae^{-bx}$ is used to fit these data. The fitted values are indicated in Figure 2 by the lines. The points are the measured rate after viscosity correction and the lines are the calculated using the fitting function. The longitudinal relaxation rate of bound BSH, R^b (i.e. Y_0) is evaluated from this equation. With the calculated value of R^b , the binding fraction f at each concentration of the three proteins are calculated using the equation:

$$R_1^o = R_1^b f + R_1^u (1 - f)$$

since R_1^u and R_1^o are the longitudinal relaxation rate of BSH with and without albumin present, and are obtained from direct measurement. With the binding fraction f the concentration of bound and unbound BSH ($[B]^b$ and $[B]^u$) is then calculated.

With the known concentration of bound and unbound BSH, the binding constant K (mole $^{-1}$) and number of binding sites n on serum albumin is calculated using the binding equation (where $[SA]_0$ is the concentration of serum albumin).

$$\frac{[B]^b}{[B]^u [SA]_0} = kn - k \frac{[B]^b}{[SA]_0}$$

The number of binding sites (n) and the binding constant K (mole $^{-1}$) are evaluated from the slope and the intercept.

Figure 2. The longitudinal relaxation rate vs. the concentration of the serum albumins.

The results are listed in Table 4. The number of binding sites is about 3 to 5, which is similar to that obtained using gel-filtration and other methods by Dr. William F. Bauer (INEL). The binding constant is about 3 (mole⁻¹) at 295°K and 1.5 (mole⁻¹) at 310°K, which means that the binding effect decreases as temperature increases. Exception was observed on BSA, which shows a binding constant of 4.87 (mole⁻¹) at 310°K. The inconsistency may come from the purity and water content of this sample.

Task 3: Real-Time Measurement Dosimetry Research

The ISU accelerator has been down for maintenance during this period, so there is no prog-

ress to report on neutron yields from thick lithium targets.

Task 4: Analytical Dosimetry Software Development

Floyd Wheeler traveled to Petten during October under a collaborative agreement with the European Collaboration (EC) on BNCT in order to install a copy of the rtt_MC analytical dosimetry software for use in the EC program. This was done in response to a formal request made by Dr. Detlef Gabel, President of the EC, to Admiral James Watkins, Secretary of Energy. At a later date it is also planned to transfer a copy of the bnct_edit medical image processing software to the EC in response to the same request.

Table 4. Binding parameters of BSH.

A. The binding fraction of BSH at various concentration of serum albumin.						
f	DSA	295°K HSA	BSA	DSA	310°K HSA	BSA
1%	.26 ± .01	.31 ± .04	.34 ± .03	.23 ± .02	.21 ± .02	.42 ± .03
2%	.46 ± .03	.55 ± .07	.63 ± .03	.41 ± .03	.35 ± .03	.63 ± .02
3%	.60 ± .03	.70 ± .06	.74 ± .05	.54 ± .05	.48 ± .03	.79 ± .03
4%	.71 ± .03	.83 ± .03	.83 ± .03	.64 ± .05	.59 ± .03	.87 ± .03
5%	.79 ± .02	.89 ± .03	.89 ± .03	.74 ± .02	.68 ± .03	.93 ± .01
B. The binding constant and the number of binding sites to albumin.						
k(mole ⁻¹)	2.89	3.11	3.69	1.80	1.27	4.87
n	3.40	4.15	4.70	3.48	3.42	5.14

TECHNICAL SUPPORT CORE ACTIVITIES

Task 1: ICP-AES Analyses of Boron in Biological Samples

Samples Received 1711
 Samples Prepared for Analysis ~1126
 Samples Analyzed ~1188

Backlog

Awaiting Preparation ~3004
 Prepared Awaiting Analysis ~740

Task 2: Boron Compound Purity Determinations

Two separate shipments of chemicals were received for analysis from Boron Biologicals. The first batch, Na₂¹⁰B₁₂H₁₁SH, (Na₂¹⁰BSH) contained a total of twenty - 1 g ampules. One

vial was opened for the purity determinations. This vial (BNCT 456) represented the Boron Biologicals Lot number 082792. One vial from this lot was also archived. The second batch included 20 g of $\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$, (Na_2BSH) packaged in four - 5 g ampules. One vial (BNCT 466) was opened for the purity determinations. This vial represented the Boron Biologicals Lot number 93092. Two grams of the opened vial were transferred under nitrogen to a crimp top vial for archive purposes and the remainder was stored for general use. The samples received from Boron Biologicals were subjected to the routine INEL tests for purity analysis: high performance liquid chromatography (HPLC), NMR spectroscopy, carbon, hydrogen, nitrogen (CHN), analysis and ICP-atomic emission spectroscopy (AES).

