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In the third year, we have procured specialized cryo-valves, low-temperature components and Oxford cryostat. All these components are assembled with the HTS coil. The complete HTS probe is under final evaluation. We have concentrated on in vivo NMR spectroscopy and micro-imaging studies of MCF7 wild type and MCF7/ADR drug resistant tumors. A localized NMR spectroscopy technique ISIS (image-selected in vivo spectroscopy) was implemented. Detailed in vivo ^{31}P spectra were obtained. Many phosphate metabolites are identified and they are similar to the in vitro results. During tumor progression, the phosphocreatine and adenosine triphosphate gradually decrease while the inorganic phosphate increases. The control signals of non-involved muscle show constant intensities. The phosphomonoester and phosphodiester signals are too weak for an accurate measurement. For the microscopic imaging of mouse, it demonstrated many small capillaries. The image resolution of 30 μm is accomplished.

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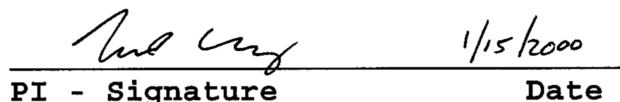

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TABLE OF CONTENTS

I.	Front Cover	1
II.	SF298 Form	2
III.	Foreword	3
IV.	Table of Contents	4
V.	Introduction	5
VI.	Body	6-11
VII.	Conclusions	11
VIII.	References	12-14
IX.	Appendices	15-20

V. Introduction

Conventional mammography has been shown to play an important role in detection and staging of breast cancer in older women. For younger women who frequently have radiodense breast tissue or women with silicon implants, rendering breast cancer diagnosis with conventional mammography is problematic. When mammographic findings and clinical findings concur regarding the possibility of a lesion being malignant, usually a fine-needle aspiration biopsy will be performed for definitive diagnosis. The false positive rate is high; only 20-30% of lesions suspicious for cancer at mammogram is actually positive for cancer at biopsy. In general, mammography is limited to detect a tumor several millimeters or larger in size. Because of difficulty with early detection, clinicians are sometimes limited to treat larger size cancers, which in many cases have already metastasized. Accurate definition of tumor size, number, and margins is highly critical in the clinical determination of conservation treatment versus mastectomy. A role exists for an imaging method that can improve sensitivity for detection of small lesion and to improve the specificity for better staging of the disease. To provide the best chance of overall survival, breast cancers need to be accurately staged for systemic treatment and optimal conservation surgery. Traditionally, the gold standards for such assessments are clinicopathological staging and histopathological typing and grading of malignancy. In the classical histopathological approach problems exist inherently, predominantly, the accuracy of the initial biopsy procedure and the variable skills applied to its histological assessment. Development of a new modality to remove sampling errors, improve specificity and produce a grading of tissues that relates to establish biological criteria would be very useful.

Over the last few years, magnetic resonance imaging (MRI) and spectroscopy (MRS) have emerged as one of the most promising clinical tools to fill the gap between clinical needs and information obtained by conventional breast imaging and pathological methods. Preliminary results indicate that MRI may be more sensitive than conventional x-ray mammography in detecting small lesions. Cancers have typical metabolic characteristics in ^{31}P and ^{1}H MRS including high levels of phospholipid metabolites and a cellular pH more alkaline than normal. Although these alone are not unique for cancer they are very useful diagnostic information in appropriate clinical settings. MRS is capable of distinguishing benign and malignant lesions in a particular anatomical site and to be a specific diagnostic discriminant in a particular situation. It has been demonstrated to be useful to improve the specificity of the MR imaging of breast. Some metabolic characteristics appear to be prognostic indices and correlate well with the response of treatment. The improvement of specificity will reduce the number of biopsies performed to confirm false-positive mammographic findings and more effectively assess the results of treatment. Many of these progresses are based on the advances of nuclear magnetic resonance (NMR) studies of perfused breast cancer cells and tumor-bearing animal models. One of the major limitations of the application of NMR methods both in vitro and in vivo is its low sensitivity. The sensitivity determines the ultimate cancer detection capability and the resolution in image and in spectrum. In this study, a high temperature superconductor (HTS) working at very low temperature will be used to reduce electronic noise and significantly improve the sensitivity of detection. It will dramatically increase the sensitivity and improve the resolutions. The improvement will be verified by comparing the sensitivity with that of a conventional probe.

The improvement of detection sensitivity will provide a more accurate diagnosis, and it may become possible for early prediction of tumor response to therapy. The probes will be constructed with $YBa_2Cu_3O_7$ material and tested in two well defined experiments: an in vitro cell metabolism study on a 9.4 T spectrometer and an in vivo tumor bearing animal study on a 4.7 scanner. In the cell metabolism study, the breast cancer cell line MCF7 and its variants will be studied in terms of characteristic differences of their ^{31}P spectra during growth phase and under effects of Tamoxifen. In the in vivo animal study, MCF7 cells and its variants will be grown as xenografts on nude mice. The differences of ^{31}P spectra during progress of tumor and responses to Doxorubicin and Tamoxifen will be studied. The high-resolution proton imaging experiments of vasculature of tumor will be conducted using both conventional copper probe and the proposed HTS probes.

VI. Body

In the first year of the project, the design of self-resonant probes for high-resolution NMR has been completed. The receiver coil uses thin-film, high temperature superconductor (HTS), $YBa_2Cu_3O_7$. The transmitter coil is a standard room-temperature coil. The probe is designed to fit either a 9.4 T machine for in vitro cell study or a 4.7 T machine for animal study. The coils are detachable so that different coil can be substituted in and out of the different machines and for different nuclei. Three identical cell perfusion apparatus for the NMR study of breast cancer cell metabolism have been constructed and tested. The apparatus was tested using known standard compounds. To study the metabolism of breast cancer cells for an extended period of time, the cells are continuously perfused with nutrients. During perfusion, the breast cancer cells are restrained in agarose gel-thread matrices. A protocol for making agarose gel-thread matrices containing MCF7 breast cancer cells is established. Besides the above-mentioned tasks, some of the infrastructure and preparation works necessary for conducting the proposed research have been accomplished including relocation of a 400 MHz machine and renovation of laboratories.

In the second year of this project, we have fabricated and tested a HTS coil, as well as, studied the ^{31}P spectroscopic differences of MCF 7 cells and its variants and their responses to Tamoxifen and Doxorubicin. Based on the test done at 77 ^{0}K, the HTS coil has a resonance frequency 401.6 MHz and the Q value for the coil is 650. This is better than we expected in the design. Since the coil is going to be mounted in the cryostat on a sapphire substrate and cooled below 40^0 K, the Q should increase further. We have studied the differences in the ^{31}P NMR spectroscopic profiles for drug sensitive MCF7 cancer cells and their multidrug resistant variant MCF7/ADR cells. The cells are embedded in agarose gel threads and perfused with growth medium during the NMR studies. Many detailed phosphorus metabolites have been identified. There may be some subtle differences in the spectra of the two cell lines. However, the differences are not conclusive using the conventional probe. We have successfully demonstrated drug sensitive MCF7 cells, which were dramatically affected by 2 μM Doxorubicin within two hours perfusion and not responsive to Tamoxifen up to 12 hours. In contrast, 2 μM Doxorubicin was without any effect on multidrug resistant MCF7/ADR cells and the ^{31}P NMR spectrum did not differ appreciably after addition of Doxorubicin. In order to have a highest S/N in the in vivo studies, a great deal of efforts have

been made to ensure a reliable NMR system and a contamination free cell culture environment. The scan parameters are optimized. The magnetic field drift during the long acquisition time is negligible. All the potential cell contamination sources are eliminated.

