In the period 1969-1986, this project was directed to the evolution of target-specific labeled chemicals useful for nuclear medical imaging, especially radioactive indicators suited to tracing adrenal functions and localizing tumors in the neuroendocrine system. Since 1986, this project research has focused on the chemistry of positron emission tomography (PET) ligands. This project has involved the evaluation of methods for radiochemical syntheses with fluorine-18, as well as the development and preliminary evaluation of new radiopharmaceuticals for positron emission tomography. In the radiochemistry area, the ability to predict fluorine-18 labeling yields for aromatic substitution reactions through the use of carbon-13 NMR analysis was studied. Radiochemical yields can be predicted for some structurally analogous aromatic compounds, but this correlation could not be generally applied to aromatic substrates for this reaction, particularly with changes in ring substituents or leaving groups. Importantly, certain aryl ring substituents, particularly methyl groups, appeared to have a negative effect on fluoration reactions. These observations are important in the future design of syntheses of complicated organic radiopharmaceuticals. In the radiopharmaceutical area, this project has supported the development of a new class of radiopharmaceuticals based on the monoamine vesicular uptake systems. The new radioligands, based on the tetrabenazine structure, offer a new approach to the quantification of monoaminergic neurons in the brain. Preliminary primate imaging studies support further development of these radioligands for PET studies in humans. If successful, such radiopharmaceuticals will find application in studies of the causes and treatment of neurodegenerative disorders such as Parkinson's disease.

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I. New Methods of \(^{18}\text{F}\)Fluorination.

(a) Production of \(^{18}\text{F}\)Fluoride ion. We have constructed and fully tested small volume (1 ml), high pressure water target for the routine production of \(^{18}\text{F}\)fluoride ion using the \(^{18}\text{O}(p,n)^{18}\text{F}\) nuclear reaction on enriched water. This target design has proven exceptionally successful, and can be used to prepare large quantities of radionuclide (700 mCi) in short times (20 min). To maximize reliability and allow for periodic cleaning (without concommitant downtime for activation products to decay) we have actually constructed three identical targets which are rotated on a periodic basis.

(b) New precursors for nucleophilic aromatic \(^{18}\text{F}\)fluorination. We have prepared and fully examined a series of aryl trimethylammonium trifluoromethanesulfonates as easy to prepare, stable, and highly reactive precursors for the synthesis of \(^{18}\text{F}\)aryl fluorides. The use of the cationic precursors allows both the use of lower reaction temperatures and the simple isolation of neutral, \(^{18}\text{F}\)-labeled products (easily separated from charged precursors). We also examined aryl dimethylsulfonium trifluoromethanesulfonates as precursors, but these proved unsuitable for high yield synthesis of aryl \(^{18}\text{F}\)fluorides. This method of fluorination proved important in the development of \(^{18}\text{F}\)GBR 13119 and \(^{18}\text{F}\)GBR 12909, new radiopharmaceuticals we have used in animal and human studies of the dopamine reuptake system. Furthermore, the synthesis and application of these cationic precursors has been adopted and duplicated by at least two other research groups worldwide.

(c) \(^{18}\text{F}\)Fluorination of thiophene rings. We have extended the nucleophilic aromatic substitution reactions with \(^{18}\text{F}\)fluoride ion to include preparation of fluorothiophenes. The reaction of \(^{18}\text{F}\)fluoride with three thiophene-2-carboxaldehydes was examined. Substitution of the 5-bromo compound gave reasonable yields, but the 4-bromo derivative proved unreactive, and the nitro substituted thiophene gave small amounts of the \(^{18}\text{F}\)fluorothiophene but mostly decomposition products. These results are consistent with the literature on nucleophilic substitution in thiophene rings.

(d) Preparation of \(^{18}\text{F}\)trifluoromethyl groups. A new and potentially powerful method for the synthesis of no-carrier-added \(^{18}\text{F}\)trifluoromethyl groups has been devised, in collaboration with scientists from Parke-Davis Co. Reaction of nca \(^{18}\text{F}\)fluoride ion with bromodifluoromethyl groups yields the \(^{18}\text{F}\)trifluoromethyl groups in good radiochemical yield. This reaction does not require high temperatures or Lewis acid catalysts. This is potentially a method for labeling a wide variety of pharmaceuticals which contain trifluoromethyl substituents.

