S.M. Larson

DOE/ER/60407-T3

DE93 007142

CURRENT REPORT 2/87-9/89

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

~~Appendix 1B~~

IMPROVING CANCER TREATMENT WITH CYCLOTRON PRODUCED RADIONUCLIDES

PROGRESS REPORT

2/87 - 9/89

TABLE OF CONTENTS

IA. IN VIVO MEASUREMENT OF AMINO ACID TRANSPORT AND PROTEIN SYNTHESIS IN CANCER

IA1. Objectives

The **long-term objectives** of this project are (a) to develop non-invasive techniques for measuring amino acid transport and protein synthesis *in vivo* using ^{11}C- and ^{13}N-labeled amino acids; and (b) to use such methods to study the *in vivo* amino acid metabolism of tumors and normal tissues of the cancer-bearing host.

Specific aims during the period of the grant:

(a). Develop and validate a direct-injection, multiple indicator method for *in vivo* measurement of transport in skeletal muscle and peripheral tumors.
(b) Develop and validate a similar method for liver and hepatic tumors.
(c). Extend the method to measure protein synthesis in skeletal muscle.

IA2. Scope of Investigation

IA2a. *Overview*

The experimental approach used in this investigation is based on the **Multiple Indicator Dilution (MID) method** (1). In this method a mixture containing a permeant tracer of interest (e.g., an amino acid) and one or more reference indicators (e.g., intravascular and extracellular tracers) is injected as a bolus into the direct, arterial blood supply of the tissue region of interest (ROI). Data are collected by sampling the venous outflow from the ROI or recording the amount of tracer remaining in the ROI vs. time ("residue detection"). The data are fitted with a mathematical model which includes parameters corresponding to blood flow, capillary and cellular transport, and, in some cases, binding or metabolic interactions.

IA2b. *Instrumentation for Multitracer Transport Studies*

Two systems for simultaneous recording of multiple time-activity curves (TACs) at different photon energies were developed. The first is a high-energy gamma (HEG), rectilinear scanner used as a stationary, **dual-detector, scintillation probe.** We outfitted the HEG with collimators of various resolution and connected it through a six-channel interface to a personal computer. Software was written for real time data acquisition and display.

We also developed a **dual-detector, gamma camera system** capable of separate, simultaneous, dynamic imaging of three different photon energies per detector. This we refer to as the MEDIS (for

"multiple energy dynamic imaging system"). Its features are described in Section IE, below. The system is equipped both with 511 keV and Tc-99m collimators.

IA2c. *Specific Aim 1: Transport Studies in Peripheral Tissues*

Methodology

The **technical problems** which had to be **overcome in developing the transport measurement technique** are summarized in **Table 1.** Because extraction of circulating molecules from blood into tissue is affected by blood flow as well as tissue permeability, it is **necessary to measure regional flow.** This is done with a plasma indicator (^{99m}Tc human serum albumin (HSA)), using the **height/area method** (2) in conjunction with a determination of the **regional partition coefficient** (distribution volume/tissue mass) for HSA. The partition coefficient is obtained by measuring (a) regional plasma volume from steady-state images and blood samples, and (b) regional tissue mass from a transmission image (3).

A number of items listed in Table 1 require explanation. Inter-tracer crosstalk refers to the fact that higher-energy radiolabels contribute counts to lower-energy data channels (e.g., an amino acid labeled with ^{13}N contributes counts to the channel used to record ^{99m}Tc HSA). Correction for **tracer recirculation** simplifies data interpretation by reducing the problem to a first-pass measurement and eliminating interference from recirulating metabolites. Knowledge of the **tracer input function**, or at least the total first-pass input, is necessary for the blood flow and transport calculations (this is the "height" in the height/area method). The first-pass input function can be determined from the time-activity curve (TAC) of a tissue region of interest (ROI), provided the input duration is less than the minimum transit time of tracer through that ROI. This condition is adequately met in limb studies by using the TAC of the entire gamma camera image. "**Vascular transit noise**" refers to interference from radiolabel in arterial or venous blood which crosses the tissue ROI without perfusing it. This problem can be avoided in gamma camera studies by confining the first-pass input to the tissue or organ of interest and including that entire region within the field of view. **Transport parameters** are computed by fitting the TACs with a kinetic model. We previously used a graphical, exponential-fitting technique developed by Sejrsen, *et al.* (4,5). However, this approach is somewhat arbitrary (1). We have recently implemented a software package for a multicapillary, multitracer, axially-distributed model developed by J.B. Bassingthwaighte (6). The multicapillary feature permits the model to account for **blood flow heterogeneity** (1).

TABLE 1

TECHNICAL PROBLEMS IN TRANSPORT AND BLOOD FLOW MEASUREMENTS

Problem	Solution
1. **Bolus arterial injection:**	
a) duration	a) High activity concentrations; special, preloaded, injection line.
b) flow perturbation	b) Shown negligible using laser doppler flowmetry.
2. **Inter-tracer crosstalk**	Corrected by subtraction of normalized TACs* corresponding to interfering channels; normalization factors determined in limb phantom.
3. **Tracer recirculation**	Corrected by subtracting normalized, contralateral TAC; normalization determined in limb phantom.
4. **Input function determination**	Gamma camera; whole-image TAC measures input function.
5. **Vascular transit noise** (VTN)	Gamma camera; TAC for entire tissue region receiving first-pass input is immune to VTN.
6. **Blood flow measurements:**	
a) plasma volume	a) Quantitate absolute activity of plasma indicator in steady state (3)
b) tissue mass	b) Transmission measurement (3).
7. **Transport measurements:**	
a) tracer reflux, tissue to blood	a) Fit data with axially-distributed model including intravascular, interstitial and intracellular spaces (6).
b) separation of capillary from cell transport	b) " " " " " "
8. **Stationarity:**	Closely maintain physiologic parameters in anaesthetized animals.
a) blood flow	a) Shown stable by laser-doppler flowmetry
b) metabolism	b) Hindlimb amino acid fluxes shown stable
9. **Heterogeneity**	
a) tissue	a) Using labeled microspheres, measured tracer distribution among skin, fat, bone and muscle of hindlimb arising from arterial injections. Muscle contributes 90% of the signal.
b) blood flow	b) Fit data with multi-capillary model (6).

*Time-activity curves.

To validate the methodology, we developed an isolated, perfused, hindlimb model in the rabbit and used it to demonstrate themethod's sensitivity to capillary and cellular transport (5). Using the new gamma camera system, we recently performed studies in rabbits to compare (a) blood flow measurements by our method with the standard, radiomicrosphere method (injection into the left atrium (7)), and (b) transport measurements by our method with the standard, venous-sampling, MID method (1). Data from these experiments are currently being analyzed.

Amino Acid Transport in Skeletal Muscle

In collaboration with Dr. M.F. Brennan, Chief of Surgery, Memorial Hospital and Director of the MSKCC Surgical Metabolism Laboratory, **we have used the new methodology to investigate the hypothesis that amino acid transport has a role in the regulation of peripheral metabolism in cancer cachexia and simple starvation.** We have studied the effect of prolonged starvation and acute refeeding on amino transport in skeletal muscle of the dog, using ^{11}C *a*-aminoisobutyric acid (AIB), an analogue substrate for A-type amino acid transport (7,8). We have also examined the effect of cancer-induced cachexia on the transport of ^{13}N L-glutamine (Gln) in skeletal muscle of tumor-bearing rabbits cf. pair-fed controls (10-12). Others have recently identified an active transport system for Gln in muscle (26), and suggested that impaired glutamine transport contributes to the large decrease of muscle intracellular glutamine observed in cachexia (27).

IA2d. *Specific Aim 2: Transport Studies in Tumor-Bearing Liver*

Previously-obtained results on blood flow and amino acid transport in human colorectal hepatic metastases (13) have now been published in part (14).

We have also made progress in developing a **recirculation correction method for HA-injection studies.** The method is based on the realization that the liver (plus intrahepatic tumors) acts as a source of systemic venous input of radiolabel following tracer injection into the HA. The circulatory time delay between hepatic efflux and return of label to the liver permits this hepatic venous input to be computed from the slope of the whole-liver TAC. The other necessary pieces of information are the systemic IV bolus response functions (i.e., TACs resulting from bolus systemic IV injection of the tracer) for liver and tumors. These are measured by supplementing the HA injection study with a second, systemic IV injection, imaging study.

IA2e. *Specific Aim 3: Protein Synthesis in Skeletal Muscle*

We have performed studies to determine whether our arterial-injection, first-pass technique can be extended to measure the rate of incorporation of circulating amino acids into protein of

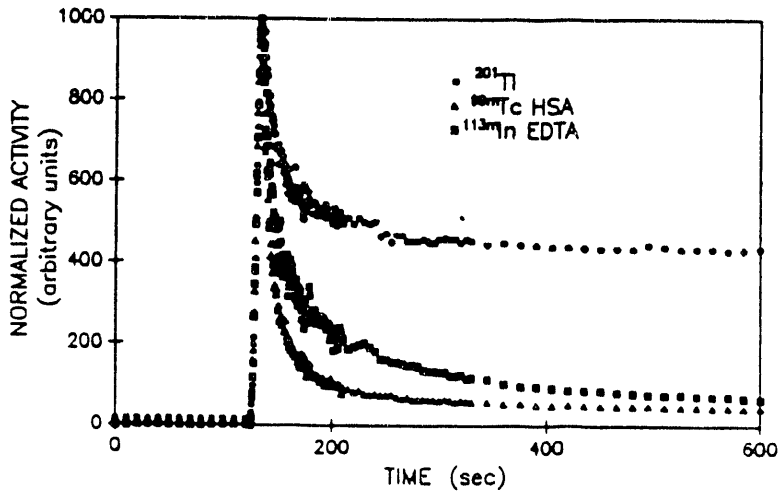
skeletal muscle. Because it is thought not to be metabolized in muscle (15), we chose to investigate phenylalanine (Phe) as a potential tracer of amino acid incorporation into protein in that tissue. L-Phenylalanine, labeled either with ^{13}N, ^{3}H or ^{14}C, was injected into the femoral artery of our isolated, perfused, rabbit hindlimb preparation, and muscle tissue was biopsied 1 hr. later. Activity bound to protein (acid-insoluble fraction) was then determined as a percentage of whole-tissue activity.

IA3. Results

IA3a. *Multitracer Dynamic Imaging*

The characteristics of the MEDIS are described in Section IE and in an enclosed abstract (Bading '89). Figure 1 illustrates the system's ability to perform multiple indicator transport studies. The figure shows time-activity curves (TACs) measured with the system in the thigh of a rabbit following coinjection of ^{201}Tl (permeant tracer), ^{99m}Tc HSA (plasma indicator) and ^{113m}In EDTA (extracellular indicator) into the femoral artery. The data have been corrected for inter-tracer crosstalk and recirculated radiolabel. The ^{99m}Tc retained after 400 sec reflects a small percentage of extravasation or binding to vascular endothelia (16); this component is fitted and subtracted prior to computing blood flow (5).

FIGURE 1.
MULTITRACER STUDY WITH MEDIS



IA3b. *Specific Aim 1: Transport Studies in Peripheral Tissues*

Methodology

Validation of the method for measuring absolute ^{99m}Tc activity densities in thigh tissue (HSA partition coefficient measurements), as well as studies verifying the interpretation of ^{13}N amino acid TACs in rabbit thigh are described in two enclosed publications

(3,5). We believe that all of the technical problems associated with the method have been adequately resolved (ref. Table 1). Data are reasonably consistent with published values, where available (Table 2). As noted previously, studies directly comparing our method with standard methodologies are in progress.