In the third year, we concentrated on the in vivo animal study. The MCF7 wild type human breast cancer cells and its drug resistant variants are grown as solid tumor xenographs in athymic nude mice as the animal model. This in vivo animal study is a continuation of our previous in vitro cell study. The results from the animal model will be used to confirm whether the differences seen in vitro are also observed in the in vivo spectra obtained for the tumors growing in nude mice. The in vivo NMR imaging and spectroscopy studies of the solid tumor provide information regarding (i) heterogeneity and microvasculature of tumor, (ii) energy metabolism, (iii) tumor pH, (iv) tumor hypoxia, (v) observed predictive response to antiestrogens and Doxorubicin even before regression by tumormeter measurements. Besides this in vivo animal study, we also have completed the integration of the Oxford Spectrostate cryostat with the HTS probe (Figure 1). The whole system includes HTS coil, mounting facilities, the fine tuning paddle and a copper impedance matching loop and the preamp.

One of the problems in the in vivo NMR study of tumor is the tumor heterogeneity. The conventional RF probe has relatively poor detection sensitivity; it requires obtaining signals from a large volume. For a large sample volume, it may contain different tissue types such as tissues from the peripheral of tumor and the tissues from necrotic center. Consequently, the biochemical profile obtained by NMR may be a result of a mixture of different tissue types and tumor cells at different growing phases. One of the goals of using the proposed HTS probe is to be able to obtain NMR signals from a much smaller volume due to the significant gain in detection sensitivity. Smaller volume of the HTS probe from a well-defined region may help to identify the heterogeneous characteristics of the tumor. Some of the proposed diagnostic and predictive tumor markers can then be tested more reliably. In order to obtain NMR signal form a well-defined region, we have implemented a localized NMR spectroscopy technique, image-selected in vivo spectroscopy (ISIS) on our system. Figure 2 illustrates the results of the ISIS technique using a test phantom. The test phantom includes two cylindrical vials, one small and one large. The small vial contains phosphoric acid (H_3PO_4) and it is placed inside the large vial. The large vial contains triphenylphosphate ($(C_6H_5)_3PO_4$). Three localized spectra taken from locations which contains either phosphoric acid or triphenylphosphate or both. Three almost clean spectra from the intended area demonstrate the excellent volume selection. This localized NMR spectroscopy technique will be used to study the differences of biochemical profiles from the peripheral and necrotic center of the tumor during the tumor growing phase and the initial response to the Tamoxifen and Doxorubicin.

For the in vivo study, a MCF7 wild type drug sensitive breast cancer cell line and its drug resistant variant MCF7/ADR are used first to study the differences of energy metabolism of breast tumors. Healthy female athymic nude mice (~30gm) are anesthetized by i.p. injection of 7 to 1 ratio of ketamine and xylazine at 10 mg/kg, before cells implantation and NMR scans. A four-turns RF coil is placed on the tumor (Figure 3) for the ^{31}P spectroscopy studies. An external reference methylene diphosphonic acid is placed on the other side of the

coil. The size and the position of the coil allow the maximum signal detection sensitivity. The relative position of the coil to the tumor is important. In Figure 3, it demonstrates the detection sensitivity drops as the sample place further away from the coil. The sensitivity also depends on the shape of RF pulse. The sech 90^0 pulse has a better penetration than the hard pulse. The sensitivity for both pulses will be significantly deteriorated beyond one coil radius distance.

Cancer cell suspensions ($5x10^6$ cells) from monolayer cultures in exponential phase of growth are injected into the left hind leg of mice subcutaneously using a 21 gauge needle. The tumor-free right hind leg of the mouse was used as control. The cancer cells are grown as solid tumor xenographs. A series of NMR studies are performed on the tumor as well as on the control leg. Figure 4 shows the result from a study of MCF7/ADR drug resistant tumor during the growth phase. Figure 4 are four spectra from day 4, 6, 8 and 12 after the cell implantation. The repetition time of scan is one second. The RF pulse width is 22 μsec. Each spectrum is a summation of three 300 scans. The total scan time is 15 minutes. A 5 Hz line broadening is applied to the spectrum. The spectrum clearly demonstrates many phosphate metabolites such as inorganic phosphate Pi (5.0 ppm), phosphocreatine PCr (0 ppm), γ-adenosine triphosphate γ-ATP (-2.3 ppm), α-adenosine triphosphate α-ATP (-7.5 ppm), and β-adenosine triphosphate β-ATP (-16.0 ppm). The phosphocreatine peak is chosen to be the reference. Small amount of phosphomonoester (PME) and phosphodiester (PDE) can be seen near Pi. Both PCr and ATP gradually decrease as tumor progresses and at same time the Pi increases. For the normal control, Figure 5 shows spectra from the tumor-free right hind leg on the fourth and twelfth days. The overall signal-to-noise ratio of the spectrum is better than that of the tumor. PCr and ATP signals are stronger than that in the tumor and Pi is much smaller. An external reference methylenediphosphonic acid is at 20.2 ppm. The reference intensity changes on each day are due to the change of the relative position of the reference to the RF coil. Although all the spectra show excellent signal-to-noise ratio, the measurement of absolute values of each phosphate is difficult. This is primarily due to the NMR setup and detection sensitivity may not be identical every time. It is always a challenge for a quantitative measurement. The contributing factors include coil tuning, sample shimming, RF pulse calibration and the relative position of the animal to the coil. In order to have a more consistent reference, we have set up an experimental protocol in our later experiments so that the position of the reference to the coil is fixed relative to the coil. This ensures the constant intensity of the reference.

In the third year we also started a study of high resolution MRI imaging of capillary of mouse. The goal of this study is to examine capillary density in tumors and the relative permeability or leakiness of the capillaries in the different tumors before and during the different stages of treatment with Tamoxifen and/or Doxorubicin. Since the improvement of sensitivity of HTS probes, a microscopic imaging about 15 μm pixel size is possible within a reasonable imaging time. A conventional RF coil was first used to image the vasculature near a wound on a nude mouse to perfect the imaging protocol. A one-turn RF surface coil is used for RF excitation and signal receiving. It is gently placed against the mouse skin. The arrangement is the same as in the spectroscopy study shown in Figure 3. The mouse is anesthetized by i.p. injection of ketamine and xylazine as in the spectroscopy studies. The NMR machine used was a Varian 4.7 T, 33 cm horizontal bore magnet system. The NMR

machine has an active gradient system capable to produce 60 G/cm magnetic field gradient. A spin-echo imaging technique was used. The repetition time is one second and the echo time is 22 μsec. The field-of-view is 1.5 cm x 1.5 cm. The number of phase encoding steps is 512. The in-plane spatial resolution is approximately 30 μm. In Figure 6 many new small blood capillaries near the wound can easily be identified. Using a three-dimensional display of a series of these microscopic images is used to illustrate the vasculature structure of blood vessels in the tumor.