(e) Synthesis of \(^{18}\text{F}\)fluoro ethers. In an unusual reaction, we have examined the isotopic substitution of 1,1-difluoro-2,2-dichloroethyl ethers by \(^{18}\text{F}\)fluoride ion to yield the corresponding carrier-added, fluorine-18 labeled ethers. This reaction is an isotopic substitution; reaction in the ring was ruled out by successful synthesis of the benzene analog, and the product obtained was identical by all chromatographic methods with authentic starting material. This substitution by nucleophiles is unprecedented in the literature of perfluoroalkyl ethers.

(f) New approach to fluorocatechols. Aromatic nucleophilic substitution has emerged as the best method for high specific activity fluorine-18 labeling of PET radiopharmaceuticals. This reaction requires an electron-activating substituent on the aromatic ring; this substituent must subsequently form a part of the intended radiopharmaceutical, be altered to a more desireable substituent, or be completely removed. As part of this research we have developed methods for changing such activating groups to phenols, exemplified by the oxidation of benzaldehydes to phenols, and have reported methods for synthesis of NCA fluorine-18 labeled fluorophenols and fluorocatechols. This is the only example of reactions of this type in fluorine-18 radiochemistry. More recently we have developed a new method which allows the removal of aldehyde activating groups, yielding simple fluorine-18 substituted ring systems.
reaction sequence uses decarbonylation with Pd-C to convert intermediate [18F]fluorobenzaldehydes to the corresponding aryl [18F]fluorides.

(g) Predicting Yields in Nucleophilic Aromatic Substitution Reactions. Following a published report showing a correlation between 13C-NMR chemical shift and radiochemical yields in nucleophilic aromatic substitution reactions with [18F]fluoride, we have examined the general applicability of this correlation, particularly in regards to different ring substituents and different leaving groups on the aromatic ring. A number of appropriately substituted benzaldehydes and benzonitriles were either purchased or synthesized, and subjected to reaction with no carrier added [18F]fluoride ion. Yields were determined under controlled reaction conditions and reaction times. These yields were then correlated with 13C-NMR chemical shifts, using latter values either measured in our laboratories, reported in the literature, or calculated de novo. We have found that there is a good correlation between 13C-NMR chemical shift and the radiochemical yield within a group of methoxy or dimethoxy substituted benzaldehydes. However, two types of compounds clearly did not fit the correlation line: a trimethylammonium substituted benzaldehyde, and two methyl-substituted benzaldehydes. We have preliminary evidence that the reasons for this is that the kinetics of [18F]fluorination of these derivatives is completely different: the trimethylammonium substituent is displaced far more rapidly the nitro or fluoro leaving groups, and the methyl groups are slowing down the nucleophilic aromatic substitution reaction. The faster kinetics of the trimethylammonium group is precedent in the literature, but the explanation for the deleterious effects of the methyl group is not clear. These studies have import for researchers trying to predict the yields obtainable in fluorine-18 labeling of complex drug structures.

II. Applications of Robotics

We have undertaken and completed evaluation of a robot as a PET radiopharmaceutical dispensing station. We believe we are the only institution which has developed and implemented a robotic approach to PET radiopharmaceutical dispensing. At the Society of Nuclear Medicine Meeting in Toronto, Canada, in June of 1993, a commercial concern (BNFL Engineering, England) unveiled a robotic dispensing system for technetium radiopharmaceuticals. Together, these two robotic systems demonstrate the potential value of such an approach to reduction of radiation doses to technical personnel.

This robot and its associated hardware systems have been developed to replace the human operator in the receiving and dispensing of individual doses of PET radiotracers in the Nuclear Pharmacy. The robot system is comprised of the robot arm and its control system, specially modified pieces of commercially available hardware, as well as specifically designed ancillary mechanical apparatus. The entire assembly is located in a standard three foot chemical hood situated in the Nuclear Pharmacy (Fig 3,4). Operational compliance is assured through active confirmation provided by optical sensing of task completion. Error recovery algorithms have been developed to minimize operator intervention in the event of error detection. Personnel protection is provided via a "light curtain" which immediately halts robot operation when the light field is interrupted. Users interact with the robot system via a menu driven operator interface. Operational menu selections include receiving the tracer agent for immediate use, receiving the tracer agent for eventual use, retrieving a previously received and stored tracer for use now and retrieving a stored tracer for re-assay. Convenience menu selections include a time decayed listing of all stored tracers and a clean-up option to ready the system for a new day.