HPLC Analysis: The chromatography was performed on a $250 \times \frac{1}{4} \times \frac{1}{3}$ mm column packed with 120-5 C_{18} , 5 μm particles of Nucleosil®. The mobile phase was approximately a 55% methanol-45% water with ~20 mM tetra butyl ammonium sulfate (TBAS) ion pairing reagent flowing at 0.5 mL/min. The target TBAS con-

centration was 25 mM, however there was some precipitate that was filtered out so the 20 mM concentration is an estimate only. The TBAS concentration was changed from 5 mM to the 20-25 mM range to improve the general peak shape of the BSH^{2-} as well as to achieve a better resolution of BSH^{2-} and $\text{B}_{12}\text{H}_{12}^{2-}$. Although, the higher concentration of ion pairing reagent appeared to provide better separation of the initial components, retention of all components was dramatically increased and the later eluting peaks had retention times of 60 min or more, therefore, the methanol content had to be increased to 55% to achieve elution in a reasonable length of time. However, it appears that at 55% methanol the upper limit of TBAS solubility becomes a problem.

The result of the chromatographic analysis are shown in Table 5 and representative sample chromatograms are shown in Figure 3. As usual, the reference sample (BNCT 202) was prepared as an individual sample and the test samples (BNCT 456 and 466) were prepared in triplicate. Duplicate chromatograms are obtained for all of the standards and samples.

Figure 3. Reversed phase, ion pair HPLC chromatograms of the new samples (BNCT 456, BNCT 466), the reference sample (BNCT 202) and standards of BSS^{4-} and BSSO^{4-} .

The presence of the as yet unidentified late eluting peaks is apparent in BNCT 202 and BNCT 466. Even with the new conditions, the analyses were unclear regarding the presence of $B_{12}H_{12}^{2-}$ since the $B_{12}H_{12}^{2-}$ peak shape appeared to be quite distorted even though the peak shape of BSH^{2-} was much better than in some previous analyses. The NMR analyses did not suggest that significant levels of $B_{12}H_{12}^{2-}$ were present but may hint that low levels may exist in the sample. The new conditions also seemed to degrade the BSS^{4-} peak as it tailed badly, possibly because of column degradation.

ICP-AES Analysis: Elemental analysis for B, S, and Na by ICP-AES were performed on aliquots of all samples and standards prepared for HPLC. The results of these analyses are presented as mole ratios in Table 6. Once again,

the B/S and B/Na ratios are slightly high indicating that there may potentially be a slight excess of B although these values are quite typical of those seen in past analyses.

CHN Combustion Analysis: A nitrogen atmosphere was utilized as much as possible for preparation of the samples used in the CHN combustion analyses. The CHN analysis results are presented in Table 7. Samples from BNCT 202 and 456 appear to have a somewhat significant level of carbon containing contaminants. The carbon levels of BNCT 202 are quite typical of past values. It is interesting to note at this point that upon opening the vials from BNCT 466 a sharp petroleum-like odor was present. Occasionally, a strong sulfur odor is present, however at this time the petroleum-like pungency was overpowering. Often the

Table 5. Oxidation products in BNCT 456, BNCT 466 and the reference sample (BNCT 202) as determined by HPLC.

Sample	Compound	BSS ⁴⁻ (wt%)	BSSO ⁴⁻ (wt%)
202	$Na_2B_{12}H_{11}SH$	1.49 ± 0.05	0.13 ± 0.01
456	$Na_2^{10}B_{12}H_{11}SH$	0.14 ± 0.04	0.03 ± 0.04
466	$Na_2B_{12}H_{11}SH$	1.37 ± 0.27	0.48 ± 0.07

All concentrations have been corrected for B isotopic abundance.

Table 6. ICP-AES analysis of BSH^{2-} compounds.

Sample	Compound	Mole Ratios		
		B/S	B/Na	Na/S
202	$Na_2B_{12}H_{11}SH$	12.6	6.30	2.01
210	$Cs_4^{10}B_{24}H_{22}S_2$	11.2		
212	$Cs_4^{10}B_{24}H_{22}S_2O$	13.2		
456A	$Na_2^{10}B_{12}H_{11}SH$	13.1	6.86	1.91
456C	$Na_2^{10}B_{12}H_{11}SH$	13.1	6.74	1.94
466A	$Na_2B_{12}H_{11}SH$	13.1	6.67	1.97
466B	$Na_2B_{12}H_{11}SH$	12.8	6.65	1.97
466C	$Na_2B_{12}H_{11}SH$	12.9	6.70	1.93

Table 7. CHN combustion analysis results.