Key Research Accomplishments

The accomplishment in the third year is highlighted in the following Statement of Work.

I. Probe Design (10/96-09/98, 24 months)

162 MHz ^{31}P Probe for Cell Metabolism Study on 9.4 T machine

1. Detail the design of HTS probe for 9.4 T NMR machine (complete)
2. **Procure specialized cryo-valves and low temperature mechanical and electrical components (complete)**
3. **Order cryogenic preamplifier and test its specifications (complete)**
4. Fabricate the components of HTS probe (complete)
5. **Assemble the HTS probe (complete)**
6. Construct conventional copper probe for comparison (complete)
7. Evaluate components and test the operation characteristics of HTS probe. Determine the SNR gain as function of temperature on conductive and non-conductive samples (in progress)

81 MHz ^{31}P Probe and 200 MHz ^{1}H Probe for In Vivo Study on 4.7 T machine

Procedures are the same as 9.4 T case. The cryogenic system and preamplifier will be shared in both probes. The HTS coils in these probes will be different. Different geometry, sizes and electronic designs of the HTS coils are necessary to accommodate the sample sizes and resonance frequency differences. The procurement of cryostat and preamplifier has completed. The testing of these devices is conducted at Quantum Magnetics. A conventional copper coil has been constructed and tested.

II. Cell Metabolism Study (02/97-07/98, 18 months)

The cell metabolism study and in vivo animal study have begun. The initial phase of the studies will be using conventional room temperature probes. It will serve as comparison studies.

1. To construct the perfusion apparatus and setup the perfusion experiment (complete)
2. Obtain ^{31}P spectra of MCF7, MCF7/III, MCF7/LCC2 and MCF7/LY2 in agarose gel in order to compare the SNR improvement by HTS probe. (in progress)

3. **To determine the lowest cell density which still can have a good SNR in a reasonable acquisition time. A study of MCF7 cell proliferation in Matrigel from a low starting cell density will be conducted. (in progress)**
4. To study of the effect of Tamoxifen and growth effectors on these breast cancer cell lines. (complete)

III. In Vivo Animal Study (04/98-09/00, 30 months)

1. **To obtain in vivo ^{31}P MRS spectra during growth and progression of MCF-7, MCF-7/MIII, MCF-7/LCC2 and MCF-7/ADR tumors in athymic nude mice using the conventional probe as well as the HTS probe. Following groups will be studied: (in progress)**
   (i) Normal athymic nude (10 animals).
   (ii) Mice with MCF-7 tumors in one leg (10 animals).
   (iii) Mice with MCF-7/ADR tumors in one leg (10 animals).
   (iv) Mice with MCF-7/LCC2 tumor in one leg (10 animals).
   (v) Mice with MCF-7/MIII tumor in the other leg (10 animals).

2. **To study the ^{31}P MRS of the different tumors at different times after treatment with Doxorubicin and Tamoxifen used singly or in combination. (began)**
   To study Tamoxifen:
   (i) Mice with MCF7 tumor in one leg and MCF7/MIII tumor in the other leg (15 animals).
   (ii) Mice with MCF7 tumor in one leg and MCF7/LCC2 tumor in the other leg (15animals).
   (iii) Mice with MCF7/MIII tumor in one leg and MCF7/LCC2 tumor in the other leg (15 animals).
   (iv) Mice with MCF-7 tumor in one leg and MCF-7/ADR tumor in the other leg (15animals).
   To study Doxorubicin:
   (I) Mice with MCF-7 tumor in one leg and MCF-7/ADR tumor in the other leg, but treated with one or three cycles of Doxorubicin treatment (30 animals).
   (ii) Same as group (I) but treated with one or three cycles of Doxorubicin in combination with Tamoxifen (30 animals).

3. **Utilize a high resolution MRI and a gradient-echo dynamic contrast enhancement technique to examine capillary densities in tumors and the relative permeability or leakiness of the capillaries in the different tumors before and during the different stages of treatment with Tamoxifen and/or Doxorubicin. These will be done at the same time when task (2) is performed. (same time as (2)) (in progress)**

Reportable outcomes

1. Two high temperature superconductor NMR probes are constructed. Significant improvement of signal detection sensitivity was observed.
2. Three cell perfusion apparatus are constructed. The perfusion apparatus is used for continuous supply of nutrients to the cancer cells inside the NMR machine during the in vitro study.

3. One postdoctoral fellow, Dr. Dongsheng Liu, and one MD/PhD student Mr. Emmanuel Agwu are supported by this grant.
4. Two U.S. Army training grants received based on the research opportunity with this grant:

- "NMR Sensitivity Improvement Using High Temperature Superconductor for RF Probe" (DAAG55-98-1-0187)
- "A Training Program in Breast Cancer Research Using NMR Techniques" (to be funded by 07/2000)

VII. Conclusions

In the third year, we have procured the specialized cryo-valves, low temperature components and Oxford cryostat. The HTS probe is assembled with the cryostat at Quantum Magnetics Inc (subcontractor). The whole HTS probe is under final evaluation for its operation characteristics. This year we particularly concentrated on the in vivo localized spectroscopy and micro-imaging studies. A localized spectroscopy technique ISIS (image-selected in vivo spectroscopy) technique has been implemented and tested. The intended selected volume is well defined. To study the progress of tumor a series of the in vivo ^{31}P spectra taken from MCF7/ADR drug resistant tumor on 4, 6, 8, and 12 days after cell implantation. The spectra clearly show the gradual decreases of phosphocreatine and ATP. The spectra also show the increase of inorganic phosphate. The potential malignant markers such as phophomonoester and phosphodiester signals are weak. We hope this may dramatically improve later by replacing the conventional RF probe with the proposed HTS probe. The spectra from the non-involved control leg demonstrate no metabolic changes during this period. The consistent spectral intensities also demonstrate the consistency of the NMR machine. The results from these in vivo ^{31}P spectroscopic studies are similar to the results from the in vitro cell studies. The signal-to-noise ratio of the in vivo animal studies is better. This is primarily due to more cells involved in the in vivo studies. In this year, we have also studied the small blood vessels of mouse using NMR microscopic imaging technique. The in-plane spatial resolution is 30 μm. A series of high quality detailed images reveal the fine microscopic structures of mouse capillaries. We will continue to concentrate on the in vivo studies in the coming year, particularly, we will compare the sensitivity improvements by using the HTS probe.