The robot itself is a Unimate PUMA Mark II series six axis arm developed by Unimation Inc., a Westinghouse company. It uses an adapted DEC PDP-11/73 computer as its imbedded controller and includes a teach pendant. We have installed a 128 Kbyte RAM board as well as a pair of 8 Input/8 Output boards for our application. All ancillary mechanical systems are controlled via these boards and Humphrey Series S410 stackable pneumatic control valves coupled with Compact Air Products, Inc pneumatic cylinders. Additionally, all remote sensing is accomplished with either through beam or reflective fiber optics Photoelectric Controls from Banner Engineering Corporation.

The following is a brief description of the specially developed hardware components that support the operation of the robot system. The equipment is permanently attached to the floor or supporting struts of the overall structure.

RABBIT MODIFICATIONS: The standard pneumatic rabbit that has been in use is about 3-1/2 inches long, 1-1/4 inches in diameter and includes a simple screw-on base. This base was replaced with a snap-on type that includes a socket to receive a standard multi-dose vial and a special slot to enable a guillotine like blade to be used to hold the base during separation from the body.
RECEIVING STATION: This device is affixed to the rabbit delivery line and includes a sliding trap door and an elevating cup for the assembled rabbit to drop into. Upon successful reception of the rabbit the robot is able to remove the rabbit with the internally housed dose vial from this station and place it into the separation station.

SEPARATION STATION: This device includes a vertically displaceable three finger gripper to grasp the rabbit body and a guillotine like blade to hold the base. Successful rabbit separation allows the multi-dose vial to be grasped by the robot and removed from the base for further processing.

VIAL ARRAY: This station is a ten well leaded enclosure with individual elevators designed as a shielded storage area for dose vials. As sequential doses are received the robot control program fills the vial array and designates each well position by either isotope or compound type.

SYRINGE HOLDERS: These devices are aluminum bodied enclosures designed to house 5 and 10 ml Becton Dickinson style sterile syringes. They feature a pivoting access door for loading and unloading new syringes, a conical hole docking port for alignment and stabilization in the Syringe Pump station and a square edged protrusion to block axial movement in the Syringe Shield. They were developed to enable the robot to handle the syringes without direct contact between the robot gripper and the syringes.

SYRINGE SHIELDS: These hinged clam-like lead body devices are designed to shield the PET Technologists from the loaded syringe containing the radioisotope. They are moved in and out of the robot cage as well as transported from the Nuclear Pharmacy to the scanning room. Additionally, they are designed to mount in the socket of the Auto Injector System for remote administration of the patient dose in the scanner room. When mounted in the robot cage the top lid is hinged open to provide access for the robot grippers to grasp the Syringe Holder and to manipulate it as it reads a patient dose. When transporting and injecting the dose the top is locked close to prevent unshielded personnel contact with the radioisotope.

VIAL CUP STATION: This simple device serves two purposes. One is for establishing a precise and repeatable elevation position for the multi dose vial. The other is to facilitate fitting the multi dose vial into the Capintec elevator socket.

CAPINTEC STATION: This station incorporates a commercially available dose calibrator well with a simple elevator system that includes a special socket. The socket is designed to accept the plunger end of the Syringe Holder and to maintain its rotational configuration as well as to receive the Vial Cup. In addition, the socket allows for the insertion of a bare syringe in a normal needle first mode for manual use of the dose calibrator.

WEIGHING STATION: A commercially produced small foot-print balance with an RS-232 interface has been modified to include a small light plastic receptacle for precision placement of the multi dose vial.

DRAW-OFF STATION: This apparatus is formed from a commercially available syringe pump that also includes an RS-232 interface. The normal V-block syringe slot was modified to accommodate the Syringe Holders by designing a saddle like fixture which incorporates a conical locking plunger to securely attach the Syringe Holder in a vertical orientation. Because operation of this system requires both compression and extension of the syringe plunger there is another short stroke gripper which engages and holds the plunger base. In addition, there is also a device to grasp and hold a multi dose vial in an inverted position. This device displaces downward to allow the syringe needle to pierce the resealable rubber septum of the dose vial. It then withdraws upwards decoupling from the needle to allow the robot to remove both the vial and the Syringe Holder for further processing.