TABLE 2.

Comparison of Blood Flow and Transport Measurements in Skeletal Muscle with Published Values

Quantity	Published reference	Experimental model and tracer reference's	Experimental model and tracer our's	Measured values reference's	Measured values ours
Blood flow (ml/min/100g)	(21-22)	dog, μspheres	dog, ^{99m}Tc HSA	4-30	5-39
	(7)	rabbit, "	rabbit, "	3-19	5-23
Extracellular fluid volume (ml/100g)	(23)	dog, $^{35}SO_4$	dog, ^{113m}In EDTA	16±1	15±2
Capillary PS* (ml/min/100g)	(24)	human, fructose	rabbit ^{13}N L-glutamine	5.9**	5.4**
Cellular PS (ml/min/100g)	(25)	^{3}H L-glutamine perfused rat hindlimb	^{13}N L-glutamine perfused rabbit hindlimb	<13±3	3.5±0.6
	(26)	^{14}C AIB perfused rat	^{11}C AIB intact dog	<1.2±0.8	0.33±0.06

* PS = permeability x surface area (transport parameter)
** Values correspond to plasma flow range 7-13 ml/min/100g.

Amino Acid Transport Studies in Skeletal Muscle

Our findings in starvation and cancer cachexia *in vivo* are as follows:

1. In the intact dog, **A-type transport is not a major mechanism of amino acid entry into skeletal muscle in either the normal or chronically-starved states** (9). This unexpected result is consistent with recent findings elsewhere (17).

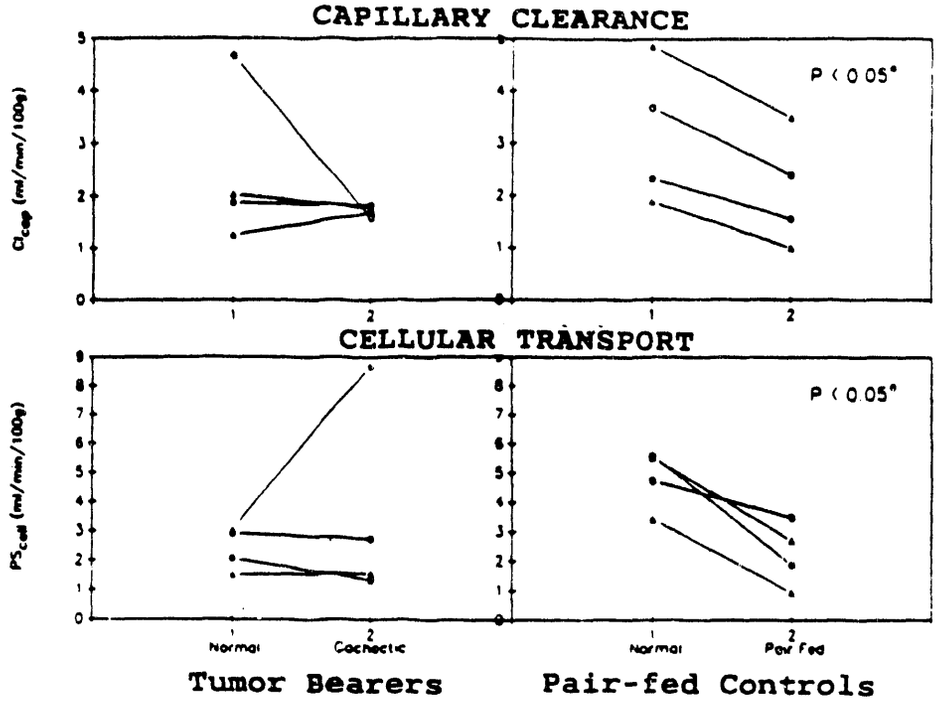
2. Chronic nutrient deprivation reduced peripheral blood flow and amino acid clearance into skeletal muscle of both the dog (8,9) and rabbit (12). This did not happen in cancer-cachectic rabbits (10-12) (Figure 2). A similar pattern of change/non-change has also been observed in fasted and cancer-cachectic humans (18,19). **These observations suggest that reduced peripheral flow and nutrient clearance are part of the normal adaptation to starvation; their non-occurence in cancer cachexia may reflect an aspect of maladaption not previously recognized.**

3. Cellular transport of L-glutamine (Gln) was reduced in nutrient-deprived rabbits, but not consistently altered in cancer-cachectic rabbits (10-12) (Figure 2). **Others have hypothesized**

that impairment of Gln transport contributes to muscle wasting in cachexia (27). Our findings do not support this idea, but rather suggest that reduction of Gln transport occurs as part of the normal, energy-conserving response to starvation.

FIGURE 2.

GLUTAMINE CAPILLARY CLEARANCE AND CELLULAR TRANSPORT IN RABBITS



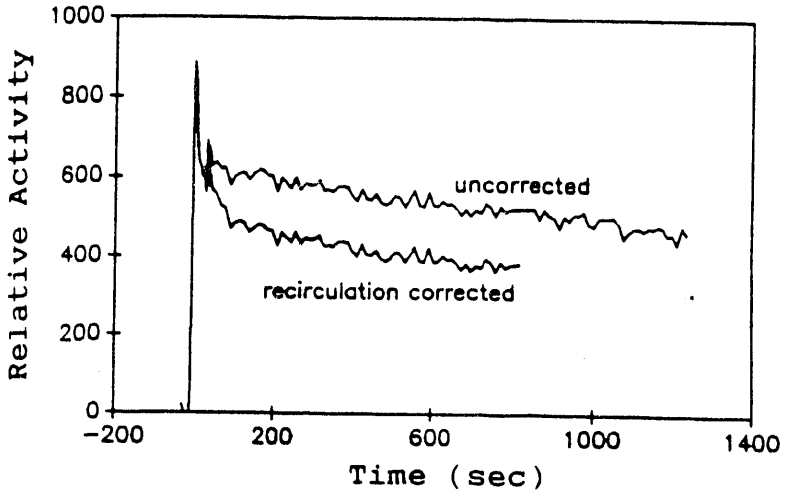
*Paired t-test.

IA3c. *Specific Aim 2: Transport Studies in Tumor-Bearing Liver*

We showed that human colorectal hepatic metastases receive relatively little nutritive blood flow from the portal vein cf. the hepatic artery, and that these tumors efficiently extract amino acids and ammonia from the blood (14).

Preliminary results with the recirculation correction for HA-injection studies are illustrated with data from one of our patient studies in Figure 3. The patient, who had catheters into both her HA and superior vena cava (SVC), was first given a bolus injection of 10 mCi of ^{13}N L-glutamate into the SVC, and dynamic gamma camera images data were acquired for 20 min. The study was then repeated with 4 mCi of ^{13}N glutamate injected into the HA. The heart, which was included in the camera field of view, served as a reference from which to adjust for differences in vascular transit times between the IV and HA studies. The figure shows uncorrected and corrected TACs from a tumor in the right hepatic lobe.

FIGURE 3.
Recirculation Correction for Hepatic Arterial Injection Studies
Example: Time-Activity Curves for ^{13}N L-Glutamate in a Human Hepatic Tumor



IA3d. Specific Aim 3: Protein Synthesis in Skeletal Muscle

A necessary criterion for measuring amino acid incorporation into protein by our method is that, by the end of the experiment, most (≥80%) of the activity remaining in tissue be bound to protein (20). In our first two experiments with phenylalanine (Phe), we used ^{13}N Phe and found that 60% and 80% of the radiolabel remaining in muscle 1 hr. after femoral artery injection was bound. We followed up on this promising result by repeating the measurement several times with ^{3}H-labeled L-Phe. (^{13}N Phe was not available because of the cyclotron renovation.) This time only 30-40% of the remaining label was bound, a result which was subsequently verified with ^{14}C L-Phe.

IA4. Conclusions

We have succeeded in developing a rigorous, radiotracer imaging, method for measuring capillary and cellular transport *in vivo* **in skeletal muscle.** The method can be used repeatedly in the same laboratory animal and is also suitable for use in humans. Using it, **we have obtained several new pieces of information about peripheral blood flow and amino acid transport in starvation and cancer cachexia.**

We have demonstrated the feasibility of measuring molecular transport in human hepatic tumors by dynamic radionuclide imaging. Our recirculation correction technique for HA-injection studies appears to give reasonable results, but must be verifed. We plan

to do this by computer simulation and by direct comparison with an alternative method of recirculation measurement in an animal model. **We have not succeeded in identifying an amino acid with kinetics suitable for measuring protein synthesis in skeletal muscle by ourarterial bolus, first-pass technique.** Phenylalanine was studied, but its free-pool turnover rate appears to be too slow to permit accurate determination of incorporation into protein.

IA5. Participating Personnel

Name	Title/Affiliation/Role	Dates	% Effort
J.R. Bading, Ph.D.	Asst. Lab. Member, BL* Principle Investigator	'87-'89	80
A.S. Gelbard, Ph.D.	Assoc. Lab. Member, BL Radiochemist	'88-'89	20
J.D. Fissekis, Ph.D.	Research Assoc., BL Radiochemist	'87-'88	20
G.R. DiResta, Ph.D.	Research Assoc., BL Biomed Engineer	'87-'89	10
M.F. Brennan, M.D.	Member, DS** Co-investigator	'87-'89	5
M.T. Corbally, M.D.	Research Fellow, DS	'87	100
K.C. Conlon, M.D.	Research Fellow, DS	'87-'88	100
E.W. McDermott, M.D.	Research Fellow, DS	'88-'89	100

*Biophysics Laboratory, Sloan-Kettering Institute
**Department of Surgery, Memorial Hospital

IA6. Publications

Journal Articles

Ridge JA, Bading JR, Gelbard AS, Benua RS, Daly JM. Perfusion of colorectal heaptic metastases: Relative distributions of flow from the hepatic artery and portal vein. Cancer 1987;59:1547-1553.

Bading JR, DiResta GR, Corbally MT, Conlon KC. Fiberglass limb phantoms: Fabrication and use for quantitative scintigraphy. Med Phys 1988;15:215-20.

Conlon KC, Bading JR, DiResta GR, Corbally MT, Gelbard AS, Brennan MF. Validation of transport measurements in skeletal muscle with N-13 amino acids using a rabbit isolated hindlimb model. Life Sci 1989;44:847-59.

Abstracts

Bading JR, Corbally MT, Fissekis JD, DiResta GR, Brennan MF. Effect of starvation on C-11 α-aminoisobutyric acid (AIB) transport in skeletal muscle. J Nucl Med 1987;28:650.

Bading JR, Conlon KC, Gelbard AS, Corbally MT, Banket WJ,

muscle transport in starvation. The Physiologist 1988;Aug:A133. (abstract)

Conlon KC, Bading JR, Gelbard AS, Corbally MT, DiResta GR, Vydelingum NA, Brennan MF. *In vivo* skeletal muscle glutamine transport in cancer. The Physiologist, 1988;Aug:A133. (abstract)

Conlon KC, Bading JR, McDermott EW, Gelbard AS, Brennan MF. Calillary clearance and cellular transport of glutamine decline in starvation but not in cancer cachexia. Proc Am Assoc Cancer Res 1989;30:17. (abstract)

Bading JR, Wilson HH, Nupnau WA, Gregg GM. A multiple-energy gamma camera system for dynamic transport studies. J Nucl Med 1989;30:852. (abstract)

Master's Thesis

Corbally MT. **Development of a Novel Technique to Study Amino Acid Transport and Regional Blood Flow In-Vivo in Skeletal Muscle and It's Application During Brief and Prolonged Starvation in the Dog.** (M. Ch. Thesis, National University of Ireland, University College, Galway, Ireland, 1988).