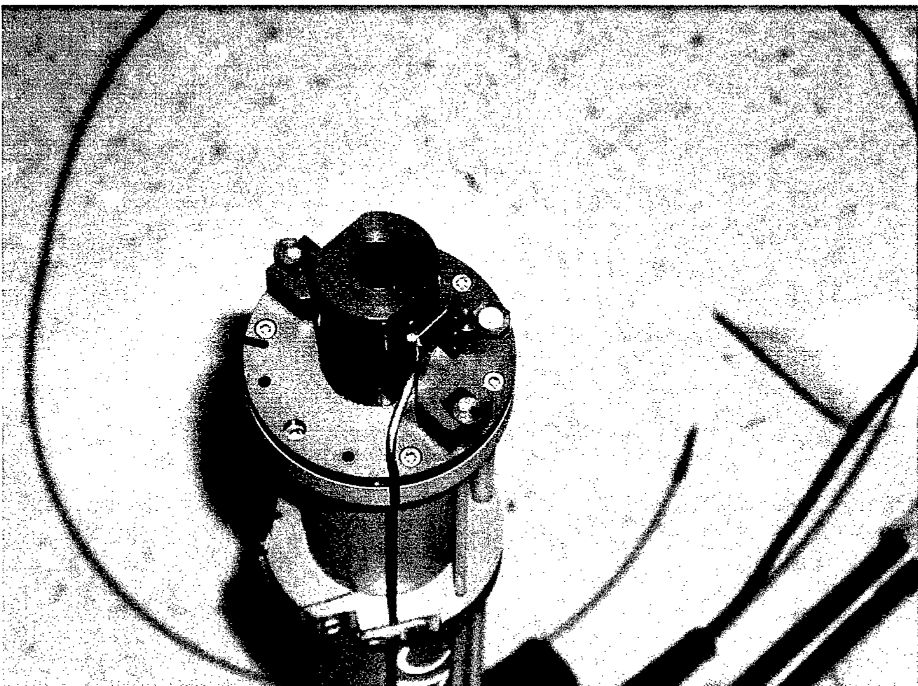
VIII. REFERENCES

1. Michal Neeman, Hadassa Degani Metabolic Studies of Estrogen- and Tamoxifen-treated Human breast Cancer Cells by Nuclear Magnetic Resonance Spectroscopy. Cancer Research 49, 589-594. 1989.
2. Stelling CB, Wang PC, Lieber A, etal, Prototype coil for magnetic resonance imaging of the female breast, Radiology 154(2): 457-62,1985.
3. Harms SE, Flaming DP, Present and Future Role of MR imaging, Syllabus; A categorical Course in Physics Technical Aspects of Breast Imaging RSNA, 1992.
4. Harms SE, Flaming DP, MR imaging of the breast, J. Mag. Res. Imaging 3:277-283, 1993.
5. Harms SE, Flaming DP, Hesley KL, etal, Magnetic Resonance Imaging of the breast, Mag. Res. Quarterly, 8(3):139-155, 1992
6. El Youself SJ, O'Connell DM, Magnetic resonance imaging of the breast, Mag. Res. Annual, 177-195, 1986.
7. Kuelson MH, El Youseif SJ, Goldberg RE, Ballance W, Intracustic Papillary Carcinoma of the breast: mammographic, sonographic and MR appearance with pathologic correlation, J. Comp. Ass. Tomography 11(6):1074-1076, 1987.
8. Partain CL, Kulkarni MV, et al, Magnetic resonance imaging of the breast: Functional T1 and Three-dimensional Imaging, Cardio Vas, Intervent, Radiology 8:292-299, 1986.
9. Santyz GE, Henkelman M, Bronskill M, Spin-locking for magnetic resonance imaging with application to human breast, Mag. Res. Med. 12:25-27, 1989.
10. Adams AH, Brookeman JR, Merickel MB, Breast lesion discrimination using statistical analysis and shape mesures on MRI, Compt. Med. Imaging and Graphics 15(5):339-349, 1991.
11. Harms SE, Flamig DP, Hesley KL, Evans WP, Cheek JH, Ptters GN, Knox SM, Savino DA, Netto GJ, Wells RB, Jones SE. Fat-suppressed Three-dimensional MR Imaging of the Breast,RadioGraphics 13:247-267, 1993.
12. Fischer U, Vosshenrich R, Probst A, Burchhardt H,Grabbe E, Preoperative MR-mammography in diagnosed breast carcinoma, Rofo-Fortschr Geb Rontgenstr Neuen Bildgeb Verfahr 161 (4):300-306 1994.
13. Kuhl C, Specificity of dynamic contrast-enhance MR mammography, Syllabus of Workshop in Breast MR, Soc. Magnet. Reson. p5 June 1995.
14. Aisen, A.M., and Chenevert, T.L. MR spectroscopy: clinical perspective. Radiology 173, 593-599 (1989).
15. Bottomley, P.A. Human in vivo NMR spectroscopy in diagnostic medicine: Clinical tool or research probe? Radiology 170, 1-15 (1989).
16. Daly, P.F., and Cohen, J.S. Magnetic resonance spectroscopy of tumors and potential in vivo clinical applications: A review. Cancer Res. 49, 770-779 (1989).
17. Glickson, J.D. Clinical NMR spectroscopy of tumors. Invest. Radiol. 24, 1011-1016 (1989).
18. den Hollander, J.A., Luyten, P.R., Marien, A.J.H., Segebarth, C.M., Baleriaux, D.F., de beer, R., and Van Ormondt, D. Potentials of quantitative image-localize human ^{31}P nuclear magnetic resonance spectroscopy in the clinical evaluation of intracranial tumors. Mag. Reson. Quarterly 5, 152-168 (1989).
19. Radda, G.K., Rajagopalan, B., and Taylor, D.J. Biochemistry in vivo: An appraisal of

