9102-034

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U.S. DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM

COVER PAGE

(THIS PAGE MUST BE THE FIRST PAGE OF THE APPLICATION)

1. Name of Institution: University of Southern California
2. Department: (Radiology (Division of Basic Medical Imaging Sciences)
3. Street: 1985 Zonal Ave. 4. City: Los Angeles
5. County: Los Angeles 6. State: CA 7. ZIP: 90033 8. Congressional District: 25

Principal Investigator: Walter Wolf, Ph.D.

9. Telephone: Area Code 213 Office: 342-1405 Home: [redacted]
10. Title of Application: Purchase of a Biomedical NMR Imaging Spectrometer
11. Area of Proposed Research (Select ONE) Biomedical/Environmental*

A. Biomedical/Environmental
B. Chemical/Coal Science
C. Geosciences
D. Materials Research
E. Plant Science/Microbiology
F. Other

12. Research Subcategory (See Section II of DOE/URI FY 91 Announcement) Nuclear Medicine
13. Total DOE Funding for Research in Selected Area (During the last two fiscal years): $ 350,477
14. Estimated Purchase Price of Equipment: $ 722,000 15. Amount requested from DOE: $ 536,000

List all Federal agencies which are currently considering proposals from the institution involving the same or similar equipment.

16. Agency: _____ Agency Proposal Number: _____
17. Agency: _____ Agency Proposal Number: _____

NOTE: The institution is responsible for immediately informing the URI program manager in writing if a proposal involving similar or related equipment is submitted to a federal agency prior to the announcement of DOE's URI awards.

18. List and federal agency which has provided funds to the institution during the past two years for the same or similar equipment.

Agency: _____ Amount of Funds: _____

19. Please check one of the following:
- ☒ I authorize outside peer review of this proposal.
- ☐ I do not authorize peer review of this proposal.**

Signature of Principal Investigator: [signature] Date: December 7, 1990

Name and Title of Institutional Official (President or Designee)
Cornelius J. Pings
Senior Vice President
Academic Affairs And Provost

Signature: Cornelius J. Pings
Date: 12/19/90
Area Code/Telephone: (213) 342-2346

20. Is Applicant Delinquent on any Federal Debt? ☐ Yes (If, "Yes," attach an explanation) ☒ No

* Note – The application will be evaluated by reviewers in this field.
** Note – May prevent full consideration of this application.

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BOX No. H-182-18 Bldg. 2714-H
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UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM

Application submitted by

Walter Wolf, Ph.D.

for the Purchase of a Biomedical Imaging Spectrometer

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Faculty Resumes: Walter Wolf, Ph.D.	
Manbir Singh, Ph.D.	
Cary A. Presant, M.D.	
Kenneth L. Servis, Ph.D.	
David Z. D'Argenio, Ph.D.	
Victor Waluch, M.D., Ph.D.	
R. Ricardo Brechner, M.D., Ph.D.	

Appendices: **A:** PNAS and J.Clin. Oncol. articles

B: Relevant abstracts published in the Annual Proceedings of the Society of Magnetic Resonance in Medicine

C: Specifications of the 4.7T, 31 cm bore Biomedical NMR Imaging Spectrometer

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SUMMARY

We are requesting funds towards the purchase of a horizontal bore high field magnet with combined spectroscopy and imaging capabilities. This instrument is requested for the development of the Center for the Noninvasive Study of Drug Biodistribution, Targeting and Metabolism, by complementing the capabilities of USC investigators in high field (above 6T) NMR spectroscopy (chemistry) and lower field (human) MRI imaging (0.5-2T) and spectroscopy (1.5-2T). The 4.7T NMR requested will allow us to carry out, in a well coordinated and integrated manner, animal studies to explore and develop the unique capabilities of this noninvasive technique for studies of body chemistry and morphology. The PI and his coworkers have a special interest in the noninvasive study of drug metabolism and organ pharmacokinetics, and have been the first to show that NMR spectroscopic kinetic analysis of drugs can be carried out *in vivo* in organs and tumors of patients undergoing chemotherapy. The NMR requested will allow us to carry out both co-clinical and fundamental studies on living animals and gain novel insight into the chemical, biochemical and pharmacological changes that occur at selected organs of a living system when it has been subject to a variety of physiological, pathophysiological and pharmacological stimuli. In addition to the obvious integration of the various field levels of NMR, we will also integrate this modality with PET (positron emission tomography), another key method capable of noninvasive monitoring of body chemistry. Finally, this instrument will also allow us to fully utilize our novel and unique approach to Pharmacokinetics, that has allowed us to estimate the amount of the active form of a drug at its target site when organ/tissue pharmacokinetic data can be generated.

Specifically, the experiments that are planned for this instrument include:

a) Measuring the kinetics of 5-fluorouracil in tumors, using ^{19}F NMRS, and correlating such measurements with the animal's response, as well as with the effect of various modulators (e.g. leucovorin, levamisole, methotrexate, etc)

b) Developing and testing methods for improving and enhancing spatial localization and quantitation techniques

c) Initiating studies to expand these noninvasive pharmacological studies to other drugs, using both F-19 and proton spectroscopy

The program for which this instrument is requested is a direct outgrowth of DOE's sponsored energy research, and will greatly contribute to the PI's DOE-sponsored research, as well as to that of those other investigators who have been active contributors to DOE's research programs. NMR, as a noninvasive technique using stable isotopes, is a highly complementary technique to the use of radioactive materials. In line with DOE's mission of expanding the use of technology in Nuclear Medicine, the

instrument requested will help open new vistas in Nuclear Medicine studies, by expanding its role and capabilities into pharmacological problems and issues. As an example, the development of the proposed ^{19}F studies (to be coordinated closely with ^{18}F studies of the same drugs) are expected to be translatable in the near future to other stable/radioactive nuclide pairs, such as $^{13}C/^{11}C$, as well as correlative studies of $^{1}H/^{11}C$. Other major applications of this new noninvasive technology to biomedical problems, in closely correlative and complementary studies with techniques based on nuclear and ionizing radiation, are also likely. Other USC faculty members who will also be active participants in the use of the requested instrument include investigators from the Schools of Pharmacy, Medicine and Engineering. In addition, strong support for all these investigators and their projects will continue to be provided, both by basic scientists in imaging and NMR, and by clinicians in radiology, oncology, neurology and other areas of medicine, whose work will also be strongly stimulated and expanded by the availability of the spectrometer requested.

The projects proposed by Profs. Wolf and Singh, supported by DOE, are discussed in detail, and illustrate how this new noninvasive technique may help to open new vistas in studies of biochemistry, physiology and pathophysiology.

USC is one of the major US Universities training graduate students (1902 enrolled in Ph.D. programs, 311 Ph.D's graduating last year). A significant number of USC's graduate students will continue to be most actively involved, both in the projects described below, and in those that are expected to evolve as the capabilities of using NMR spectroscopy for performing noninvasive studies of body chemistry become more widely recognized as a novel, exciting and unique tool. At least 12 of the students who have graduated or are about to graduate from the Ph.D. programs associated with the PI and the core faculty, are among those who have been involved in areas directly related to those research programs whose capabilities will be greatly expanded by the acquisition of the high field NMR biomedical imaging spectroscopy system. Indeed, several of the PI's former students have developed their own position of leadership in directly related research areas. Finally, the availability of this instrument will allow us to implement novel vistas in research and graduate programs in noninvasive studies and in medical imaging.

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U.S. DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM
BUDGET PAGE

ESTIMATED COSTS

Instrumentation	Requested of DOE	Institution's Cost Sharing (1)	Other Federal Funds (2)	TOTAL
A. Purchase Price (3)	$ 482,000	$ 240,000		$ 722,000
Maintenance (4)	$ 54,000	XXXXX	XXXXX	$ 54,000
Subtotal:	$ 536,000	$ 240,000		$ 776,000
B. Other Allowable Costs				
1. Shipping/Handling	XXXXX	$ 19,000		$ 19,000
2. Building/Laboratory Renovation	XXXXX	$ 31,000		$ 31,000
Subtotal:		$ 50,000		$ 50,000
C. TOTAL	$ 536,000	$ 290,000 (5)		$ 826,000

NOTES:

(1) Non-Federal funds only. (However, may be provided by a third party.)

(2) Estimate funds to be obtained from other Federal agencies for purchasing the instrument, etc.

(3) Only the purchase price of the instrumentation is eligible for DOE funding through this program.

(4) See discussion of eligible maintenance costs on page 10.

(5) Only those costs specified above are eligible as cost sharing. Installation, operation, maintenance, travel and training costs, or faculty and student salaries, etc. are ineligible as cost sharing. Review discussion of eligible and ineligible costs on pages 10 and 11.

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A. Purchase Price (List components and unit prices.)

Description/Vendor	Quantity	Total Estimated Unit Price	Requested of DOE	Institution's Cost Sharing	Total
Biomedical NMR Imaging Spectrometer	1	$ 722,000	$ 482,000	$ 240,000	$ 722,000
Additionally, maintenance costs for the first two years after the end of the first year warranty is being requested.					
The vendor that has so far offered to contribute to USC's cost sharing portion is Otsuka Electronics, USA. Other vendors have been contacted, and negotiations are in progress for similar arrangements.					
The complete specifications for the system requested are appended					
	1	$ 722,000	$ 482,000	$ 240,000	$ 722,000
Subtotal:					

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OMB CONTROL NO. 1910-1400

DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM
GRANT AND CONTRACT SUMMARY FORM

Institution Name:

Current Grant/Contract/Subcontract No. (and contractor name if subcontract)	Principal Investigator	Title	Contract Date: From MO	From YR	To MO	To YR	Total Award Value	$ AMT Awarded for FY Period 1988 to 1990	DOE Technical Monitor/Location
DE-FG03-84ER-60219	Walter Wolf, PhD	Radiopharmacokinetics: Utilization of Nuclear Medicine Techniques in the Noninvasive Study of Drug Distribution	03	84	07	91	861,330	210,000	Donald W. Cole, Jr.
DE-FG03-86ER-60416-2	Gerald Huth, PhD	Development of Silicon Avalanche Internal Gain Radiation Detection Structures; Study of New Quantum Concepts for the Detection of Optical Wavelength Radiation	01	86	11	88	277,818	-0-	Gerald Goldstein
DE-FG03-86ER-60416-3	Gerald Huth, PhD	Development of Detectors for Ionizing Radiation Based on High Tc Superconductivity	01	86	11	89	910,000	140,477	Gerald Goldstein
DE-FG03-87ER-60504	P.P.Lambropoulos PhD	Theoretical Problems in Laser Spectroscopy of Atoms and Molecules	01	87	01	93	312,016	157,301	Paul Duhamel

Provide information only on those research projects which are directly related to the selected principal research area and which were active during the period from October 1, 1988 to September 30, 1990. Do not include grants or contracts for the following: (1) workshops, (2) education/training projects or other non-research projects, (3) facilities, (4) instrumentation, (5) those projects completed before October 1, 1988.

See discussion on page 8 for additional requirements relating to subcontracts.

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RELATED FEDERAL AGENCY GRANTS AND CONTRACTS

Agency/Agency Contact	Principal Investigator	Grant/Contract No.	Title	Grant/Contract/Dates FROM MO	FROM YR	TO MO	TO YR	Total Award Value
The only Federal Agency supporting the work of the PI has been the Department of Energy, where our current grant is completing year 09 in 1990. An NIH grant application was approved, but not funded. A revised application is pending.								
This work has, however, been supported by private agencies and foundations								
M & H Hoover Foundation	Walter Wolf, PhD		^{19}F NMR Spectroscopic Studies of 5-Fluorouracil in Humans and in Rabbits as the Tool for Monitoring Individual Variations of the Metabolism and the Biodistribution of this Drug	08	87	06	90	$ 67,882
American Cancer Society	Walter Wolf, PhD	RD 261	Noninvasive Monitoring of 5-Fluorouracil Biodistribution and Metabolism	01	88	12	88	$ 74,775
Natl.Inst.Health	Walter Wolf, PhD	CA48255-01A2	Metabolic Imaging of Drugs using ^{18}F PET and ^{19}F NMR	04	90	03	95	$ 1,612,684 (pending)
Natl.Inst.Health	Manbir Singh, PhD	CA28105-08	An Electronically Collimated Gamma Tomography System	07	87	05	90	$ 255,000
Kaprielian Inn. Res.Fund	Manbir Singh, PhD Co-PI		New Windows on the Human Brain	07	89	05	94	$ 1,200,000

Only provide information on research projects which are directly related to the selected principal research area.

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NARRATIVE

TECHNICAL MERIT AND ACCOMPLISHMENTS OF USC'S RESEARCH PROGRAM

1) *General Overview*

The PI and his collaborators have been, for years, funded by DOE in the development of novel methods for performing noninvasive studies of body chemistry, with special emphasis on drug metabolism and biodistribution. These studies have documented how nuclear medicine and NMR techniques can be used uniquely for performing noninvasive studies of body chemistry, and strongly suggest that the combined use of NMR and other noninvasive methods should be extended to a number of further biochemical, physiological and pharmacological studies in whole animals and in humans. Indeed, a key concept that has evolved from these DOE-funded studies is the realization that the *precision with which we might capture the dynamics of a process in a living system is inversely proportional to the degree of perturbation that such a system has undergone*. Thus, noninvasive [e.g., non perturbing] studies of biochemical, physiological and pharmacological events in a living system are no longer a luxury: they are a necessity in order to correctly measure and observe such processes.

The PI and his coworkers are currently using the only wide bore NMR imaging spectrometer that exists in the Southern California basin, a 4.7T CSI at the Huntington Research Institute. This instrument has been made available to us once a week, although instrumental failure caused this facility to be out of operation for several months at a time. While this arrangement has and is allowing us to perform a limited number of studies, as detailed below, it is not a satisfactory solution given the nature and the scope of our programs, and those of other investigators at USC: there are obvious limitations of instrument availability and scheduling, given the expanded needs of the investigators at the Huntington Research Institute; this instrument, several years old, does not have the flexibility for incorporating several of the new sequences that need to be tested; sequential animal studies, determined by biochemical needs, can not be scheduled at present; finally, it is not accessible to other USC investigators. Thus, the core faculty, who represent a true interdisciplinary team, is therefore desperately in need of a much more comprehensive utilization of the biomedical NMR imaging spectrometer requested, both for spectroscopy and localization. The key to our interest is that such an instrument provides unique capabilities and will contribute significantly to further expanding USC's ability of developing methods and procedures for performing truly noninvasive studies of living systems, specially at the mammalian level. Concomitant with the qualifications of the USC faculty, the projects proposed range from the purely physical to the totally biological, and as documented by their research

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productivity and funding, the faculty is well experienced in the development of new research ideas and methods in these areas.

The PI and his coworkers have, by now, established a well proven track record in "hands-on" experience in both human and animal NMRS studies. We documented in 1986 (W1) that the metabolism of 5-fluorouracil (5FU) (and hence, by extension, of other drugs) could be observed, noninvasively and 'on-line' in the liver of patients undergoing chemotherapy with 5FU. More recent work in both human (W2,W3,W4) and animal tumor models (W5,W6), has documented that 5FU is retained in some tumors with a $t_{\frac{1}{2}}$ that is significantly longer than this drug's $t_{\frac{1}{2}}$ in blood. We have used the term *trapping* for this phenomena, and shown that a good correlation appears to exist between the degree of trapping of 5FU in a tumor, and a patient's response to chemotherapy (W3,W4). These first leads have now been expanded and we have documented that we can also observe, in animals, how a modulator (methotrexate) affects the metabolism of 5FU in an experimental animal tumor model (W7). The studies we propose to perform expand and exploit these leads, and are intended to allow us to make the fullest use of this new methodology for gaining a better understanding of the mechanism of action of drugs, and for using such information in basic science and clinical studies.

However, such studies would not have been possible without a first rate interdisciplinary team that had been developing the Radiopharmacokinetics program, including both basic scientists and clinicians. This team includes basic scientists (Prof. Kenneth L. Servis) with a well-established "hands-on" experience in high-resolution chemical NMR, basic scientists (Prof. Manbir Singh) developing new vistas in the use of neuromagnetism in biomedical applications, as well as pharmacokineticists (Prof. David D'Argenio) and mathematicians (Prof. Alan Schumitzky), developing algorithms that have allowed us to test and analyze various multicompartmental models of 5FU, using the **radiopharmacokinetic** methods developed as part of our DOE-funded programs. R. Ricardo Brechner, M.D., Ph.D., who had done his Ph.D. with Prof. Wolf, is now sharing responsibility for the clinical NMR studies at the St. Vincent Medical Center, as well as working with Prof. Singh on spectroscopic and imaging studies at the LAC/USC Medical Center.

As stated above, instrumentation for high-resolution (chemical) NMR is readily available at USC, including several FT NMR spectrometers: a Varian XL-200, an IBM WP-270SY, a JEOL FX-900, a Brucker AM-360 on the University Park Campus, and a 200 MHz Varian Gemini on the Health Sciences Campus. USC has also well qualified faculty in these and directly related areas, some of which are actively involved in the current proposal. For clinical (human) NMRS studies, two units are currently in

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active use: the 2T Helicon at St. Vincent's Medical Center, and the 2T Gyroscan at the LAC/USC Imaging Center. Two additional MRI units have and used for these projects: the 1.5T Magnetom at Pomona Valley Community Hospital (where most of published human work had been performed), and a 1.5T Magnetom at the VA West Los Angeles Medical Center, thereby covering the major regions of the Los Angeles metropolitan basin (east, central and west). The VA facility is of particular interest, inasmuch as it also has an operating PET unit, which the PI is using for correlative ^{18}F-5FU imaging and kinetic studies. Other units, in addition to the above, have shown interest in participating in clinical spectroscopy studies, including Loma Linda Medical Center, Long Beach Memorial, etc. Thus, and provided this current grant is funded, Southern California is likely to possess a major coordinated program resources in basic, animal and clinical spectroscopy, and the PI has proposed the coordination of these efforts into a "Regional Spectroscopy Center". The present proposal is both a logical and highly desirable complement to the above basic and clinical efforts.

Thus, the present request for a Biomedical NMR is for the missing link that will allow researchers in the Los Angeles basin to fully integrate their NMR interests and capabilities, from chemical and physical studies in the 5-14 T (high resolution) range, to 0.5-2.0 T for human studies. What is more important, this integration of efforts will allow us to closely correlate and interdigitate chemical, biochemical, pharmacological and clinical studies, an integral approach we have been fostering and nurturing in both our ongoing DOE research program and in new programs expanding the noninvasive studies of body chemistry.

2) *Prior work by the Prof. Wolf and Coworkers using ^{19}F-NMRS*

5-Fluorouracil (5FU) is one of the most extensively used antitumor drugs, originally reported by Heidelberger et al. (W8) in 1957, and widely used since, specially for breast (W8), stomach and colon (W9), and squamous cell carcinoma of the head and neck (W10), esophagus and anus. Its metabolism has been widely reported: most of the drug is catabolized in the liver (W11,W12) to 5,6-dihydro-fluorouracil (DHFU), alpha-fluoro beta-ureido propionic acid (FUPA) and finally to alpha-fluoro beta-alanine (FBAL). A small fraction, however, can be converted into various fluorinated nucleotides and nucleosides such as FUR, FUdR, FdUMP; some of these intermediates can, in turn, interact with thymidylate synthase, with RNA or with DNA, all of which have been implicated in the possible mode of action of this antitumor agent (W13,W14). Thus, a necessary condition for assessing whether this drug is active in that individual would be to be able to monitor in that individual, what fraction of the injected 5FU is catabolized in the liver, and which fraction of

5FU is anabolized to the active anabolite(s) inside the tumor. While tumor biopsies has been used, to measure tumor anabolism (W15), availability of such material is inherently limited, can only be collected a very few times, and is unavailable from unaffected organs (e.g., the liver). We had successfully used ^{18}F (the 2 hr, positron emitting radionuclide of fluorine) to label 5FU (W16) and to monitor drug biodistribution in animals (W17) and in patients (W18,W19), and shown, in a murine tumor model (L-1210 Lymphocytic Leukemia) that the responsive variant of that tumor accumulated 4x as much ^{18}F than the refractory variant (W20). However, and while such studies did provide valuable information, they failed to provide the key data on the chemical nature of the fluorinated products present at any of the target sites, inasmuch as nuclear imaging alone is unable to generate sufficient data to analyze the likely compartmental models we tested using our radiopharmacokinetic modeling approach (W21). Inasmuch as the high sensitivity of nuclear detection can now be complemented by NMR spectroscopy (e.g., a method that allows noninvasively a direct chemical analysis of selected fluorinated compounds present at the desired target sites) we expect to be able, by using the combination of these two techniques, to estimate and validate a reduced model (including the key compartments) aimed at understanding the biodistribution and the metabolism of 5FU.

Pilot ^{19}F NMR studies in patients. Two series of ^{19}F-NMR spectroscopic human studies have now been conducted by us in patients receiving 5FU. The studies in Erlangen (in 1986/87) included 8 patients. Of these, 7 patients received 5FU as a bolus and 1 as a slow infusion. The results obtained with the first three patients have been published (W1). Spectral data were collected from the liver in all these patients, and over the tumor in 5 of these patients. All patients receiving 5FU as a bolus (5-15 min) did document the presence of both 5FU and FBAL in the liver, although their residence times and rates of metabolism varied, both as a function of the dose administered, as well as of inter-patient variations. Liver half-lives of 5FU ranging from 15 to 30 min were determined in the first three patients who had received 1000 mg/m^2, whereas more rapid catabolism appears to occur in patients receiving 600 mg/m^2. This could suggest a nonlinear kinetics in the catabolism of this drug.