IA7. Literature Cited

1. Bassingthwaighte JB, Goresky CA. Modeling in the analysis of solute and water exchange in the microvasculature. In: Handbook of Physiology, Vol IV, ed. Renkin EM, Michel CC (Am Physiol Soc, Bethesda, Md, 1984) pp.549-626.
2. Lassen NA, Perl W. Tracer Kinetic Methods in Medical Physiology. (Raven Press, New York, 1979) pp.77-78.
3. Bading JR, DiResta GR, Corbally MT, Conlon KC. Fiberglass limb phantoms: Fabrication and use for quantitative scintigraphy. Med Phys 1988;15:215-20.
4. Sejrsen P. Capillary permeability measured by bolus injection, residue and venous detection. Acta physiol scand 1979;105:73-92.
5. Conlon KC, Bading JR, DiResta GR, Corbally MT, Gelbard AS, Brennan MF. Validation of transport measurements in skeletal muscle with N-13 amino acids using a rabbit isolated hindlimb model. Life Sci 1989;44:847-59.
6. Bassingthwaighte JB. A concurrent flow model for extraction during transcapillary passage. Circ Res 1974;35:483-503.
7. Neutze JM, Wyler F, Rudolph AM. Use of radioactive microspheres to assess distribution of cardiac output in rabbits. Am J Physiol 1968;215:486-95.
8. Bading JR, Corbally MT, Fissekis JD, DiResta GR, Brennan MF. Effect of starvation on C-11 α-aminoisobutyric acid (AIB) transport in skeletal muscle. J Nucl Med 1987;28:650.

9. Corbally MT, Bading JR, Fissekis J, DiResta GR, Conlon KC, Brennan MF. *In vivo* C-11 α-aminoisobutyric acid (AIB) skeletal muscle transport in starvation. The Physiologist 1988;Aug:A133. 1988. (abstract)
10. Bading JR, Conlon KC, Gelbard AS, Corbally MT, Banket WJ, Brennan MF. Effect of cancer cachexia on N-13 L-glutamine transport in rabbit skeletal muscle. J Nucl Med 1988;29:902.
11. Conlon KC, Bading JR, Gelbard AS, Corbally MT, DiResta GR, Vydelingum NA, Brennan MF. *In vivo* skeletal muscle glutamine transport in cancer. The Physiologist, 1988;Aug:A133. (abstract)
12. Conlon KC, Bading JR, McDermott EW, Gelbard AS, Brennan MF. Calillary clearance and cellular transport of glutamine decline in starvation but not in cancer cachexia. Proc Am Assoc Cancer Res 1989;30:17. (abstract)
13. Bading JR, Daly JM, Gelbard AS, Benua RS. A kinetic imaging study of human metastases by hepatic artery injection of N-13 L-glutamate. J Nucl Med 1985;26:109. (abstract)
14. Ridge JA, Bading JR, Gelbard AS, Benua RS, Daly JM. Perfusion of colorectal heaptic metastases: Relative distributions of flow from the hepatic artery and portal vein. Cancer 1987;59:1547-1553.
15. Barrett EJ, Revkin JH, Young LH, Zaret BL, Jacob R, Gelfand RA. An isotopic method for measurement of muscle protein synthesis and degradation *in vivo*. Biochem J 1987;245:223-8.
16. Sejrsen P, Paaske WP, Henriksen O. Capillary permeability of ^{131}I-albumin in skeletal muscle. Microvasc Res 1985;29:265-81.
17. Hundal HS. Which system transports alanine in rat muscle? J Physiol 1986;380:68P. (abstract)
18. Albert JD, Legaspi A, Horowitz GD, *et al.* Peripheral tissue metabolism in man with varied disease states and limited weight loss. J Surg Res 1986;40:374-81.
19. Lundholm K, Bennegard K, Eden E, Svaninger G, Emery PW, Rennie MJ. Efflux of 3-methylhistidine from the leg in cancer patients who experience weight loss. Cancer Res 1982;42:4807-11.
20. Phelps ME, Barrio JR, Huang SC, *et al.* Criteria for the tracer kinetic measurement of cerebral protein synthesis in humans with positron emission tomography. Ann Neurol 1984;15(Suppl):S192-S202.
21. Cronenwett JL, Zelenock GB, Whitehouse WM, *et al.* The effect of sympathetic innervation on canine musle and skin blood flow. Arch Surg 1983;118:420-4.
22. Gelman S, Fowler KC, Smith LR. Regional blood flow during isofluorane and halothane anesthesia. Anesth Analg 1984;63:557-65.
23. Barratt TM, Walser M. Extracellular volume in skeletal muscle of the rat and dog: A comparison of radiosulphate and radiobromide spaces. Clin Sci 1968;35:525-36.
24. Trap-Jensen J, Lassen NA. Capillary permeability for smaller hydrophilic tracers in exercising skeletal muscle in normal man and in patients with long-term diabetes mellitus. In: Capillary Permeability, ed. Crone C and Lassen NA (Munksgaard, Copenhagen, 1970) pp.135-52.

25. Hundal HS, Rennie MJ, Watt PW. Characteristics of acidic, basic and neutral amino acid transport in perfused rat skeletal muscle. J Physiol (in press).
26. Hundal HS, Rennie MJ, Watt PW. Characteristics of L-glutamine transport in perfused rat skeletal muscle. J Physiol 1987;393:283-305.
27. Rennie MJ, Hundal HS, Babij P, *et al*. Characteristics of a glutamine carrier in skeletal muscle have important consequences for nitrogen loss in injury, infection and chronic disease. Lancet (II) 1986;8154:1008-1012.

IB. ANGIOGENESIS IN HUMAN GLIOMA: CORRELATIONS WITH BLOOD FLOW AND TRANSPORT OF C-11 AIB

IB1. Objectives

This project has as its primary goal the development of a rabbit/rat corneal implant method to study human tumor angiogenesis and metabolism. The method is intended to determine the impact of chemotherapeutic strategies directly on human glioma tumors.

IB2. Scope of Investigation

We have identified, characterized and evaluated the necessary equipment and procedures which would be used to measure blood flow and the accumulation of radio-labeled compounds (^{11}C-AIB, ^{18}F-FDG) in superficial tumors growing in rabbit cornea and in a rat ear pouch. Specifically, we have: a) developed the tumor propagation, and implant procedures for the cornea and ear sites; the surgical procedures to permit multiple isotope studies in the same animal, b) validated the instrumentation to measure tumor blood flow, c) used phantom models to characterize the solid state CdTel β detectors used to follow the time-activity dynamics of positron-emitting(β^+) labeled compounds and d) performed rat/rabbit studies with animal and human glioma tissue to evaluate the method *in toto*.

IB3. Results

IB3a. *Blood Flow Methodology*

A laser-Doppler flowmetry [LDF] technique was evaluated in conjunction with a "clearance of locally generated hydrogen technique [CLGH]" to ascertain LDF's ability to provide rapid, continuous, quantifiable blood flow measurements (1). The combination LDF-CLGH probe was used in calibration studies with rat parietal cortex. LDF output was recorded simultaneously against CLGH (2) or the standard inhalation hydrogen method (3) as cerebral blood flow was varied by changing the rat's pCO_2 levels. The slope of the linear relationship between LDF versus CLGH or Inhalation H_2 clearance represents the LDF calibration factor for the tissue. Excellent linearity was obtained between LDF and both hydrogen clearance techniques. Calibration factors of 9.63 and 8.02 $mlmin^{-1}100gm^{-1}/hHz$ were determined for the TSI, Inc. BPM403 LDF device. These calibration results and the associated plots were published as part of a recent human brain tumor blood flow study(4). This calibrated LDF probe was used to rapidly map the tumor flow prior to isotope studies.

IB3b. *Beta Probe Characterization Studies*

A new, dual channel solid state beta probe system was fabricated for our laboratory by RMD, Inc. We made circuit enhancements to

this unit to provide multi-channel analyzer outputs, analog recorder outputs, and bias voltage adjustment. The probe utilizes a 2mm thick cadmium telluride detector with an active diameter of 5mm. The dual probe system requires an IBM personal computer or compatible for operation. System software was written to acquire data from the two channels, decay correct, display (in real time) count rate versus time and create data files for later analysis.

The β probe was evaluated in a series of phantom studies for its ability to measure the β^+ emitted by C-11, N-13 and F-18 in a superficial tumor. These studies: 1. characterized the probe with respect to tissue depth of sensitivity, positron signal/photon noise performance, response time, and spatial resolution; and 2. assessed the system's ability to measure the positrons in the presence of gamma ray interference for the corneal implant study geometry . The results of these studies are presented in the table below and indicate that the system is indeed capable of measuring the positrons emitted from a 3-5 mm diameter, 2mm thick tumor chip growing in the rat/rabbit cornea or rat ear pouch. Phantom work for the ear pouch location is currently underway.

Preliminary Eye Probe - Instrumentation Phantom Data	
1. Positron penetration depth (in saline)	2.5mm
2. Line spread function: Full width half max. for slit width = .22mm	4.5mm
3. Response time: positive step function	.11sec
negative step function	.23sec

IB3c. *Animal Models*

Animal tumor model development was pursued to: a) evaluate the advantages/disadvantages of tumor implant location and b) determine the best operative strategy to permit pre and post chemotherapy isotope studies. This work was conducted using both human glioma implanted into immune suppressed animals and rat 91-gliosarcoma implanted into normal animals. The rat gliosarcoma was used to facilitate the methodological development with inexpensive, normal rats.

To date, 7 (n=6 rats) ear pouch and 2 (n=1 rabbit) corneal pouch isotope uptake studies have been successfully conducted. We were unable to propagate human glioma in the athymic rat ear pouch nor have we been able to elicit angiogenesis from rabbit VX-2. We are currently exploring conditioning procedures to further immune suppress the athymic rat.

The 7 ear pouch isotope studies were conducted with ^{18}F-FDG provided by the North Shore Hospital Cyclotron. Arterial blood sampling was performed as part of the study. Our intention was to duplicate the autoradiographic FDG protocol, but to survive the

animal for a repeat study. This objective required careful placement of arterial and venous catheters, volume pre-loading of the animal prior to the study, and following sterile procedures. A repeat FDG study with blood sampling was recently carried out with our most recent animal two weeks after its first study.