clinical magnetic resonance spectroscopy. Mag. Reson. Quarterly 5, 122-151 (1989).
20. Steen, R.G. Response of solid tumors to chemotherapy monitored by in vivo ^{31}P nuclear magnetic resonance spectroscopy: A review. Cancer Res. 49, 4075-4085 (1989).
21. Vaupel, P., Kallinowski, F., and Okunieff P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: A review. Cancer Res. 49, 6449-6465 (1989).
22. Negendank W, Studies of Human Tumors by MRS : a Review, NMR Biomed. 5:303-324 1992.
23. Barfuss, H. et al. In vivo Magnetic Resonance Imaging and Spectroscopy of Humans with a 4 T Whole Body Magnet. NMR in Biomed. 3:31-45, 1989.
24. Hetherington, HP, Pan, JW, Mason GF, et al. High Resolution ^{1}H Spectroscopic imaging of Human Brain at High Field Quantitative Evaluation of Gray and White Matter Metabolite Differences, 12th Ann. Meeting Soc. Mag. Res. Med. p. 127, 1993.
25. Posse, S., Cuenod, CA, Balaban, RS, Le Bihan, D. Anomalous Transverse Relaxation in ^{1}H Spectroscopy in Human Brain at 4 Tesla. 12th Ann. Meeting Soc. Mag. Res. Med. p. 371, 1993.
26. Ugurbil, K. Insensitive Adiabatic RF Pulses. Syllabus of 12th Ann. Meeting Soc. Mag. Res. Med. 195-202, 1993.
27. W.A. Edelstein, G.H. Glover, C.J. Hardy, R.W. Redington. Magn. Reson. Med. 3, 604, 1986.
28. R.D. Black, T.A. Early, P.B. Roemer, O.M. Mueller, A Mogro Campero, L.G. Turner, G. A. Johnson. A High-Temperature Superconducting Receiver for NMR Microscopy. Science 259:793-795, 1993.
29. D.I. Hoult and R.E. Richards, "The signal-to-noise ratio of the nuclear magnetic resonance experiment," J.Mag.Res. 24, 71-85, 1976.
30. Black R.D., P.B. Roemer, W.A. Edelstein, S.P. Souza, A. Mogro Campero, L.G. Turner, Scaling Laws and Cryogenic Probes for NMR Microscopy in Proceedings of the Tenth Annual Meeting of the Soc. Magn. Reson. Med. San Fransisco, CA, August 10-16, 1991.
31. Vogl, T., Peer, F., Schedel, H., Reiman, V., Holtmann, S., Rennschmid, C., Sauter, R., and Lesner, J. ^{31}P-spectroscopy of head and neck tumors - Surface coil technique. Magn. Reson. Imaging 7, 425-435 (1989).
32. Koutcher, J.A., Ballon, D., Graham, M., Healey, J.H., Casper, E.S., Heelan, R., and Gerweck, L.E. ^{31}P NMR spectra of extremity sarcomas: diversity of metabolic profiles and changes in response to chemotherapy. Magn. Reson. Med. 16, 19-34 (1990).
33. Shinkwin, M.A., Lenkinski, R.E., Daly, J.M., Zlatkin, M.B., Frank, T.S., Holland, G.A., and Kressel, H.Y. Integrated magnetic resonance imaging and phosphorous spectroscopy of soft tissue tumors. Cancer 67, 1849-1858 (1991).
34. Glaholm, J., Leach, M.O., Collins, D.J., Mansi, J., Sharp, J.C., Madden, A., Smith, I.E., and McCready, V.R. In vivo ^{31}P magnetic resonance spectroscopy for monitoring treatment response in breast cancer. Lancet I, 1326-1327 (1989).
35. Ng, T.C., Grundfest, S., Vijayakumar, S., Baldwin, N.J., Majors, A.W., Karalis, I., Meaney, T.F., Shin, K.H., Thomas, F.J., and Tubbs, R. Therapeutic response of breast carcinoma monitored by ^{31}P MRS in situ. Magn. Reson. Med. 10, 125-134 (1989).
36. Twelves, C.J., Lowry, M., Porter, D., Graves, P., Smith, M.A., and Richards, M.A. ^{31}P MR spectroscopy of breast cancer in vivo: metabolite characteristics and influence of estrogen receptor status and tumor grade. Soc. Mag. Res. Med. Abstr., p. 1216 (1990).

37. Kalra, R., Hands, L., Styles, P., Wade, K., Greenall, M., Smith, K., Harris, A., and Radda, G.K. Localized ^{31}P spectroscopy of human breast cancer correlations of phosphomonoester and phosphodiester with epiderman growth factor expresion. Soc. Mag. Res. Med. Abstr., p. 1217 (1990).
38. Redmond, O.M., Stack, J.P., O'Connor, N.G., Codd, M.B., and Ennis, J.T. In vivo phosphorous ^{31}P magnetic resonance spectroscopy of normal and pathological breast tissue. Br. J. Radiology 65, 210-216 (1991).
39. Mimnaugh, E.G., Fairchild, C.R., Fruehauf, J.P., and Sinha, B.K. Biochemical and pharmacological characterization of MCF-7 drug-sensitive and Adr^R multidrug resistant human breast tumor xenografts in athymic nude mice.Bichem. Pharmacol. 42:391-402, 1991.
40. Cohen, J.S., Lyon, R.C., Chen, C., Faustino, P.J., Batist, G., Shoemaker, M., Rubalcaba, E. and Cowan, K.H. Differences in phosphate metabolite levels in drug-sensitive and resistant human breast cancer lines determined by ^{31}P magnetic resonance spectroscopy. Cancer Res. 56:4087-4090, 1986.
41. Garwood, M. Introduction to Basic Principles of Spectral Localization. Syllabus,11th Ann. Meeting, Soc. Mag. Res. Med. p. 222-231, 1992.
42. R.J. Ordidge, M.R. Bendall, R.E. Gordon, and A. Connelly, MAgnetic resonance in biology and medicine (Govil, Khetrapal, and Saran, Eds.), p.387, Mcgraw-Hill, New Delhi, 1985.
43. Bottomley PA, Hardy CJ, Roemer RB, Weiss RG, Problems and expediencies in human 31P spectroscopy, The definition of localized volumes, dealing with saturation and the technique-dependence of quantification NMR in Biomedicine, 2:284-289, 1989.
44. Granot J, Selective volume excitation using stimulated echoes (VEST), Applications to spatially localized spectroscopy and imaging, J. Magn. Res. 70,488-492 (1986).
45. Kimmich R and D. Hoepfel, Volume selective multipulse spin-echo spectroscopy, J. Magn. Reson. 72, 379 (1987).
46. Frahm J, K.D. Merboldt, and W. Hanicke, Localized proton spectroscopy using stimulated echoes, J. Magn. Reson. 72, 502 (1987).
47. Ordidge RJ, A. Connelly, and J.A.B. Lohman, Image selected in vivo spectroscopy (ISIS), A new technique for spatially-selective NMR spectroscopy, J. Magn. Reson. 66, 283 (1986).