We have now extended these observations by evaluating the pharmacokinetics of 5FU in the tumors of 16 patients with carcinoma of the breast, colon, endometrium, cervix and kidney, using ^{19}F-NMRS, following administration of 5FU 600 mg/m^2 (W3,W4). In these studies we detected a long-lived tumor pool of 5FU in tumors of 8 out of the 16 patients studied. The half-life of this tumor pool of "trapped" 5FU was 0.33-1.2 hrs, significantly longer than the half-life of 5FU in blood (5-10 minutes). Neither

the anabolites of 5FU (fluorinated nucleosides, nucleotides, RNA or thymidilate synthase), nor the catabolites, e.g., fluorobetaalanine (FBAL), were detectable by ^{19}F NMRS. Of the 8 patients evaluable for antitumor response who received only 5FU or 5FU plus leucovorin, 5 of the 5 patients who accumulated ("trapped") free 5FU in their tumors had clinically documented tumor responses. All of the 3 patients who did not show detectable 5FU after the first 2 spectra failed to respond to therapy. The $t_{1/2}$ of 5FU in the tumors of those patients that responded to 5FU ranged from .33 to 1.2 hrs, while the $t_{1/2}$ of 5FU in the patients who did not respond was in the order of 4-10 min, a value corresponding to that of 5FU in the blood pool. In one patient studied twice, the intratumoral $t_{1/2}$ of 5FU given alone was 22 minutes, and after treatment with 5FU plus leucovorin was 20 minutes, not a statistically significant difference. No anabolites of 5FU were detected in either study.

Correlations of the Clinical Response of Evaluable Patients with ^{19}F-NMRS

	Results of Therapy			
Tumor Trapping*	5FU plus Leucovorin		Other 5FU Combination Therapy	
of 5FU	> 50% Response	No Response	> 50% Response	No Response
YES	5	0	1	1
NO	0	3	2	2

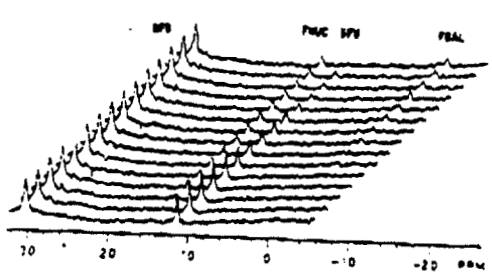
*Tumor trapping of 5FU is defined as retention with a $t_{1/2}$ of 20 min or greater.

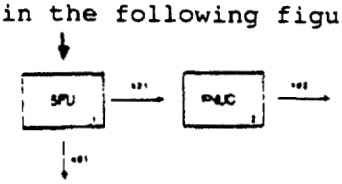
Pilot NMRS studies in animal tumor models: Following the first *in vivo* ^{19}F NMRS by Griffith et al (W23) in mice and a 1.89 T vertical bore NMR system, we attempted (in collaboration with Griffith) to monitor the L-1210 lymphocytic tumor in mice (W24); Hull et al. studied mice bearing both the sarcoma-180 and the M5076 tumors (W25); Wolf et al. studied control rats and rabbits (W5,W6), while Nunnally et al. had studied rabbits at high doses (W26).

Proper selection of animal tumor models is critical, inasmuch as they should mimic and predict what has been/will be happening in patients. **The selection of a proper animal system and a proper tumor model is not a trivial issue.** Two animal systems will be used in this work. Preliminary work (W5) had suggested that the metabolism of 5FU in the rabbit liver parallels well that of the human liver, while a very different metabolic pattern was observed in rats and mice. The only detectable compounds seen in the livers of rabbits were 5FU and FBAL, even at very high doses (at least one order of magnitude higher than what would be used in patients). One possible reason for the species differences in the handling of this drug could be in their differential enzyme levels and ability to synthesize metabolizing enzymes. In

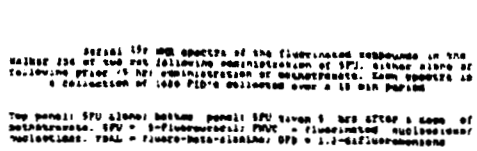
the liver of mice one of the intermediate catabolites (FUPA) was detectable (in addition to FBAL), and the catabolism of 5FU, even at low doses, was much faster than in humans and rabbits. In rats, not only were both these catabolites seen in the liver (FUPA and FBAL), but also the anabolites (nucleosides/nucleotides). Our ongoing tumor studies have shown that the ^{19}F NMR spectra detected in the VX2 tumor in the rabbit also parallel the ^{19}F NMR spectra detected in human tumors, whereas more compounds, including the FNUC peak, are detectable in the Walker 256 adenocarcinoma tumor model in the rat.

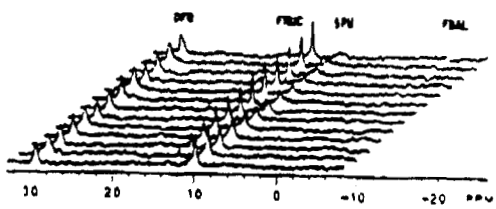
While recognizing its limitations as a model, we believe that the use of the Walker 256 carcinosarcoma in rats - a tumor reported to be responsive to 5FU (W27) - has been producing results that may have significance as models for human studies. Griffiths et al. had detected the FNUC peak by NMRS (W28), and Wolf et al. have fully confirmed such findings (W6,W7). Recent work has shown that MTX modulation has a significant effect on the metabolism of 5FU: rats bearing the Walker 256 tumor, when pretreated with MTX, exhibited a marked increase in the rate of formation of the FNUC peak, and of its relative amount, shown in the following figure:





2 Compartment Model 1B for 5FU Metabolism





A simple 2 compartment subsystem model (see above) has been used to analyze these data: k_{21} represents the rate of conversion of 5FU to FNUC, k_{01} the rate of catabolism of 5FU and its elimination from the tumor, and k_{02} its rate of conversion to the active anabolites (incorporation into RNA, binding to thymidilate synthase).

Rate constants estimated using the reduced subsystem model 1B (x 10^{-3})

	5FU alone (n=6)	MTX 5 hr (n=6)	MTX 22 hr (n=4)
k_{21}	6.4±2.3	15.4±5.0	11.6±3.6
k_{01}	11.6±4.9	.003±.002	2.4±4.2
k_{02}	.12±.29	1.4±.9	1.9±.96

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Using an F test for k_{21}, the difference between the rats receiving only 5FU and those pretreated with MTX, was $p<0.01$ for MTX5hrs, and $p<.02$ for MTX22 hrs. For the two MTX treatment groups there is no statistically significant difference even at the 5% level. These reults suggest that more detailed studies are needed to determine which scheduling regimen will maximize the MTX modulation in the Walker 256 model, so that the pharmacologically optimal modulation can be tested in humans for its maximum therapeutic potential. The mean k_{01} value for 5FU alone is statistically significantly different from the mean values for the rats that had received MTX at 5 and 22 hrs prior to 5FU: $p<.001$ and $p<.02$, respectively. For k_{02}, the F-test is statistically significantly different at the 1% level ($p<.01$). We have also begun to validate these estimations: when rats bearing the Walker 256 were sacrificed at 2 hours following treatment with 5FU alone, or following predosing with MTX, and their tumors extracted, a significant increase was noted in the FNUC present in the acid-soluble fraction: from 464±68 nmol/g to 1027±304 nmol/g, as well as for the 5FU isolated from the RNA fraction: from 106±24 to 257±64 nmol/g. These results clearly illustrate the power of this method, and suggest that it should be used to test the effect of modulators on the metabolism of 5FU. As noted in the discussion of the clinical studies to be performed, the clinical effect of MTX on enhancing 5FU response is variable in the large number of reported studies. The present preclinical results suggest that most clinical studies may have been performed with suboptimal pharmacologic understanding and suboptimal clinical scheduling. Therefore, these pilot preclinical studies will be used in the design of the clinical NMRS studies proposed intended to determine if it is possible to enhance the therapeutic efficacy of MTX using dosing schedules whose effects can be monitored, and thereby, optimized. Finally, the work we have pioneered is now being taken up by other groups. Prior, Maxwell and Griffith (W29) have extended their studies and fully confirmed our observations that ^{19}F NMRS can indeed monitor drug metabolism *in vivo*, and Jynge et al. (W30) have now extended such studies to another fluorinated drug, fleroxacin, a new trifuloroquinoline antibiotic (W31).

3) *^{19}F NMRS studies with 5-Fluorouracil* (Walter Wolf, Ph.D.)

Some of the specific noninvasive pharmacological and pharmacokinetic studies we wish to **continue** performing using the biomedical imaging spectrometer will address the following questions:

a) What is the **quantitative** correlation between trapping of 5FU in a tumor and the response of that tumor to chemotherapy, and is such a correlation truly predictive?

b) What are the specific effects that modulators of 5FU action, such as leucovorin, levamisole or interferon have on the kinetics of 5FU trapping and metabolism?

c) What are the best methods that can be used to enhance the sensitivity of detection of fluorinated compounds *in vivo* and their spatial localization, as well as collecting information from more than one tumor region?

d) How can we begin extending such studies to other drugs, both using ^{19}F and perhaps ^{1}H, specially if we they have protons that absorb above 7 ppm?

As an example of studies on the effect of various condition on the metabolism of 5FU, a tumor bearing Sprague Dawley rat will be administered light anesthesia (Ketamine, 50 mg/Kg and Xylazine, 10 mg/Kg, IM) and will be placed in the specially designed rat holder for the 4.7 T NMR spectrometer. An IV catheter will be placed in the animal, allowing drug administration at the desired time, and maintenance of the IV anesthesia. The following sequence will now be carried out:

1) Position coil over the tumor
2) Acquire a proton "scout" image (e.g., a very fast and rough image) of the region monitored by the surface coil, and verify that the rat's tumor is well positioned in relation to the surface coil. Reposition rat, if necessary.
3) Shim, either globally, or locally on the region to be studied, using a DRESS technique.
4) Switch to the ^{19}F frequency and collect a background ^{19}F spectra from that one organ/tissue
5) Inject the desired dose of 5FU (from 15 to 150 mg/kg)
6) Collect serial spectra over the tumor during drug administration and for 2-6 hours thereafter

Specific variables will include: a comparison of the kinetics of the ^{19}F containing compounds present when 5FU is administered alone, or after the prior administration of leucovorin, methotrexate, levamisole or interferon, at various times before 5FU injection. These studies will be conducted in succession, so that each rabbit acts as its own control. For example, a rat may receive first a therapeutic dose of 5FU (in the 20-40 mg/Kg range), NMR spectra will be recorded, then the second drug will be administered, and a new series of spectra will be recorded following administration of a second dose of 5FU to that same rabbit. This second study may be performed the same day, or one or two days later. Inasmuch as preliminary studies suggest that the parameters of a 3 compartment subsystem model for 5FU can be estimated from the NMR data in the rat tumor (where both 5FU and FNUC can be detected) (W31), it will be of great interest to determine which parameters are

affected by the action of the various modulators: 5FU uptake, conversion to nucleosides/ nucleotides, incorporation into the active products (RNA, thymidilate synthase), or catabolism and excretion. These estimations will be possible in the Walker 256 tumor of rats, when using NMRS alone, and appear to be possible in human and rabbit tumors, when combining ^{19}F NMRS with ^{18}F PET.

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4) *Spectroscopic Localization and Quantitation Studies* (Manbir Singh, Ph.D.)

Although the requested instrument wil be used primarily for ^{19}F spectroscopic studies, studies using ^{31}P, ^{13}C and ^{1}H spectroscopy are also planned. The spectroscopic localization and quantitation aspects described below are therefore illustrated with ^{19}F studies, although they could be readily adapted readily to the other nuclei.

Spectroscopic Localization

To maximize sensitivity, surface coils are used in our ongoing ^{19}F studies, and will continue to be used with the requested instrument. Unlike he saddle shaped coils used in conventional imaging, surface coils produce non-uniform field distributions. The greatest sensitivity is at the surface, diminishing with distance in accordance with the Biot-Savart law. When the surface coil is used to excite as well as receive, the flip angles change as a function of distance from the surface coil, and this factor must also be included in the sensitivity optimization procedures.

Instead of using the standard rf excitation pulses, an adiabatic pulse can be designed for use with a surface coil to achieve almost uniform flip angle throughout the excited region. A preliminary experiment using an adiabatic half-pulse on the 1.5T magnet system produced almost a factor of two sensitivity gain in a simple phantom. This pulse sequence will be developed further with the requested system, tested with phantoms and animals, and refined for eventual implementation in human studies.

In conjunction with sensitivity optimization, techniques to localize the region of interest, pertinent to our spectroscopic applications, will be investigated. A few possible approaches with their pro and cons are summarized below.

Depth-resolved surface spectroscopy (DRESS)

A 90 degree selective excitation pulse, applied in the presence of a B0 gradient along the depth axis of the surface coil, results in the excitation of a plane parallel to the area of the surface coil (S1). The lateral extent of this plane, however, is determined by the sensitivity profile of the surface coil and is not well defined since the isocontours curve backwards, although this may not be a major problem because there is almost no background uptake of ^{19}F in our applications. The gradient is turned off, and a gradient refocussing pulse is then applied to generate the signal via a gradient echo. The duration of the signal is limited by T_2^*, i.e., the effective T_2 value in the presence of field inhomogeneities. Thus, the major problem with DRESS that we may encounter in our work is that turning gradients on

and off produces eddy currents requiring a waiting period before data can be acquired, resulting in the loss of signal from short T2* components. The *in-vivo* T_2* values of relevant ^{19}F components are not known to us at the present time.

Volume selective excitation (VSE)

Like DRESS, VSE utilizes selective rf pulses in the presence of B0 gradients to define a sensitive volume. The difference lies in the manner in which the sensitive region is formed. Composite pulses are applied along each axis in the presence of gradients such that at the end of the sequence, nuclei within a selected cubical volume are left with their original longitudinal magnetization, whereas nuclei elsewhere have their magnetization tilted in the transverse plane (S2). The transverse magnetization dephases rapidly producing no signal. Subsequently, a 90 degree read pulse with the gradients turned off provides a localized signal from the selected region. The advantage of this method over DRESS is that it allows for additional time (proportional to T_1 rather than T_2*) to switch gradients and to wait for the eddy currents to dissipate. The disadvantage is that high powered rf pulses are required to produce accurate flip angles, implying that a body rf coil is essential to produce accurate flip angles. If the flip angles are not accurate, nuclei outside the region of interest may contribute a much larger net signal than that from the selected region, rendering the technique practically useless.

Image selected *in-vivo* spectroscopy (ISIS)

The above limitation of VSE is reduced in ISIS which does not require an accurate flip angle, and surface coils could therefore be used to excite and receive. A combination of frequency selective inversion pulses in the presence of B0 gradients is used to generate positive and negative signals from the selected and surrounding regions (S3). A series of eight experiments, with resulting FIDs added while maintaining their proper sign, produces localization in cubical volumes. Like VSE, the data acquisition window is controlled by T_1 decay. Thus, gradient switching and eddy currents are less of a problem than DRESS.

At this stage it appears that ISIS or approaches related to ISIS may be the best choice for our work. Test-object and animal studies will be conducted to evaluate ISIS as well as related approaches with the requested instrument to determine the optimum technique for ^{19}F human studies.

Phase-encoded spectroscopic localization

In situations such as encountered in our work where signals are inherently weak, an alternative to the volume localization techniques described above may be to acquire

first the signal from a larger region and then subdivide it into smaller "point" or localized regions through mathematical postprocessing. We propose to investigate a spatial encoding technique for spectroscopy, similar to imaging, where a gradient would be applied along the depth axis of a surface coil to encode phase. Data are acquired after turning the gradient off, and the sequence repeated N times, with N different gradients, similar to the procedure used in phase encoded imaging. The lateral resolution in this technique would be limited by the sensitivity profile of the surface coil. After each gradient pulse, the FID would be acquired on M points, and a 2-D Fourier transform with the NxM data points performed to obtain spectra from N successive slices oriented parallel to the area of the surface coil. The depth resolution is determined by N and the chemical shift resolution by M. The value of N in practice will depend on the sensitivity. We expect that it should be possible to set N in the 8-16 range for the proposed ^{19}F studies.

It may also be possible to perform phase encoded spectroscopy using a B1 gradient (S4). The B1 gradient can be changed in steps by changing the duration and/or strength of the current through the surface coil. Data acquired after each step are phase encoded in one-dimension, and a subsequent Fourier Transform localizes spectra on slices approximately parallel to the area of the surface coil. Again, since the B1 isocontours curve backward, the delineated slices are not as well defined as those obtained from B0 gradients. However, this method is relatively simple and does not require switching B0 gradients.

^{19}F Spectroscopic Quantitation

A quantitation of 5FU and its products in-vivo is needed to enable development of models for explaining the kinetics of drug uptake, washout and chemical transformation as described in several sections of this proposal. Quantitative ^{19}F spectroscopy in conjunction with quantitative ^{18}F PET imaging is expected to provide synergistic information in developing accurate pharmacokinetic models that will have great significance in the chemotherapeutic treatment of an individual patient. Initially, we propose to develop techniques to perform a relative quantitation of ^{19}F spectra with respect to a standard, and in parallel, investigate the possibilities of, and problems inherent to, absolute quantitation.

The relative quantitation approach relies on measuring accurately the area of peaks assigned to the various chemical components that can be visualized in the spectra. The following general procedure, adapted from (S5), will be investigated.

Let A, V, S, f(T1), f(T2) denote the area of a peak, the volume from which the signal is detected, the sensitivity of the surface coil, a function of T1, and a

function of the effective T2 (including field inhomogeneities) respectively. The quantitated concentration 'C' of a specified chemical component is then determined from the following equation.

$$C = \frac{A.V'.S'.f(T1)'.f(T2)'}{A'.V.S.f(T1).f(T2)}$$

where primes are used to denote values from a standard. 'A' is measured from the area of the peaks, and 'V' is determined by the sensitivity profile of the coil in conjunction with the rf exitation pulse (normal or adiabatic) and the particular spectroscopic localization technique used. As mentioned earlier, we are still searching for a suitable spectroscopic localization technique for our application. The sensitivity profile of the surface coil may be delineated from an equivalent proton image since the resonance frequencies of protons and ^{19}F differ by 5% only. The sensitivity factor 'S' depends, among other factors, on the 'Q' value of the coil, which in turn depends on how well it is tuned. The functions of T_1 and T_2 are dependent on the particular pulse sequences used. Optimal placement of a standard for reference is still under investigation. Various test-objects will be studied, and several standards for generating the reference signals, for example, small beads containing known amounts of 5FU, FBAL or 5FUR (as a representative of the nucleosides/nucleotides that appear at the FNUC peak) will be investigated to evaluate the quantitation procedure.

5) *Imaging Research* (Manbir Singh, Ph.D.)

There are two ongoing projects related to development of instrumentation and techniques in NMR imaging that we wish to pursue with the requested instrument. The first pertains to echo planar imaging and the second to flow imaging. These are briefly described below.

T_2 Effects in Echo Planar Imaging

A major limitation of conventional magnetic resonance imaging (MRI) techniques is the relatively long data acquisition time required for collecting a complete set of samples to reconstruct tomographic or three-dimensional images of the human body. This limitation is set mainly by the pulse repetition rate TR as constrained by the relatively long (~1s) longitudinal relaxation time T_1 of biological tissue. The data acquisition time in a typical spin echo head scan, for example, is on the order of minutes, and longer acquisition times are normally required for other regions of the body. Respiratory, cardiac and bodily motion, as well as flowing blood and fluids in the body represent potential sources of error in the images reconstructed from data acquired over such long scan times.

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Several innovative approaches have been developed in MRI to reduce the data acquisition time. These may be divided in two broad categories. One category is based on reducing TR in conjunction with small flip angles (instead of the commonly used 90 deg flip angle). By optimizing the flip angle and utilizing gradient echoes, it is possible to cut the scan time to a few seconds with a small penalty in the signal-to-noise ratio (SNR). The second category, which can complete data acquisition in about 50ms for a single slice, is based on the echo-planar imaging (EPI) approach, first described by Mansfield in the mid-1970s (S6). In a current version of the EPI approach, an initial slice-selective 90 deg rf pulse is followed by an oscillating gradient field along one axis (e.g., the y-axis) and a constant or a sharp pulsed ("blip") gradient along an orthogonal axis (e.g., the x-axis) to generate a train of gradient echoes (S7). Each echo within this train represents a row in k-space, i.e., a row within the two-dimensional (2-D) Fourier Transform (FT) of the object. If appropriate x and y phase encoding gradients are applied before the oscillating gradient field, the k-trajectory in the above sequence traces a rectilinear path through the planar k-space, sequentially sampling the entire k-space following a single 90 degree excitation, thereby generating what is referred to as an "instant snapshot" image.

An inherent limitation of the EPI technique, in addition to the hardware and gradient power requirements, is the constraint imposed on the data acquisition time by T_2^* of the excited region. The acquired k-space samples are sequentially weighted by T_2^* along the k-trajectory. Due to magnetic field inhomogeneities, T_2^* may lie in the 10-100ms range for some biological tissues, where its value is comparable to the data acquisition window. Short T_2 components impart a transient weight to the acquired data, thereby distorting the measurements and the resulting images produced by an Inverse Fourier Transform (IFT) of the k-space samples.