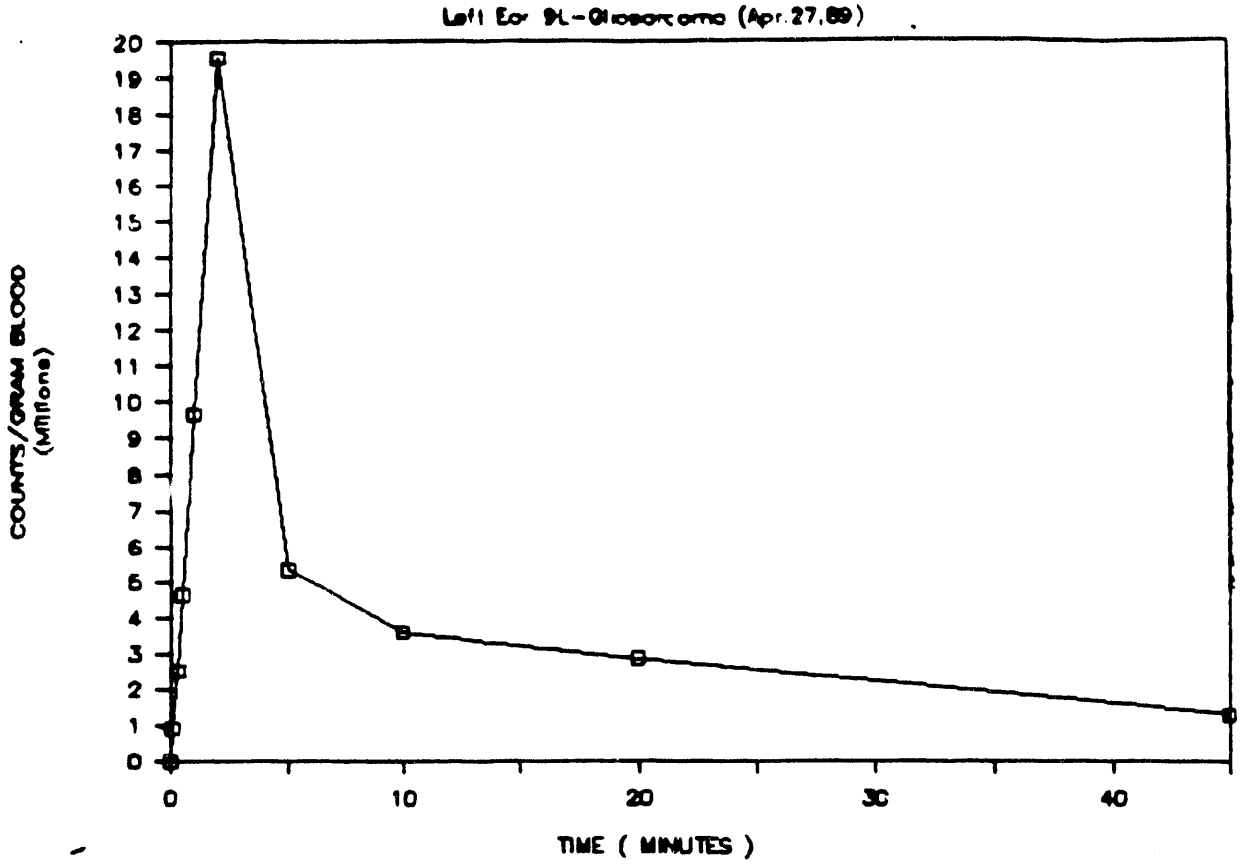
The tumor's uptake of FDG was observed continuously for 45 minutes post injection with dual β probes. A bare probe was placed above the tumor to measure its (β^+ + gamma) signal and the second, covered with 1.6mm lead, was placed behind the ear to measure the gamma component of the signal. This gamma component represents the interference from the tumor and the animal's body, while the β^+ came from the superficial tumor. The two signals were subtracted from each other to obtain the β^+ time-activity profile. Accumulation of the FDG throughout the entire observation period was observed in 3 studies. The time-activity curves and the associated blood curve from one such study is presented in Fig. 1. The remaining studies displayed an initial uptake followed by a slow decline.

In two ear pouch studies we simultaneously followed the gamma ray output from the tumor with our HEG scanner to validate the time-activity profile observed by the β^+ measurements. We observed, however that while the direction of the HEG measured profile was the same as the β^+ profile, the magnitude was different. Figure 2 displays the time-activity curves from one of these validation studies. This figure is also representative of the 4 studies which did not continually accumulate the FDG. Recent phantom studies indicate that the differences between the HEG and β probe results was due in part to the probes' orientation relative to the animal's skull, and to each other.

We successfully implanted a human glioma into a rabbit corneal pouch. Angiogenesis was observed and 2 ^{11}C-AIB uptake studies were conducted, one week apart. In both studies, accumulation of the AIB did not occur throughout the 20 minute observation period, rather it rose initially then slowly declined. The LDF flow profile of the implant demonstrated that the tumor had a higher blood flow than the surrounding area. Arterial blood sampling was not performed either study. Post mortum evaluation performed following the second isotope study concluded that the tumor was predominantly glioma and genetically human, but had became infiltrated with inflammatory cells shortly after vascularization.

SBIR Grant Award

In conjunction with RMD, Inc. and on the basis of the results to date, the decision was made to apply for an SBIR device development grant to pursue the development of a novel solid state positron probe. This device incorporates a gamma ray detector mounted behind a sensitive beta detector. Such a probe will measure the positron emission from a superficial tumor in the presence of gamma ray background directly without the geometry problems associated with the dual probe approach we used in this work. Th SBIR grant,


THE PLOT OF CPM/GRM BLOOD VS. TIME
Lewis Rat 9L-Gliosarcoma (Apr.27,89)
COUNTS/GRAM BLOOD (Millions)
TIME (MINUTES)

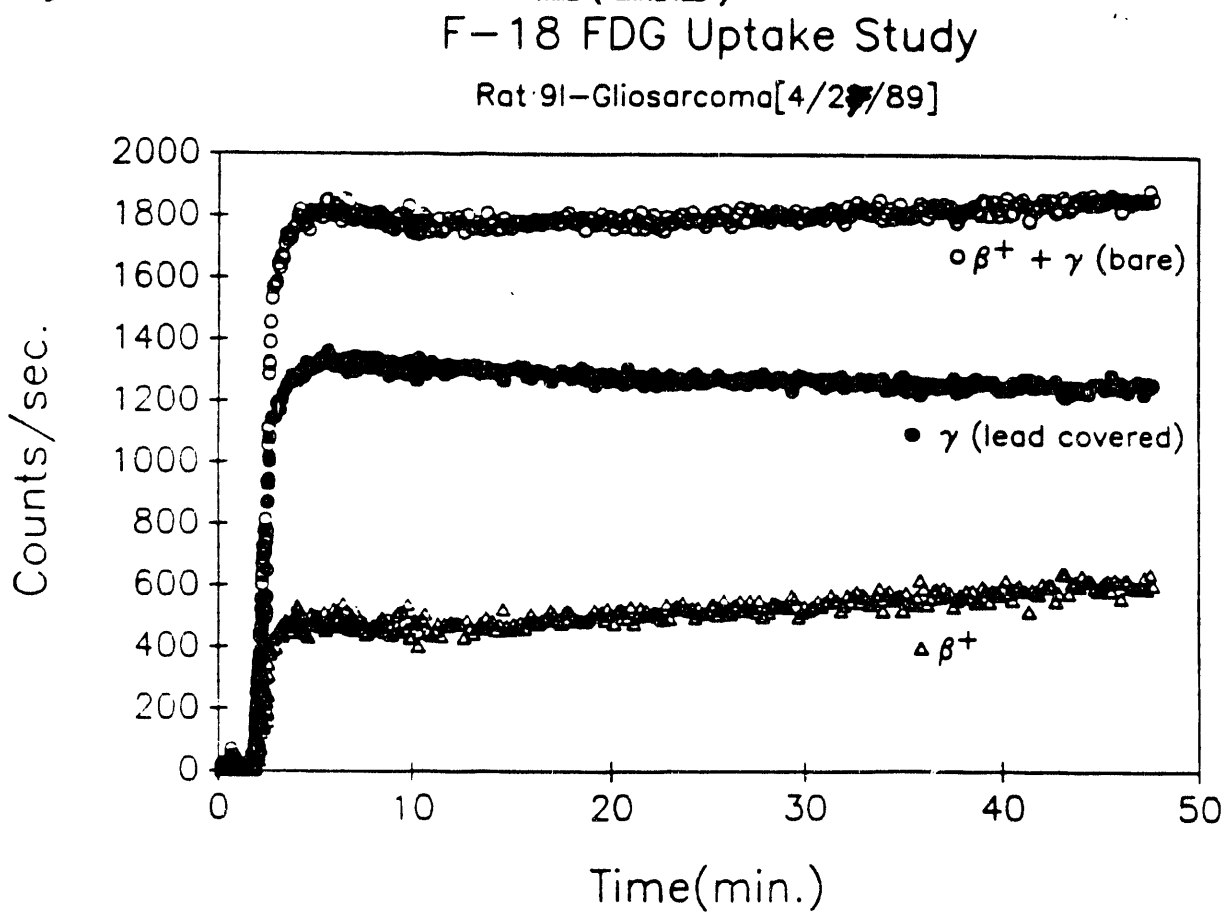

F-18 FDG Uptake Study
Rat 9l-Gliosarcoma[4/2?/89]
Counts/sec.
Time(min.)
β+ + γ (bare)
γ (lead covered)
β+

FIGURE 1

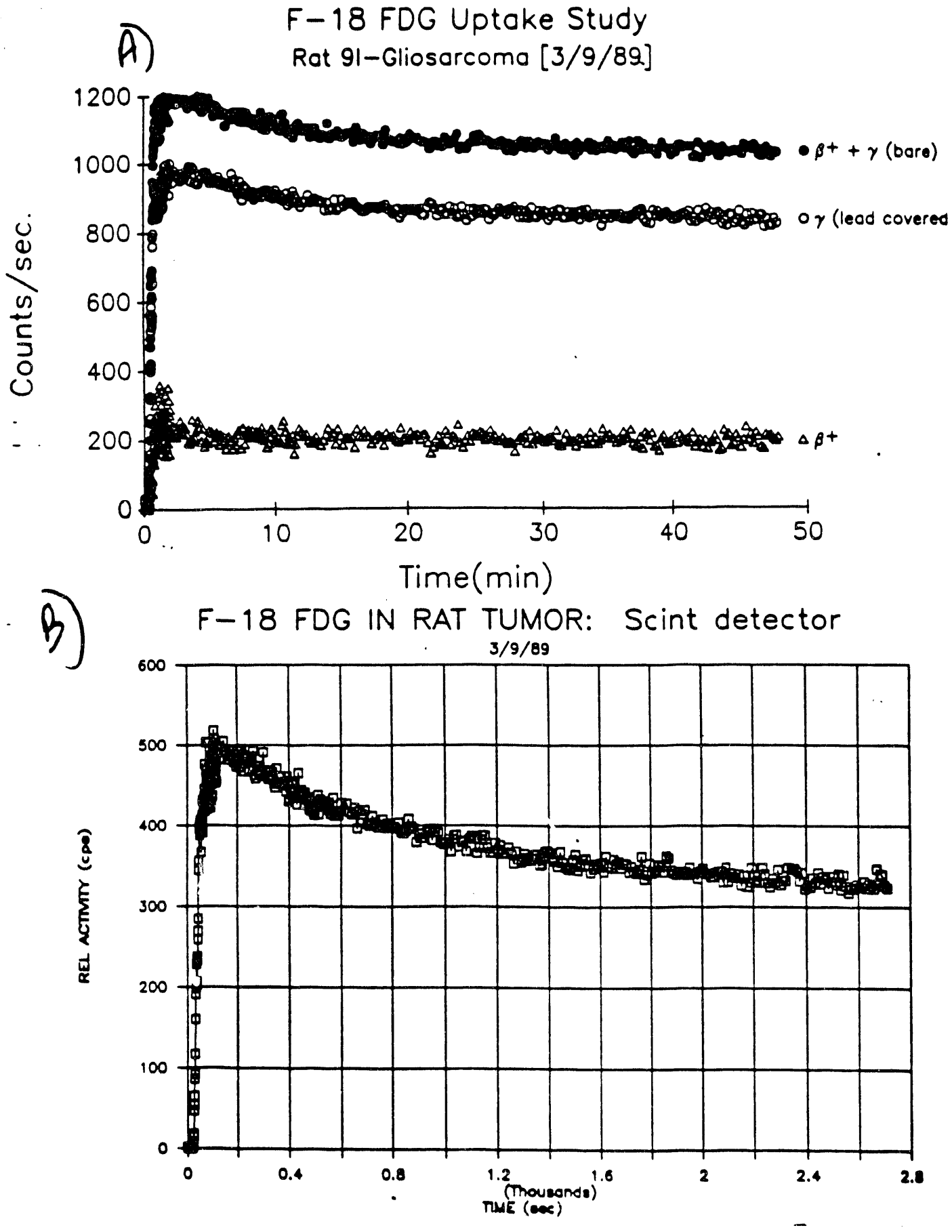

A)
F-18 FDG Uptake Study
Rat 91-Gliosarcoma [3/9/89]
Counts/sec.
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Time(min)
β+ + γ (bare)
γ (lead covered
β+
B)
F-18 FDG IN RAT TUMOR: Scint detector
3/9/89
REL ACTIVITY (cps)
600
500
400
300
200
100
0
0
0.4
0.8
1.2
1.6
2
2.4
2.8
(Thousands)
TIME (sec)

FIGURE 2

"Positron Probe For Corneal Tumor Bioassay, # R43 CA47711-01A1" was awarded 8/89.