IX. Appendices



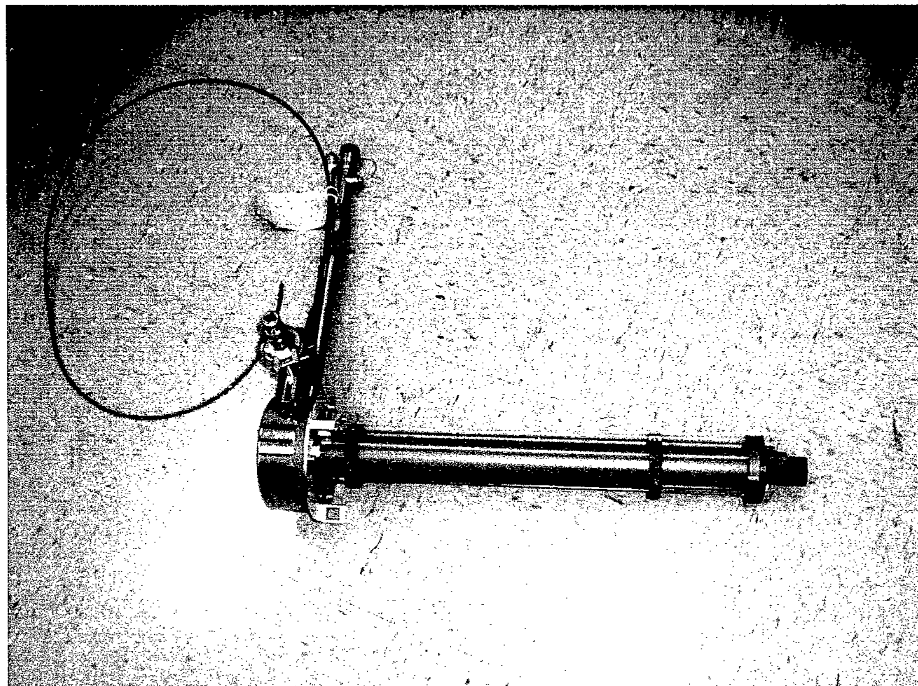
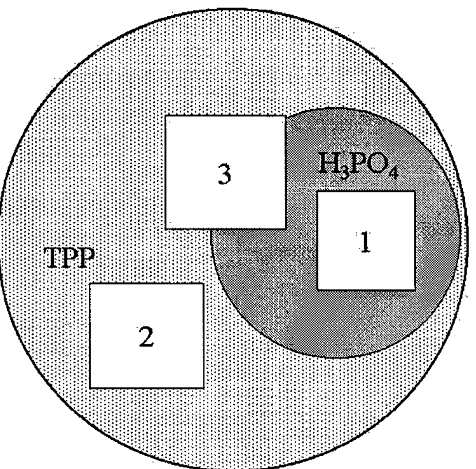


Figure 1. A picture of the complete high temperature superconductor NMR probe assembly including HTS coil and the Oxford Spectrostate Cryostat. This is completed by the subcontractor Quantum Magnetics, Inc. (San Diego, CA).



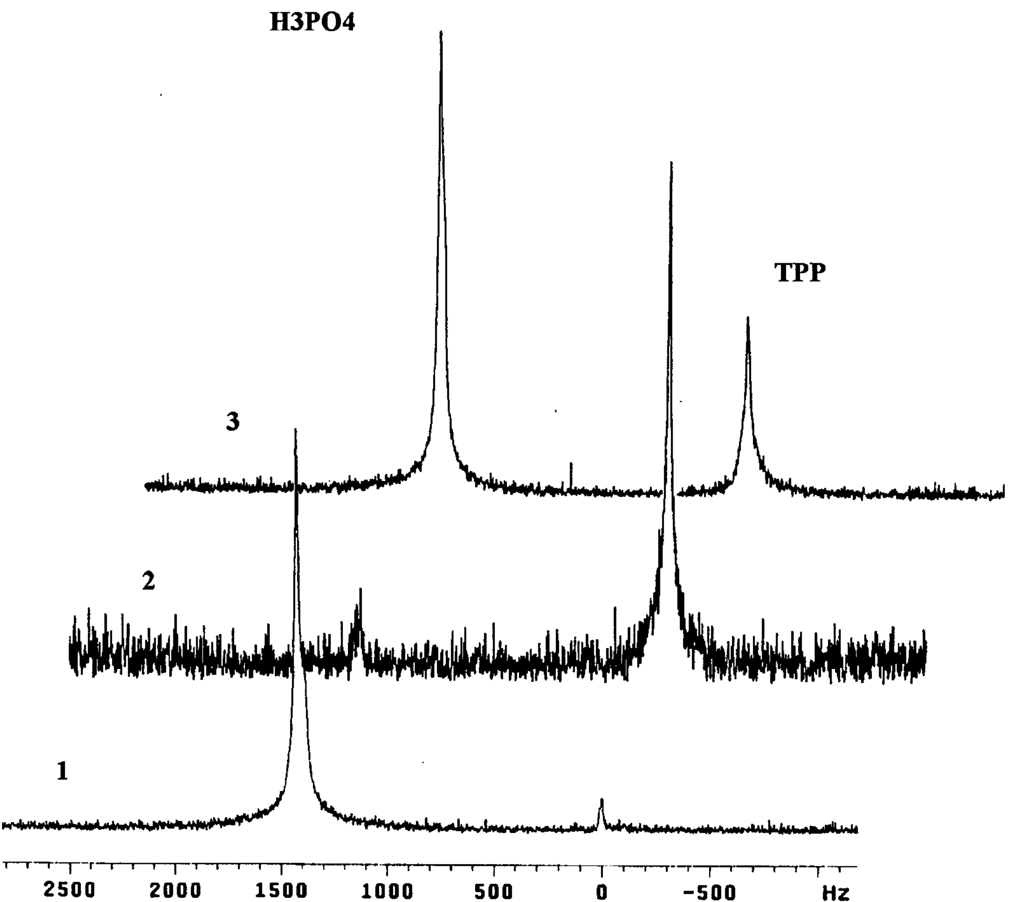
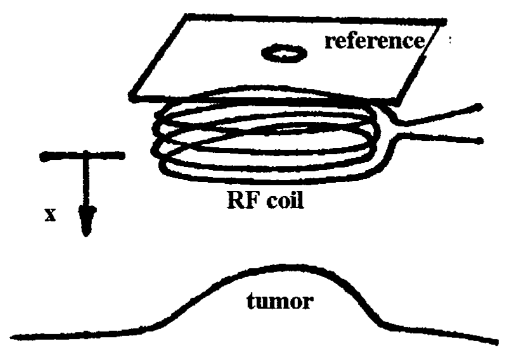


Figure 2. Image-selected in vivo spectroscopy (ISIS). A test phantom includes two cylindrical vials. The small vial contains phosphoric acid (H_3PO_4). The large vial contains triphenylphosphate ($(C_6H_5)_3PO_4$). The spectrum 1 is taken from a box from this small vial. The spectrum 2 is taken from the large vial. The spectrum 3 is taken from a volume containing both phosphoric acid and triphenylphosphate. The phosphoric acid peak is at 17.6 ppm down field from the triphenylphosphate peak. This demonstrates the volume selectivity of ISIS.