A possible strategy to reduce the effects of T_2 is to shorten the data acquisition window. The acquisition time, however, cannot be reduced arbitrarily because it is limited by the switching rate of the gradients, the sampling rate of the analog to digital converters, and the truncation errors that would result from a short acquisition window, ultimately limiting the resolution in the images. We are investigating several approaches to correct for distortions in echo planar images caused by short T_2 components (S8-S11). In an iterative approach (S10,S11), which appears to be very promising, the values of T_2 are initially estimated from a set of images produced by the inverse Fourier Transform of the geometric mean of Hermitian symmetric points. The estimated T_2 values are then used to compute k-space data, which, when compared with the true data, provide error datasets and corresponding

images to iteratively refine the estimates of T_2. Images corrected for T_2* decay are thereby generated at specified echo times. Computer simulation studies of several phantoms show good convergence under a variety of conditions. This procedure should enable wider data acquisition windows to be utilized in echo planar or spin echo images, leading to better resolution or better signal to noise ratio. The procedure could also be applied to spectroscopy to reduce T_2* related artifacts and enable wider data acquisition windows.

The requested instrument will not have enough gradient power to perform standard echo planar imaging over the full field of view of the magnet. However, it should be possible to generate enough power within a small central region to enable us to verify our concepts by using a small object or small animals. We propose to develop the necessary sequences and algorithms to perform echo planar imaging of small objects. In addition we will also pursue fast imaging techniques such as 'RARE' sequences (S12), which are similar in concept to echo planar, but can be implemented using standard hardware. Our preliminary experiments suggest that RARE would also require T_2 corrections similar to echo planar, and we have investigated techniques to enable correction under a variety of pulse sequences (S12).

Flow related effects

Moving protons produce either time-of-flight or phase related effects in NMR imaging. Phase shifts are produced by movements of the protons along the y or x axis, i.e., the axes along which the phase encoding and the read-out gradients are applied in typical imaging pulse sequences. These phase shifts produce many non-linear effects on the resulting images. On the one hand, these effects may result in several types of artifacts as well as a loss in the signal. On the other hand, the same phase shifts may be controlled to produce images depicting desired flow characteristics, giving NMR its unique ability to image and eventually quantify *in-vivo* flow.

The flow induced phase shifts depend on several variables related to a coupling of the flow parameters with the parameters of the specific imaging pulse sequence used in a study. To visualize the effect of phase shifts on spin echo images acquired under a variety of conditions, we have initiated a computer simulation study using a phantom containing stationary as well as flowing regions (S13). The role of parameters such as flow direction, mean velocity, velocity modulation, gradient pulses, echo time and data sampling time in producing artifacts and the reduction of these artifacts by a flow compensating pulse have been examined. It is shown that,

due to the finite data sampling window, the flow compensating pulse cannot fully correct for phase shifts even for first-order motion (S13).

We propose to use the requested instrument to perform test-object and animal studies of basic flow phenomena and relate the experimental results to our ongoing theoretical studies. Of paricular interest are the study of turbulent and pulsatile motion which would have numerous clinical applications, for example, in imaging the heart or in angiography. Also, our studies will allow us to develop methods to reduce motion related artifacts in practical imaging. In addition, we also propose to extend our work to image diffusion and perfusion related effects using test-objects and animals.

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QUALIFICATIONS OF THE FACULTY

The faculty participating in this proposal have all documented their interest and have active involvement in the use of NMR techniques in biomedical and clinical work. Thus, Prof. Wolf has been dedicated his research, funded by DOE, to the development of methods and techniques that would allow the measurement, in living systems, of how much of a drug is **required** at the target site to exhibit activity. Such information is not available from blood measurements, but requires measurement at the drug's site of action. Such work has required the formation of a team, composed by specialists in various disciplines, that would collaborate on the DOE-funded programs. This team includes Prof. Manbir Singh, who, with a background in Physics, has specialized the development of novel approaches in Medical Imaging; Prof. Kenneth L. Servis, who has expanded his interests from chemical NMR spectroscopy into their application to biomedical problems; Prof. Cary A. Presant, who as an eminently successful practicing oncologist provides key clinical insights and expertise into this work; Prof. David Z. D'Argenio, whose work in the development of novel computational methods for pharmacokinetics has found a logical field of application in the development of Radiopharmacokinetics; Prof. Alan Schumitzky, who has and is providing unique new insights into the application of mathematical concepts into the solution of the computational problems encountered in the development of the Radiopharmacokinetic technique; Prof. Kenneth K. Chan, whose understanding of pharmacokinetics as applied to cancer allows for a good correlation between our methods and those currently in use; and R. Ricardo Brechner, Assistant Professor of Research Radiology, whose original training as an MD, followed by a Ph.D. in Biomedical Engineering, bring his dual understanding and expertise in medical imaging and data processing, as well as pharmacology and radiopharmacokinetics, to the development of this new field.

Conventional methods of analysis that require sampling inroduce a significant perturbation into the system, thereby modifying the very phenomena we are trying to measure. The PI has categorized this notion, analogous to Heissenberg's Principle of Uncertainty, as the Biological Principle of Uncertainty. However, if and when we can measure events which do not perturb the system (e.g., noninvasively), then we may be able to gain information heretofore unavailable. NMRS is particularly suited to the study of drugs, inasmuch as such materials are administered, for pharmacological reasons, in significant amounts, and because they may possess, in their molecule, unique markers suitable for noninvasive NMR detection and measurement. ^{19}F is one

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such marker, and there are over 40 drugs now in the pharmacopeial armamentarium that are fluorinated. The PI has worked for several years with 5-fluorouracil, and shown that its metabolism can be detected, using NMRS, in the liver of humans and animal models; that there are significant species differences; that 5FU can be detected in the tumors of rabbits, rats and humans, and that trapping of 5FU may offer a direct correlation between NMRS measurements and prediction of clinical response. Thus, Prof. Wolf has a documented interest and track record in the use of NMR in clinical, biochemical and pharmacological studies.

The other investigators are all highly qualified and with active experience and expertise in NMR studies, including basic high field (Servis, Singh, Chan), clinical (Waluch, Presant) and animal systems (Servis, Singh). Further support to our group is provided by Dr. Presant's medical oncology colleages, and David Z. D'Argenio, Ph.D., for computational methods for the radiopharmacokinetic analyses. Although not directly related is the work that Prof. Singh has started in neuromagnetic imaging, one further utilization of magnetic phenomena in biomedical research.

LIFE SPAN, OPERATION AND MAINTENANCE OF THE BIOMEDICAL NMR IMAGING SPECTROMETER

While it is difficult to predict the probable life-span of the type of instrument requested, the PI has a track record of maintaing and utilizing, for many years, any equipment acquired. Thus, we are still using some of the nuclear equipment purchased in the 1960's, and the PI's E-3 ESR spectrometer was only retired recently, after more than 15 years of active service.

Operationally, this instrument will be operated by the PI, the coinvestigators, and their graduate students and post-doctorals. The PI plans to have one technician or postdoctoral assigned to be responsible for the operation and maintenance of this important resource. Funding for such an individual will come from the grants of the PI and of his coinvestigators. Currently, the PI is paying $450/day for access to the only other wide bore NMR spectrometer in the Los Angeles basin. Assuming 20 days of operation of the biomedical NMR imaging spectrometer, and if the same rates were to be charged, this would result in an annual "income" of over $100,000. No final decision has yet been made on how to share the NMR's operating expenses, including the technician/postdoctoral, but obviously such sharing is required. It is expected that as the potential of this technology becomes more widely appreciated and understood by the scientific community, the rate of demand for the utilization of this instrument will grow to the point where an additional wide-bore NMR might be needed in 3-5 years.

The operation of the NMR facility will be supervised by a Committee selected from among the users listed in this proposal (Profs. Chan, Presant, Servis, Singh, Waluch and Wolf), as well as new users that may be added subsequently.

INSTITUTIONAL PLANS FOR COST-SHARING

The cost-sharing plan proposed by USC is that we are requesting from DOE a grant for 67% of the cost of the NMR, plus the maintenance for years 02 and 03, whereas USC, through funds available to the PI and the co-investigators, would provide the other 33%, plus the costs of transportation, building renovation and payment of sales taxes, as well the operating and maintenance of the NMR after year 3. Otsuka Electronics, USA, has offered to provide $240,000 towards the purchase of the biomedical NMR imaging spectrometer. This has provided the basis of the matching needed to request the current grant.

USC, as a private University, does not have a source of internal funds available for capital equipment purchases. Indeed, it meets the requirements that have led Federal agencies, such as DOE, to providing the funds required for acquisitions of necessary research equipment. USC has, on the other side, been quite successfull in raising funds for its programs, as well as for individual faculty members.

USC will pay for the transportation of the biomedical NMR imaging spectrometer, the renovations required for its installation, the taxes that will have to be paid, as well as any further expenses that are needed to keep this important equipment operational.

DISCUSSION OF HOW WE BELIEVE THAT WE HAVE ADDRESSED THE COMMENTS BY THE REVIEWERS OF THE PRIOR APPLICATION

Inasmuch as this proposal had been reviewed previously in 1989/90 (9002-195), it would appear appropriate to address some of the questions that were raised in such reviews. Although these reviewes were unanimously highly favourable, the proposal was not funded. One of the few negative comments was that no description of the PET related programs were included in the write up. This is because no funds are requested for such studies. They are fully described in the regular DOE program, and we wonder if the reviewers may have access to those. This same reviewer comments about resolution and sensitivity. Indeed, ths is one of the precise reasons for our requesting this instrument, instead of continuing to operate, on a very part-time basis, at the Huntington Research Institute. We wish to implement the localization techniques that have been discussed in Prof. Singh's section of this proposal, as

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well as be able to operate on a daily schedule, rather than be limited to a single day a week. More on that below. Finally, this reviewer questions the availability of graduate students in the NMRS area. Because of our integration and collaboration with Biomedical Engineering, Chemistry and Radiology, we have full access to personnel and students in those areas. While we expect to recruit graduate students for this program, we have had excellent success in training graduate students in Medicinal Chemistry/Pharmacy for undertaking and performing NMRS studies. They are fully qualified.

Another reviewer has asked about the NIH proposal. This has now been approved and recommended for funding, with a good priority (19%). Although it was not funded in the December 1990 round, we have been advised that there is a very high expectation for it to be funded in February 1991. Another question of the reviewers relates to the notions of tracers. While, in nuclear medicine, we need to radiolabel materials and use [radioactive] tracers, and while such studies can be extended to NMR with nuclides such as ^{13}C, a stable isotope of carbon 12, the proposed work does not use **tracers**. Rather, is makes use of the full power of the atoms already **present** in the very molecules that we are interested in measuring. Thus, ^{19}F has an abundance of 100%: all natural fluorine is ^{19}F, and we are therefore able to measure fluorinated drugs and their fluorinated metabolites without any further addition of any tracers. Furthermore, we now intend to extend such studies to other drugs, given the significant progress being made in ^{1}H spectroscopy *in vivo*. While most of the current work has and is being done in the methyl region (1-3 ppm chemical shift) and has focused, as in the case of the ^{31}P work, on physiological compounds, most drugs have protons in the aromatic and heterocyclic regions (6-12 ppm). We believe that drug studies are now possible in this region, allowing us to extend these concepts of noninvasive pharmacology to most drugs whose dosages are in NMR-detectable amounts. We have not proposed detailed experiments for such an much broader extension of the pharmacological NMRS studies because we have not yet had time to collect the necessary preliminary data. This will be possible if we have our own magnet, which could be scheduled for a number of such exploratory studies. A even more critical reason for our own magnet, as opposed to using, on a one day a week, the Huntington instrument, is that we are not currently able to perform true longitudinal studies: studying the same animal in successive days, and studying animals the day their tumor have reached the optimal level of growth. Thus, our own magnet is no longer a question of convenience: it is a necessity to perform studies in a rigorous, systematic manner. We have documented what we can do under difficult conditions. We are now requesting support to show what we can do under much better conditions.

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In addition of their contribution to cost-sharing for the acquisition of the Biomedical NMR spectrometer, we have been advised by Otsuka that they wish to collaborate with us in a more sustained manner. Their corporate structure has been reorganized, and Dr. Ray Nunally has joined them as Director for Research. New software specially suitable for pharmacological studies should now be made available for this 4.7T magnet, as is being made available for the 2T Siemens magnets, now operating on the SP2 software.

Finally, we have deleted all projects other than those from Dr. Singh and myself: Dr. Lewis has left USC, Drs. Colletti and Turk are using the clinical Gyroscan system for some of their studies, and while Dr. Chan continues interested in the *in vivo* interaction of drugs with receptors and enzymes, he recognizes the need to collect preliminary data. This will be possible once our system is available. Indeed, it will be possible for a number of other investigators at USC to get such preliminary data, and thereby expand, significantly, the research capabilities of this institution.

CURRICULUM VITAE

Name: **Walter Wolf**, Ph.D.

Place and Date of Birth: [redacted], Germany, [redacted]

EDUCATION: [redacted]

PROFESSIONAL EXPERIENCE: Professor of Pharmacy, USC, 1970-present

Senior consulting Radiopharmacologist, LAC/USC Medical Center, 1987-present

Director, Radiopharmacy Program, University of Southern California, 1969-1988

Director, Radiopharmacy Services, LAC/USC Medical Center, 1971-1987.

Visiting, Assistant and Associate Professor, USC, 1959-1970.

Research Associate, University of Southern California , 1959-1962.

Research Associate, Amherst College, Amherst, MA, 1958-1959.

Associate Professor, Organic Chemistry, University Concepcion, Chile, 1956-1958.

Stagiaire, then Attache de Recherches, CNRS, Paris, France, 1955-1956.

Consultant: Int. Atom. Energy Agency, US Veterans Administration. Member: Soc. Magnetic Reson. Med., Soc. Nuclear Med., Am. Assoc. Cancer Research, Am. Chem.Soc.; Fellow, Acad. Pharm. Sci.; Foreign Corresp. Member, Acad. Pharmacie, France

143 Publications in peer reviewed journals, 4 books, 171 abstracts in scientific conferences. The most recent and **relevant** publications include:

125. Fluorine-19 NMR Spectroscopic Studies of the Metabolism of 5-Fluorouracil in the Liver of Patients Undergoing Chemotherapy. W. Wolf, M.J. Albright, M.S. Silver, H. Weber, U. Reichardt and R.Sauer, Mag. Reson. Imaging, 5 (No. 3), 165-169, 1987.
127. Simulation of linear compartment models with application to nuclear medicine kinetic modeling. D.Z. D'Argenio, A. Schumitzky and W. Wolf, Comp. Prog. in Biomed., 27, 47-64, 1988.
128. A Non-Invasive Study of Drug Metabolism in Patients as Studies by F-19 NMR Spectroscopy of 5-Fluorouracil. Walter Wolf, Michael S. Silver, Michael J. Albright, Horst Weber, Ulrich Reichardt and Rolf Sauer. pp. 491-493, in "Physiological NMR Spectroscopy: From Isolated Cells to Man", Sheila M. Cohen and Jeffrey R. Alger, eds., New York Academy of Sciences, New York, 1988.
129. Noninvasive Drug Monitoring, Using Nuclear Medicine and Nuclear Magnetic Resonance Techniques. W. Wolf. Nuclear Medicine/Nuklear Medizin: New Trends and Possibilities in Nuclear Medicine, 770-774, 1988.
133. Considerations Towards The Structural Identification of Large Compartmental Models By Subsystem Analysis. D. Young, A. Schumitzky and W. Wolf. Indian Journal of Nuclear Medicine. 4, 193-197, 1989.
134. Compartmental Biodistribution of a Monoclonal Antibody Against Human Lung Adenocarcinoma Grown in Athymic Mice. J. Shani, S. bin Mohd, W. Wolf and L.E. Walker. Intl. J. Radn. Appl. Instr. Part B: Nucl. Med. Biol., 16, 33-40, 1989
137. Noninvasive Monitoring of Drug Biodistribution and Metabolism: Studies with Intraarterial Pt-195m-Cisplatin. J. Shani, J. Bertram, C. Russell, R. Dahalan, D.C.P. Chen, R. Parti, J. Ahmadi, R.A. Kempf, T.K. Kawada, F.M. Muggia and W. Wolf. Cancer Research 49, 1877-1881, 1989.
139. Tumor Trapping of 5-Fluorouracil: *in vivo* ^{19}F-NMR Spectroscopic Pharmacokinetics in Tumor-bearing Humans and rabbits. Walter Wolf, C.A. Presant, K.L. Servis, A. El-Tahtawy, M.J. Albright, P.B. Barker, R. Ring III, D. Atkinson, R.L. Ong, M. King, M. Singh, M. Ray, C. Wiseman, D. Blayney, and J. Shani. Proc. Natl. Acad. Sci., 87, 492-496, 1990.
142. Human Tumor 5-Fluorouracil Trapping: Clinical Correlations of in-vivo ^{19}F Nuclear Magnetic Resonance Spectroscopy Pharmacokinetics. C.A. Presant, W. Wolf, M.J. Albright, K.L. Servis, R. Ring III, D. Atkinson, R.L. Ong, C. Wiseman, M. King, D. Blayney, P. Kennedy, A. El-Tahtawy, M. Singh and J. Shani. J. Clin Oncol. 8, 1868-73, 1990

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CURRICULUM VITAE

Name: **Manbir Singh**, Ph.D.

Place and Date of Birth: [redacted] India, [redacted]

EDUCATION: [redacted]

PROFESSIONAL EXPERIENCE: Associate Professor of Radiology, USC, 1988-present
Associate Professor of Research Radiology, USC, 1983 -1988
Assistant Clinical Professor of Radiology, USC, 1978 - 1983
Physicist, Medical Imaging Science Group, USC, 1977 - 1978
Visiting Scientist, Biophysical Sciences Unit, Mayo Clinic, 1976 - 1977
Postdoctoral Scholar, University of California, Los Angeles, 1973 - 1976

47 Peer-Reviewd Publications, 17 other publications and 18 abstracts The most recent and pertinent are:

47. Human Tumor 5-Fluorouracil Trapping: Clinical Correlations of in-vivo ^{19}F Nuclear Magnetic Resonance Spectroscopy Pharmacokinetics. C.A. Presant, W. Wolf, M.J. Albright, K.L. Servis, R. Ring III, D. Atkinson, R.L. Ong, C. Wiseman, M. King, D. Blayney, P. Kennedy, A. El-Tahtawy, M. Singh and J. Shani. J. Clin Oncol. 8, 1868-73, 1990
46. Wolf, W, Presant, CA, Servis, KL, El-Tahtawy, A, Albright, MJ, Barker, PB, Ring, R, Atkinson, D, Ong, R, Singh, M, Ray, M, Wiseman, C, Blayney, D and Shani, J.: Tumor Trapping of 5-Fluorouracil: *in vivo* ^{19}F-NMR Spectroscopic Pharmacokinetics in Tumor-bearing Humans and rabbits. Proc. Natl. Acad. Sci., 86, 492-496, 1990
41. Singh, M., Brechner, R.R., Oshio, K., Leahy, R., and Henderson, V., SQUID Neuromagnetic Reconstruction of Brain Activity, SPIE 1351, 417-426, 1990
40. Singh, M. and Brechner, R.R.: First Experimental Test Object Study of Electronically Collimated SPECT. J. Nucl. Med., 31, 178-186, 1990
39. Singh, M. and Brechner, R.R.: SQUID Tomographic Neuromagnetic Imaging. Int. J. Imag. Systems & Technol., 1, 218-222, 1990
38. Oshio, K. and Singh, M.: A Computer Simulation of T2 Decay Effects in Echo Planar Imaging. Magnetic Resonance in Medicine, 11, 389-397, 1989
37. Singh, M:, Leahy, R., Brechner, R.R. and Yan, X.: Design and Imaging Studies of a Position Sensitive Photomultiplier Based Dynamic SPECT System. IEEE Trans. Nucl. Sci. NS 36, 1132-1137, 1989
36. Brechner, R.R. and Singh, M.: Comparison of an Electronically Collimated System and a Mechanical Cone-Beam System for Imaging Photons. IEEE Trans. Nucl. Sci. NS 36, 649-653, 1988
35. Singh, M., Horne, C., Maneval, D., Amartey, J. and Brechner, R.: Non Uniform Attenuation and Scatter Correction in SPECT. IEEE Trans. Nucl. Sci. NS-35, 767-771, 1988
34. Singh, M., Leahy, R., Brechner, R. and Hebert, T.: Noise Propagation in Electronically Collimated Single Photon Imaging, IEEE Trans. Nucl. Sci. NS-35, 772-777, 1988
33. Hebert, T., Leahy, R. and Singh, M.: Fast Maximum Likelihood Estimation for SPECT. IEEE Trans. Nucl. Sci. NS-35, 615-619, 1988
32. Jeffs, B., Leahy, R. and Singh, M.: An Evaluation of Methods for Neuromagnetic Image Reconstruction. IEEE Trans. Nucl. Sci. BME-34(9), 712-723, 1987
31. Singh, M. and Horne, C.: Use of a Germanium Detector to Study Scatter Correction in SPECT. J. Nucl. Med. 28, 1853-1860, 1987
30. Brechner, R., Singh, M. and Leahy, R.: Computer Simulated Studies of Tomographic Reconstruction with an Electronically Collimated Camera for SPECT. IEEE Trans. Nucl. Sci. NS-34(1), 369-372, 1987
29. Brechner, R. and Singh, M.: Reconstruction of Electronic Collimated Images Obtained from Single Photon Emitters Using a Spherical System of Coordinates. IEEE Trans. Nucl. Sci. NS-33(1), 583-586, 1986
28. Singh, M., and Doria, D., Single Photon Imaging with Electronic Collimation, IEEE Trans. Nucl. Sc. NS-32(1), 843-847, 1985. (Invited paper)
27. Singh, M., Doria, D., Huth, G.C., and Beatty, J., Reconstruction of Images from Neuromagnetic Fields. IEEE Trans. Nucl. Science NS-31 (1): 585-589, 1984.
26. Singh, M., and Henderson, V.W., Feasibility of Neuromagnetic Imaging of Cognitive Processes. Annals of Neurology 14: 144, 1983.