IB4. Conclusions

The data indicate that:

1. Tumor blood flow can be measured using the combination LDF-CLGH probe.

2. The RMD beta probe measures the β^+ emission from ^{11}C, ^{18}F, ^{13}N labeled compounds accumulated within a superficial tumor with statistically acceptable count rates in spite of a significant gamma component which must be corrected for.

3. The instrumentation and software for measuring positron emission is available for use with the corneal or ear implanted glioma tumors.

4. Human glioma can be grown within the rabbit cornea, however rejection will occur after the tumor is highly vascularized, typically 3-4 weeks.

5. The ear site was found to be substantially easier to work with, less traumatic to the animal and capable of supporting larger tumors, however it was not useful for observing the initial angiogenesis processes.

This work has demonstrated the feasibility of the key aspects of the proposed method. Implementation of the method to evaluate the impact of chemotherapeutic strategies on tumor tissue awaits the availability of the novel positron probe to minimize the gamma component, and a suitable mathematical model for the compound being observed. Application to human tumor heterographs, however, awaits a successful propagation scheme using a fully immune suppressed animal.

IB5. Contributing Personnel

G.R. DiResta, Ph.D. - Biophysics Laboratory
J. Fissekis, Ph.D - Biophysics Laboratory
J.R. Bading, Ph.D. - Biophysics Laboratory
E. Arbit, M.D.- Department of Surgery/Neurosurgery

IB6. Publications

DiResta GR, Kiel JW, Reidel GL, Kaplan P, and Shepherd AP Hybrid Blood Flow Probe for Simultaneous H2 Clearance and Laser-Doppler Velocimetry. Am. J. Phys. 253:G573-581, 1987

DiResta GR, Arbit E, and Bedford RF. Application of Laser Doppler Velocimetry to the Measurement of Brain Tumor Blood Flow. Memorial Sloan-Kettering Cancer Center Research Colloquium, June 17, 1987:p20

DiResta GR, Arbit E, Lau N, and Galicich JH. Measurement of Regional Cerebral Blood Flow using a Combination Laser Doppler - Hydrogen Clearance Probe. Int. Conf. CBF & Met., Montreal, Que., June 20-25, 1987, Abstract #0833

DiResta GR, Corbally MT, Sigurdson E, Haumschild DJ, Ridge JA. Application of a Combination Laser-Doppler Velocimeter-Local Hydrogen Clearance Probe to Determine Small Bowel Blood Flow. Fourth World Conference for Blood Flow. Osaka, Japan, July 31-Aug. 2, 1987

IB7. Literature Cited

1. DiResta GR, Kiel JW, Reidel GL, Kaplan P, and Shepherd AP Hybrid Blood Flow Probe for Simultaneous H2 Clearance and Laser-Doppler Velocimetry. Am. J. Phys. 253:G573-581, 1987

2. Wodick R, Lübbers DW, Grossman U, Skolasinska K, and Pointek E, Determination of the mean flow velocity of blood in tissue by application of locally generated hydrogen as an indicator. Pflügers Arch. 374,p91-96 1978

3. Aukland K. Hydrogen polarography in measurement of local blood flow: theoretical and empirical basis. Ingvar DH, Lassen ISA (eds) Regional Cerebral Blood Flow, Copenhagen, Minksgaard, 1965:p42-45

4. Arbit E, DiResta GR, Bedford RF, Shah NK, and Galicich JH. Intraoperative Measurement of Cerebral and Tumor Blood Flow with Laser-Doppler Flowmetry. Neurosurgery 24:166-170, 1989

IC. USE OF F-18 FLUOROPYRIMIDINES FOR DESIGN AND EVALUATION OF REGIONAL AND SYSTEMIC CHEMOTHERAPEUTIC STRATEGIES IN HUMAN ADENOCARCINOMAS OF THE GASTROINTESTINAL TRACT

IC1. Objectives

The main goal of this project is to develop and use scintigraphy of [F-18]-labeled 5-fluorodeoxyuridine (FUDR) and 5-fluorouracil (FU) for quantitative evaluation of strategies for chemotherapy of human colo-rectal adenocarcinoma and other adenocarcinomas of the GI tract. A partial specific aim is to develop a reliable and efficient synthetic method for the preparation of {F-18]-FUDR suitable for patient studies.

IC2. Scope of the investigation

With respect to the partial specific aim described above we undertook the development of the required chemical methodology for obtaining FUDR and FU labeled with F-18. these syntheses have been accomplished. The needed nuclide, [F-18]-F_2 was generated by the $^{20}Ne(d,alpha)\ ^{18}F$ reaction. For the fluorination of uracil and of 2`-deoxyuridine we employed a one-pot synthetic prodecure. The respective products were isolated by semi-preparative chromatography.

IC3. Results

We performed a total of eleven experiments for the synthesis of [F-18]-5-fluorouracil and twenty five such experiments for [F-18}-5-fluoro-2`-deoxyuridine. For the production of the radionuclide (F-18), the target was filled with a mixture of 0.1% F_2 in Ne. After a bombardment at 25uA for one hour, about 40mCi of activity were trapped in the reaction vessel. Several chromatographically pure preparations of [F-18] labeled FU and FUDR have been obtained. An improved HPLC system has been developed for the separation of [F-18]-FUDR from UDR. We now employ Hamilton PRP-1 packing which shows greater stability towards aqueous solvents and good resolving power. Good resolution was observed within a broad range of pH (2.5 to 5.5). A sample of [F-18]-FUDR purified by HPLC was concentrated to contain 5.5 mCi of the material in 0.5 ml of isotonic water within approximately two hours from EOB. this activity concentration is considered to be adequate for in vivo experiments in small animals (rabbits).

We have designed and constructed a remote control apparatus for the synthesis of [F-18]-FU and of -FUDR. Random limulus tests of eleven preparations using this apparatus were all negative. The chemical and radiochemical purity of such preparations has been checked with HPLC and also with

autoradiography on TLC. Preliminary biodistribution studies in three different nude mouse models bearing human hepatoma were done. Tumor tissue accumulated [F-18] from both FU and FUDR. (1) Tumor/Liver ratios were below one for all three tumor lines.

IC4. Conclusions

The synthesis of [F-18]-FU and of [F-18]-FUDR have been accomplished. Sterile, apyrogenic preparations of both compounds are obtained on a routine basis at specific activities adequate for experimentation on small animals. We have designed and built a remote control apparatus which we use in the preparation of these compounds.

IC5. Participating Personnel

Year	Name	% T & E
1987-88	John Fissekis, Ph.D.	50%
	Chris Nielsen, tech.	50%
1988-89	John Fissekis, Ph.D.	10%
	Chris Nielsen, tech.	50%
1989-90	Chris Nielsen, tech.	50% for four months;

IC6. Publications

Abstracts

Conti P.S., Sordillo P.P., Fissekis J.D., Nielsen C.M., Chung J. Development of F-18 5-Fluorouracil and fluorodeoxyuridine as tracers of chemotherapeutic agents for liver cancer. Radiology 1987;165 (Supplement):98.

Conti P.S., Sordillo P.P., Fissekis J., Nielsen C.M., Chung J. Comparative uptake of positron emitting biomolecules in hepatoma heterotransplants. J Nucl Med 1988;29 (Supplement):903.

ID. ENZYMATIC SYNTHESIS OF METABOLITES LABELED WITH N-13 OR C-11.

ID1. Objectives

The **long-term objectives** of this project are to enzymatically synthesize metabolites labeled with the positron emitting radionuclides N-13 or C-11 and to make these compounds available in sufficiently high radiochemical and biological purity as to be suitable for clinical studies in human subjects and metabolic studies in animal systems.

ID2. Scope of Investigation

ID2a. *Overview*

During this report period L-[^{13}N]glutamate, L-^{13}N]alanine, and L-[*amide*-^{13}N]glutamine were prepared on a regular basis for biological studies. We planned to develop a new method for labeling tyrosine and phenyalanine with ^{13}N. The procedure utilized phenylalanine dehydrogenase purified from Bacillus sphaericus. We determined the specificity of this reaction for the synthesis of L-[^{13}N]DOPA. We studied the feasibility of preparing L-[*amine*-^{13}N]glutamine by coupling the glutamate dehydrogenase and glutamine synthetase reactions.

Study of the short-term metabolic fate of ^{13}N labeled ammonia and amino acids in the normal rat liver was undertaken (also supported by U.S. Public Service grants AM 163791 and CA-34603). The metabolic fate of ^{13}N derived from L-[^{13}N]glutamate was studied in the Walker-256 tumor (also supported by CA-34603).

ID3. Results and Conclusions

We had previously developed a method for the enzymatic synthesis of L-[^{13}N]tyrosine that made use of the glutamate dehydrogenase and aspartate aminotransferase activities to transfer the newly synthesized [^{13}N]amino group from glutamate to *p*-hydroxyphenyl pyruvate, the α-keto acid analog of tyrosine. Labeled tyrosine was separated from ^{13}N labeled ammonia and glutamate, the intermediates in these reactions, by differential elution of these compounds from a Poropak-Q column. This procedure took at least 20 min and the yields were limited. We have since collaborated with Dr. Y. Asano of the Sagami Chemical Research Center in Japan to produce ^{13}N aromatic amino acids in high yields using the newly isolated enzyme phenylalanine dehdrogenase. We have immobilized this enzyme and used it to catalyze the synthesis of high yields (30-70 mCi) of either L-[^{13}N]phenylalanine or L-[^{13}N]tyrosine by the reductive

[^{13}N]amination of the respective α-keto acids. We also prepared L-DOPA by the exchange reaction between L-DOPA and [^{13}N]ammonia in the presence of NAD^+ and phenylalanine dehydrogenase. Nine percent of the label was tranferred to L-DOPA.

We have prepared L-[*amine*-^{13}N]glutamine from [13N]ammonia by sequentially coupling the glutamate dehydrogenase and glutamine synthetase reactions. The resultant L-[*amine*-^{13}N] was used in parallel with L-[*amide*-^{13}N]glutamine to study the metabolism of glutamine in the rat liver. Although we are able to carry out metabolic fate studies with the synthesized L-[*amine*-^{13}N]glutamine, we encountered problems with the synthetic method employed. We found that the stability of different batches of sheep brain glutamine synthetase vary greatly. Moreover, in preparing L-[*amine*-^{13}N]glutamine from L-[^{13}N]glutamate, it was found that glutamate dehydrogenase activity was a major contaminant in the sheep brain glutamine synthetase preparation. This resulted in considerable exchange of ^{13}N label between glutamate and ammonia. This exchange was inhibited by adding guanosine triphosphate to the reactionmixture. We have recently used glutamine synthetase purified by affinity chromatography from Escherichia coli for the synthesis of L-[*amide*-^{13}N]glutamine in studies involving amino acid transport in skeletal muscle. The yields have been considerably high (>60 mCi) and we now plan to use this enzyme for the synthesis of L-[*amine*-^{13}N]glutamine.

