DOE/ER/60892--3

DE92 013583

# SYNTHESIS AND *IN-VIVO* DETECTION OF BORONATED COMPOUNDS FOR USE IN BNCT

Comprehensive Progress Report

August 1, 1989 - July 31, 1992

George W. Kabalka, Ph.D.

Department of Radiology
University of Tennessee Medical Center at Knoxville
Knoxville, Tennessee 37920



January, 1992

PREPARED FOR THE U.S. DEPARTMENT OF ENERGY
UNDER GRANT NUMBER DE-FG05-893460892

## TABLE OF CONTENTS

## I. OBJECTIVE

The primary objective of the D.O.E. program at The University of Tennessee Graduate School of Medicine is the development of effective molecular medicine for use in neutron-capture therapy (NCT). The research focuses primarily on the preparation of new boron-rich NCT agents and the technology to detect them *in-vivo*. The detection technology involves the development of effective magnetic resonance imaging (MRI) and spectroscopy (MRS) techniques for verifying and measuring NCT agents *in-vivo*. The synthetic program is directed toward the design of novel boron NCT (BNCT) agents which are targeted to the cell nucleus and gadolinium liposomes targeted to the liver. The U.T. - D.O.E. program is unique in that it has access to both state-of-the-art whole-body and microscopy MRI instruments. In addition, the U.T. - D.O.E. researchers actively collaborate with colleagues at other D.O.E. facilities (Brookhaven National Laboratory, Oak Ridge National Laboratory, and Los Alamos National Laboratory).

An important goal of the D.O.E. program at U.T. is to provide training for students (predoctoral and postdoctoral). The University of Tennessee is one of the very few institutions in the world where students have "hands-on" access to both modern scientific equipment and medical imaging modalities such as the clinical MRI units. The academic nature of the program facilitates collaborative interactions with other D.O.E. programs and helps to insure the continued availability of skilled scientists dedicated to the advancement of molecular medicine techniques.

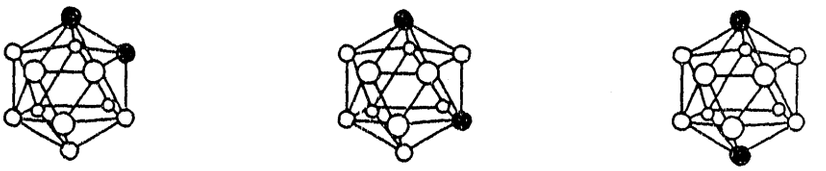
## II. RESEARCH ACCOMPLISHMENTS

I am pleased to report that the D.O.E. NCT Program at Tennessee has been a productive one. Eleven journal articles resulted from the work and fourteen scientific presentations were made; they are compiled in **Section III**.

### A. SYNTHESIS OF NEUTRON CAPTURE THERAPY AGENTS

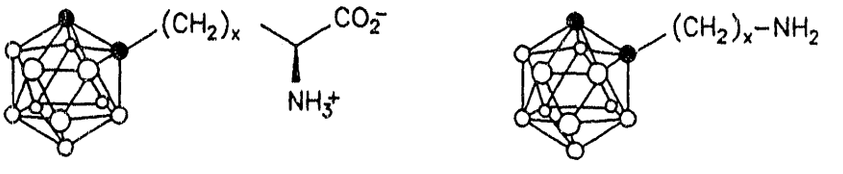
#### 1. Carboranyl Precursors

Carboranes contain ten boron atoms in a three-dimensional space equivalent to a benzene ring; consequently, the carborane isomers can be utilized to prepare a variety of boron-rich agents for potential use in boron-neutron-capture therapy.

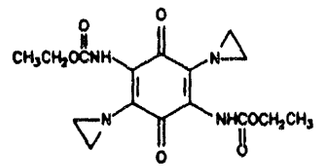


[Where ○ =BH, ● =CH]

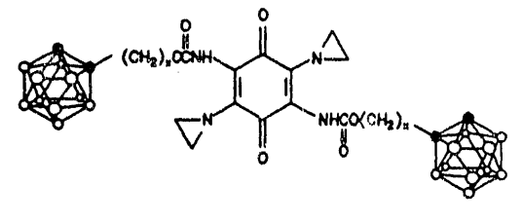
We are currently developing synthetic methodology suitable for preparing amino acids and other physiologically active compounds of potential use in BNCT.