CURRICULUM VITAE

Name: Cary A. Presant, M.D.

Place and Date of Birth: [redacted] NY, [redacted]

EDUCATION: [redacted]

PROFESSIONAL EXPERIENCE: Professor of Clinical Medicine, USC School of Medicine, Los Angeles, CA 1982-present

Private Practice in Oncology and Hematology, Los Angeles, CA 1982-present

Queen of the Valley Hospital, West Covina, CA: Chairman, Tumor Board, 1982-present. Chairman, Cancer Committee, 1984-present. Director, Oncology Program, 1984-present

Associate Director, City of Hope Cancer Research Center, Duarte, CA 1980-82

City of Hope Medical Center, Director of Medical Oncology, 1979-1982

Chief, Section of Medical Oncology, Mallinckrodt Institute of Radiology, 1976-1979

Assistant Professor of Medicine and Radiology, Washington University School of Medicine, 1976-1979

Assistant in Medicine (1969-70), Instructor in Medicine (1970-1973) and Assistant Professor of Medicine, Washington University School of Medicine, 1973-1976. Research in lymphatic activation, lectin binding on tumor cell membranes, and clinical investigations.

125 Publications in peer reviewed journals, and over 130 abstracts in scientific conferences. The most recent key publications include:

120. Human Tumor 5-Fluorouracil Trapping: Clinical Correlations of in-vivo ^{19}F Nuclear Magnetic Resonance Spectroscopy Pharmacokinetics. C.A. Presant, W. Wolf, M.J. Albright, K.L. Servis, R. Ring III, D. Atkinson, R.L. Ong, C. Wiseman, M. King, D. Blayney, P. Kennedy, A. El-Tahtawy, M. Singh and J. Shani. J. Clin Oncol. 8, 1868-73, 1990
119. Presant, CA, Ksionski, G. and Crossley, R.: In-111 Labelled Liposomes for Tumor Imaging. Clinical Results of the International Liposome Imaging Study. J. Liposome Research. In Press, 1990
118. Presant, CA, Wiseman, C, Blayney, D, et al: Proposed Criteria for Serial Evaluation of Quality of Life in Cancer Patients. J. Natl. Cancer Inst. 82, 322-323, 796, 1990.
117. Presant, CA, Blayney, D, Proffitt, RT et al: Preliminary Report: Imaging of Kaposi Sarcoma and Lymphoma in AIDS with Indium-111 Labelled Liposomes. Lancet 335, 1307-1309, 1990.
116. Wolf, W, Presant, CA, Servis, KL, El-Tahtawy, A, Albright, MJ, Barker, PB, Ring, R, Atkinson, D, Ong, R, Singh, M, Ray, M, Wiseman, C, Blayney, D and Shani, J.: Tumor Trapping of 5-Fluorouracil: *in vivo* ^{19}F-NMR Spectroscopic Pharmacokinetics in Tumor-bearing Humans and rabbits. Proc. Natl. Acad. Sci., 1989, 87, 492-496, 1990.
115. Presant, CA, Forseen, EA, Proffitt, R.T. et al. Liposome targeted chemotherapy. Murine and Human evidence for targeting and initial clinical results. Abstracts, 6th. NCI-EORTC Symposium on New Drugs in Cancer Therapy, Amsterdam, 1989.
114. Forssen, E.A., Coulter, D.M., Presant, CA and Proffitt, R.T.: Chemotherapy of solid tumors in-vivo using site-directed daunorubicin liposomes. Proc. Am. Soc. Canc. Res. 30, 604, 1989.

CURRICULUM VITAE

Name: **David Z. D'Argenio**, Ph.D.

Place and Date of Birth: [redacted] New York, [redacted]

EDUCATION: [redacted]

PROFESSIONAL EXPERIENCE: Associate Professor of Biomedical Engineering, USC, 1985 - present

Assistant Professor of Biomedical Engineering, USC, 1979 - 1985

Postdoctoral Research Fellow, USC School of Medicine, 1978-1979

Consultant, Laboratory of Applied Radiopharmacokinetics, USC, 1977-1978

Predoctoral Trainee, Biomedical Engineering, USC, 1975-1978

Research Assistant, Bioengineering Program, Pennsylvania State University, 1974-1975

Recent Publications:

5. D'Argenio, D.Z. and Katz, D. Sampling Strategies for noncompartmental estimation of mean residence times. J. Pharmacokin. Biopharm. 11, 435-446, 1983.
6. D'Argenio, D.Z. and K. Khakmahd. Adaptive control of Theophylline therapy: Importance of blood sampling times. J. Pharmacokin. Biopharm. 11, 547-559, 1983.
7. Katz, D. and D.Z. D'Argenio. Discrete approximnation of multivariate densities with application to Bayesian estimation. Computational Stat. Data Anal. 2, 37-36, 1984.
8. Siebes, M., D.Z. D'Argenio and R.H. Selzer, Computer Asssessment of Hemodynamic Severity of Coronary Artery Stenosis from Angiograms. Computer Methods and Prog. in Biomed. 21, 143-152, 1985
9. Katz, D. and D.Z. D'Argenio. Implementation and evaluation of control strategies for individualizing dosage regimens, with application to the aminoglycoside antibiotics. J. Pharmacokin. Biopharm. 14, 523-537, 1986
10. Brechner, R.R., D.Z. D'Argenio, R. Dahalan and W. Wolf, Non-Invasive Estimation of Bound and Mobile Platinum Compounds in the Kidney Using a Radiopharmacokinetic Model. J. Pharm. Sci., 53, 873-877, 1986.
11. Zimmerman, R.C., J.B. SooHoo, J.N. Kramer and D.Z. D'Argenio. An evaluation of variance approximation techniques for non-linear photosynthesis-irradiance models. Marine Biology 95, 209-15, 1987
12. D'Argenio, D.Z., A. Schumitzky and W. Wolf, Simulation of linear compartment models with application to nuclear medicine kinetic modeling. Comp. Prog. in Biomed., 27, 47-64, 1988.
13. D'Argenio, D.Z. and Katz, D. Application of stochastic control methods to the problem of individualizing intravenous theophyline theram. Biomed. Measurement Informat. and Con. 2, 115-122, 1988
14. D'Argenio, D.Z. and Marmarelis. Experimental design for biomedical system modeling. Encyclopedia of Systems and Control: Biological Control Systems. Pergamon Press, London, pp. 486-490, 1988
15. D'Argenio, D.Z. and M. Van Guilder. Design of experiments for parameter estimation involving uncertain dynamic systems, with application to pharmacokinetics. Proc. 12th. World Congress on Scientific Computation, Paris, France, pp. 511-513, 1988.
16. Katz, D. and D.Z. D'Argenio. Stochastic control of pharmacokinetic systems: Open-loop feedback strategies. Proc. 1st. IFAC Symp. on Modelling and Control in Biomedical Systems, Venice, Italy, pp. 560-566, 1988.
17. Kurland, I.J. and D.Z. D'Argenio. A minimal model of liver glycogen metabolism: Feasibility for predicting flux rates. J. Theor. Biol. 135, 345-358, 1988.
18. D'Argenio, D.Z. and D.C. Maneval. Estimation approaches for modeling sparse data systems. Proc. 1st. IFAC Symp. on Modelling and Control in Biomedical Systems, Venice, Italy, pp. 377-382, 1988.
19. Maneval, D.C., D.Z. D'Argenio and W.Wolf: A Kinetic Model for Tc-99m DMSA in the Rat. Nuclear Medicine/Nuklear Medizin, 16, 29-34, 1990.

CURRICULUM VITAE

Name: **Kenneth K. Chan**, Ph.D.

Place and Date of Birth: [redacted]

EDUCATION: [redacted]

PROFESSIONAL EXPERIENCE: Associate Professor of Pharmacy, USC, 1979-present

Director, Pharmacoanalytic Laboratory, LAC/USC Com. Cancer Ctr., 1979-present

Assistant Professor of Pharmacy, USC, 1974-1979

Head, Pharmacokinetic Laboratory, John Wesley County Hospital, 1973-1979

Assistant Clinical Professor of Pharmaceutical Chemistry, USC, 1973-1974

Research Associate, USC, 1972-1973

Teaching Assistant, University of California, San Francisco, 1968-1972

Teaching Assistant and Research Assistant, Univ. of Calif., Davis, 1965-1967

Research Biochemist, VA Hospital, Palo Alto, 1964-1965

Recent Publications:

31. Chlebowski, R.T., A. Brezechwa-Ajdukiewwicz, A. Cowden, J.B. Block, M. Tong and K.K. Chan. Dozorubicin at 75mg/mb for hepatocellular carcinoma: Clinical and pharmacokinetic results. Cancer Treatment Rept. 68, pp. 487-491, 1984.
32. Hengst, J.C.D., K.K. Chan and M.S. Mitchell. Inhibition of proliferation without affecting the generation of cytotoxicity in the human mixed lymphocyte reaction. Cellular Immunol. 90, pp. 281-294, 1985.
33. Moran, R.G., P.D. Colman, A. Rosowsky, R.A. Forsch and K.K. Chan. Structural features of 4-amino antifolates required for substrate acitivity with mouse liver folate plyglutamate synthetase. Mol. Pharmacol. 27, pp. 156-166, 1985.
34. Chan, K.K., M.B. Bolger and K.S. Pang. Statistical moment theory in chemical kinetics. Anal. Chem. 57, pp. 2145-2151, 1985.
35. Watson, E., P. Dea and K.K. Chan. Kinetics of phosphoramide mustard hydrolysis in aqueous solution. J. Pharm. Sci. 74, pp. 1283-1292, 1986.
36. Chan, K.K., S.C. Hong, E. Watson and S.K. Deng. Identification of new metabolites of phosphoramide and nor-nitrogen mustard and cyclophosphamide in rat urine using ion cluster techniques. Biomed. Environ. Mass Spectrom. 13, pp. 145-154, 1986.
37. Stamp, J.J., E. Siegmund, T. Cairns and K.K. Chan. Chemical ionization mass spectrometry of carbamate pesticides. A major dissociation pathway. Anal. Chem. 58, p. 873-881, 1986.
38. Chan, K.K., E. Watson and S.C. Hong. The application of stable isotopes in antineoplastic drug research. In Current Topics in Pharmaceutical Sciences. eds. Breimer and Speiser, Elsevier. The Netherlands, pp. 249-267, 1985.
39. Hong, P.S.C. and K.K. Chan. Identification of alcophosphamide, a metabolite of cyclosphosphamide in the rat using chemical ionization mass spectrometry. Biomed. Environ. Mass Spectrom. 14, pp. 167-172, 1987.
40. Richardson, J.L., G.S. Marks, C.A. Johnson, J.W. Graham, K.K. Chan, J.N. Selser, C. Kishbaugh, Y. Barranday and A.M. Levine. Path model of multidimensional compliance with cancer therapy. Health Psychol. 6(3), pp. 183-207, 1987.
41. Levine, A.M., J.L. Richardson, G. Marks, K. Chan, J. Graham, J.N. Selser, C. Kishbaugh, D.R. Shelton and C.A. Johnson. Compliance with oral therapy in patients with hematologic malignancy. Clin. Oncology 5, pp. 1469-1476, 1987.
42. Lee, Y.J. and K.K. Chan. Metabolic interaction between methotrexate and mAMSA in the rabbit. Cancer Res. 48, pp. 5106-5111, 1988.
43. Chan, K.K. and A. Barrientos. Analysis of clomesone in plasma by electrolytic conductivity detection. J. Chromatogr. 428, 331-339, 1988.
44. Hong, P.S.C. and K.K. Chan. Pharmacokinetics of 4-hydroxycyclophosphamide in the rat. Drug Metab. Disp. (Accepted for publication).
45. Hong, P.S.C. and K.K. Chan. Analysis of 4-hydroxycyclophosphamide by gas chromatography-mass spectrometry in plasma. J. Chromatogr. (In press), 1989.

CURRICULUM VITAE

Name: **Kenneth L. Servis**

Place and Date of Birth: [redacted] Indiana [redacted]

EDUCATION: [redacted]

PROFESSIONAL EXPERIENCE:

Dean, Academic Records and Registrar, USC, 1989-present

Professor, Department of Chemistry, USC, 1983-present.

Associate Professor, Department of Chemistry, USC, 1968-1983.

Visiting Associate Professor, University of Zagreb, 1974.

Institute Fellow, Liquid Crystal Institute, Kent State University, 1974.

Visiting Scientist, Bell Laboratories, 1973.

Summer Research Fellow, Oak Ridge National Laboratory, Summer, 1971.

Institute Fellow, Massachusetts Institute of Technology, 1970.

Assistant Professor, University of Southern California, 1965-1968.

Analytical Chemist, Materials Laboratory, Dept. of Civil Engineering, Purdue University.

52 Peer Reviewed Publications. The most recent include:

52) C.A. Presant, W. Wolf, M.J. Albright, K.L. Servis, R. Ring III, D. Atkinson, R.L. Ong, C. Wiseman, M. King, D. Blayney, P. Kennedy, A. El-Tahtawy, M. Singh and J. Shani: "Human Tumor 5-Fluorouracil Trapping: Clinical Correlations of in-vivo ^{19}F Nuclear Magnetic Resonance Spectroscopy Pharmacokinetics". J. Clin Oncol., 8, 1868-73 (1990).

51) E. Ryzen, K.L. Servis and R.K. Rude: "Effect of intravenous epinephrine on serum magnesium and free intracellular red blood cell magnesium concentrations measured by nuclear magnetic resonance". J. Am. Coll. Nutr. 9, 114-9, (1990).

50) W. Wolf, C.A. Presant, K.L. Servis, A. El-Tahtawy, M. J. Albright, P. B. Barker, R. Ring III, D. Atkinson, R. Ong, M. King, M. Singh, M. Ray, C. Wiseman, D. Blayney, and J. Shani: "Tumor Trapping of 5-Fluorouracil: *in vivo* ^{19}F-NMR Spectroscopic Pharmacokinetics in Tumor-bearing Humans and rabbits". Proc. Natl. Acad. Sci., 87, 492-496, (1990).

49) E. Ryzen, K.L. Servis, P. DeRusso, A. Kershaw, T. Stephen and R.K. Rude: "Determination of intracellular free magnesium by nuclear magnetic resonance in human magnesium deficiency". J. Am. Coll. Nutr. 8, 580-87, (1989).

48) P. Baine, R.L. Domenick and K.L. Servis, "Rate of Degenerate Rearrangement of the 2-Methyl-2-Norbonyl Cation studied by ^{13}C Selective Pulse Transfer and Two Dimensional NOESY Spectroscopy", Mag, Reson. in Chemistry, 25, 1035 (1987).

47) D.A. Fortsyth, J.H. Botkin, J.S. Purchase, K.L. Servis and R.L. Domenick, "Large Intrinsic Nuclear Magnetic Resonance Isotope Shifts Associated with Bending Motion along the Bridging Coordinate in Carbocations", J. Amer. Chem. Soc. 109, 7270 (1987).

46) K.L. Servis, R.L. Domenick, D.A. Forsyth and Y. Pan, "Deuterium Isotope Effects on the Carbon-13 Chemical Shifts in 2-Substituted 2-Norbornyl Cations", J. Amer. Chem. Soc. 109, 2763 (1987).

45) K.L. Servis and R.L. Domenick, "Origin of Deuterium Isotope Effects on Carbon-13 Chemical Shifts", J. Amer. Chem. Soc., 108, 2211 (1986).

44) F. Berchier, Y.M. Pai, W.P. Weber, and K.L. Servis, "Deuterium Isotope Effects on Silicon-29 Chemical Shifts", Mag. Reson. in Chem., 24, 679 (1986).

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42) Y.M. Pai, K.L. Servis and W.P. Weber, "Preparation of Oligomeric and Polymeric , -bis(Trimethylsiloxy)-Polymethylchlorosiloxanes and their Reactions with Alkyllthium Reagents, "Organometallics, 5, 683 (1985)

41) Y.M. Pai, W.P. Weber and K.L. Servis, Silicon-29 NMR Studies of Polymethylhydrosiloxanes, J. Organomet. Chem., 288, 267 (1985).

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CURRICULUM VITAE

Name: **Victor Waluch**, M.D, Ph.D.

Place and Date of Birth: [redacted] Germany, [redacted]

EDUCATION: [redacted]

PROFESSIONAL EXPERIENCE: Clinical Associate Professor of Radiology, USC, 1989-present

Chief of MRI/MRS, Department of Radiology, St. Vincent Medical Center, Los Angeles, 1984-present

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Resident in Diagnostic Radiology, Stanford University Hospital, 1980/1983 Staff, Physics and Computing, University of California Lawrence Livermore Laboratory, Livermore, CA 1972-1976

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Chemistry

Tumor trapping of 5-fluorouracil: *In vivo* ^{19}F NMR spectroscopic pharmacokinetics in tumor-bearing humans and rabbits

(VX2 tumor/magnetic resonance imaging/chemotherapy)

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ABSTRACT **The pharmacokinetics of 5-fluorouracil (5FU) were studied *in vivo* in patients with discrete tumors and in rabbits bearing VX2 tumors by using ^{19}F NMR spectroscopy. The human studies were conducted in a 1.5-T Magnetom magnetic resonance imager (Siemens), and the rabbit studies were conducted in a 4.7-T GE/Nicolet 33-cm bore magnet. Free 5FU was detected in the tumors of four of the six patients and in all VX2 tumors but not in normal rabbit tissues. No other metabolites were seen in these tumors, contrary to the extensive catabolism we had previously documented using ^{19}F NMR spectroscopy in both human and animal livers. The tumor pool of free 5FU in those human tumors that trapped 5FU was determined to have a half-life of 0.4–2.1 hr, much longer than expected and significantly longer than the half-life of 5FU in blood (5–15 min), whereas the half-life of trapped 5FU in the VX2 tumors ranged from 1.05 to 1.22 hr. In this initial experience, patient response to chemotherapy may correlate with extent of trapping free 5FU in the human tumors. These studies document that NMR spectroscopy is clinically feasible *in vivo*, allows noninvasive pharmacokinetic analyses at a drug–target tissue in real time, and may produce therapeutically important information at the time of drug administration. Demonstration of the trapping of 5FU in tumors provides both a model for studying metabolic modulation in experimental tumors (in animals) and a method for testing modulation strategies clinically (in patients).**

The pharmacokinetic monitoring of drugs in human tumors has been limited by the invasive nature of sample acquisition. Most pharmacokinetic analyses have been performed on serial serum or plasma samples that measure only the circulating drug compartment, not the concentration of the drug or its metabolites in the target tissue of interest. Drugs radioactively labeled (intrinsically) with a γ-ray emitter can be monitored quantitatively by γ-ray camera detection (single photon or positron-emission tomography), but such detection captures the total pool of all labeled compounds present without distinguishing between chemical species.

Nuclear magnetic resonance spectroscopy (NMRS), on the other hand, is a well established chemical technique used for the identification and characterization of chemical substances and, more recently, for metabolite identification (1). Although this technique has been extensively used on sample analysis *in vitro* (1), this method has not yet been demonstrated to be unequivocally useful in the analysis of human tumors *in vivo*.

We have been using ^{19}F NMRS to analyze the time course and the metabolism of fluorinated compounds in living systems, inasmuch as this NMR-sensitive nucleus is present in a significant number of drugs, such as 5-fluorouracil (5FU). Prior publications have shown that such noninvasive analyses could be accomplished in mice (2), and we had extended this work to show that the time course of 5FU uptake and catabolism could be monitored in the uninvolved normal liver of cancer patients receiving 5FU as part of their treatment (3). We have now extended such studies by monitoring the time course of 5FU uptake into tumor tissues in both animal models and patients to assess objectively and individually the degree of drug targeting and metabolism at specific tumor sites.

Our results show that the pharmacokinetics of 5FU uptake and metabolism can be measured *in vivo* in human tumors (breast and colon carcinoma) in real time and that the rabbit VX2 carcinoma (a pharmacological model widely used by others for 5FU-related studies) can serve as an animal model for helping to analyze and understand the data from human tumors. These results show that, contrary to expectations, there is retention ("trapping") of free 5FU in some human tumors. Such tumor trapping of free 5FU may have significant clinical implications for assessing the effectiveness of 5FU treatment, for selection of chemotherapy in individual patients, and for improved evaluation of metabolic modulation of 5FU chemotherapy *in vivo*. More broadly, these data suggest that *in vivo* NMRS may have significant potential, both in understanding human biological processes and in guiding therapeutics.

METHODS

The present study has been done by using ^{19}F NMRS and described methods (3, [¶¶]). For animal studies, 10^7 VX2 tumor cells were implanted in the right flank of New Zealand White rabbits, and these animals were studied 2 weeks postimplantation. The rabbit to be studied was anesthetized by intramuscular administration of a mixture of xylazine and ketamine. An i.v. catheter was placed in the marginal right ear

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Abbreviations: 5FU, 5-fluorouracil; NMRS, nuclear magnetic resonance spectroscopy; FID, free induction decay; T_2^*, effective transverse relaxation; FβAla, 2-fluoro-3-aminopropionic acid.