The short-term metabolic fate of ammonia and several amino acids was also studied. A bolus injection of tracer quantities of labeled metabolite was introduced into the portal vein of anesthetized rats and HPLC analyses were carried out on deproteinized freeze-clamped liver extracts 5-60 sec after administration.

With [^{13}N]ammonia as the injectate it was found that: (1) label is exchanged rapidly among components of the glutamate dehydrogenase, aspartate aminotransferase and glutamate dehydrogenase reactions; (2) only a small fraction of portal blood-derived ammonia is metabolized to glutamine; and (3) despite considerable label in citrulline at early time points, no radioactivity was detected in carbamyl phosphate, suggesting very efficient conversion to citrulline little free carbamyl phophate accumulating in the rat liver. No tracer evidence could be found for the paticipation of the purine nucleotide cycle in ammonia production from aspartate.

With ^{13}N labeled glutamate, alanine or glutamine (amide or amine) as the injectate it was found that: (1) there were zonal differences in uptake of alanine and glutamate from the portal vein *in vivo*. By 30 sec after administration of either L-[^{13}N]glutamate or L-[^{13}N]alanine, label in liver

glutamate was comparable; yet by 1 min 9 times as much label was present in liver glutamine (amine) following L-[^{13}N]glutamate administration than following L-[^{13}N]alanine administration. Conversely label in liver urea at 1 min was more pronounced after L-[^{13}N]alanine administration despite (a) comparable total pool sizes of glutamate and alanine in the liver; and (b) label incorporation from alanine into urea must occur via prior transfer of alanine nitrogen to glutamate. The rate of turnover of L-[*amide*-^{13}N]glutamine was considerably slower than that of L-[^{13}N]alanine or L-[^{13}N]glutamate, presumably due in part to the higher concentration of glutamine in that organ.

We have studied the metabolic fate of ^{13}N from L-[^{13}N]glutamate in the Walker-256 tumor implanted under the renal capsule by the method first decsribed by Gullino and Grantham (J. Natl. Cancer Instit. 27: 679-93, 1961). Our results demonstrate that the tumor takes up the L-[^{13}N]glutamate more slowly than the kidney does, which is the host organ. Futhermore, the tumor metabolized L-[^{13}N]glutamate more slowly than the kidney (25.5% of label compared to 37 % in the kidney were in metabolized form). Of the metabolites formed from labeled glutamate in the tumor, 70 % was present in aspartate, 18 % in glutamine, 5 % in alanine, 4 % in urea and 2 % in ammonia. In the kidney, label was present in aspartate (49 %), glutamine (25 %), alanine (5 %), urea (8 %) and ammonia (12 %). Thus, the data reveals that tumor tissue had a more limited metabolic fate than the kidney with aspartate as the predominant product. Analyses of renal arterial and venous blood revealed only minute amount of labeled aspartate and that the label could only be transferred to aspartate within the tumor and kidney and that the label did not egress from these tissues to an appreciable extent. Specific activities of enzymes associated with glutamate metabolism were determined in extracts of both tumor and renal tissue. It was found that levels of glutamate dehydrogenase and alanine aminotransferase were very low in the tumor tissue. In the case of glutamate dehydrogenase, renal tissue values were 10 to 20 times higher than tumor values. Alanine aminotransferase activities in tumor tissue were 1/5 to 1/10 of those demonstrated by renal tissue extracts. Although the aspartate aminotransferase activity was three times higher in renal tissue than in the tumor, levels of this enzyme were sufficient enough in both tissues to enable rapid attainment of equilibrium label among the components involved. The much lower concentrations of glutamate dehydrogenase and alanine aminotransferase in the tumor, as compared with the kidney may provide less competition for the transfer of label from glutamate to aspartate.

Since the measurement of changes in uptake of label fron L-[^{13}N]glutamate after treatment with methotrexate has proven useful in determining the response of tumors to

chemotherapy, we studied the effect of methotrexate on the metabolic fate of label from glutamate in control and treated Walker-256 carcinosarcoma. Two hours after treatment with methotrexate (dose of 30 mg/kg), L-[^{13}N]glutamate was injected into the renal artery and the animals were sacrificed 5 min post injection. Acid soluble extracts of tumor and kidney were analyzed by HPLC. Analysis of tumor and renal tissue extracts revealed that more glutamate was metabolized in treated animals. In the tumor extract of methotrexate treated animals, aspartate accounted for only 28 % of the metabolized glutamate (compared to 66.4 % in untreated tumor). There was an increase of label in urea (accounting for 25.5 % as compared with the untreated value of 3.7 %), glutamine (23.1 % , up from the untreated value of 17.2 %), alanine (up from 4.9 % to 7.6 %) and ammonia (an increase from 1.8 % to 7.6 %). Kidney extracts of methotrexate treated animals demonstrated a decrease of label transfer from glutamate to aspartate. Labeled aspartate accounted for 37.5 % of the metabolized portion of labeled glutamate, compared to 46.2 % in control animals. Small increases of label were noted in urea and ammonia. The changes in accumulation of label in various metabolites within methotrexate treated tumor tissue may reflect direct changes in metabolism or may be a measurement of the cytotoxic effect of the drug. These studies further demonstrate how the use of ^{13}N as a tracer for *in vivo* metabolic investigations can provide useful information on both normal and abnormal cellular metabolic processes.

ID5. Participating Personnel

Alan Gelbard, Ph.D. Assoc. Lab. Member Biophysics Laboratory	40% Effort 1987-89
Sabina Filc-DeRicco, M.S. Research Technician	100% Effort 1987-89
Arthur Cooper, Ph.D. Research Professor, Biochemistry, Cornell Univ. Medical College	10% Effort 1987-89

ID6. Publications

Journal Articles

Cooper A.J.L., Nieves E., Coleman A.E., Filc-DeRicco S., Gelbard A.S. Short-term metabolic fate of [^{13}N]ammonia in rat liver in vivo. J Biol Chem 1987;262:1073-1080.

Ridge J.A., Bading J.R., Gelbard A.S., Benua R.S., Daly J.M. Perfusion of colorectal hepatic metastases. Cancer 1987;59:1547-1553.

Cooper A.J.L., Nieves E., Rosenspire K.C., Filc-DeRicco S., Gelbard A.s., Brusilow S.W. Short-term metabolic fate of 13N-labeled glutamate, alanine, and glutamine(amide) in rat liver. J Biol Chem 1988;263:12268-12273.

Calderon J., Cooper A.J.L., Gelbard A.S., Mora J. 13N isotope studies of glutamine assimilation pathways in neurospora crassa. J Bacteriology 1989;171:1772-1774.

Conlon K.C., Bading J.R., DiResta G.R., Corbally M.T., Gelbard A.S., Brennan M.F. Validation of transport measurements in skeletal muscle with N-13 amino acids using a rabbit isolated hindlimb model. Life Sciences 1989;44:847-859.

Gelbard A.S., Cooper A.J.L., Asano Y., Nieves E., Filc-DeRicco S., Rosenspire K.C. Methods for the enzymatic synthesis of tyrosine and phenylalanine labeled with nitrogen-13. Appl Radiat isot 1989 (in press).

Book Chapters

Cooper A.J.L., Nieves E., Filc-DeRicco S., Gelbard A.S. Short-term metabolic fate of [13N]ammonia, L-[13N]alanine, L-[13N]glutamate and L-[amide-13N]glutamine in normal rat liver in vivo. (Awarded first prize as best paper by the Dutch Academy of Liver Disease). Proceedings 6th Intenational Symposium on Ammonia Metabolism. Maastricht, The Netherlands, April 27-29, 1987. Advances in ammonia metabolism and hepatic encephalopathy. P.B. Soeters, J.H.P. Wilson, A.J. Meijer and E. Holm, eds. pp 11-25. Elsevier Science Publisher 1988.

Cooper A.J.L., Gelbard A.s. Metabolism of [13N]ammonia in normal and hyperammonemic rat brain. Proceedings 6th International Symposium on Ammonia Metabolism. Maastricht, the Netherlands, April 27-29, 1987. Advances in ammonia metabolism and hepatic encephalopathy. P.B. Soeters, J.H.P. Wilson, A.J. Meijer and E. Holm, eds. pp 420-32. Elsevier Science Publisher 1988.

Cooper A.J.L., Lai J.C.K., Gelbard A.S. Ammonia and energy metabolism in normal and hyperammonemic rat brain. Proceedings Symposium Intr Soc Neurochem and Amer soc Neurochem. May 26-29, 1987. Miami FLA. Neurology and Neurobiology Vol. 39. The Biochemical Pathology of Astrocytes. MD Norenberg, L Hertz, A Schousboe, eds. pp 419-434. AR Liss, NY Pub, 1988.

Abstracts

Cooper A.J.L., Gelbard A.S. Metabolism of [13N] ammonia in normal and hyperammonemic rat brain. J Hepatology 1987;4:S11.

Cooper A.J.L., Nieves E., Filc-DeRicco S., Gelbard A.S. Short-term metabolic fate of [13N]ammonia, L-[13N]alanine, L-[13N]glutamate and L-[amide-13N]glutamine in normal rat liver in vivo. J Hepatology 1987;4:S12.

Ficl-DeRicco S., Rosenspire K.C., Nieves E., Gelbard A.s. Metabolic fate of L-[N-13]glutamate in a transplantable rat tumor. J Nucl Med 1987;28:594.

Bading J.R., Conlon K.C., Gelbard A.S., Corbally M.T., Banket W.J., Brennan M.F. Effect of cancer cachexia on N-13 L-Glutamine transport in rabbit skeletal muscle. J Nucl Med 1988;29:902.

Conlon K.C., Bading J.R., Gelbard A.S., Corbally M.T., DiResta G.R., Vydelingum N.A., Brennan M.D. In vivo skeletal muscle glutamine transport in cancer. the Physiologist, August 1988.

Filc-DeRicco S., Gelbard A.S. Effect of methotrexate on the metabolic fate of N-13 label from l-glutamate in a transplantable rat tumor. J Nucl Med 1988;29:1320.

Gelbard A.S., Cooper A.J.L., Asano Y., Filc-DeRicco S. Use of phenylalanine dehydrogenase for the synthesis of l-phenylalanine, l-tyrosine and l-dopa labeled with nitrogen-13. J Nucl Med 1988;29:931.

Conlon K.C., Bading J., McDermott e.W., Gelbard A.S., Brennan M.F. Capillary clearance and cellular transport of glutamine decline in starvation but not in cancer cachexia. 1989 Proceedings of the American Assn for Cancer Research (in press).

IE. SYNTHESIS OF AMINO ACIDS LABELED WITH C-11

IE1. Objectives

The basic objective of this project was to produce sterile preparations of C-11 amino acids as required by the research program. Its specific aims were as follows:

(a). to fulfill the requirements for sterile pyrogen free preparations of [C-11]-labeled alpha-amino isobutyric acid (AIB) needed in studies on patients with various tumors and in others on amino acid transport, protein biosynthesis and angiogenesis in experimental animals,

(b). to produce, as needed, some [C-11]-labeled branched alpha-amino acids most likely [C-11]-valine and -leucine, for studies of protein biosynthesis in vivo,

(c) to refine and optimize the synthesis of ornithine labeled with C-11 at the carboxyl group and also develop a synthesis for a w-methylene-labeled ^{11}C ornithine.