The methodology involves the conversion of simple carboranes into more complex, reactive organometallic reagents which can then be utilized to prepare agents which will target the nuclei of tumor cells. Specific examples include the projected syntheses of boron analogs of known intercolators such as Diazaquone (AZQ) which have been proven effectiveness in chemotherapy.



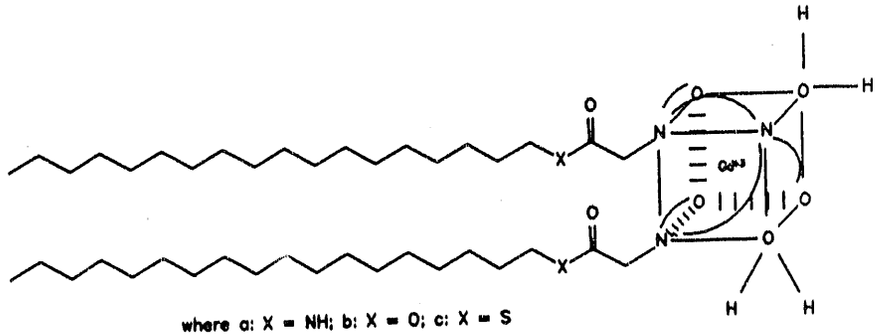
Diaziquone
AZQ
NSC-182986



AZQ
Analog

## 2. Gadolinium NCT Agents

We have synthesized and carried out biodistribution studies of gadolinium labeled liposomes (GLL) which were developed in our laboratory. Gadolinium (like boron-10) has an excellent neutron cross section and is considered to be of potential use in neutron capture therapy. GLL are constructed by adding gadolinium based amphiphiles, such as the one illustrated on the next page, to liposome preparations.



where a: X = NH; b: X = O; c: X = S

Since liposomes can be selectively targeted to organs by coating them with various proteins or by sizing them, we prepared a series of liposomes and investigated their retention in the livers of Balb/c mice. The GLL tested were constructed by linking the Gd-DTPA head group to the stearyl chains via amide (Gd-DTPA-SA), ester (Gd-DTPA-SE), and thioester (Gd-DTPA-ST) bonds.

*[See Section IIIa, articles 4, 5, 7, 8 and 9;*
*Section IIIb, abstracts 2, 4, 7, 9]*

## B. MULTINUCLEAR MAGNETIC RESONANCE IMAGING

Generation of MR images for fast relaxing nuclei such as boron is often hampered by the fact that the transverse relaxation times ($T_2$) are considerably shorter than the available time-to-echo (TE). The minimal TE achievable in MR imaging is limited by the need to apply phase-encoding and dephasing/rephasing read-out gradients which have finite rise times imposed by the hardware limitations. Currently, echo times of

2.5 ms are attainable using conventional spin-warp imaging techniques and it may be possible to shorten TE to approximately 1 ms by reducing the resolution requirements. However, boron-11 and boron-10 possess intrinsic transverse relaxation rates *in-vivo* which are so short that even after 1 ms most of the signal has already decayed, resulting in an unacceptably low signal.