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[¶¶]Wolf, W., Servis, K.L. & El-Tahtawy, A., Annual Meeting of the Society of Magnetic Resonance in Medicine, August 17–21, 1987, New York, abstr. 587.

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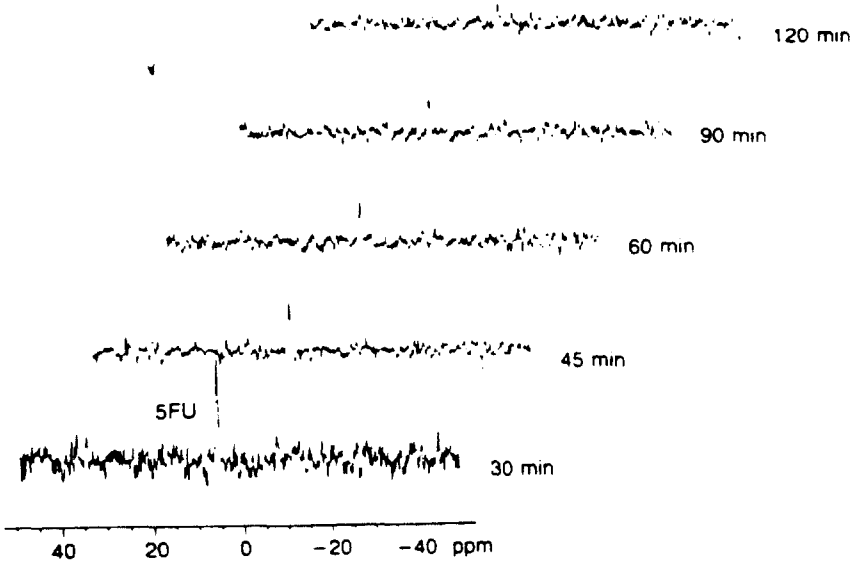

FIG. 1. Stacked plot of ^{19}F NMR spectra of the VX2 tumor (implanted in New Zealand White rabbit flank) after i.v. administration of 5FU at 100 mg/kg at 30, 45, 60, 90, and 120 min postinjection. Each spectrum was the result of 1680 FIDs acquired at 20 kHz spectral width with a delay time of 250 μsec and a pulse (15 μsec) optimized for maximum signal at 188.360061 MHz. A 1.5-cm surface coil was used. All spectra were corrected to baseline and smoothed with a gaussian filter matched to the T_2^* of the FID.

vein, so that drug could be administered after positioning and proper shimming. The tumor was positioned over a 1.5-cm surface coil, well-centered in a 33-cm bore 4.7-T NMR spectrometer. After administration of 5FU at 100 mg/kg as a bolus, 1680 free induction delays (FIDs) were acquired at 20-kHz spectral width with a delay time of 250 μsec and a pulse (15 μsec) optimized for maximum signal at 188.360061 MHz. All spectra were corrected for baseline and smoothed with a gaussian filter matched to the effective transverse relaxation time (T_2^*) of the FID. An external sample of 5FU was used to verify the identity of the peaks observed.

Patients were studied by positioning each patient in a 1.5-T Magnetom, with the tumor located over or beneath a 15-cm surface coil. The patient was placed so that the tumor was centered in the magnet, and a fast low angle shot (FLASH) image was acquired to verify positioning. An i.v. catheter was inserted into the patient's arm, and after proper shimming, a bolus injection of 5FU at 600 mg/m^2 was given i.v. A total of 256 FIDs were acquired at 5-kHz spectral width—repetition time = 1 sec, delay time = 200 μsec, with the radiofrequency pulse optimized for 4-cm depth, at 60.012500 MHz. All spectra were corrected to baseline and smoothed with a gaussian filter matched to the T_2^* of the FID. Series of consecutive spectra, each collected over a 4.17-min period, were obtained during the first hour after drug administration.

At the conclusion of 1 hr, some patients were repositioned to acquire further spectra from the liver under the same acquisition conditions. Four to six consecutive 4.17-min spectra were collected.

RESULTS

An example of a sequential series of spectra obtained from the animal studies is illustrated in Fig. 1. These spectra document that while free 5FU is present in the region monitored by the surface coil, no anabolites or catabolites of 5FU were detected up to 3 hr after drug injection. The half-life of free 5FU in the VX2 tumor of the rabbit was estimated at 1.05–1.22 hr. By comparison, the half-life of 5FU in the blood of humans and rabbits ranges from 5–15 min (4, 5). In a study conducted as part of this work, we measured the half-life of 5FU in equivalent tissue regions of control rabbits (without tumors) with the NMRS method and obtained values from 6.5 to 9 min. These values compare well with the half-life of 5FU in rabbit blood, as reported by Kar *et al.* (5), with use of standard HPLC techniques.

Note that no fluorinated nucleosides or nucleotides were detected in the VX2 tumor of the rabbit *in vivo*, even when tumor uptake of free 5FU was increased 3- to 8-fold after intraarterial drug administration (6). Such anabolites were seen, however, after excision of these rabbit tumors and collection of ^{19}F NMR spectra *ex vivo*.*** One possible explanation is that conversion to 5-fluorodeoxyuridine monophosphate (which is then complexed to thymidylate synthetase) and to fluorinated RNA had occurred *in vivo*, but that after resection these complexes underwent hydrolysis to low molecular weight, NMR-visible compounds *in vitro*. NMR is not a very sensitive technique, and only compounds present in significant concentrations are detectable. Under our current conditions of measurement and sensitivity, we have determined that concentrations of 5FU below 0.25 mM are no longer detectable. We anticipate that instrumental and operational improvements of NMRS may significantly improve that limit of detection.

Data for six of the patients studied are presented in Table 1. A stacked plot of the ^{19}F NMR spectra collected over the tumor of patient 6, who had a colon carcinoma that had metastasized to the liver, is shown in Fig. 2, illustrating detectable amounts of 5FU. No other detectable metabolites (anabolites, catabolites) were seen, even when all these spectra were summed. The possible nature of the second peak observed in this patient, 1.8 ppm downfield from 5FU, is discussed below. At the end of the above acquisitions, the patient was repositioned with the surface coil over his uninvolved liver, and four spectra were recorded starting at 1 hr after drug administration. Only signals due to 2-fluoro-3-aminopropionic acid (FβAla) could be detected, which were of lower intensity than those of the 5FU signal detected in the tumor region.

Similarly, the spectra of both patients 1 and 2 revealed the presence of free 5FU in their tumors, again with no anabolites or catabolites detectable by noninvasive NMRS. The spectra from patient 3 failed to reveal any detectable accumulation in the tumor of either 5FU, its anabolites, or catabolites over the first 90 min after drug injection. The spectra from patient 4 were collected over the liver tumor and, because of the location of this neoplasm, included tumor as well as uninvolved liver tissue. Although a 5FU signal was detected after drug administration, this signal disappeared rapidly, suggesting that it probably came from uninvolved liver tissue or from

***El-Tahtawy, A., Servis, K. L. & Wolf, W., Annual Meeting of the Society of Nuclear Magnetic Resonance in Medicine, August 12–18, 1989, Amsterdam, abstr. 411.

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Table 1. Correlation between ^{19}F NMR spectroscopic results and chemotherapeutic response

Patient number	Tumor			^{19}F resonance*			Chemotherapy†	Chemotherapeutic response‡ (%)
	Type	Location	Size, cm	5FU	FNUC	FβAla		
1	Rectal adenocarcinoma	Presacral space	3.5 × 5	+	−	−	5FU, Leuco	Partial response (81)
2	Breast carcinoma	Breast	5 × 4.3	+	−	−	5FU, Doxo, Cyclophos	Partial response (50)
3	Breast carcinoma	Breast	Exfol	−	−	−	5FU, Meno, Cyclophos	Partial response (95)
4	Carcinoma of colon	Liver	5 × 5	−	−	+§	5FU, Leuco	Progression of disease
5	Endometrial carcinoma	Lung	5 × 5	−	−	−	5FU, Leuco	Partial response (53)
6	Carcinoma of colon	Liver	10 × 18	+	−	−	5FU, Leuco	Partial response (73)

*Compounds detectable by *in vivo* ^{19}F NMRS. 5FU, peak corresponding to the free 5FU. FNUC, nucleosides and nucleotides generated from 5FU; the chemical shift differences from these compounds are too small for separation by *in vivo* NMRS. Exfol, exfoliating. A + notation for 5FU indicates its presence beyond the first few spectra after drug administration (each spectrum requires 4.17 min of collection time); hence, the $t_{1/2}$ significantly exceeds that of 5FU in the blood pool. A + notation for FNUC and FβAla indicates their presence in *any* spectra collected over the tumor.

†Current chemotherapeutic regimens. Some patients may have received prior chemotherapeutic agents. Leuco, leucovorin; Doxo, doxorubicin; Cyclophos, cyclophosphamide; Meno, menogaril.

‡Percent reduction in sum of products of cross-sectional diameters of all measured tumors. Partial response is >50% reduction in sum, with no new tumors.

§The FβAla detected in this patient was probably due to signals coming from the nontumorous liver tissue surrounding the tumor. No FβAla was detected in two studies in patient 6, consistent with the fact that his liver tumor was large and was, therefore, probably not contaminated with (nontumorous) liver signals.

the blood present in the volume detected by the surface coil. A strong signal from FβAla was detected subsequently, well in agreement with our prior healthy-liver studies (3). Interestingly, the 5FU signal from patient 6 had a significantly longer half-life than those signals from patient 4 (who also had a colon carcinoma with liver metastases). The spectra from patient 5 revealed 5FU, with no detectable anabolites or catabolites.

A preliminary analysis of the response to chemotherapy is consistent with a positive correlation between tumor 5FU trapping, as measured by ^{19}F NMRS and at least partial response to 5FU-containing chemotherapy (Table 1). Patient 1 had an 81% partial response to 5FU alone and in combination with leucovorin. Patient 2 had a 50% partial response to 5FU alone and in combination with cyclophosphamide and doxorubicin. Patient 3 had a partial response (95% reduction in tumor area), but she received a combination of 5FU, cyclophosphamide, and menogaril. Patient 4 had previously had a partial response to 5FU plus leucovorin but had begun to progress at the time of this study and, therefore, had 0% response to 5FU at the time of analysis. Patient 5 had a 53% partial response to 5FU plus leucovorin. Patient 6 had a 73% response to 5FU plus leucovorin. Thus, of four patients with tumor trapping of 5FU, all four had significant chemotherapeutic responses. Of two patients with no detectable 5FU trapping, one patient treated with 5FU was resistant to therapy and the other patient, treated with non-5FU chemotherapy (as well as 5FU), responded.

The pharmacokinetics of free 5FU in tumor tissues was analyzed by serial evaluation of the relative intensity of the 5FU peaks to determine clearance rates. As an example, a data plot of free 5FU in the tumor of patient 1 and in the VX2 tumor in a rabbit is shown in Fig. 3. Regression analysis of the relative intensity of the 5FU peak in the tumor of patient 1 gave an elimination half-life of 1.3 ± 0.5 hr, compared with 1.1 ± 0.3 hr in the VX2 tumor. In contrast, the elimination half-life in normal tissues of a rabbit without tumors (Fig. 3) was only 6.5 ± 2.2 min, a value well within the range of the half-life of 5FU in the blood of rabbits (5) or humans (4).

As an example of NMRS application to the study of 5FU modulation, patient 6 was studied twice. (*i*) On the first study,

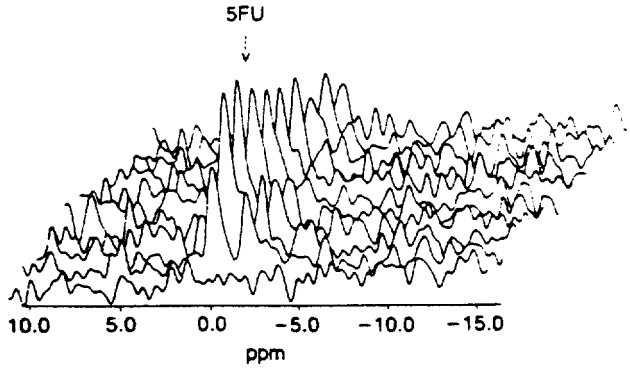

FIG. 2. Stacked plot of ^{19}F NMR spectra taken over the liver metastasis of the colon carcinoma of patient 6 from 0 to 55 min after i.v. administration of 5FU at 600 mg/m^2 as a bolus. The patient was positioned in a 1.5-T Magnetom before injection. Each spectrum was the result of 256 FIDs acquired at 10-kHz spectral width with the 90° pulse optimized for 4 cm at 60.012500 MHz and a 1-sec repetition rate. All spectra were corrected for baseline and smoothed with a gaussian filter matched to the T_2^* of the FID. Series of consecutive spectra, each collected over a 4.17-min period, were obtained during the first hour after drug administration.

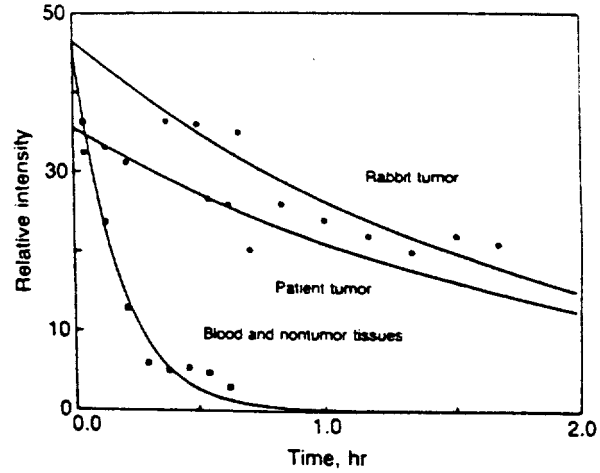

FIG. 3. Comparative kinetics of 5FU in rectal adenocarcinoma of patient 1 (●), in VX2 tumor of rabbit (○), and in an equivalent volume of the rabbit (including blood and surrounding tissues) (■)—all measured by ^{19}F NMRS. These three curves were fitted to a one-exponential model; half-life for free 5FU in a region of a nontumorous (control) rabbit that included blood and tissues was 6.5 ± 2.2 min, in the rectal adenocarcinoma of patient 1 was 1.3 ± 0.5 hr, and in VX2 tumor of a New Zealand White rabbit was 1.1 ± 0.4 hr.

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which was also his first 5FU dose, he only received the standard bolus injection of this drug. (*iii*) In the second study, conducted 6 weeks later, this patient received leucovorin plus 5FU. The kinetics of intratumoral 5FU were unchanged by leucovorin: the $t_{1/2}$ of intratumoral 5FU was 22 ± 3 min in the first study and 20 ± 3 min 4 weeks later, when calcium leucovorin (500 mg/m^2) was given as an i.v. infusion over 2 hr; after 1 hr, 5FU at 600 mg/m^2 was given as a bolus injection. No peaks attributable to fluorinated nucleosides/nucleotides could be detected in this patient. These results, if confirmed, suggest that leucovorin potentiation of 5FU chemotherapy response in colon carcinoma is not due to a universal enhancement of 5FU uptake, prolongation of intracellular 5FU half-life, or marked enhancement of the conversion of 5FU to nucleosides or nucleotides. More patients are obviously needed to further validate these preliminary observations.

Another interesting observation was made in these two studies of patient 6: in his first study, shown in Fig. 2, a second peak, 1.8 ppm downfield from 5FU, was seen. The patient exhibited significant clinical edema, an observation also seen upon shimming, which revealed a much higher water/fat ratio in this proton spectra than expected. The short half-life of the second peak (4.9 min) is consistent with 5FU in the extracellular fluid (blood, interstitial fluids). The second study, where the patient's edema had been significantly reduced by diuretics, exhibited no such second peak.

DISCUSSION

The antitumor action of 5FU depends on the uptake of this drug into tumors, on its transformation into its nucleosides and nucleotides, on the covalent binding of 5-fluorodeoxyuridine monophosphate to thymidylate synthetase, and on the incorporation of 5-fluorouridine into RNA (7). Thus, the first step required for 5FU to exhibit antitumor activity is its uptake into the tumor cell, a process considered to be controlled by passive diffusion (7). However, several recent studies have suggested the existence of a facilitated diffusion (8) or an active transport process (9).

One problem inherent to such studies is that measuring the nature of drug metabolites in tumor tissues requires that the animal be sacrificed so that tissue samples can be removed and analyzed or that biopsy material or surgical specimens from humans be available. In either case, the data obtained from one individual were limited to a single time point and did not allow for studies where the dynamics of the system could be captured from a single individual. Noninvasive methods that allow chemicals to be measured in living systems are ideally suited to generate such crucial types of information. Drugs that contain fluorine in their molecular structure lend themselves particularly well to such noninvasive methods of monitoring. The fluorine atom is 100% ^{19}F, and this atom has an NMR sensitivity second only to that of the proton (84%) and a very large chemical shift range (over 200 ppm); furthermore organic compounds do not occur naturally in the human body. A major limitation of NMRS, however, is that, although a technique of exquisite resolution, it has limited sensitivity, requiring concentrations >0.5 mM, and only molecules that can tumble rapidly are readily detectable. Thus, large fluorinated molecules (including the 5FU-containing thymidylate synthetase ternary complex and 5FU-containing RNA) would not be detectable under the present conditions.

Prior studies using NMRS have only demonstrated 5FU in human tumors *in vitro* and concurrent studies (in abstracts) in tumors in liver (10, †††), where the relative concentrations of 5FU in tumors vs. liver could not be separated. In the reported experiments we have demonstrated a method for noninvasively analyzing the pharmacokinetics of free 5FU in human tumors *in vivo* and show the close correlation and concordance of results with those obtained in the rabbit VX2 tumor.

Detectable amounts of a previously unknown, relatively long-lived trapped pool of free 5FU were measured in four of the six patients that could be properly evaluated, and this free 5FU had a half-life of elimination of ≈1 hr. The similarity of this phenomenon in human tumors to results in the VX2 tumor should make the latter model useful for exploring the effects of various metabolic modulations on the extent of 5FU trapping and changes in 5FU half-life, in the generation of increased amounts of nucleosides and nucleotides, and in the amount of catabolism to FβAla. We also note that the VX2 tumor is not a tumor model highly responsive to 5FU chemotherapy; the data of Kar *et al.* (5) and our own preliminary observations suggest that the VX2 tumor in rabbits is mildly-to-moderately responsive to 5FU therapy, in the general range of response comparable with that of most human solid tumors. The VX2 tumor in rabbits is not highly responsive to 5FU in that complete remissions are not seen after 5FU at 150 mg/kg i.v. Therefore, the VX2 tumor could be useful not only to discover and study modulators of *in vivo* 5FU tumor metabolism, but also to detect enhanced 5FU antitumor effects from addition of such modulators.

Better understanding of these results can perhaps be gained by conceptualizing a model for 5FU biodistribution and metabolism (Fig. 4), expanded from one we had earlier

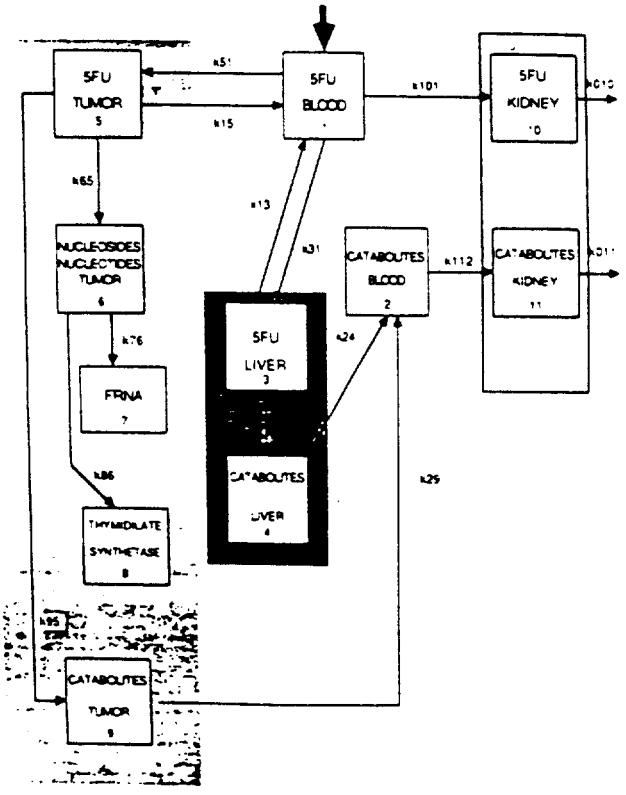

FIG. 4. Compartmental model of 5FU distribution and metabolism in tumor-bearing patients or other mammal. Shaded areas represent tumor, liver, and kidneys, each of which may contain several compounds. Catabolites of 5FU (FβAla, 5-fluoroureidopropionic acid, and 5,6-dihydrofluorouracil) have been grouped together. The low-molecular-weight anabolites generated from 5FU expected in the tumor (nucleosides and nucleotides) have also been grouped together in box 6. Each compartment is either directly measurable or can be estimated by radiopharmacokinetic analysis (11).

†††Semmler, W., Bachert-Baumann, P., Guckel, F., Lehner, B., Schlag, F. & von Kaick, G., Annual Meeting of the Society of Magnetic Resonance in Medicine, August 20–26, 1988, San Francisco, abstr. 258.