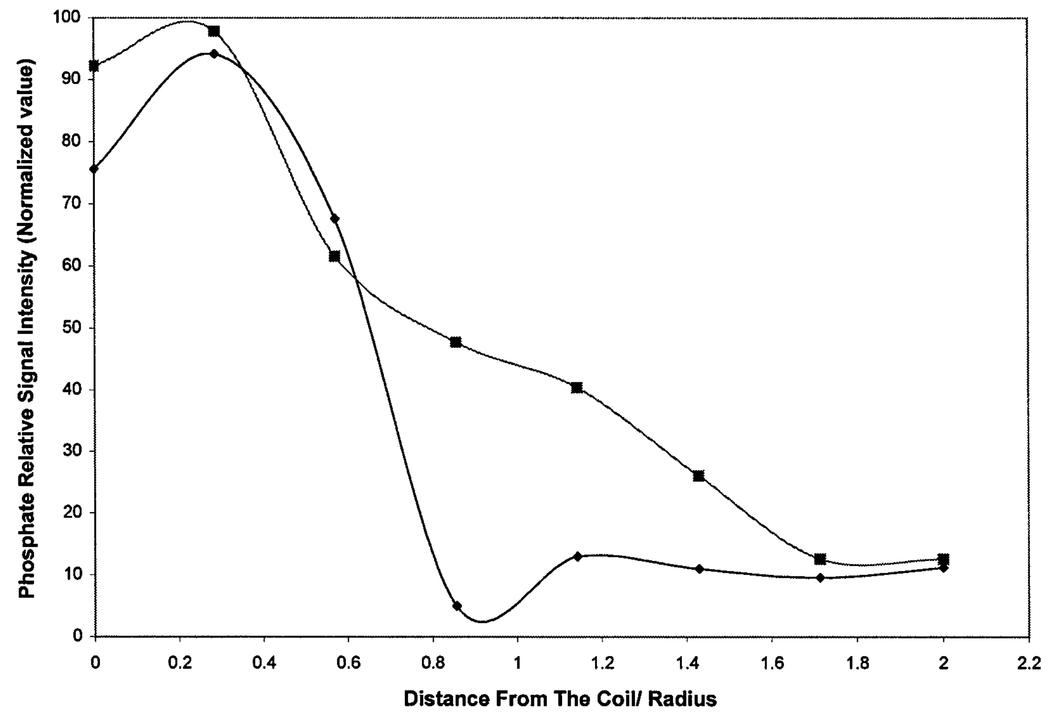


Figure 3. A. A four-turn Rf coil for the in vivo ^{31}P spectroscopy study. The coil is gently placed on the tumor. An external reference methylenediphosphonic acid placed on the other side of the coil. B. A diagram shows the RF power as a function of distance away from the coil. The intensity of the hard pulse drops faster than the sech90 pulse. This demonstrates the importance of relative position of the target area to the coil.

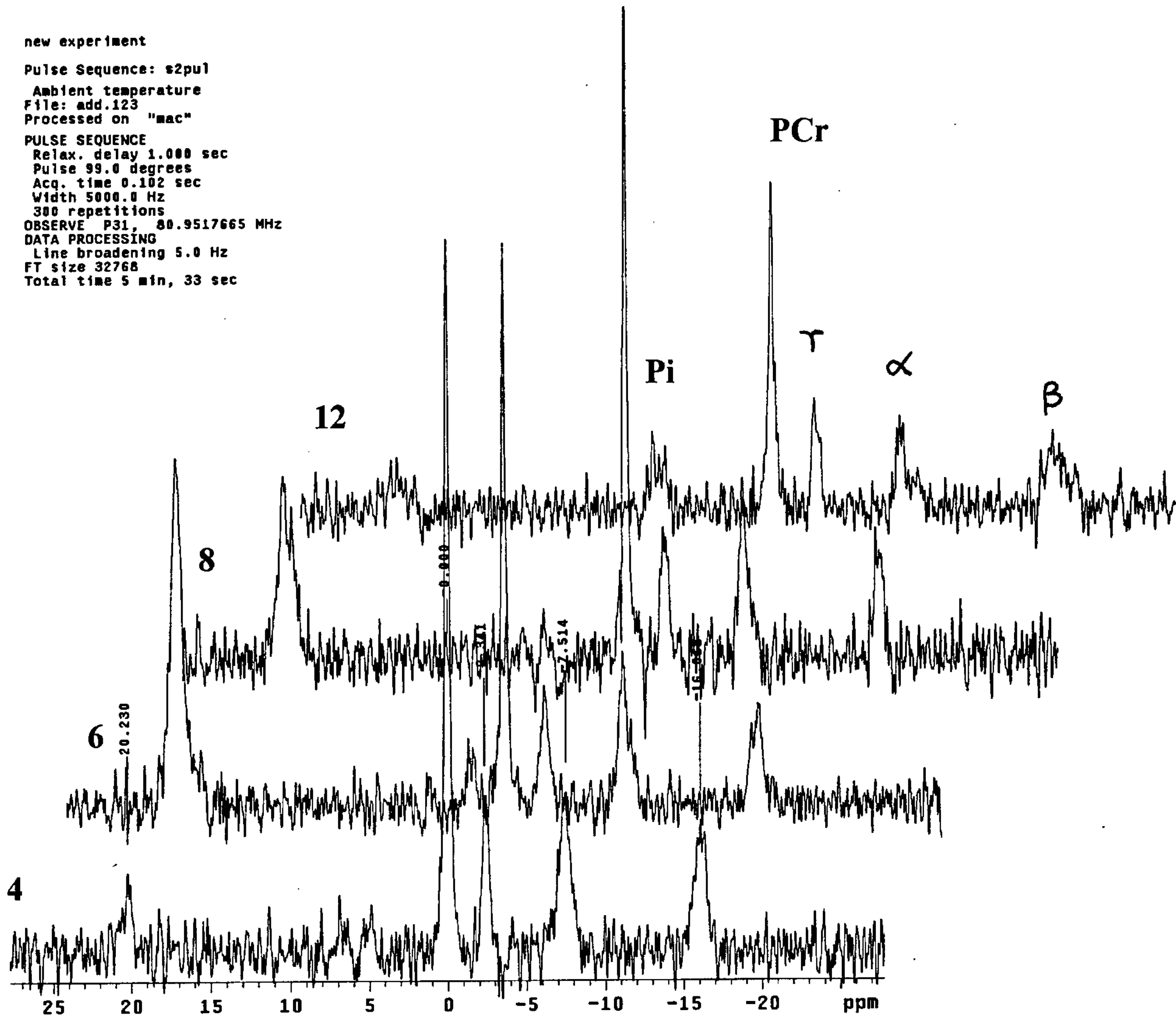


Figure 4. A series of in vivo NMR spectra (day 4, 6, 8, 12) from a tumor grown by implanting $5x10^6$ MCF7/ADR drug resistant cells on the left hind leg. The phosphate metabolites such as Pi (5.04 ppm), PCr (0 ppm), -ATP (-2.34 ppm), -ATP (-7.51 ppm), and -ATP (-16.07 ppm) can clearly be identified. The overall high energy phosphates both PCr and ATP gradually dropped at the same time the inorganic phosphate continued to increase. The peak at 20.23 ppm is from an external reference, methylenediphosphonic acid.