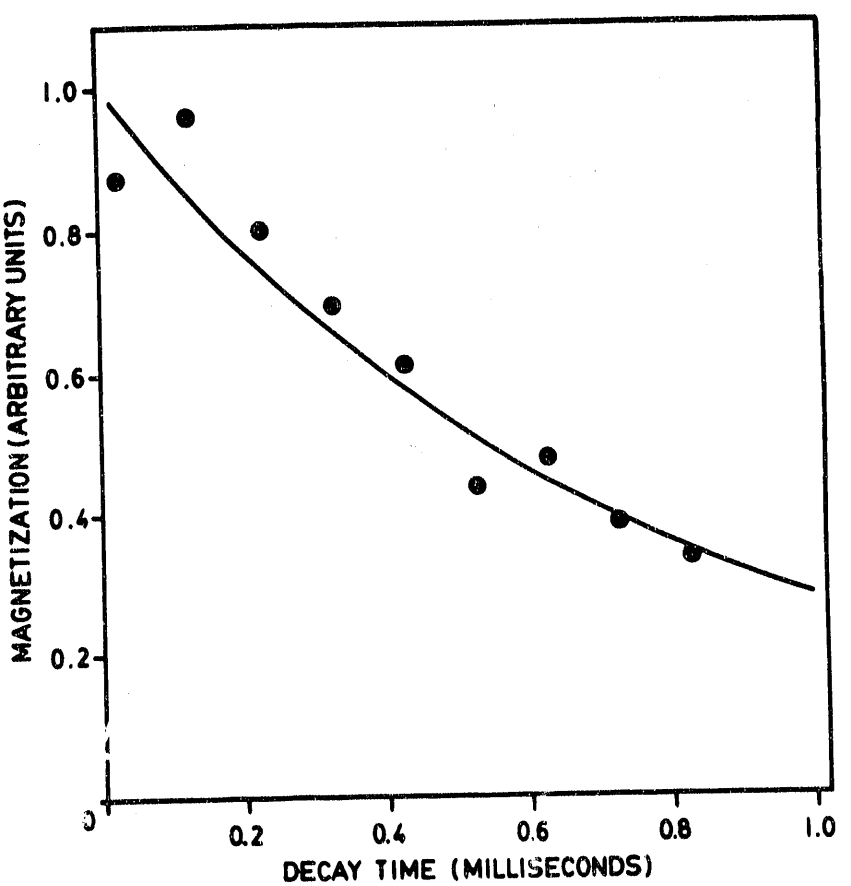



**Figure:** ***In Vivo*** **measurement of boron-11 transverse relaxation ($T_2$) in the rat.**

To overcome problems due to the short $T_2$ of boron-11, we designed a back-projection technique from which we were able to generate the world's first boron-11 MRI image of a rat that had been infused with a BNCT agent. The spectra were acquired in the presence of variably orientated gradients and these spectra were used as input for reconstructing filtered back-projection images such as the one shown below.

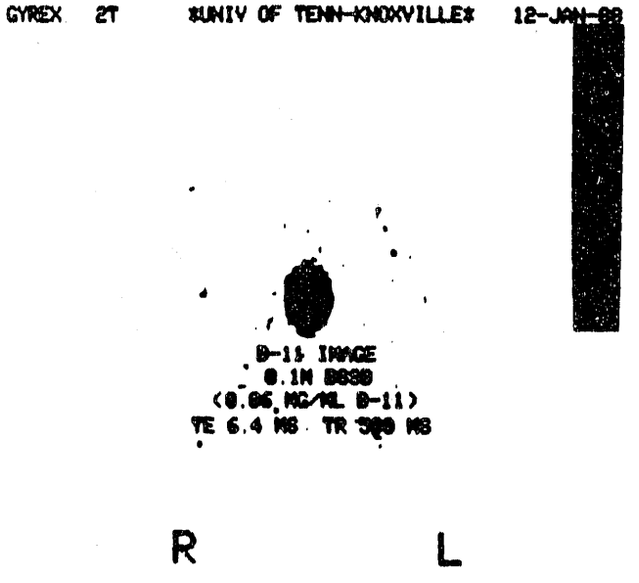



**Figure: First boron-11 image of a BNCT agent at a concentration used in preclinical BNCT studies which was obtained by reconstruction from 20 projections on a 64 x 64 matrix.**

*[See Section IIIa, articles 1, 2, 3 and 4; Section IIIb abstracts 1,2,3 and 4]*

Though successful, back-projection boron-11 MRI is time consuming and provides only modest spatial resolution. We then developed an alternate imaging method for nuclei with very short $T_2$ relaxation times. As in backprojection, the technique utilizes the FID, rather than the echo signal, but involves only phase-encoding of the signal without frequency encoding. The method was tested by obtaining boron-11 images of aqueous boron phantoms, and was used to map the boron-11 distribution in an intact Fischer 344 rat infused with a therapeutic dose of dimeric sulfhydryl dodecaborane agent by our colleagues (Slatkin and Micca) at Brookhaven National Laboratory.