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proposed.[‡‡] These models suggest that the first step—namely, transfer of 5FU from blood compartment to tumor—appears to depend on the local concentration of drug in the early postadministration phase because 5FU is very rapidly cleared from the blood. That free 5FU is seen in the tumors studied for at least 1 hr—well after it has been almost completely cleared from the blood—suggests that once 5FU is taken up by tumor tissue, diffusion back into the blood (if any) is much slower. Thus, the present work supports the hypothesis that 5FU enters into selected tumors by either a facilitated-diffusion or an active-transport process (8, 9), after which its diffusion out of the tumor is very slow.

Our results also appear consistent with the hypothesis that the free drug is trapped in the tumors, whereas it is not trapped in tissues (of rabbits) that are not proliferating rapidly, such as muscle, bone, skin, etc. Possibly the trapped pool of free 5FU represents not only the originally accumulated free 5FU, but also some 5FU that had been accumulated, converted to nucleotide, and then degraded back to free 5FU.

We note that our results conflict with other studies that have shown a long retention of free 5FU in the sarcoma 180 and M5076 tumors in mice,[‡‡‡] but not in the murine Lewis lung carcinoma (2). A report (in abstract only) on *ex-vivo* chemical analysis by Sorensen *et al.* (12) suggests that the half-life of free 5FU and of fluorodeoxyuridine monophosphate in human tumors is much longer than previously thought.

Assessing the therapeutic significance of drug trapping is important. Perhaps the presence of drug trapping and metabolism may predict tumor sensitivity to 5FU therapy, as indicated by the apparent agreement between trapping and response (or lack of trapping and lack of response) in five of the six patients evaluated (Table 1). The possible effect of other drugs on the extent of trapping and anabolism may help assess the degree of synergism between drugs in an individual patient. *The key observation is that it is now possible to measure the time course of a therapeutic agent (as an unequivocal chemical species) in its intended target site in humans* in vivo, and we believe that such measurements may indicate therapeutically important information at the time of drug administration.

Another question that these noninvasive studies might address is the degree and the rate at which 5FU is converted to its nucleosides/nucleotides in specific tumors and the activation steps required before this drug can exercise its antitumor action. Whether other drugs, known to interact synergistically with 5FU, can affect either tumor trapping of 5FU and or its conversion to any effective nucleosides and nucleotides also deserves answer.

Of broader significance is the potential application of ^{19}F NMR spectroscopic pharmacokinetics to explaining the behavior of other fluorinated drugs *in vivo* in humans. The distribution and effectiveness of 5-fluoro-2′,3′-dideoxycytidine as an antiviral agent in AIDS can be studied (13), as can the relative effect of route of drug administration on drug targeting. The method described in this work could be applied to a number of other fluorinated drugs, especially chemotherapeutics (floxuridine, Ftorafur, 5′-deoxy-5-fluorouridine, difluoromethylornithine).

Finally, such noninvasive pharmacokinetic studies can be theoretically extended to any drug by using either selected ^{1}H peaks or the ^{13}C content (natural or enriched), even though such studies are at present technically very difficult.

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Human Tumor Fluorouracil Trapping: Clinical Correlations of In Vivo ^{19}F Nuclear Magnetic Resonance Spectroscopy Pharmacokinetics

By Cary A. Presant, Walter Wolf, Michael J. Albright, Kenneth L. Servis, Robert Ring III, Dennis Atkinson, Richard L. Ong, Charles Wiseman, Mark King, Douglas Blayney, Peter Kennedy, Ahmed El-Tahtawy, Manbir Singh, and Jashovam Shani

We previously reported that fluorouracil (5FU) accumulation and metabolism in human livers and tumors can be studied by in vivo nuclear magnetic resonance spectroscopy (NMRS). We have extended these observations by evaluating the pharmacokinetics of 5FU in the tumors of 11 patients with carcinoma of the breast, colon, endometrium, cervix, and kidney, using ^{19}F-NMRS in a 1.5 Magnetom (Siemens Medical Systems, Cerrito, CA) magnetic resonance imaging unit (MRI). These NMRS measurements detected a long-lived tumor pool of 5FU in six of 11 tumors in our patients including carcinomas in the pelvis, breast, lung, and liver. The half-life ($T^1/_2$) of this tumor pool of "trapped" 5FU was 0.33 to 1.3 hours (20 to 78 minutes), much longer than the $T^1/_2$ of 5FU in blood (5 to 15 minutes). Neither the anabolites of 5FU (fluorinated nucleosides, nucleotides, 5FU-RNA, or 5FU-thymidylate synthase) nor the catabolites (eg, fluorobetaalanine [FBAL]) were detectable by ^{19}F NMRS. Patient response to chemotherapy appeared to correlate with the extent of trapping of free 5FU in the human tumors: in the seven patients receiving 5FU, or 5FU or FUdR plus leucovorin, four of four patients whose tumors trapped 5FU responded to fluorinated pyrimidine chemotherapy, whereas three patients in whom there was a failure to detect tumor trapping were resistant to 5FU. We conclude that NMRS is clinically feasible, and enables investigators to study 5FU pharmacokinetics and metabolism in tumors in vivo. ^{19}F-NMRS of 5FU allows for in vivo evaluation of 5FU metabolic modulation and might be able to guide therapeutic decisions.

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FLUOROURACIL (5FU) is a widely used antitumor drug effective in reducing tumor size in 15% to 20% of patients with carcinomas of the breast and gastrointestinal tract, and to a lesser extent in a wide variety of other tumors. Despite the fact that it has just passed its third decade of use since its clinical introduction by Curreri et al in 1958,[1] clinicians are still unable to predict which patients will respond to the drug. Further, despite many observations of different methods of modulating 5FU metabolism to effect increased antitumor response, there is no direct method of assessing the biochemical basis of modulation in vivo.

We have been using nuclear magnetic resonance spectroscopy (NMRS) to study 5FU in animals and humans, since it is a noninvasive method for evaluation of drug metabolism.[2] Stevens et al originally showed that ^{19}F-NMRS can detect 5FU in the liver of mice,[3] work that was extended to the study of 5FU metabolism in the livers[4] and tumors[5] of rats (Walker 256) and rabbits (VX2), as well as that of mice bearing various tumors.[6,7] These studies suggested that significant interspecies differences could be observed in the nature of the ^{19}F products detected.

We concluded[4] that the metabolism of 5FU in the liver of nontumored rabbits correlated with that observed in the uninvolved livers of patients

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receiving 5FU. More recent data have suggested that the metabolism of 5FU in the VX2 tumor in rabbits may also correlate with the metabolism of 5FU observed in human tumors.[8] It is also important to note that when the Walker 256 and the VX2 tumors were excised and their ^{19}F-NMR spectra measured ex vivo, we observed significant differences compared with the products observed in vivo.[9] These differences could be ascribed to a rapid catabolism of fluorinated RNA and/or the ternary complex of 5FU with thymidylate synthase (TS) into the nucleosides and/or free 5FU. It is also of interest that, while only 5FU is detectable in vivo in humans and in the rabbit VX2 tumor, anabolites of 5FU are detectable in vivo in the Walker 256 experimental tumor in rats. Such results strongly suggest that in vivo observations may have a superior accuracy for the noninvasive pharmacologic monitoring of 5FU and therefore avoid the potentially confounding effects of enzymatic changes resulting during the biopsy process. Further, in vivo NMRS measurements might document dynamic processes in living systems under conditions that could be readily performed in patients.

Our prior human and animal model studies documented that 5FU uptake and catabolism to fluorobetaalanine (FBAL) could be evaluated in uninvolved liver.[2,4] Most recently, we reported that ^{19}F-NMRS could measure 5FU uptake into and retention by a variety of human cancers in vivo.[8]

We have extended those pilot observations and now report on our initial clinical experience with NMRS in 11 patients. In this report, we characterize the nature of 5FU uptake and the discovery of a "trapped" pool of intratumoral 5FU, defined as a pool of 5FU whose disappearance half-life ($T^1/_2$) is longer than its $T^1/_2$ in peripheral blood. We also present the initial correlations between the $T^1/_2$ of 5FU in tumors and antitumor response to 5FU. This report includes and extends the analyses in our previously published abstracts and preliminary publication.[10]

METHODS

Patients were eligible for this study if they had a measurable tumor of at least 2 cm diameter and had received, were receiving, or were scheduled to receive 5FU, 5-fluorodeoxyuridine (FUdR), or a 5FU-containing chemotherapy regimen. Patients with claustrophobia were excluded. The tumor had to be located in a body location within 8 cm of the skin in an area that could be positioned over a surface coil or over which a surface coil could be placed. Voluntary informed consent was obtained from all patients.

Patient studies were performed by positioning the patient in a 1.5T Magnetom (Siemens Medical Systems, Cerritos, CA), with the tumor located over or beneath a 15 cm surface coil. The patient was positioned so that the tumor was centered in the magnet. An intravenous (IV) line was placed in the patient's arm, and following proper shimming and collection of a background ^{19}F-NMR spectrum, a bolus injection of 600 mg/m^2 5FU was given IV. A total of 256 free induction decays (FIDs) (either 512 or 1,024 data points/FID) were acquired at 5 kHz spectral width with the radiofrequency pulse optimized for 4 cm depth, at 60.012500 MHz. Apodization of the FIDs was done with a Gaussian filter matched to the T_2* of the data. Series of consecutive spectra, each collected over a 4.17-minute period, were obtained during the first hour before drug administration. The assignment of the peaks was based on their chemical shifts, using 5FU as an external reference sample and the ^{1}H water peak as the internal reference. Phantoms containing 5FU, FBAL, and fluorouridine were used to verify chemical shift positions, and the signal was maximized for a specific depth; that transmitter voltage was then used for a tumor at a similar depth. The spectra from the liver, where both 5FU and FBAL could be detected, further confirmed these assignments.

At the conclusion of 1 hour, some of the patients were repositioned to acquire further spectra from the liver, using the same acquisition conditions. Four to 6 consecutive 5-minute spectra were collected. The IV line was then removed and patients returned home.

The peak intensities of the 5FU peak were analyzed by a pharmacokinetic computer program, ANALAB,[11] assuming a single-compartment drug distribution model. The $T^1/_2$'s of the intratumoral 5FU pools were compared between patients, and with pharmacologic data from our prior studies.

RESULTS

A stack plot of the ^{19}F-NMR spectra collected over the tumor of patient no. 8, having a cervical carcinoma involving the presacral space, is shown in Fig 1. The only detectable ^{19}F-NMR signal has a chemical shift that corresponds to 5FU. No other detectable metabolites (eg, anabolites, catabolites) were observed, even when all spectra were summed. The $T^1/_2$ of 5FU was 41 ± 5 minutes in this tumor. At the end of the above acquisitions, the patient was repositioned with the surface coil over the liver, and four spectra were recorded starting at 1 hour after drug administration. Only signals due to FBAL could be detected in the liver of that patient, consistent with our previous observations of the metabolism of 5FU in that organ.[2]

The results of the ^{19}F spectra collected over the tumors, and the clinical results of all patients are

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minutes,[9] a value well in agreement and within the range of the T½'s of 5FU in plasma.[12] We had also determined that 5FU undergoes a slight chemical shift in the presence of plasma proteins (1 ppm), as well as at lower pH values. Because this patient presented with significant edema during this first study, we believe that this second, short-lived peak represented free 5FU in the plasma or in the interstitial fluid.

Despite the absence of NMR-detectable anabolites, antitumor partial responses were observed with 5FU and/or 5FU-leucovorin or FUdR-leucovorin, suggesting that the steady-state concentrations of 5-fluoro-deoxyuridine monophosphate (5FdUMP), 5-fluorouridine triphosphate (5FUTP), or 5-fluorodeoxyuridine triphosphate (which must have been produced in order for 5FU to exert its cytotoxic effect), are currently below the limit of in vivo NMR detectability. Because such products were not observable on either the single spectra or on the summed spectra, one can estimate that their total concentration was less than 10% of that of the 5FU concentration present in the tumor.

In order to determine if leucovorin was likely to have altered the accumulation or the T½ of 5FU, patient no. 9 had two NMRS studies performed (Fig 2). The first study was conducted while receiving 5FU alone, whereas in the second study, performed 4 weeks later, this patient also received leucovorin. In this single patient study, only 5FU was detected in both NMRS studies. Further, the T½ of 5FU was similar in both

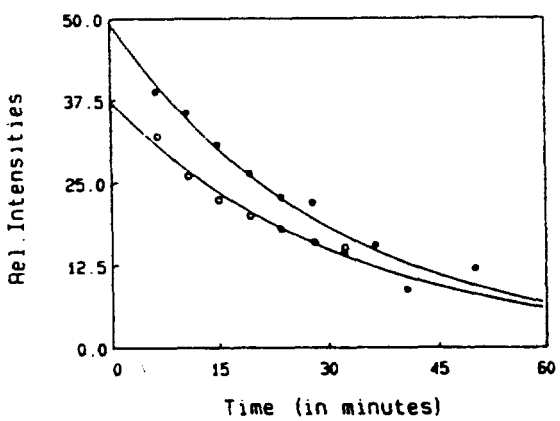

Fig 2. Clearance of 5FU based on ^{19}F-NMRS spectra of a colon carcinoma liver metastasis in patient no. 6. (●) Clearance alone of 5FU 600 mg/m². The T½ of 5FU is 20.9 ± 1.7 minutes. (○) Clearance following leucovorin 500 mg/m² plus 5FU 600 mg/m².

Table 2. Correlation Between Trapping of Free 5FU in the Tumor and Patients' Response to Chemotherapy

Therapy	Presence of Trapped 5FU Pool in Tumor	No. of Patients: Partial Response	No. of Patients: No Response
5FU (± modulator)	Yes	4*	—
	No	—	3
5FU + other chemotherapy	Yes	1	—
	No	1	1
Total	Yes	5	—
	No	1	4

*One patient received treatment with FUdR rather than 5FU, although trapping was detected following administration of 5FU. See text and Table 1.

studies: 20.9 ± 1.7 minutes after 5FU alone, and 22.6 ± 1.8 minutes after 5FU plus leucovorin. Although further studies are needed, these results, if confirmed, suggest that the effect of leucovorin on intracellular 5FU trapping is, if any, very small.

We correlated the antitumor responses seen in patients with the presence of 5FU trapping (Table 2). Ten of the 11 patients studied were assessable; the other patient died of an intercurrent illness prior to repeat tumor evaluation. In the seven patients receiving fluoropyrimidines alone, 5FU plus leucovorin, or FUdR plus leucovorin, four patients whose tumors trapped 5FU all had partial responses and three patients whose tumors failed to detect trapping both had progression of disease. Although the surface coil placed over the tumor in patient no. 11 demonstrated detectable levels of 5FU, its clearance T½ (15.5 minutes) is more in the range of blood clearance values (5 to 15 minutes) than of the tumor T½'s (20 to 78 minutes) measured in the present study (Fig 3).

In order to determine if 5FU trapping could correlate with response to FUdR as well as 5FU, patient no. 10 was evaluated before FUdR therapy. This patient had renal adenocarcinoma that had become resistant to megestrol acetate (Megace; Bristol-Myers Co, Evansville, IN) based on progressive hilar nodal and pulmonary metastases.[19] F-NMRS showed trapping of 5FU by tumor. The patient was treated with weekly FUdR and leucovorin and had an 80% partial response in her measurable hilar nodal metastasis after 3 months. This single result suggests that 5FU trapping might also predict for sensitivity to chemotherapy with FUdR, but compara-

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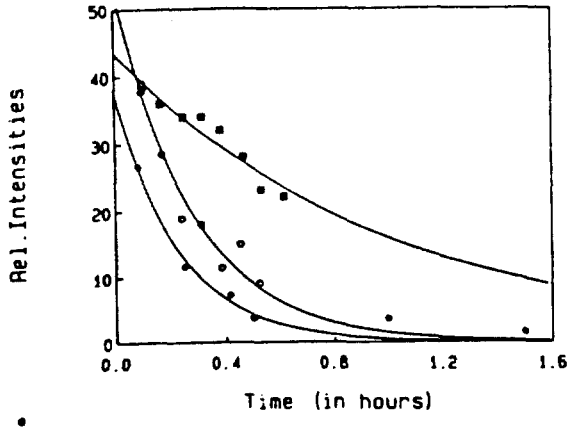

Fig 3. Tumor clearance of 5FU of patients no. 11 and 10, and blood clearance from another patient (high-performance liquid chromatography). (○) Clearance of 5FU 600 mg/m² from patient no. 11; T½ is 15.5 ± 2.9 minutes; (▩) Clearance of 5FU from patient no. 10; T½ is 41.3 ± 5.5 minutes. (●) Clearance of 5FU from blood of a patient (ER05); T½ is 9.6 ± 1.4 minutes.

tive studies of 5FU-NMRS and FUdR-NMRS are required.

The results in the three patients who received combination chemotherapy are more difficult to interpret, since no simple correlation can be made between 5FU trapping and cytotoxicity. Nevertheless, the single patient whose carcinoma of the breast trapped 5FU responded to cyclophosphamide, 5FU, and doxorubicin. One patient whose esophageal carcinoma did not accumulate 5FU showed progressive disease after 5FU and cisplatin.

The remaining patient is of considerable interest. Her breast cancer failed to detect 5FU trapping. However, she had a 95% reduction in tumor area after 5FU, cyclophosphamide, and menogaril. This suggests that the absence of detectable tumor trapping of 5FU does not infer resistance to all chemotherapeutic drugs, and that perhaps (as would be logical) 5FU trapping is a process that may correlate directly with 5FU responsiveness.

DISCUSSION

This study suggests that the ^{19}F-NMRS technique may provide in vivo information of significance to pharmacologic studies of 5FU in cancer patients. We believe that this method extends the current state of the art in cancer pharmacology by permitting continuous in vivo monitoring of 5FU and its metabolites. In contrast, prior studies have been constrained by the necessity, through tumor biopsy, of obtaining data at only one time point. Such data, in addition to failing to detect the intratumoral T½ of 5FU, were also subject to the effects of rapid enzymatic changes in the nature of the fluorinated products present in the tissue sampled.

The additional advantage of "real-time" data, available immediately at the time of 5FU administration, might have valuable implications for real-time clinical decision making during chemotherapy. For example, if further studies confirm preliminary findings that trapping correlates with antitumor response, patients whose tumors fail to exhibit detectable trapping of 5FU might be considered for other chemotherapy regimens not including 5FU.

The antitumor activity of 5FU is dependent on cellular uptake, biochemical conversion into nucleosides and nucleotides, on the covalent binding of 5FdUMP to thymidylate synthase, and on the incorporation of 5-fluorouridine into RNA.[13] The mechanism of cellular uptake had been assumed to occur by passive diffusion.[13] However, several recent studies have suggested the existence of a facilitated diffusion[14] or an active transport process.[15] Since the T½ of 5FU in blood following a bolus injection is only 5 to 15 minutes,[16] and that of 5FU in those tumors where it is trapped ranges from 20 to 78 minutes, one can conclude that the present results are consistent with either a facilitated diffusion or an active transport mechanism, but not with a simple passive diffusion process, which would require rapid equilibration of the intratumoral 5FU and that of the drug in the blood.

Although ^{19}F-NMRS has shown itself to be a meaningful tool in detecting, in vivo, 5FU and its metabolites in tumors and in the liver, one limitation inherent to this technique is its relative insensitivity when compared with biochemical analysis or positron emission tomography. Our inability to detect, noninvasively and in vivo, nucleoside and/or nucleotide pools in tumors that respond to 5FU illustrates this limitation. At present, we estimate that the lower limit of detection of 5FU is approximately .5 mmol/L in the sensitive volume of the surface coil.

The failure to demonstrate 5FU in normal and neoplastic tissues in four patients is consistent with the conclusion that nonneoplastic tissues do not trap 5FU. These results are also consistent with our prior studies of the biodistribution and

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imaging of ^{18}F-5FU in animals and in patients in which the only localization detected was in tumors and in the excretory organs (liver and kidneys).[17,18] Accumulation and retention of 5FU had been observed in some murine tumors (M5076 and sarcoma 180), but not in Lewis lung carcinoma, although these authors had not related this to the trapping phenomenon discussed in the present study. Our in vivo results confirm a recent ex vivo report showing long T½'s of 5FU and 5FdUMP in human tumors.[19]

We have demonstrated tumor trapping in six of the 11 patients studied to date. This is most likely due to cellular pathophysiologic alterations associated with the tumor process. However, it might also be modulated by altered tumor blood flow or perfusion, either due to pathologic neovascularization or prior radiotherapy. Previous ^{18}F studies[20] had shown that decreased tumor blood flow resulted in decreased ^{18}F accumulation in tumors, following administration of ^{18}F-5FU.

Our initial results are consistent with the hypothesis that 5FU trapping correlates well with 5FU antitumor response, but rigorous tests of that hypothesis depend on examining the association between trapping and response in a large number of patients with sound statistical evaluation. A multicenter trial in the Southwest Oncology Group is planned to conduct this clinical experiment.