Sample	wt% C	wt% H	wt% N
202*	0.81 ± 0.11	6.27 ± 0.04	0
456	0.90 ± 0.05	6.19 ± 0.04	0
466	0.40 ± 0.04	5.80 ± 0.24	0
Expected		5.5	

*202 stock vial

vial headspace has a definite smell that is usually described as the smell associated with sulfur compounds. Currently the vial headspace is undergoing GC-MS analysis to determine the source of the odor. Preliminary results indicate the possible presence of H_2S and the confirmed presence of CH_3SH , $\text{CH}_3\text{-S-CH}_3$ or $\text{CH}_3\text{CH}_2\text{SH}$, $\text{CH}_3\text{-S-S-CH}_3$, and $\text{CH}_3\text{-S-S-CH}_2\text{CH}_3$. Further investigations on the gaseous components found in the BSH^{2-} vials will be conducted in the future.

NMR Spectroscopy: The ^{11}B NMR of BNCT 466 and the ^{10}B NMR of BNCT 456 were unremarkable and indicate that BSH^{2-} is the primary constituent.

Fourier Transform Infrared Spectroscopy (FTIR) Spectroscopy: The Boron Biologicals samples and the reference sample were prepared for FTIR analysis entirely under nitrogen. The actual quantity of sample in each pellet was not accurately determined, consequently, some pellets may have had more sample material in them than others thus, the spectra in Figure 4 have been normalized for clarity and to offset the sample mass differences. All single-beam IR spectra were ratioed to a single-beam spectrum of a blank KBr pellet. Sample BNCT 466 appears to have less water than BNCT 202 or 456. Samples from BNCT 202 and 456 indicate a slightly higher presence of organic carbon at $2900\text{-}3000\text{ cm}^{-1}$, which correlates with the CHN analysis.

Figure 4. Infrared spectra of reference sample BNCT 202, and test samples BNCT 456, BNCT 466.

Task 3: Intra- and Intercellular Boron Analyses

Secondary Ion Mass Spectrometry (SIMS): In July a collaborative effort between Charles Evans and Associates and WSU researchers to perform a SIMS experiment with BSH infused rats was initiated. A laboratory rat was intravenously infused with ^{11}BSH (100 mg B/kg body weight) and the boron allowed to distribute to the tissues for about one hour. This rat and a control rat (not administered with boron) were then euthanized and the liver and three regions of the brain (cerebral hemispheres and brainstem) removed and immediately cryopreserved using a liquid nitrogen metal mirror fixation unit. At the time of writing this report, only the liver tissues from the infused and control rats were analyzed by SIMS and no ICP-AES data were available for comparison.

Cryofixation, as opposed to chemical fixation, is the only method by which both ultracellular and intracellular element contents can be simultaneously preserved. The frozen hydrated tissues were then cryoultramicrotomed at about -140°C using no embedding medium, including the "low temperature" types or freeze substitution technique, which may inadvertently cause some form of chemical change to occur in the tissues. Freeze-drying was performed on the cryosections and the freeze-dried sections placed onto high-purity silicon wafers for SIMS analysis. Although not performed during this test experiment, it is imperative that conventional transmission electron microscopy (CTEM) is performed in parallel to the SIMS analysis to evaluate the quality of the cryosections with respect to induced structural artifacts from the cryopreparation procedures.

Brain homogenate has been used as the matrix standard in this study because our primary goal is to accurately quantify boron in brain tumors. Figure 5 shows boron calibration curves of ^{11}B -boric acid and ^{11}BSH in brain homogenate and ^{11}BSH in gelatin. The absolute boron concentrations were quantified by ICP-AES. A similar experiment using the isotopically enriched form of these compounds was also performed. Irrespective of the boron isotope used, the slope of the calibration plots for BSH in brain homogenate was consistently lower than with boric acid. However, similar slopes are observed for the plots of ^{11}B -boric acid in brain homogenate and ^{11}BSH in gelatin, suggesting

that the sampled homogenate analyzed was more aqueous containing unbound boron. Similar experiments in aqueous media are being performed to confirm this hypothesis.