In the new method both spatial dimensions are phase-encoded by the usual amplitude cycling of gradients along the corresponding directions. The time during which these gradients are phase-encoding the signal is extremely short, 200 $\mu$s. The time-domain signal is then Fourier transformed, in the usual manner, to yield an x-y image. An extension to three spatial dimensions can be achieved by adding a third phase-encoding gradient along the z-axis.

The minimal performance time of the proposed sequence is longer than that of a conventional spin-warp sequence since at least one scan for each resolution element is required. But this is not an obstacle for the imaging of boron-11 *in-vivo* since the necessary number of scans

needed for adequate signal averaging will usually exceed the minimal number of experiments required by the imaging process.

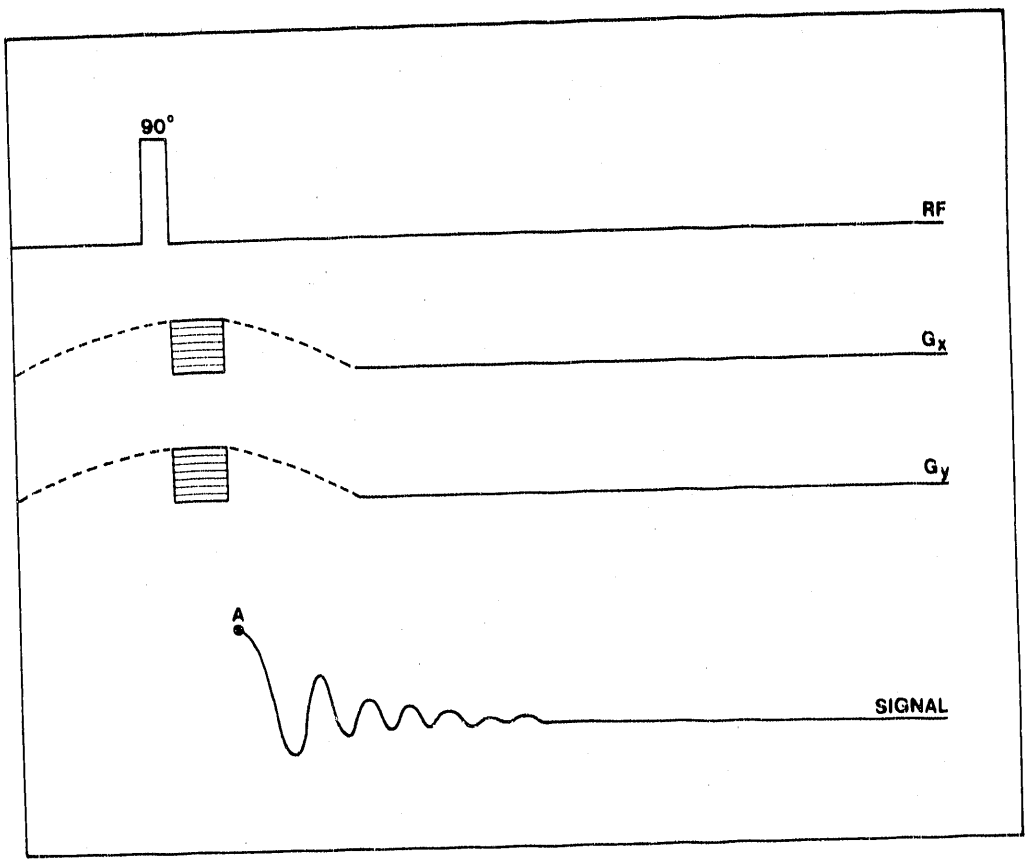



**Figure:** **Pulse sequence for two-dimensional imaging of a species with a very short $T_2$. The phase-encoding gradients are turned on prior to the rf pulse and turned off after collection of the first data point (A).**

Imaging experiments were performed on a Gyrex 2T, 90cm bore, whole-body MR imaging unit operating at 26 MHz for boron-11 using a 6-turn solenoidal coil (10 cm diameter) adjusted to the size of the rat. All imaging experiments were conducted at ambient temperatures. The animals used were Fischer 344 male rates infused with sodium-$\mu$-disulfide-bis(undecahydro-*closo*-dodecaborate), $Na_4B_{24}H_{22}S_2$, at

Brookhaven National Laboratory; the rats were and shipped by air freight to Knoxville where the imaging experiments were performed.