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SOCIETY OF MAGNETIC RESONANCE IN MEDICINE, AUGUST 1990

ORGAN PHARMACOKINETIC MODELING IN A RAT TUMOR MODEL USING ^{19}F NMR SPECTROSCOPIC DATA

A. El-Tahtawy, D.Z. D'Argenio and W. Wolf, Radiopharmacy Program and Dept. of Biomedical Engineering, University of Southern California, Los Angeles, CA

In-vivo ^{19}F-NMR spectroscopy (NMRS) has allowed us to study noninvasively the kinetics of uptake, retention and metabolism of 5-fluorouracil (5FU) in a rat (Walker 256) tumor model. These data have been used to evaluate and to estimate the parameters of various pharmacokinetic subsystem models for 5FU. The *in-vivo* NMRS studies were performed using the 4.7T CSI GE spectrometer at the Huntington Research Institute. The data were then analyzed using the ADAPT-II program operating on a VAX system. 10^7 cells of the Walker 256 adenocarcinoma were implanted into the right thigh of 150 g Sprague Dawley rats, allowing the tumor to grow for 7-10 days to a size of 3-4 cc. After the rats were anesthetized with Rompan/Ketamine, they were placed on an imaging table and the tumor centered over a 1.5 cm surface coil tuned to ^{19}F, centered inside the magnet, and following shimming to a ^{1}H line-width of less than 50 Hz, a bolus dose of 150 mg/Kg of 5FU was administered by the IV route. To acquire the ^{19}F spectra 1680 FID's were collected at 20 KHz spectral width with a pulse [15 usec] optimized for maximum signal, at 188.360061 MHz. An external sample of 40 umoles of 1,2-difluorobenzene was used as an external reference standard for both quantitation and as chemical shift reference.

We had shown previously (1,2), that both 5FU and its anabolites - fluoronucleotides/sides (FNUC) are measurable *in-vivo* in this tumor model using NMRS. A key catabolite of 5FU, Fluorobetaalanine (FBAL), was also observed at 1-2 hours post drug administration. Because even a reduced, 11-compartment conceptual model of 5FU (1) is too complex to analyze given the sparse data that can be generated noninvasively, we have begun by testing various subsystem models that may represent the fate of 5FU in that tumor. A more detailed discussion in given in a concurrent abstract (3). As an example, the following rate constants were estimated when analyzing two such models using the data from rat A31390:

Rate Constant	Model 1B	Model 2A2
k01	.053	.0064
k02	.010	.0082
k21	.0072	.0071
k12	-	.0016

These parameters were then used in model 2A to estimate the amounts of 5FU, FNUC and HMWA present at various times.

Time	5FU	FNUC	HMWA
55 min	65%	27%	7.7%
125 min	34%	35%	31%

To begin validating these models, rats bearing the Walker 256 adenocarcinoma were sacrificed at 60 and 130 minutes post 5FU administration, their tumors excised, and the acid-soluble and the RNA fractions were collected and analyzed by ^{19}F NMRS in a Brucker NMR spectrometer operating at 240 MHz. The values obtained were:

	Time	5FU	FNUC	HMWA
Rat A	60 min	63%	35%	1.84%
Rat B	130 min	20%	66%	13%
Rat C	130 min	17%	62%	21%

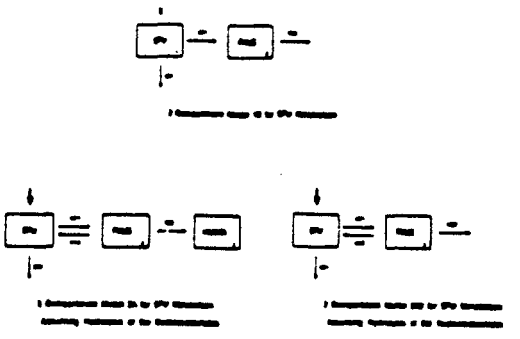

Figure 1: Selected compartmental subsystem models of 5FU metabolism in mammalian tumors.

These preliminary correlations between the values estimated in some rats and the tissue extracts from other rats appear encouraging, given that there is a significant degree of interanimal variability, and that this first model tested (2A) may not be the best representation of the metabolic events occuring in a given rat's tumor. Detailed estimations from all models, as well as of more complex models, is in progress, as well as direct correlations in the same animal.

1) Wolf, W., Servis, K.L. and El-Tahtawy, A., Abstracts of the 8th. SMRM, 411, 1989, New York.
2) Wolf, W., Presant, C.A., Servis K.L. et al., Proceedings of the National Academy of Sciences, 87, 492, 1990
3) Wolf, W., El-Tahtawy, A., D'Argenio, D.Z. and Presant, C.A. separate abstract submitted to this meeting.

1063162

Noninvasive ^{19}F NMR Spectroscopic Studies of Drug Targeting and Metabolism in Rabbit and Rat Tumor Models.

A. El-Tahtawy, K.L. Servis and W. Wolf, Radiopharmacy Program and Department of Chemistry, University of Southern California, Los Angeles, CA

In-vivo ^{19}F-NMR spectroscopy (NMRS) has been used to study the kinetics of uptake, retention and metabolism of 5-fluorouracil (5FU) in rabbit (VX2) and rat (Walker 256) tumors, as potential models for human tumors. All in-vivo studies were performed in a 4.7T CSI GE spectrometer, and ex-vivo in a 270 MHz IBM WP270SY. A comparison of IV vs. IA delivery of 5FU to the VX2 carcinoma in rabbits revealed that while the amount of initial uptake by the tumor was significantly enhanced (3 to 8 fold), no differences could be detected in the kinetics of free 5FU in that tumor, nor did this tumor model reveal any NMR-detectable metabolites. The $t_{1/2}$ of free 5FU in the VX2 tumors ranged from 1-2 hrs, whereas its $t_{1/2}$ in the blood of these same rabbits was measured at 6-8 min. Fluoronucleotide/side (FNUC) signals are however detected ex-vivo in these VX2 tumors following their excision, probably due to the hydrolysis of the NMR-invisible macromolecules, with no subsequent changes, even after weeks at 4°C. A trace of FBAL, again not observed in in-vivo, was also detectable.

A different pattern is observed in the Walker 256 carcinosarcoma. Not only is free 5FU seen in-vivo in that rat tumor, but also a FNUC signal 4.5 ppm upfield, and fluorobetaalanine (FBAL) 18.6 ppm downfield. On excision of this tumor, significant hydrolysis of FNUC to 5FU was observed, with no detectable change in the intensity of the FBAL signal. These ^{19}F-NMR spectra in rat tumors differ significantly from those observed in rat livers (1).

The significance of these results may be analyzed by using a compartmental model approach. The above results suggest that the activities of the enzymes involved in the tumoral metabolism of 5FU differ significantly between the VX2 and the Walker 256 tumors. While the rate and degree of tumor uptake of 5FU appears to be primarily determined by its local concentration in the blood on first-passage, the intra-tumoral $t_{1/2}$ of free 5FU would reflect the competing effects of the rate limiting anabolism of this drug to its nucleosides/tides (required for its subsequent incorporation into RNA and/or thymidilate synthetase), as opposed to its diffusion out of the tumor and/or its catabolism. The full mechanistic and therapeutic significance of the intratumoral $t_{1/2}$ of 5FU would thus depend on how this parameter could be correlated to these competing metabolic proceses.

It is of interest to note that the only ^{19}F-NMR signal that had been detected in (some) human tumors was that of free 5FU (2), following IV administration of this drug. Thus, it needs to be determined whether the rabbit (and its VX2 tumor) is a valid animal model for studying targeting and intra-tumoral metabolism of 5FU, given the apparent similarities between human and rabbit ^{19}F-NMR spectral patterns in both their livers and tumors.

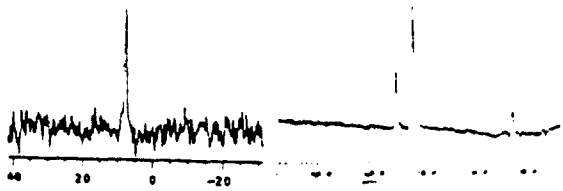

Fig.1: Left, in vivo ^{19}F NMR spectra in VX2 tumor of rabbit. Right: spectra following excision.

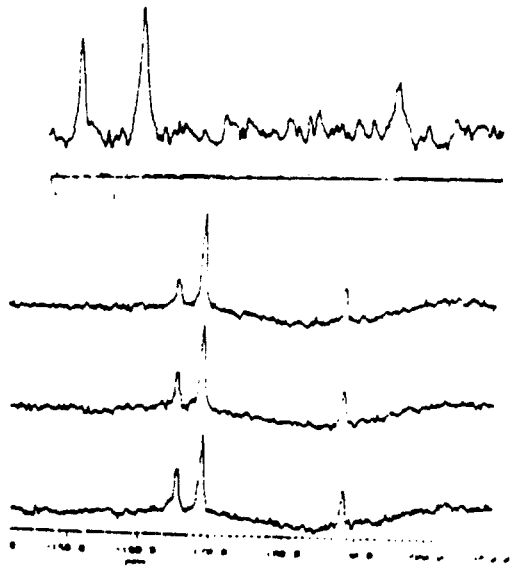

Figure 2: Top, in-vivo ^{19}F NMR spectra in Walker-256 tumor in rat. Bottom, spectra at 0, 3 and 5 hrs. excised.

1) W. Wolf, K.L. Servis and A. El-Tahtawy, Abstracts of the 6th. SMRM, 587, 1987, New York.

2) W. Wolf, C.A. Presant, M.J. Albright, et al., Abstracts of the 74th. RSNA, #209, 1988, Chicago.

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SOCIETY OF MAGNETIC RESONANCE IN MEDICINE

Sixth Annual Meeting • New York Hilton Hotel, New York, NY, U.S.A. • August 17-21, 1987

COMPARATIVE NONINVASIVE ORGAN MONITORING OF DRUG BIODISTRIBUTION AND METABOLISM IN HUMANS AND RODENTS: F-19 NMR SPECTROSOCOPIC STUDIES WITH 5-FLUOROURACIL.

W. Wolf, K.L. Servis and A. El-Tahtawy, Radiopharmacy Program and Department of Chemistry, University of Southern California, Los Angeles, CA

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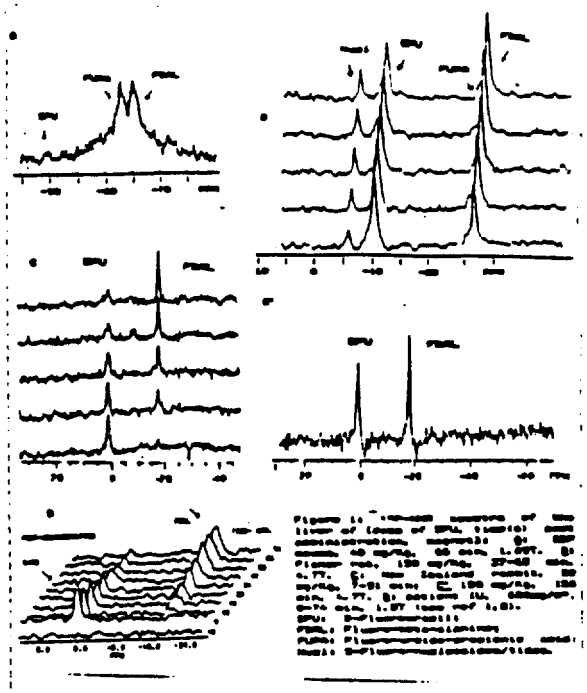
10631 64

In work we presented at SMRM (1) last year, we documented that it is possible to monitor the metabolism of drugs such as 5-fluorouracil (5FU) non-invasively in patients using F-19 NMR spectroscopic methods. Following administration of 600 mg/m^2 of 5FU, the standard chemotherapeutic dose, to patients being treated for sarcomas of the head and neck, we documented both that the only products that could be detected in their livers were 5FU and fluoro-beta-alanine (FBAL) and that the half-lives of the drug catabolites in their livers differ significantly from patient to patient (the t1/2 of 5FU ranged from 15 to over 30 min) (2). While drug anabolism to the active nucleosides/tides could not be observed in the liver of these patients, such products could be observed in tumors of some of the patients studied (3).

We now wish to report a series of comparative studies of 5FU metabolism in rabbits and rats, complementing prior NMRS studies in mice at 1.89 and 2.4T (4,5,6). While mice had been shown to metabolize 5FU very rapidly, and lead to both FBAL and 5-fluoroureido-propionic acid (FUPA) in their livers, no FUPA could be detected in rabbits in the present study (at 4.7T). The metabolism of 5FU in rats reveals that, in their livers, FBAL is again the major metabolite, and that small, but observable concentrations of FUPA can also be detected. What is interesting is that a more significant amount of the anabolite(s) can be observed in rat liver, not seen either in rabbit or human livers at the doses given, but seen previously in small amounts in the liver of mice (5) following administration of 130 mg/Kg of 5FU.

Bolus injection of 5FU to rabbits and to rats at doses ranging from 30 to 150 mg/Kg also appears to confirm the strong dose dependence of the drug's metabolism: there is rapid catabolism of 5FU to FBAL, as detected by the spectra of the livers of both rabbits and rats, when the drug is administered at low doses (similar to those used in human chemotherapeutic regimens). The metabolism of the drug appears to be significantly slowed down when the drug is administered at the higher doses to control rabbits, thereby confirming that in-vivo the detoxification enzyme system appears to be saturable. Figure 1 summarizes the spectra obtained to date in the liver of mice (A:40 mg/Kg, 1.89T), rats (B:150 mg/Kg), rabbits (C:50 mg/Kg; C':150 mg/g) (both at 4.7T) and humans (D: 600mg/m^2, 1.5T).



A major question such studies may be able to help answer is one of the key problems in pharmacology: how to define which experimental animal model will mimic best human drug metabolism. The present results suggest that non-invasive monitoring of drug metabolism can be carried out in a variety of animal species, and that the rabbit appears to be a suitable animal model that may allow us to help understand the mechanism and the reasons why individual patients may respond differently to 5FU chemotherapy.

1) Wolf, W., Silver, M., Weber, H., et al., Abst. Soc. Mag. Reson. Med. p. 653, 1986

2) Wolf, W., Albright, M., Silver, M. et al., Mag. Res. Imag. 5, 1987.

3) Wolf, W., Albright, M., Silver, M. et al., Radiology 161(P), 315, 1986

4) Stevens, A.N., Morris, P.G., Iles, R.A. et al, Brit.J. Cancer 50, 113, 1984

5) Hull, W.E., Port, R., Osswald, H. et al., Abst. Soc. Mag. Reson. Med. p. 594, 1986.

6) Wolf, W., Griffiths, J.R., Silver, M. et al. J. Nucl. Med. 27, 737, 1986.

Keywords:

1. 5-Fluorouracil
2. F-19 NMR Spectroscopy
3. Drug Metabolism
4. Rodents (rats, rabbits, mice)

Please type or print clearly the name and complete mailing address of the first author.

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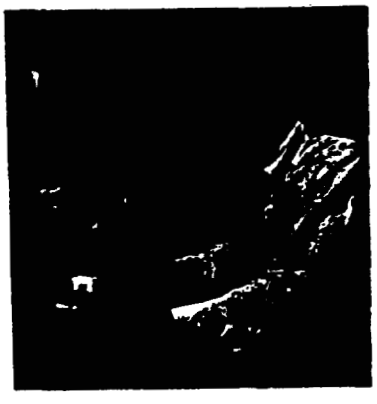
VIVOSPEC:
SYSTEM SPECIFICATIONS

APPENDIX C



RF UNIT

Frequency	5 - 300 MHz
Synthesizer	PTS-500 Computer Controlled
Frequency Step	0.1 Hz
Phase Control	0 - 360 Degrees
Phase, Discrete Steps	0, 90, 180, 270 Degrees
Phase, Variable Steps	0.1 Degree Resolution
Output Power	Computer Controlled, 12 Bit DAC
Power Control	0 - 100 %
Linearity of Power Control	±1%
RF Amplifier Power Output	1000W
Preamplifier	Broadband
Noise Figure	< 2dB

COMPUTER SYSTEM

Computer	DEC VAX Station 3200, 24 MByte RAM Standard, Up To 64 MByte Optional, 350 MByte Hard Drive, 44 MByte Removable Cartridge Drive, 600 MByte Optical Drive
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Digital Interface	Controls 2 Synthesizers: Frequency, Amplitude, And Phase For 2 RF Channels, 4 Gradient Channels, All With 12 Bit Resolution, 14 Bit A/D At 100 KHz, Audio Filter 51.2 KHz In 200 Hz Steps, Local Memory Buffer 64 KByte, External Gating Input

GRADIENTS

Gradient Coils	2 Gauss/cm Minimum
Rise Time (10 - 90 %)	1 msec Maximum (Uncompensated)
Power Supply	TECHRON Model 7570

HETERONUCLEAR DECOUPLER

Frequency	5 - 300 MHz
Synthesizer	PTS-500, Computer Controlled
Frequency Step	0.1 Hz
Phase Control	0 - 360 Degrees
Phase, Discrete Steps	0, 90, 180, 270 Degrees
Phase, Variable Steps	0.1 Degree Resolution
Output Power	Computer Controlled, 12 Bit DAC
Power Control	0 - 100 %
Linearity of Power Control	±1%
RF Amplifier Power Output	100W CW

STANDARD MAGNET CONFIGURATIONS

Field (T)/Bore (cm)	2/31	2/30.5	2.4/40	4.7/20	4.7/31	4.7/40	7/20
Bore With RT Shims And Gradients (cm)	26.5	26	33	15	26.5	31	15
Helium Evaporation (ml/hr)	50	50	50	50	55	50	50
Nitrogen Evaporation (ml/hr)	400	400	400	400	450	500	400
Half Length (mm)	350	275	570	396	460	735	575
5 Gauss Line — Radial From Center (m)	3.4	3.2	4.7	3.7	5.3	6.4	5.1
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1. The prospective primary participant certifies to the best of its knowledge and belief, that it and its principals:

a. Are not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency;

b. Have not within a three-year period preceding this proposal been convicted of or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State or local) transaction or contract under a public transaction; violation of Federal or State anti-trust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;

c. Are not presently indicted for or otherwise criminally or civilly charged by a governmental entity (Federal, State or local) with commission of any of the offenses enumerated in paragraph 1.b. of this certification; and

d. Have not within a three-year period preceding this application/proposal had one or more public transactions (Federal, State or local) terminated for cause or default.

2. Where the prospective primary participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

University of Southern California
Organization Name

Award Number

Cornelius J. Pings, Sr. Vice President for Academic Affairs and Provost
Name and Title of Authorized Representative

Cornelius J. Pings
Signature

12/19/90
Date

(See Reverse)

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PCDFA-083

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Instructions for Certification

1. By signing and submitting this proposal, the prospective primary participant is providing the certification set out below.

2. The inability of a person to provide the certification required below will not necessarily result in denial of participation in this covered transaction. The prospective participant shall submit an explanation of why it cannot provide the certification set out below. The certification or explanation will be considered in connection with the department or agency's determination whether to enter into this transaction. However, failure of the prospective primary participant to furnish a certification or an explanation shall disqualify such person from participation in this transaction.

3. The certification in this clause is a material representation of fact upon which reliance was placed when the department or agency determined to enter into this transaction. If it is later determined that the prospective primary participant knowingly rendered an erroneous certification, in addition to other remedies available to the Federal Government, the department or agency may terminate this transaction for cause or default.

4. The prospective primary participant shall provide immediate written notice to the department or agency to whom this proposal is submitted if at any time the prospective primary participant learns that its certification was erroneous when submitted or has been erroneous by reason of changed circumstances.

5. The terms "covered transaction," "debarred," "suspended," "ineligible," "lower tier covered transaction," "participant," "person," "primary covered transaction," "principal," "proposal," and "voluntarily excluded," as used in this clause, have the meanings set out in the Definitions and Coverage sections of the rules implementing Executive Order 12549. You may contact the department or agency to which this proposal is being submitted for assistance in obtaining a copy of those regulations.

6. The prospective primary participant agrees by submitting this proposal that, should the proposed covered transaction be entered into, it shall not knowingly enter into any lower tier covered transaction with a person who is debarred, suspended, declared ineligible, or voluntarily excluded from participation in this covered transaction, unless authorized by the department or agency entering into this transaction.

7. The prospective primary participant further agrees by submitting this proposal that it will include the clause titled "Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transaction," provided by the department or agency entering into this covered transaction, without modification, in all lower tier covered transactions and in all solicitations for lower tier covered transactions.

8. A participant in a covered transaction may rely upon a certification of a prospective participant in a lower tier covered transaction that it is not debarred, suspended, ineligible, or voluntarily excluded from the covered transaction, unless it knows that the certification is erroneous. A participant may decide the method and frequency by which it determines the eligibility of its principals. Each participant may, but is not required to, check the Nonprocurement List (Telephone No. [financial assistance administrator]).

9. Nothing contained in the foregoing shall be construed to require establishment of a system of records in order to render in good faith the certification required by this clause. The knowledge and information of a participant is not required to exceed that which is normally possessed by a prudent person in the ordinary course of business dealings.

10. Except for transactions authorized under paragraph 6 of these instructions, if a participant in a covered transaction knowingly enters into a lower tier covered transaction with a person who is suspended, debarred, ineligible, or voluntarily excluded from participation in this transaction, in addition to other remedies available to the Federal Government, the department or agency may terminate this transaction for cause or default.

1063167

CERTIFICATION REGARDING LOBBYING

This certification is required by Section 319 of Public Law 101-121 and the OMB Governmentwide Guidance for New Restrictions on Lobbying; Interim Final Guidance, as published in the *Federal Register* on December 20, 1989.

NOTE: Based on OMB guidance dated March 23, 1990, and Civilian Agency Council Letter No. 90-04 dated April 4, 1990, this certification applies only to the instant transaction and not to all transactions.