III. New radiopharmaceutical development.

(a) Dopamine reuptake blockers. We have prepared, fully characterized, and extensively examined in animal models the new radioligands [18F]GBR 13119 and [18F]GBR 12909. These are selective, high affinity ligands for the dopamine reuptake site. These radiotracers could be successfully utilized to image the DA uptake sites in animals, and [18F]GBR 12909 was further developed and brought into clinical trials. We have also synthesized a number of fluorine-18 and radioiodine-labeled derivatives of the parent compound, [18F]GBR 13119. For the fluorine-18 labeled derivatives, the changes in the structure were intended to reduce the lipophilicity of the drug; the effects of such changes in the structure had been previously examined in in vitro binding assays. The two new derivatives, lacking either one or two methylene groups, were synthesized using the methodology developed for preparation of [18F]GBR 12909 and [18F]GBR 13119. Regional brain distribution was determined in mice, according to previously developed protocols. Surprisingly, both new derivatives showed essentially no localization in
the striatum, and hence no selective binding to the dopamine uptake carrier. This was despite reasonably
good in vitro binding affinities to be expected for these compounds. It is thus clear that structural
modification of the GBR series of compounds will be limited in the search for an optimal in vivo imaging
agent.

As part of this project we have introduced [18F]GBR 12909, a dopamine uptake inhibitor, into
clinical trials for brain imaging. The effects of endogenous transmitter on in vivo binding have not been
previously examined. We have therefore studied, in mice, the effects of acute and chronic treatment with
dopaminergic drugs which should alter endogenous levels of dopamine. Treatment with acute or chronic
pargyline, a monoamine oxidase (MAO) inhibitor, did not affect in vivo binding of [18F]GBR 13119,
despite the known effects of this drug to raise extracellular dopamine levels. Acute amphetamine did
decrease the in vivo binding of [18F]GBR 13119, but it is not possible to distinguish between the ability
of amphetamine to release dopamine and its ability to block dopamine reuptake. Reserpine, a drug known
to acutely and chronically deplete tissue levels of dopamine, had interesting effects on [18F]GBR 13119
binding. Acute reserpine had no effect on the binding of this radioligand, but chronically reserpinized
animals showed a statistically significant decrease in [18F]GBR 13119 binding sites. This would be
consistent with down-regulation of the dopamine uptake system, as a compensatory mechanism for the
lower tissue dopamine levels. This has been previously seen in the norepinephrine and serotonin uptake
systems. Down-regulation of dopamine uptake sites would have important ramifications for the
interpretation of PET scans using dopamine reuptake inhibitors: whereas they have previously been
considered as possible markers of neuronal loss, the in vivo decreases should now be considered as
resulting from either decreased dopamine uptake density per neuron, loss of neurons, or both.

(b) Norepinephrine reuptake blockers. We have prepared and examined in mice a specific high
affinity ligand for the norepinephrine reuptake site, [11C]nisoxetine. Although this radiotracer showed
regional specificity and pharmacological specificity expected of a NE reuptake inhibitor, this compound
shows high non-specific binding which may limit its usefulness in vivo using PET.
(c) GABA reuptake inhibitors. In a collaboration with Parke-Davis company we have prepared
several GABA reuptake compounds as potential mapping agents for this site. These compounds utilized
the chemical synthesis methods developed for the GBR class of compounds.
(d) Tetrabenazine and related benzoisoquinolines. We have prepared two new carbon-11 radiotracers
for the study of vesicular monoamine uptake, as a new approach to the quantification of presynaptic
neuron densities. These radiotracers, [11C]tetrabenazine and [11C]methoxydihydrotetrabenazine, have
been prepared in high yields (30-55% EOB) and high specific activities (>2000 Ci/mmol at end-of-
synthesis).