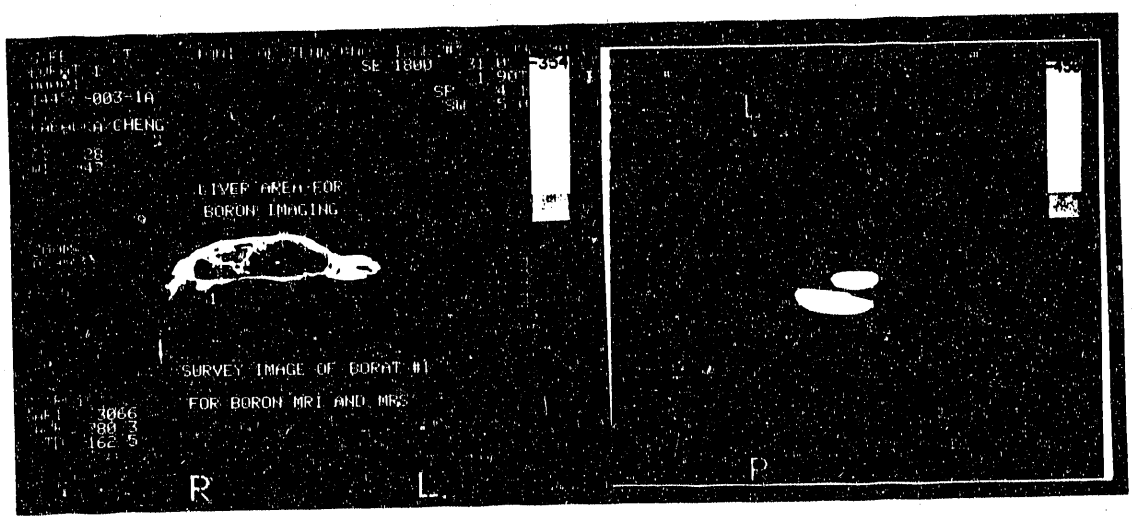



**FIGURE: Hydrogen (left) and boron-11 (right) MRI of a rat infused with a BNCT agent.**

The new method possesses a number of advantages when compared to backprojection techniques. First, the natural linewidth of the signal (which is expected to be very broad due to the short $T_2$) does not degrade the spatial resolution since no frequency encoding is employed. Second, for the same reason, the images acquired by the method are free of artifacts and degradation resulting from magnetic field inhomogeneities, susceptibility variations and chemical shifts. (Both advantages are achieved without the need to employ gradients which are large compared to the prevalent interaction strengths in the spin system.)

Third, the analog filter can be set to a much smaller frequency cutoff, compared to backprojection, which results in a substantial improvement in signal-to-noise.

*[See Section IIIa, articles 8, 9, 10, and 11; Section IIIb, abstracts 10, 11, 13, and 14]*

## III. BIBLIOGRAPHY OF PUBLICATIONS

### A. JOURNAL ARTICLES


1. G. W. Kabalka, P. Bendel, M. Davis, D. N. Slatkin, P. L. Micca, in "Boron-11 Magnetic Resonance Imaging and Spectroscopy: Tools For Investigating Pharmokinetics for Boron Neutron Capture Therapy," R. G. Fairchild and J. P Bond, in "Boron Neutron Capture Therapy,"eds., Plenum Press, New York, pp 243-249, 1989.

2. E. Holmberg, K. Maruyama, D. C. Litzinger, S. Wright, M. Davis, G. W. Kabalka, S. J. Kennel, and L. Huang, "Highly Efficient Immunoliposomes Prepared with a Method which is Compatible with Various Lipid Compositions," *BBRC,* **165**, 1272 (1989).

3. S. J. Kennel, S. Bultman, R. Lee, and G. W. Kabalka, "Rat Monoclonal Antibody Distribution in Mice: An Epitope Inside the Lung Vascular Space Mediates Very Efficient Localization", *Int. J. Radiat. Appl. Instr.* **17**, 193 (1990).