For the purpose of this study, an average value of the brain homogenate and gelatin calibration results was used for SIMS quantification in the liver tissue. Dynamic SIMS profiles acquired from the livers of the diffused and control rat are shown in Figure 6. Boron concentrations of $53\text{ }\mu\text{g B/g}$ in the diffused rat and $13\text{ }\mu\text{g B/g}$ in the control rat were observed. The boron concentration determined by SIMS-based calibration curves should be evaluated as preliminary estimates. The time-axis in Figure 6 represents sputtering time and represents the depth profile of the boron concentration (in the range of $300\text{-}400\text{ }\text{\AA}$). The observed boron in the liver sample of the control rat (no BSH infusion) probably represents the background boron from dietary intake in these preliminary studies. The boron secondary ion image of the infused rat's liver are shown in Figure 7. Boron is homogeneously distributed and is not observed in the tissue voids indicating that no diffusible boron was lost during sample preparation. The potassium secondary ion image in Figure 8 shows clusters of high intensity spots probably corresponding to cells. The observed potassium/sodium ratio of about eight suggests that the tissue was viable at the time of cryopreservation.

In conclusion, these preliminary results illustrate that with appropriate cryopreservation of tissue, quantitative imaging is possible by SIMS. Quantification using brain homogenate gave reasonable boron concentration values in the liver; a comparison with ICP-AES results will confirm the accuracy. Boron was homogeneously distributed in the freeze-dried liver section of the infused rat. The viability of the tissue at the time of cryopreservation was confirmed by the high potassium to sodium ratio. The high intensity potassium spots observed probably correspond to individual cells.

Further improvements in the cryopreservation methodology is required to obtain the higher resolution images needed for this localization study.

Figure 5. Boron calibration curves of BSH and boric acid in brain homogenate and gelatin.

Figure 6. SIMS depth profiles of boron in the liver of an infused BSH rat and control rat.

Figure 7. Boron secondary ion image of liver tissue from the infused rat.

Figure 8. Potassium secondary ion image of liver tissue from the infused rat.

Task 4: Neutron Beam Measurement Dosimetry

A plexiglas rat-phantom has been constructed as a monolithic piece by a researcher at WSU. It was trimmed down in order to make it fit into the Brookhaven Medical Research Reactor (BMRR) 5 cm diameter rat holder. The head was also changed to the right-angle orientation used at BNL. The phantom has been drilled to allow placement of dosimeters in the head, neck, chest and body. Figure 9 shows the modified phantom with the dosimeter holes. The holes are designed to take either a 6 mm long TLD 400 gamma dosimeter or a gold-copper wire for neutron flux measurement. Dr. Tom LaHann at ISU was consulted to determine the brain center, esophagus and lung locations, as well as for the skull orientation. A magnetic resonance imaging (MRI) scan of the phantom was obtained. (See Task 5.)

Calculation of expected count rates indicate that the 1.5% gold-in-copper wire used in the past at the epithermal beam port for neutron flux monitors will be satisfactory for the rat phantom placed at the thermal port. These show that for a thermal flux of 1.15×10^{10} and an epithermal flux of 2.4×10^9 neutrons/cm² sec, a five minute irradiation of a 2 mm piece of this wire (14 mg) will provide a count rate of about 280 cps from the copper and 100 cps from gold using the existing sodium-iodide detector system.

Two additional rat collars have been fabricated for use at BMRR with live rats. The purpose of these collars is to allow some leeway in positioning a rat's neck in the holder, since the centered collar may cause unacceptable bending of the spine in the head-neck region. The original collar assumed that the neck is centered in the holder cylinder, with the head at right angles, the same as shown on the rat-phantom sketch. The new collars allow displacement of the neck either 0.6 cm toward the bottom of the holder cylinder, or all the way to the bottom.

Task 5: Canine Dosimetry Calculations

A set of MRI images of the lucite rat phantom was made at the Eastern Idaho Regional Medical Center (EIRMC) for future use in support of the planned rat irradiations in the BMRR thermal beam.

Task 6: BNCT Database Management System

Database development and data entry continue at WSU.

Task 7: Georgia Tech Research Reactor Physics Support

A joint statement of work for a proposed cooperative research and development agreement (CRADA) between INEL, Neutron Technology, and The Georgia Institute of Technology was drafted and verbally agreed to by all parties. The purpose of this proposed CRADA is to design and install an advanced epithermal-neutron beam in the large horizontal biomedical irradiation port at the Georgia Institute of Technology Research Reactor. The INEL contribution to the CRADA would be to provide analytical physics and thermal-hydraulic support to the beam design and to provide experimental characterization of the resulting epithermal beam. INEL will be granted access to the medical room (and beam) for some specified period of time.

Task 8: Research Reactor/Accelerator Physics Support

No progress to report.

Miscellaneous:

Several presentations were made at the Fifth International Symposium on Neutron Capture Therapy in Columbus, OH September 13-17, 1992, including a plenary lecture on Analytic Dosimetry, and a plenary lecture on the results of the canine irradiations at BNL and Petten.

H157-RR-1102-01

Figure 9. Modified rat phantom.

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