We have determined the characterization of the pharmacokinetics and pharmacological specificity of
these radioligands in rodents. Both [11C]TBZ and [11C]TBZOME show rapid uptake and egress from
brain tissues, with specific retention of the radioligands in the tissues most heavily innervated with
monoaminergic neurons, striatum (STR) and hypothalamus (HYPO). From the kinetic curves it was
determined that the best target/nontarget ratios, operationally defined as STR/CER and HYPO/CER (CER
= cerebellum), were obtained at 10 and 15 minutes after i.v. injection for [11C]TBZ and [11C]TBZOME,
respectively. It was recognized that the cerebellum is not devoid of monoaminergic neurons, but from in
vitro studies there is a much smaller fraction present relative to the other brain regions. Our studies have
shown some interesting characteristics of the binding of these radioligands, and are summarized below:
Specific binding can be significantly reduced by pretreatment with reserpine; unexpectedly,
complete blocking of striatal binding was not observed. We have repeated these studies, and each time
find that reserpine pretreatment does not completely block [11C]TBZ or [11C]TBZOME binding in the
striatum, although it is completely effective in the hypothalamus. This raises interesting questions of the
relative affinities of these two drugs for the purported two binding sites on the monoamine transporter.
Studies with reserpine also point out the difficulties in simple analysis of tissue ratios when delivery of
radioligand is uniformly disturbed, as can be the case with reserpine.
Specific binding is completely blocked by co-injection of unlabeled TBZ. Even though our results
and the literature suggest that TBZ is essentially completely out of the brain after a couple of hours, we
find that in vivo TBZ binding is still reduced (about 20%) even 4.5 hours after injection of a large dose of
cold TBZ.
Specific binding is reduced by co-injection of ketanserin, a low affinity inhibitor of the TBZ
binding site. The amount of TBZ blocking is dose dependent, but high doses of ketanserin were fatal to
50% of the test animals.
Specific binding is not blocked by haldol, a D2 receptor antagonist. Our observation that haldol does not block [11C]TBZ binding, together with the loss of specific TBZ binding upon a presynaptic lesion (unilateral MPTP-treated monkey, (see below), and 6-hydroxydopamine-treated rats provides evidence that [11C]TBZ does not bind to dopamine receptors, even though it can be shown to have a low affinity (5 mM) in vitro.

Specific binding is unaffected by pretreatment with GBR 12935, the neuronal reuptake inhibitor.

Specific binding is blocked by i.v. injection of possible metabolites of TBZ, including α-TBZOH, β-TBZOH, and 9-desmethylTBZ (OH-TBZ, the phenol).

These preliminary results support the conclusion that in vivo [11C]TBZ and [11C]TBZOMe bind to the vesicular transport site for monoamines. Our preliminary experiments also demonstrate that drug treatments can alter radioligand delivery (as for reserpine, haldol and ketanserin by changes in radiotracer levels in cerebellum) and such phenomena need to be understood and accounted for in any many vivo studies using radioligands.

In Vivo Imaging of [11C]TBZ. We have completed the first successful in vivo imaging of monoamine vesicular transporters in living primate brain is described, using [11C]tetrabenazine ([11C]TBZ) and Positron Emission Tomography (PET). Radioligand uptake into brain is rapid, and at short time periods (10-20 minutes) the higher uptake and retention of the radiotracer in the more densely dopaminergic innervated striatum is clearly visualized. Ratios of striatum to nontarget tissues were around 2; however, it should be recognized that there is not a good non-innervated region to use as a "blank", particularly in a small monkey brain. Specific binding in striatum can be entirely blocked with co-administration of a pharmacological dose (1 mg/kg i.v.) of tetrabenazine. In a unilaterally MPTP-lesioned monkey, specific binding of radioligand was absent in the striatum on the affected side, with no effect on radiotracer distribution in the contralateral striatum. Following successful imaging of [11C]TBZ in monkeys and determination of the expected human dosimetry, permission was sought and received for the administration of [11C]TBZ to human subjects. These studies demonstrated that the vesicular monoamine transporter site could be clearly imaged in the human brain, providing an image of binding of the radiotracer in the striatum, predominantly, but also in the cortex and cerebellum.

Finally, we have sought approval for administration of [11C]methoxytetrabenazine, our radioligand of choice. Limited safety testing with the cold drug (prepared in our laboratories) was done under approval of the University of Michigan Institutional Review Board. Following this study, we have obtained approval for administration of this second, improved radioligand to humans for quantitative PET studies of the monoamine vesicular transporter. These studies began in June 1993.