4. P. Bendel, M. Davis, E. Berman, and G. W. Kabalka, "A Method for Imaging Nuclei with Short T2 Relaxation: Application to Boron-11 MR Imaging of a BNCT Agent in an Intact Rat," *J. Mag. Reson.*, **88**, 369 (1990).

5. G. W. Kabalka, "Gd-Labeled Liposomes Containing Amphipathic Agents for MRI", *Invest. Radiol.* **25**, S63 (1990).

6. G. W. Kabalka, M. A. Davis, T. H. Moss, E. Buonocore, K. Hubner, E. Holmberg, K. Maruyama, and L. Huang, "Gadolinium-Labeled Liposomes Containing Various Amphiphilic Gd-DTPA Derivatives: Targeted MRI Contrast Enhancement Agents For The Liver", *Magn. Reson. Med.*, **19**, 406 (1991).

7. G. W. Kabalka, M. A. Davis, K. Hubner, E. Holmberg, K. Maruyama, and L. Huang, "Gadolinium-Labeled Liposomes Containing Various Amphiphilic Gd-DTPA Derivatives of Varying Chain Length: Targeted MRI Enhancement Agents", *Magn. Reson. Med.*, **9**, 373 (1991).

8. G. W. Kabalka, G. Q. Cheng, P. Bendel, P. L. Micca, and D. N. Slatkin, "In-Vivo Boron-11 MRI and MRS Using $(B_{24}H_{22}S_2)^{4-}$ in the Rat", *J. Magn. Reson. Imag.*, **9**, 969 (1991).

9. G. W. Kabalka, Q. C. Cheng, P. Bendel, D. N. Slatkin, and P. L. Micca, "Boron MRI Method for Imaging BNCT Agents", Plenum Press, in press.

10. G. W. Kabalka, G. -Q Cheng, P. Bendel, "In Vivo Boron-11 MRI in the Rat", Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, *Elsevier,* Amsterdam, in press.

11. G. W. Kabalka, G. Q. Cheng, and P. Bendel, "Boron-11 MRI and MRS", in NMR Microscopy: Methods and Applications in Material Science, Agriculture, and Biomedicine, VCH Publishers, Germany, in press.

## B. INVITED PRESENTATIONS

1. "A Boron-11 Imaging Technique that Combines Chemical Shift and Spatial Localization", M. Davis, P. Bendel, E. Berman, and G. W. Kabalka, Society of Magnetic Resonance in Medicine. Annual Meeting, Amsterdam, Netherlands (August, 1989).
2. "Gadolinium-Labeled Liposomes Containing Paramagnetic Amphipathetic Agents: Targeted MRI Contrast Agents", Contrast Media Research-89, Sydney Australia (October, 1989).
3. "Use of Organoboranes in Modern Medical Imaging", G. W. Kabalka, Boron USA II Workshop, Raleigh, NC (June 1990).
4. "Gadolinium Labeled Liposomes", G. W. Kabalka, M. Davis, and L. Huang, Society of Magnetic Resonance in Medicine Meeting, New York, NY (August, 1990).
5. "A New Boron MRI Method for Imaging BNCT Agents In Vivo", Fourth International Symposium on Neutron Capture Therapy for Cancer", University of Sydney, Sydney, Australia (December, 1990).

6. "Recent Advances in Magnetic Resonance Imaging: Organ Specific Relaxation Agents and Multinuclear Imaging of BNCT Agents", Royal Northshore Hospital, Sydney Australia (December, 1990).

7. "Synthesis of Organ Specific MRI Contrast Agents", Mallinckrodt Medical Inc., St. Louis, MO (June, 1991).

8. "Gadolinium-Labeled Liposomes Containing Various Amphiphilic Gadolinium-Diethylenetriamine-pentaacetic Acid Derivatives: Targeted MRI Contrast Agents", G. W. Kabalka, E. Buonocore, M. Davis, K. Muruyama, E. Holmberg, and L. Huang, Society of Magnetic Resonance in Medicine Meeting, San Francisco, CA (August, 1991).