IE2. Scope of Investigation

Several alpha-amino acids, labeled with C-11 at the carboxyl group have been prepared over a number of years in our laboratory by the application of the Bucherer-Strecker synthesis. Some, particularly [C-11]-labeled alpha-amino-isobutyric acid (AIB) and 1-amino-cyclopentane carboxylic acid (ACPC) are produced routinely. The synthesis of others, including valine, leucine, isoleucine and ornithine, was developed on the basis of projected studies in protein biosynthesis and polyamine metabolism. The significance of [C-11]-Orn is based on its potential as a probed for studying the distribution and function of ornithine decarboxylase (ODC) in tumor of the prostate in relation to the metabolism of polyamines.

IE3. Results

In the period covered by this report we performed a total of 122 preparations of various amino acids labeled with C-11 at the carboxy group. Of these, ten preparations were used in patient studies, another eighty in animal studies and the remaining were developmental syntheses. In all instances we used a remote control process for the Bucherer-Strecker synthesis which we have described elsewhere (1). The machine has been improved to allow for the degassing of the liquid streams and in addition to permit monitoring of the

pH of column effluent by a pH flow monitor we designed and fabricated.
The radiolabeling of ornithine at C-1 has been accomplished. We have obtained several preparations of [C-11]-Orn in good radiochmical yield (up to 60mCi). The method involves the Bucherer-Strecker synthesis and has been adapted to a remote control process.

IE4. Conclusions

A remote control process for the synthesis of [C-11]-labeled alpha-amino acids developed in our laboratory has been proven to give sterile and pyrogen free preparations with consistent radiochemical yields. The process meets all our requirements for [C-11]-labeled amino acids on a production basis. It affords preparations of high specific activity, activity concentration and radiochemical purity as required by studies with small experimental animals. It is being used for the syntheses of labeled AIB, ACPC, Leu, Val, Ile, and Orn.

IE5. Contributing Personnel

Year	Name	% T & E
1987-88	John Fissekis, Ph.D.	50%
	Chris Nielsen, tech.	50%
1988-89	John Fissekis, Ph.D.	15%
	Chris Nielsen, tech.	50%
1989-90	Chris Nielsen, tech.	50% for four months;

IE6. Publications

Bading J.R., Corbally M.T., Fissekis J.D., DiResta G.R., Brennan M.F. Effect of starvation of C-11 alpha-aminoisobutyric acid (AIB) transport in skeletal muscle. J Nucl Med 1987;28:650.

Corbally M.T., Bading J.R., Fissekis J., diResta G.R., Conlon K.C., Brennan M.D. In-vivo C-11 alpha aminoisobutyric acid (AIB) skeletal muscle transport in starvation. The Physiologist, August 1988.

Sordillo P.P., DiResta G., Conti P.S., Fissekis J., Bading J.R., Nielsen C.M., Benua R.S., Laughlin J.S. Imaging studies using C-11 alpha-aminoisobutyric acid (AIB) in patients with malignant melanoma. J Nucl Med 1988;29:904.

IF. INSTRUMENTATION

IF1. Core Cyclotron Facility

In June, 1988, the Sloan-Kettering Institute (SKI) Cyclotron Core Facility was closed in order to implement a major overhaul and upgrade of the plant. This renovation was supported by a BRS Shared Instrumentation Grant (S10 RR03304-01) from the NIH Division of Research Resources ($300,000). Additional funding ($100,000) was provided by SKI.

The renovation was performed initially under the direction of Drs. Laughlin and Fissekis and, beginning in April of 1989, under the direction of Dr. Finnand Mr. Sheh. All work other than plumbing and installation of a new set of magnet cores was performed by personnel assigned to the Core Facility.

In the first phase of the operation, the cyclotron was dismantled and reassembled with redesigned and upgraded systems; some new systems were also added. These are as follows:

(1). Magnet coils. Replaced by new coils of improved design.
(2). Trimming and harmonic coils. Replaced with redesigned coils.
(3). Deflector power supply and RF oscillator unit. Repaired and modified.
(4). Cyclotron vacuum control system. Replaced with a new, upgraded system of improved design.
(5). Beam diagnostic devices. Added.
(6). Ion source and trimming/harmonic coil power supplies. Replaced.
(7). Radiation monitoring system. Replaced.
(8). Cooling water and compressed air distribution pipe networks. These systems were redesigned and replaced. New oil-less compressors at the building's central power plant now provide compressed air to the Facility.

The renovations have been completed, and the cyclotron is currently being tested and retuned. The Facility is expected to resume normal operation in October, 1989.

Participating personnel: J.S. Laughlin, Ph.D.
J.D. Fissekis, Ph.D.
A. Knott, B.S.
S. Tirelli
R. D. Finn, Ph.D.
H. Sheh

IF2. Multiple Energy Gamma Camera System

Our interest in using the Multiple Indicator Dilution method (1) to measure nutrient and drug transport *in vivo* (Section IA) has motivated us to develop a gamma camera system for multi-tracer dynamic studies. Institutional funding was obtained for this project in November, 1986. Four commercial companies - Encore Medical Systems (EMS), Nuclear Imaging Computers (NIC), Nuclear Fields, and Qualogy - were contracted to provide, respectively, two rebuilt gamma cameras, a computer and camera-computer interface, a pair of 511 keV collimators, and an optical computer disk system. A private consultant, Henry H. Wilson, Ph.D., was contracted to write the special software required for multi-channel data acquisition. All parts of the system were provided according to Dr. Bading's specifications. The cameras and computer were delivered in November, 1987, the collimators in March, 1988, and the optical disk system in June, 1988. Since that time we have added certain mechanical features (detector alignment, couch modification, supplementary support for upper detector when loaded with 511 keV collimator), and written software for image alignment and image matrix arithmetic.

We refer to the system (cameras plus computer) as the Multiple Energy Dynamic Imaging System, or MEDIS. The essential features and rationale for the system are described in an enclosed abstract (2). The system's components and modes of operation are summarized in Table 1. Figure 1 shows a schematic of the MEDIS. **Key features permitting separate, simultaneous, multi-energy acquisition are: (a) modification of the camera electronics to extract separate data-ready (Z) signals from each energy discriminator; (b) a six-channel ADC interface; and (c) Dr. Wilson's software for setup and management of the multichannel data acquisition and storage.** A special switching manifold permits images to be acquired for any possible combination of energy windows within a given detector. The buffer box conditions signals for transfer to the ADC interface; the two detectors are aligned electronically via adjustments within the buffer box. **The dual-detector configuration and alignment features permit the system to be used for quantitative imaging by the conjugate-view, or geometric mean, method** (3). However, the detectors are mounted independently and thus can also be used to simultaneously image different parts of the body.

A key problem in multi-energy imaging is **photon crosstalk**, i.e., the contribution of image counts by one radiolabel to the image channel of another radiolabel. We have measured signal:noise ratios for this effect on the MEDIS as a function of energy window settings and source geometry; data for a 511 keV source are shown in Figure 2. **The observed signal:noise ratio is acceptable, even for the worst case of the human torso.** As indicated by the figure, ^{99m}Tc is optimal for use with β^+ emitters when the source is relatively small, but higher energy labels, such as ^{131}I or ^{113m}In are better for larger sources.

FIGURE 1.

MULTIPLE ENERGY DYNAMIC IMAGING SYSTEM

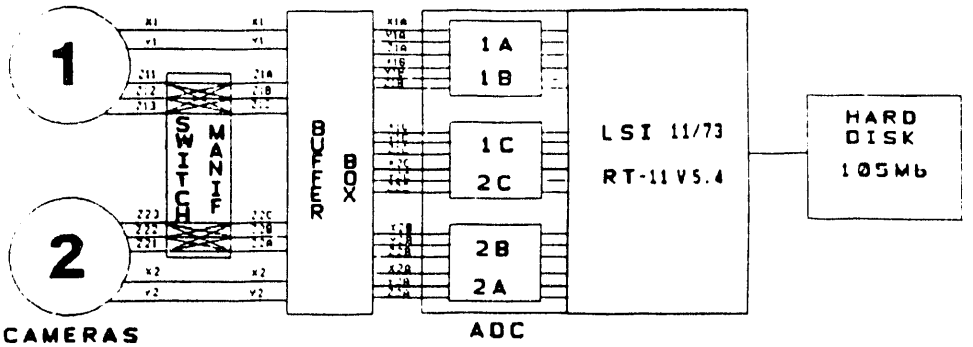
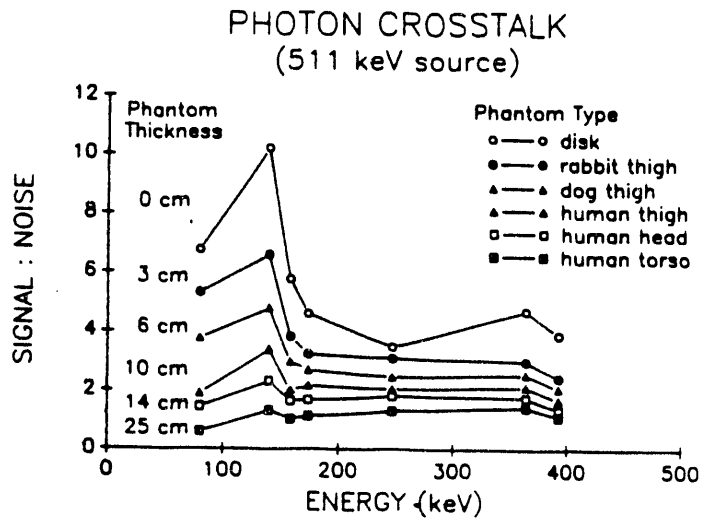


FIGURE 2.



Participating personnel: J.R. Bading, Ph.D.
G.R. DiResta, Ph.D.
B.J. Beattie

Publications:

Bading JR, **Wilson HH**, Nupnau WA, Gregg GM. A multiple-energy gamma camera system for dynamic transport studies. J Nucl Med 1989;30:852. (abstract)

Literature Cited:

1. Bassingthwaighte JB, Goresky CA. Modeling in the analysis of solute and water exchange in the microvasculature. In: Handbook of Physiology, Vol IV, ed. E.M. Renkin, CC Michel (Am Physiol Soc, Bethesda, Md, 1984) pp. 549-626.
2. Bading JR, Wilson HH, Nupnau WA, Gregg GM. A multiple-energy gamma camera system for dynamic transport studies. J Nucl Med 1989;30:852. (abstract)
3. Sorenson J. Quantitative measurement of radioactivity *in vivo* by whole body counting. In: Instrumentation in Nuclear Medicine, ed. G.J. Hine and J. Sorenson (Academic Press, New York , 1974) Vol II, pp.311-48.