Certification for Contracts, Grants, Loans, and Cooperative Agreements

The undersigned certifies, to the best of his or her knowledge and belief, that:

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b. If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.

c. The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements) and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

University of Southern California
Organization Name

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Cornelius J. Pings, Sr. Vice President for Academic Affairs and Provost
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Signature

12/19/90
Date

1063168

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Cornelius J. Pings 12/19/96 Signature
Date

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c. Are not presently indicted for or otherwise criminally or civilly charged by a governmental entity (Federal, State or local) with commission of any of the offenses enumerated in paragraph 1.b. of this certification; and

d. Have not within a three-year period preceding this application/proposal had one or more public transactions (Federal, State or local) terminated for cause or default.

2. Where the prospective primary participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

UMDNJ - New Jersey Medical School

Organization Name | Award Number

Dr. Carol Welt, Assistant Dean for Research and Sponsored Programs

Name and Title of Authorized Representative

Carol Welt | 12-10-90

Signature | Date

(See Reverse)

1063170

Appendix

Faculty Curriculum Vitae:

Raghbir Athwal,	Ph.D.	I
Harvey Ozer,	M.D.	II
Jeffrey Wilusz,	Ph.D.	III
Michael Small,	Ph.D.	IV
Frank Desposito,	M.D.	V
Burton Fine,	M.D.	IV

1063171

Raghbir S. Athwal

Education

Institution and Location	Degree	Year Conferred	Field of Study
[redacted]	[redacted]	[redacted]	[redacted]

Experience:

1985-Present Associate Professor, Department of Microbiology and Molecular Genetics, New Jersey Medical School

1978-1985 Assistant Professor, Department of Microbiology and Molecular Genetics, New Jersey Medical School

1973-1978 Research Associate, Laboratory of Biochemistry, National Cancer Institute, NIH

1971-1973 Postdoctoral Fellow, Division of Human Genetics, Dept. of Pediatrics Howard, University Medical School.

Publications: (5 most recent references [57 total])

1. Sidhu, M.S. and R.S. Athwal DNA Fingerprinting of human chromosomes by Alu-PCR (Manuscript in Preparation for PNAS)

2. Gudi, R., S.S. Sandhu and R.S. Athwal (1990) Kinetochore identification in micronuclei in mouse bone marrow erythrocytes: An assay for the detection of Aneuploidy inducing agents. Mutation Res. 234:263-268.

3. Kaur, G.P. and R.S. Athwal (1989) Complementation of DNA repair defect in Xeroderma Pigmentosum cells: by transfer of human chromosome 9. Proc. Natl. Acad. Sci. U.S.A., 86:8872-8876.

4. Vig, B.K. and R.S. Athwal (1989) Sequence of centromere separation: Separation in a quasi-stable mouse/human somatic cell hybrid. Chromosoma, 98:167-173.

5. Gudi, R., S.S. Sandhu and R.S. Athwal (1989) A genetic method to quantitate induced chromosome breaks using a mouse/human monochromosomal hybrid cell line: Identification of potential clastogenic agents. Mutation Res. 225:149-156.

6. Sandhu, S.S., R. Gudi and R.S. Athwal (1988) A genetic assay for aneuploidy: Quantitation of chromosome loss using a mouse/human monochromosomal hybrid cell line. Mutation Research, 201:423-430.

7. Athwal, R.S., M. Smarsh, B.M. Searle and S.S. Deo (1985) Integration of a dominant selectable marker into human chromosomes and transfer of marked chromosomes to mouse cells by microcell fusion. Somatic Cell and Mol. Genet. 11:2:177-187.

8. Dhar, V., B.M. Searle and R.S. Athwal (1984) Transfer of Chinese hamster chromosome I to mouse cells and regional assignment of 7 Genes: A combination of gene transfer and microcell fusion. Somatic Cell and Mol. Genet. 10:547-559.

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Name	Birthdate
Harvey L. Ozer	[redacted]

Institution and Location	Degree	Year Conferred	Field of Study
[redacted]	[redacted]	[redacted]	[redacted]

Experience:

1960-63	Fellow, Department of Genetics, Stanford Medical School (Immunogenetics, Somatic Cell Genetics)
1964	Visiting Scientist, Inst. Tumor Biology, Karolinska Institute, Stockholm, Sweden (G. Klein)
1966-69	Research Associate and Post-doctoral Fellow, NIAID, NIH
1969-72	Senior Staff Fellow, Laboratory of Biochemistry, NCI, NIH, Independent Investigator
1975	Visiting Scientist in the laboratory of Dr. E. Winocour, Department of Virology, Weizmann Institute, Israel
1972-77	Senior Scientist, Worcester Foundation for Experimental Biology, Shrewsbury, MA
1977-88	Professor of Biological Sciences and Biochemistry, Department of Biological Sciences, Hunter College, CUNY, New York, NY
1983-88	Thomas Hunter Professor of Science and Mathematics, Hunter College/CUNY
1983-88	American Cancer Society Scholar and Visiting Professosr, Department of Molecular Biology and Genetics, Johns Hopkins School of Medicine, Baltimore, MD
1986-88	Program Coordinator, Center for Gene Structure and Function, Hunter College
1988-	Professor and Chairman, Dept. of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark, NJ

Publications: (5 most recent references [57 total])

1. Dermody, J.J., Lawlor, K., Du, H., Wojcik, B., Jha, K.K., Malkas, L., Hickey, R., Baril, E., and H.L. Ozer (1988) Polyoma virus DNA synthesis in vitro: Studies with CHO, 3T3, and their tsDNA mutants. Cancer Cells 6:95-100.
2. Klein, C.E., Ozer, H.L., Traganos, F., Atzpodien, J. Doettgen, H.F., and L.J. Old (1988) A transformation-associated 130kD cell surface glycoprotein is growth regulated in normal human cells. J. Exp. Med. 167:1684-1696.
3. Dermody, J.J., Lawlor K., Malkas, L., Du, H., Orlian, M., Wojcik, B., Jha, K.K. and H.L. Ozer (1988) Mammalian cell mutants ts in DNA synthesis. Current Communications in Molecular Biology: Cell Cycle Control in Eukaryotes, pp 100-105.
4. Radna, R.L., Caton, Y., Jha, K.K., Kaplan, P., Li, G., Traganos, F., and H.L. Ozer (1989) Growth of immortal SV40 tsA-transformed human fibroblasts is temperature-dependent. Mol. Cell. Biol. 9:3093-3096.
5. Wojcik, B.E., Dermody, J.J., Ozer, H.L., Mun, B., and Mathews, C.K. (1990) Temperature-sensitive DNA mutant of Chinese hamster ovary cells with a thermolabile ribonucleotide reductase activity. Mol. Cell. Biol. 10:5688-5699.

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Jeffrey Wilusz, Ph.D.

EDUCATION

[redacted]

EMPLOYMENT EXPERIENCE

8/1/89 to present	Assistant Professor of Microbiology & Mol. Genetics UMDNJ - New Jersey Medical School Newark, New Jersey 07103
9/85 to 8/89	Postdoctoral Fellow Princeton University, Princeton, NJ 08544 Advisor: Thomas Shenk, Ph.D.

SCHOLARSHIPS/HONORS/FELLOWSHIPS

John Zdanewicz Scholarship 1977-1978
Award in Animal Science 1980
Dean's List, Cook College 1977-1981
Graduated with Highest Honors 1981
James B. Duke Graduate Fellowship 1981-1982
Departmental Merit Scholarship 1981-1985
National Science Foundation Graduate Fellowship 1982-1985
American Cancer Society Postdoctoral Fellowship 1986-1988
Howard Hughes Medical Institute Research Associate 1989

SELECTED PUBLICATIONS

Wilusz, J., Kurilla, M.G. and Keene, J.D. (1983). Proc. Natl. Acad. Sci. USA **80**: 5827-5831.

Wilusz, J. and Keene, J.D. (1984). Virology **135**: 65-73.

Wilusz,J., Youngner, J.S. and Keene, J.D. (1984). Virology **140**: 249-256.

Kiley, M.P., Wilusz, J., McCormick, J.B. and Keene, J.D. (1986). Virology **149**: 251-254.

Wilusz, J. and Keene, J.D. (1986). J. Biol. Chem. **261**: 5467-5472.

Wilusz, J. and Shenk, T. (1988). Cell **52**: 221-228.

Wilusz, J., Feig, D.I. and Shenk, T. (1988). Mol. Cell. Biol. **8**: 4477-4483.

Wilusz, J., Pettine, S.M. and Shenk, T. (1989). Nucleic Acids Res. **17**: 3899-3908.

Wilusz, J., Shenk, T., Takagaki, Y. and Manley, J.L. (1990). Mol. Cell. Biol. **10**: 1244-1248.

Wilusz, J. and Shenk, T. (1990). Mol. Cell. Biol. **10**: 6397-6407.

Takagaki, Y., Manley, J.L., MacDonald, C.C., Wilusz, J. and Shenk, T. (1990). CstF is a multisubunit factor required for polyadenylation of mammalian pre-mRNAs. Genes Dev., in press.

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Michael Barry Small

Education:

Previous Employment and Experience:

9/74-8/76 Senior Honors Research/Research Assistant, Department of Biochemistry, State University of New York at Stony Brook (Dr. Masayori Inouye, advisor)

9/77-8/78 Research Assistant, Department of Biological Sciences, Hunter College of the City University of New York

9/78-2/84 Doctoral Student in Biochemistry, Department of Biological Sciences, Hunter College of the City University of New York (Dr. Harvey L. Ozer, thesis advisor)

Identification of cellular transforming genes in chemically-transformed mouse fibroblasts by DNA-mediated gene transfer; analysis of the transformed phenotype by somatic cell hybridization; transformation of diploid human fibroblasts.

3/84-12/87 Postdoctoral research fellow, G.W. Hooper Foundation, University of California, San Francisco (Dr. J. Michael Bishop, sponsor)

1/88-6/89 Assistant Research Virologist, G.W. Hooper Foundation, University of California, San Francisco

Anti-sense RNA inhibition of HSV thymidine kinase expression in mouse cells; analysis of the transforming activity of human N-myc and c-myc by DNA transfection and retroviral infection; construction and screening of a mouse cDNA library for differentially-expressed genes in myc-transformed rodent cells.

7/89-Present Assistant Professor, Department of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School

Publications:

Small, M.B., Hay, N., Schwab, M., and Bishop, J.M. (1987) Neoplastic Transformation by the Human Gene N-myc. Molecular and Cellular Biology 7: 1638-1645

Kothari, N. and Small, M.B. Dominant-negative reversion of c-myc transformation by a mutant c-myc allele. (manuscript in preparation)

Small, M.B., Hubbard-Smith, K., and Pardinas, J.R. Telomere Length in Normal and SV40-Transformed Human Fibroblasts (manuscript in preparation)

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NAME: Franklin Desposito, MD

CURRENT POSITION: Acting Chairman, Department of Pediatrics
UMDNJ-New Jersey Medical School
Newark, New Jersey
Professor of Pediatrics
Director, Division of Human Genetics

EDUCATION: [redacted]

POSTGRADUATE TRAINING:

1957-58 Rotating Intern, Long Island Jewish Medical Center, NY
1958-61 Pediatric Resideny/Chief Resident, Long Island Jewish
1961-63 Fellow, Pediatric Hematology, U of Wisconsin Medical School, Madison, WI

PROFESSIONAL APPOINTMENTS:

1971-89 Assoc. Prof. of Pediatrics, UMDNJ-NJ Medical School
1981- Director, Division of Human Genetics, UMDNJ-NJMS
1989- Professor of Pediatrics, UMDNJ-NJMS
1990- Acting Chairman, Dept. of Pediatrics, UMDNJ-NJMS
1990- Pediatrician-in-Chief, Children's Hospital of NJ, Newark

CERTIFICATION:

1964 Am. Bd. of Pediatrics. Recertified 1986
1974 Am. Bd. of Pediatric Hematology/Oncology. Recertified 1986
1982 Am. Bd. of Medical Genetics-Clinical Genetics

PUBLICATIONS:

1. Shih, LY, Suslak, L, Rosin, I, Searle, BM & **Desposito, F**: "Gene dosage studies supporting the localization of the structural gene for galactose-1-phosphate uridyl transferase (GALT) to Band p13 of chromosome 9." Am J Med Genet, 19:539-543, 1984.
2. Suslak, L, Price, D & **Desposito, F**: "Transmitting balanced translocation information among family members." Am J Med Genet, 20:227-232, 1985.
3. Lieber, C, Bordiuk, J, **Desposito, F**: "46,XY/46XX blood chimerism with severe central nervous system defect and multiple congenital malformation." Am J Med Genet, 23:833-836, 1986.
4. McCormack, MK, Stone, N, **Desposito, F**, Boehme, CD, Kazazian, HH, Jones, RT: "Normal production of the mutant hemoglobin in heterozygotes for hemoglobin J Paris and B-thalassemia." Hemoglobin, 10:427-432, 1986.
5. Suslak, L, **Desposito, F**: "Infant with cleft lip/cleft palate." Pediatr Rev, 9:331-334, 1988.
6. Shih, LY, Kurer, HM, Chen, TH, **Desposito, F**: "Strain differences galactokinase level and susceptability to the teratogenic efect of dietary galactose in mice." Teratology, 38:175-179, 1988.

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BURTON P. FINE

EDUCATION:

[redacted]

PREVIOUS EMPLOYMENT AND EXPERIENCE:

1968 - 1969	Albert Einstein Med. Center, New York, NY Instructor
1969 - 1973	University of Medicine & Dentistry of New Jersey Assistant Professor of Pediatrics
1973 - 1990	University of Medicine & Dentistry New Jersey Associate Professor of Pediatrics
1990 - Present	Professor of Pediatrics

PUBLICATIONS: (last 3 years)

Hatori, N., Fine, B.P., Nakamura, A., Cragoe, Jr., E. and Aviv, A.: Angiotensin II effect on cytosolic pH in cultured rat vascular smooth muscle cells. *J. Biol. Chem.* 1987, **262**:5073-5078.

Khalil, F., Fine, B., Kuriyama, S., Hatori, N., Nakamura, A., Nakamura, M. and Aviv, A.: Increased atrial natriuretic factor receptor density in cultured vascular smooth muscle cells of the spontaneously hypertensive rat. *Clin. Exp. Hyperten.* 1987, **9**:741-752.

Fine, B.P., Ty, A., Lestrange, N., Maher, E. and Levine, O.R.: Diuretic-induced growth failure in rats and its reversal by sodium repletion. *J. of Pharmacol. & Exp. Therap.* 1987, **242**:85-89.

Fine, B.P., Ty, A., Lestrange, N. and Levine, O.R.: Sodium deprivation growth failure in the rat: Alterations in tissue composition and fluid spaces. *J. Nutr.* 1987, **117**:1623-1628.

Kuriyama, S., Nakamura, A., Hopp, L., Fine, B.P., Kino, M., Cragoe, Jr., E. and Aviv, A.: Angiotensin II effect on 22Na+ transport in vascular smooth muscle cells. *J. Cardiovasc. Pharmacol.* 1988, **11**:139-146.

Fine, B.P., Ponzio, N.M., Denny, T.N., Maher, E., and Walters, T.R.: Restriction of tumor growth in mice by sodium deficient diet. *Cancer Res.* 1988, **46**:3445-3448.

Fine, B.P., Vetrano, T., Skurnick, J., Ty, A.: Blood pressure elevation in young dogs during low level lead poisoning. *Tox. App. Pharm.* 1988, **93**:388-393.

Rivkees, S.A. and Fine, B.P.: The reliability of calculated bicarbonate in clinical practice. *Clin. Ped.* 1988, **27**:240-242.

Nakamura, M., Nakamura, A., Fine, B.P., Aviv, A.: Blunted cyclic GMP response to ANF in vascular smooth muscle cells in the spontaneously hypertensive rat. *Am. J. Physiol.* 1988, **255** (Cell Physiol. 24): C573-C580.

Nakamura, M., Hatori, N., Nakamura A., Fine, B.P., and Aviv, A.: Cytosolic Ca2+ attenuates ANF-induced cyclic GMP response in vascular smooth muscle cells. *J. Hypertension.* 1989, **7**:51-56.

Nakamura, A., Gardner, J., Hatori, N., Nakamura, M., Fine, B.P., and Aviv, A.: Differences of cytosolic Ca2+ regulation in skin fibroblasts from blacks and whites. *J. Cell Physiol.* 1989, **138**:367-374.

Fine, B., Hansen, K.A., Salcedo, J.R., and Aviv, A.: Calcium-Activated Potassium Channels in Human Platelets. *Proc. Soc. Exp. Biol. Med.* 1989, **192**:109-113.

Hatori, N., Gardner, J., Tomonari, H., Fine, B.P., and Aviv, A.: Differences in Na^+/H^+ antiport activity in cultured fibroblasts from blacks and whites. *Hypertension* 1990, **15**:140-145.

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1910-0400

U.S. Department of Energy

Assurance of Compliance

Nondiscrimination in Federally Assisted Programs

University of Southern California (Hereinafter called the "Applicant") HEREBY AGREES to comply with Title VI of the Civil Rights Act of 1964 (Pub. L. 88-352), Section 16 of the Federal Energy Administration Act of 1974 (Pub. L. 93-275), Section 401 of the Energy Reorganization Act of 1974 (Pub. L. 93-438), Title IX of the Education Amendments of 1972, as amended, (Pub. L. 92-318, Pub. L. 93-568, and Pub. L. 94-482), Section 504 of the Rehabilitation Act of 1973 (Pub. L. 93-112), the Age Discrimination Act of 1975 (Pub. L. 94-135), Title VIII of the Civil Rights Act of 1968 (Pub. L. 90-284), the Department of Energy Organization Act of 1977 (Pub. L. 95-91), and the Energy Conservation and Production Act of 1976, as amended, (Pub. L. 94-385). In accordance with the above laws and regulations issued pursuant thereto, the Applicant agrees to assure that no person in the United States shall, on the ground of race, color, national origin, sex, age, or handicap, be excluded from participation in, be denied the benefits of, or be otherwise subjected to discrimination under any program or activity in which the Applicant receives Federal assistance from the Department of Energy.

Applicability and Period of Obligation

In the case of any service, financial aid, covered employment, equipment, property, or structure provided, leased, or improved with Federal assistance extended to the Applicant by the Department of Energy, this assurance obligates the Applicant for the period during which Federal assistance is extended. In the case of any transfer of such service, financial aid, equipment, property, or structure, this assurance obligates the transferee for the period during which Federal assistance is extended. If any personal property is so provided, this assurance obligates the Applicant for the period during which it retains ownership or possession of the property. In all other cases, this assurance obligates the Applicant for the period during which the Federal assistance is extended to the Applicant by the Department of Energy.

Employment Practices

Where a primary objective of the Federal assistance is to provide employment or where the Applicant's employment practices affect the delivery of services in programs or activities resulting from Federal assistance extended by the Department, the Applicant agrees not to discriminate on the ground of race, color, national origin, sex, age, or handicap, in its employment practices. Such employment practices may include, but are not limited to, recruitment, recruitment advertising, hiring, layoff or termination, promotion, demotion, transfer, rates of pay, training and participation in upward mobility programs; or other forms of compensation and use of facilities.

Subrecipient Assurance

The Applicant shall require any individual, organization, or other entity with whom it subcontracts, subgrants, or subleases for the purpose of providing any service, financial aid, equipment, property, or structure to comply with laws cited above. To this end, the subrecipient shall be required to sign a written assurance form, however, the obligation of both recipient and subrecipient to ensure compliance is not relieved by the collection or submission of written assurance forms.

Data Collection and Access to Records

The Applicant agrees to compile and maintain information pertaining to programs or activities developed as a result of the Applicant's receipt of Federal assistance from the Department of Energy. Such information shall include, but is not limited to, the following: (1) the manner in which services are or will be provided and related data necessary for determining whether

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any persons are or will be denied such services on the basis of prohibited discrimination; (2) the population eligible to be served by race, color, national origin, sex, age and handicap; (3) data regarding covered employment including use or planned use of bilingual public contact employees serving beneficiaries of the program where necessary to permit effective participation by beneficiaries unable to speak or understand English; (4) the location of existing or proposed facilities connected with the program and related information adequate for determining whether the location has or will have the effect of unnecesarily denying access to any person on the basis of prohibited discrimination; (5) the present or proposed membership by race, color, national origin, sex, age and handicap, in any planning or advisory body which is an integral part of the program; and (6) any additional written data determined by the Department of Energy to be relevant to its obligation to assure compliance by recipients with laws cited in the first paragraph of this assurance.

The Applicant agrees to submit requested data to the Department of Energy regarding programs and activities developed by the Applicant from the use of Federal assistance funds extended by the Department of Energy. Facilities of the Applicant (including the physical plants, buildings, or other structures) and all records, books, accounts, and other sources of information pertinent to the Applicant's compliance with the civil rights laws shall be made available for inspection during normal business hours on request of an officer or employee of the Department of Energy specifically authorized to make such inspections. Instructions in this regard will be provided by the Director, Office of Equal Opportunity, U.S. Department of Energy.

This assurance is given in consideration of and for the purpose of obtaining any and all Federal grants, loans, contracts (excluding procurement contracts), property, discounts or other Federal assistance extended after the date hereto, to the Applicants by the Department of Energy, including installment payments on account after such data of application for Federal assistance which are approved before such date. The Applicant recognizes and agrees that such Federal assistance will be extended in reliance upon the representations and agreements made in this assurance and the the United States shall have the right to seek judicial enforcement of this assurance. This assurance in binding on the Applicant, its successors, transferees, and assignees, as well as the person whose signature appears below and who is authorized to sign this assurance on behalf of the Applicant.

12/19/90
(Date)

University of Southern California
Department of Contracts & Grants
Health Sciences Campus Office
(Name of Applicant)

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(Applicant's Telephone Number)

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