9. "NMR Dispersion Studies on the Effect of Vesicle Size Upon Relaxivity of Surface Associated Paramagnetic Chelates: Implications for the Design of Liposomal and Polymeric Contrast Media", C. Tilcock, Q. F. Ahkong, S. Koenig, R. Brown III, M. Davis and G. W. Kabalka, Society of Magnetic Resonance in Medicine Meeting, San Francisco, CA (August, 1991).

10. "In Vivo Boron-11 MRI and MRS Using $(B_{24}H_{22}S)^{4-}$ in the Rat", G. W. Kabalka, G.-Q. Cheng, P. Bendel, Society of Magnetic Resonance in Medicine Meeting, San Francisco, CA (August, 1991).

11. "In Vivo Boron MRI in the Rat", International Isotope Society, Toronto, Canada (September, 1991).

12. "Boron MRI and MRS", International Conference on NMR Microscopy, Heidelberg, Germany (September, 1991).

13. "In Vivo Boron-11 MRI in the Rat", G. W. Kabalka, G.-Q. Cheng, P. Bendel, International Isotope Society Meeting, Toronto, Canada (September, 1991).

14. "Boron MRI and MRS", G. W. Kabalka, P. Bendel, and G.-Q Cheng. International Conference on NMR Microscopy", Heidelberg, Germany, (September, 1991).

## IV. GRADUATE AND POSTDOCTORAL STUDENTS

Three postdoctoral students and three graduate students were supported by this Department of Energy NCT Program.

**A. Postdoctoral Student**

Dr. Quang-Qiang Cheng
Dr. Charles A. Anderson
Dr. N. Kesavulu Reddy

**B. Graduate Students**

Mark A. Davis (Ph.D.)
George Hondrogiannis (Ph.D. candidate)
Zhe Wang (Ph.D.)

## VII. PRESENT STATE OF KNOWLEDGE

Boron neutron capture therapy is a form of brachyoradiotherapy which is based on the properties of boron-10 and the reaction which occurs when it is exposed to neutrons. Thus successful BNCT is dependent on two factors: (1) effective delivery of a sufficient quantity of boron-10 to the targeted lesion and (2) neutron flux of sufficient energy to achieve the prerequisite nuclear reaction while minimizing damage to healthy tissue.

$$^{10}B_5 + {}^{1}n_0 \longrightarrow {}^{7}Li_3 + {}^{4}\alpha_2$$

Significant advances have been made in both these areas in recent years by chemists and physicists such that the future of BNCT in medicine appears assured, but much remains to be accomplished before BNCT is a clinical reality. For example, although physicists have developed methodology to effectively deliver epithermal neutrons, reactors appropriately modified for use in clinical protocols are still not readily available; research focused on alternative epithermal neutron sources and/or on the cost-effective modification of reactors near large medical centers will continue to be a high priority. Of even greater priority is the continued development of techniques to generate, deliver, and detect boron-rich pharmacologically active agents in a clinically suitable mode. Effective BNCT demands that the boron content of targeted tissues exceeds 15 parts per million and that the ratio of the boron in the target tissue to that in the

neighboring healthy tissue is >3 so that minimal destruction of healthy tissue is realized. Biochemists and synthetic chemists have designed a number of BNCT agents for potential use in clinical BNCT; boronic acid analogs of amino acids have shown promise, as has a series of boronated antibodies. To date, these agents have been effective in a limited number of clinical protocols. Clearly, more powerful and broadly based BNCT agents must be developed.

*In-vivo* biodistribution and pharmacokinetic studies are intimately tied to the successful development of clinically useful BNCT agents. Currently, there are no clinically useful methods for determining boron concentrations *in-vivo*. Thus biodistribution and pharmacokinetic studies must be accomplished post-mortem or in animal experiments. Certainly, continuing research on non-invasive analytical techniques such as whole-body magnetic resonance imaging and spectroscopy should remain a high priority.

The D.O.E. NCT program at The University of Tennessee Biomedical Imaging Center has successfully developed a two-prong research plan focused on the development of new tumor-specific, anticancer drugs and methodology for the non invasive, *in-vivo* detection of these agents. The chemistry of these exciting agents has only begun to develop but significant advances have been made in boron detection at The University of Tennessee; this includes the world's first *in-vivo* boron MRI of a successful BNCT agent.

# END

DATE
FILMED
7/1/92