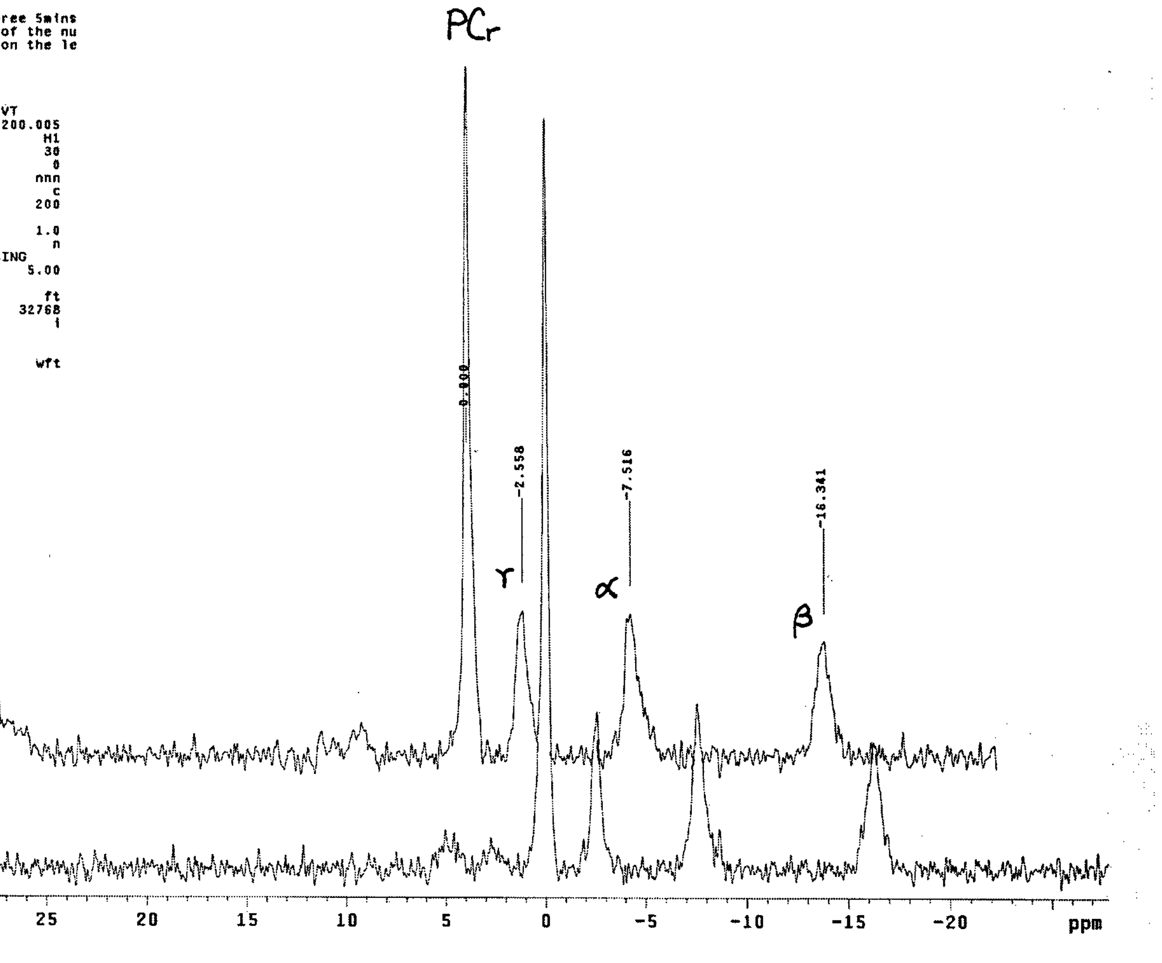


Figure 5. Two spectra form the tumor-free right hind leg of the mouse on the 4th and 12th day. This serves as a control. The overall signal-to-noise ratio is better than that of the tumor. PCr and ATP signals are stronger. Pi can barely be detected. The intensity does not change during this period of time.

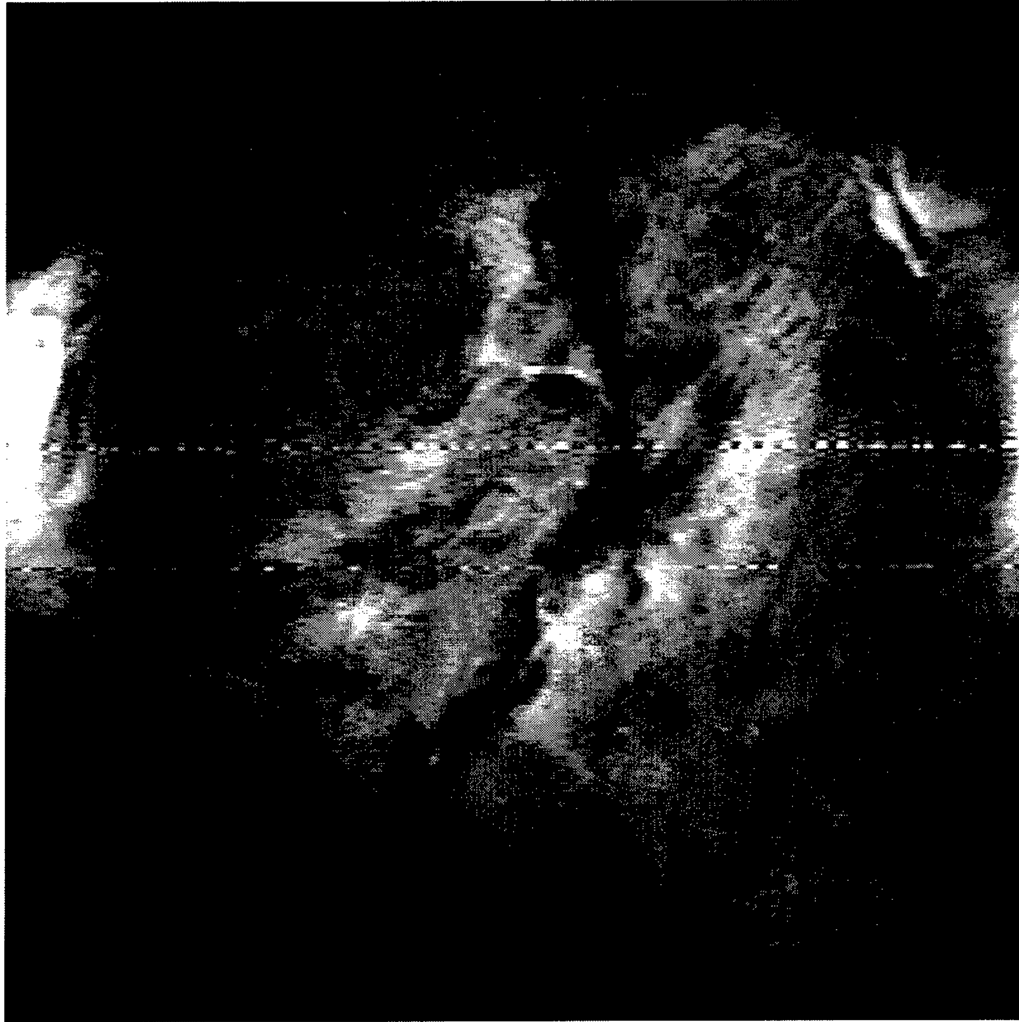


Figure 6. An NMR microscopic image of small blood vessels of mouse. This is a spin-echo image. The repetition time is 1 second and the echo time is 22 μsecond. The field-of-view is 1.5 cm x 1.5 cm. The number of phase encoding steps is 512. The spatial resolution is ~30 μm. The dark lines in the image indicate the small blood vessels.