TABLE 1.
MULTIPLE ENERGY DYNAMIC IMAGING SYSTEM

Detectors:
- 2, mechanically-independent, reconditioned Searle-Seimens gamma cameras
- new 1/2" thick NaI scintillation crystals
- 37 new, high performance, phototubes per camera

Collimation and shielding:
- one matched pair of custom-designed 511 keV collimators
- one pair of Tc-99m high-resolution collimators
- side shielding of cameras and 511 keV collimators adeqaute for 511 keV

Computer:
- dedicated nuclear medicine computer system based on LSI-11/73 processor
- special camera-computer interface permitting simultaneous dynamic acquisition from six independent data channels
- two 105 Mbyte winchester disks
- optical disk system for high density data archiving
- RT-11 Vers. 5.4 operating system
- special imaging and data processing software for multienergy studies

Data acquisition modes:
- 6-channel static, dynamic histogram, or list mode

Performance characteristics:
- spatial resolution (central (11") field of view)
  intrinsic 6 mm
  image (10 cm from collimator) 20 mm (511 keV collimators)
  8 mm (Tc-99m collimators)
- image registration error at different energies < 3 mm
- count rate capability
  camera dead time loss < 20% at 50K cps
  computer acquisition rate ≤ 80K cps

COLLECTED BIBLIOGRAPHY 1987-1989

Abstracts

1. Bading J.R., Corbally M.T., Fissekis J.D., DiResta G.R., Brennan M.F. Effect of starvation of C-11 alpha-aminoisobutyric acid (AIB) transport in skeletal muscle. J Nucl Med 1987;28:650.

2. Conti P.S., Sordillo P.P., Fissekis J.D., Nielsen C.M., Chung J. Development of F-18 5-Fluorouracil and fluorodeoxyuridine as tracers of chemotherapeutic agents for liver cancer. Radiology 1987 (Supplement);165:98.

3. Cooper A.J.L., Gelbard A.S. Metabolism of [13N] ammonia in normal and hyperammonemic rat brain. J Hepatology 1987;4:S11.

4. Cooper A.J.L., Nieves E., Filc-DeRicco S., Gelbard A.S. Short-term metabolic fate of [13N] ammonia, L-[13N] alanine, L-[13N] glutamate and L-[amide-13N] glutamine in normal rat liver in vivo. J Hepatology 1987;4:S12.

5. Corbally M.T., DiResta G.R., Sigurdson E., Brennan M.F. Local hydrogen clearance and flourescein angiography in the determination of small bowel viability. Brit Ass Ped Surg - Dublin Meeting, July 22-24, 1987.

6. DiResta G.R., Arbit E., Lau N., Galicich J.H. Measurement of regional cerebral blood flow using a combination laser doppler-hydrogen clearance probe. Int Conf on Cerebral Blood Flow and Metabolism, Montreal, Canada, June 20-25, 1987.

7. DiResta G.R., Corbally M.T., Sigurdson E., Haumschild D.J., Ridge J.A. Application of a combination laser doppler velocimeter-local hydrogen clearance probe to determine small bowel blood flow. Proceedings 4th World Congress for Microcirculation, Osaka, Japan, July 31- August 2, 1987. Microcirculation: An Update. M. Tsuchiya et al, eds. pp 381-2. Elsevier Science Publisher 1987.

8. Filc-DeRicco S., Rosenspire K.C., Nieves E., Gelbard A.S. Metabolic fate of L-[N-13] glutamate in a transplantable rat tumor. J Nucl Med 1987;28:594.

9. Starnes H.F., DiResta G.R., Stone M.D., Conti P.S., Bading J.R., Jeevanandam M., Brennan M.D. Altered protein metabolism in cancer patients with jaundice. Gastroenterology 1987;92:1783.

10. Arbit E., Kayata M., DiResta G., Lau N., Galicich J.J. Lidocaine infusion reduces ICP associated with a mass lesion. 7th Annual International Symposium on Intracranial Pressure and Brain Injury, Ann Arbor, Michigan. June 19-23, 1988.

11. Bading J.R., Conlon K.C., Gelbard A.S., Corbally M.T., Banket W.J., Brennan M.F. Effect of cancer cachexia on N-13 L-Glutamine transport in rabbit skeletal muscle. J Nucl Med 1988;29:902.

12. Conlon K.C., Bading J.R., Gelbard A.S., Corbally M.T., DiResta G.R., Vydelingum N.A., Brennan M.D. In vivo skeletal muscle glutamine transport in cancer. The Physiologist 1988;August:A133.

13. Conti P.S., Sordillo P.P., Fissekis J., Nielsen C.M., Chung J. Comparative uptake of positron emitting biomolecules in hepatoma heterotransplants. J Nucl Med 1988;29:903.

14. Conti P.S., Bading J., Benua R.S., Yeh S.D.J., Laughlin J.S. Tumor imaging with carbon-11 labeled alpha-aminoisobutyric acid (AIB). 74th Acientific Assembly of the Radiologic Society of North America, Chicago, Illinois. November 27 - December 2, 1988.

15. Conti P.S., Bading J.R., Wong D.F., Gelbard A.S., Dannals R.F., LaFrance N.D., Laughlin J.S., Wagner H.N. Potential aids in the diagnosis and management of neoplasms. TumorDiagnostik Therapie 1988;9:175.

16. Corbally M.T., Bading J.R., Fissekis J., DiResta G.R., Conlon K.C., Brennan M.D. In-vivo C-11 alpha aminoisobutyric acid (AIB) skeletal muscle transport in starvation. The Physiologist 1988;August:A133.

17. DiResta G.R., Shah N.K., Long C.W., Marx W., Arbit E., Mascott C., Mallya K., Bedford R.F. Cerebrovascular response to CO2 in edematous brain during either fentanyl or isoflurane anesthesia. Anesthesia Review 1988;15:27-28.

18. Filc-DeRicco S., Gelbard A.S. Effect of methotrexate on the metabolic fate of N-13 label from l-glutamate in a transplantable rat tumor. J Nucl Med 1988;29:1320.

19. Gelbard A.S., Cooper A.J.L., Asano Y., Filc-DeRicco S. Use of phenylalanine dehydrogenase for the synthesis of l-phenylalanine, l-tyrosine and l-dopa labeled with nitrogen-13. J Nucl Med 1988;29:931.

20. Khayata M., Arbit E., DiResta G., Lau N., Galicich J.H. ICP reduction by lidocaine: Dose response curve and effect on CBF and EEG. 7th International Symposium on Intracranial Pressure and Brain Injury. Ann Arbor, Michigan. June 10-23, 1988.

21. Shah N.K., DiResta G.R., Arbit E., Bedford R.F. Regional cerebral blood flow in malignant supratentorial tumors vs. normal brain. Anesth Analg 1988;67:S204.

22. Shah N.K., DiResta G.R., Long C., Arbit E., Bedford R.F. Perioperative intracranial hypertension in patients with supratenterial tumors: Role of CBF changes vs. importance of mass effect. Proceedings 7th International Symposium on Intracranial Pressure and Brain Injury. Ann Arbor, Michigan, June 19-23, 1988. Intracranial Pressure VII. J. T. Hoff and A.L. Betz, eds. pp 884-885. Springer-Verlag 1989.

23. Sordillo P.P., DiResta G., Conti P.S., Fissekis J., Bading J.R., Nielsen C.M., Benua R.S., Laughlin J.S. Imaging studies using C-11 alpha-aminoisobutyric acid (AIB) in patients with malignant melanoma. J Nucl Med 1988;29:904.

24. Bading J.R., Wilson H.H., Nupnau W.A., Gregg G.M. A multiple-energy gamma camera system for dynamic transport studies. J Nucl Med 1989;30:852.

25. Conlon K.C., Bading J.R., McDermott E.W., Gelbard A.S., Brennan M.F. Capillary clearance and cellular transport of glutamine decline in starvation but not in cancer cachexia. 1989 Proc. Amer. Assn. Cancer Res. 1989;30:17.

PEER - REVIEWED PUBLICATIONS

1. Cooper A.J.L., Nieves E., Coleman A.E., Filc-DeRicco S., Gelbard A.S. Short-term metabolic fate of [13N]ammonia in rat liver *in vivo*. J Biol Chem 1987;262:1073-1080.

2. DiResta G.R., Kiel J.W., Riedel G.L., Kaplan P., Shepherd A.P. Hybrid blood flow probe for simultaneous H2 clearance and laser-Doppler velocimetry. Am J Physiol 1987;253:G573-G581.

3. Ridge J.A., Bading J.R., Gelbard A.S., Benua R.S., Daly J.M. Perfusion of colorectal hepatic metastases. Cancer 1987;59:1547-1553.

4. Bading J.R., DiResta G.R., Corbally M.T., Conlon K.C. Fiberglass limb phantoms: Fabrication and use for quantitative scintigraphy. Med Physics 1988;2:215-220.

5. Cooper A.J.L., Nieves E., Rosenspire K.C., Filc-DeRicco S., Gelbard A.S., Brusilow S.W. Short-term metabolic fate of 13N-labeled glutamate, alanine, and glutamine(amide) in rat liver. J Biol Chem 1988;263:12268-12273.

6. Ridge J.A., Collin C., Bading J.R., Hancock C., Conti P.S., Daly J.M., Raaf J.H. Increased adriamycin levels in hepatic implants of rabbit vx-2 carcinoma from regional infusion. Cancer Research 1988;48:4584-4587.

7. Arbit E., DiResta G.R., Bedford R.F., Shah N.K., Galicich J.H. Intraoperative measurement of cerebral and tumor blood flow with laser-Doppler flowmetry. Neurosurgery 1989;24:166-170.

8. Calderon J., Cooper A.J.L., Gelbard A.S., Mora J. 13N isotope studies of glutamine assimilation pathways in neurospora crassa. J Bacteriology 1989;171:1772-1774.

9. Conlon K.C., Bading J.R., DiResta G.R., Corbally M.T., Gelbard A.S., Brennan M.F. Validation of transport measurements in skeletal muscle with N-13 amino acids using a rabbit isolated hindlimb model. Life Sciences 1989;44:847-859.

10. Gelbard A.S., Cooper A.J.L., Asano Y., Nieves E., Filc-DeRicco S., Rosenspire K.C. Methods for the enzymatic synthesis of tyrosine and phenylalanine labeled with nitrogen-13. Appl Radiat Isot 1989 (in press).

BOOK CHAPTERS

1. Cooper A.J.L., Nieves E., Filc-DeRicco S., Gelbard A.S. Short-term metabolic fate of [13N]ammonia, L-[13N]alanine, L-[13N]glutamate and L-[*amide*-13N] glutamine in normal rat liver in vivo. (Awarded first prize as best paper by the Dutch Academy of Liver Disease). Proceedings 6th International Symposium on Ammonia Metabolism. Maastricht, The Netherlands, April 27-29, 1987 Advances in ammonia metabolism and hepatic encephalopathy. P.B. Soeters, J.H.P. Wilson, A.J. Meijer and E. Holm, eds. pp 11-25. Elsevier Science Publisher 1988.

2. Cooper A.J.L., Gelbard A.S. Metabolism of [13N]ammonia in normal and hyperammonemic rat brain. Proceedings 6th International Symposium on Ammonia Metabolism. Maastricht, The Netherlands, April 27-29, 1987 Advances in ammonia metabolism and hepatic encephalopathy. P.B. Soeters, J.H.P. Wilson, A.J. Meijer and E. Holm, eds. pp 420-32. Elsevier Science Publisher 1988.

3. Cooper A.J.L., Lai J.C.K., Gelbard A.S. Ammonia and energy metabolism in normal and hyperammonemic rat brain. Proceedings Symposium Intr Soc Neurochem and Amer Soc Neurochem. May 26-29, 1987. Miami FLA. Neurology and Neurobiology Vol. 39 The Biochemical Pathology of Astrocytes. MD Norenberg, L Hertz, A Schousboe, eds. pp 419-434. AR Liss, NY Pub, 1988.

4. Conti P.S., Starnes H.F., Bading J.R. Carbon-11 labeled amino acid analogs as imaging agents for in vivo study of amino acid transport in tumors and organ systems. In: Absorption and Utilization of Amino Acids, ed. Friedman M (CRC Press, Boca Raton, Fla., 1989).

END

DATE
FILMED
2/24/94