(e) Radiolabeled inhibitors of brain endopeptidases. Development of radiotracers for in vivo PET (Positron Emission Tomography) studies of neurochemistry has largely centered on ligands for specific receptors, neurotransmitter transport systems, or the enzymes involved in neurotransmitter synthesis or degradation. No efforts have been made to develop radioligands for the protein endopeptidases involved in brain neuropeptide generation and degradation, and general protein processing. Following reports that specific enzymes are involved in the processing of a precursor protein to form the b-amyloid found in brains of Alzheimer's disease patients at autopsy, coupled with the observations that levels of specific peptidases (propyl endopeptidase, angiotensin converting enzyme, membrane amidoendopeptidases) may be altered in brains of Alzheimer's and Huntington's disease patients at autopsy, we have targeted CNS endopeptidases as a potential new area of radiopharmaceutical development for PET. The goal of this project is to develop suitable radioligands for the in vivo study of the distribution of endopeptidases in normal and diseased brains, with initial emphasis on propyl endopeptidase (PEP), a serine protease putatively involved in amyloid precursor protein processing. We have thus prepared PEP inhibitors of two distinct chemical classes.

PEP inhibitors: derivatives of SUAM-1221. Based on the limited structure-activity study of Atack et al (1991), we have prepared a methylamino derivative of SUAM 1221, and labeled it with carbon-11 for in vivo evaluation . The 4-amino derivative of SUAM 1221 was synthesized according to the literature method. This was then methylated with [11C]CH3I to provide the corresponding [11C]methylamino compound in good radiochemical yields (>25%) and high radiochemical purity (>95%). The yield of this reaction has not been optimized. The product was not separated from the starting material, but used as the chemically impure mixture (although radiochemical purity was >95% of the desired methylamino derivative). In preliminary mouse brain distribution studies this carbon-11 compound, even at the low effective specific activity used (because of the accompanying biologically active amino contaminant),
showed a regional brain distribution at 40 minutes after injection which is remarkably similar to that reported for PEP enzymatic activity in rat and rabbit. Levels in striatum, cortex, hippocampus and hypothalamus were significantly (p < 0.005) different from cerebellum, and the level in cortex was significantly (p < 0.05) more than in any other tissue. The cortex/cerebellum ratio is similar or greater than that previously reported using PEP enzymatic activities.

This preliminary work showed several important features. First, these compounds clearly pass through the BBB. Second, the regional distribution roughly matches PEP enzymatic activity, suggesting that one might expect a good correlation between number of enzyme molecules and enzymatic activity. Third, the regional distribution obtained can be thought of as the worst-case result; use of purified, higher specific activity material might be expected to provide even higher regional contrast than that obtained here. In all, the results obtained so far are quite encouraging.

PEP inhibitors: [11C]Y-29274. We have also successfully radiolabeled Y-29794, a high affinity (IC50 = 3 nM) of prolylendopeptidase. This compound was radiolabeled by N-alkylation of the normethyl precursor, and obtained in 10-17% yields (EOB) and high specific activity (>2000 Ci/mmol).

In preliminary mouse brain distribution studies, [11C]Y-29794 did not show any marked regional differences in distribution, but adequate brain uptake (1.5% ID/g at 5 min), and a longer half-life for washout (35 min) than the SUAM derivative. These results were consistent with the more lipophilic nature of this drug.
III. Students 1991-1993

Postdoctoral Scholars Trained:

This DOE-supported basic science research program has provided funds for the training of postdoctoral students in radiochemistry and imaging physics. The following individuals were involved in this work during the grant period.

Dr. Alaa Mourad, Ph.D. (1989-1992)
Dr. Jean DaSilva, Ph.D. (1990-1992)
Fred Buck, M.D. (1989-1990)
Ping Chiao, Ph.D. (1991-1992)
Jon Zubieta, M.D. (1991-present)
Dr. Avgui Charalambous, Ph.D. (1992-present)
Dr. Scott Snyder, Ph.D. (1993-present)

Graduate Students Trained:

The following graduate students have participated in this research project as part of their education and training:


Undergraduate Students Trained:

This grant has also provided research funds for undergraduate Thesis research of a chemistry student:

Ramesh Rengan
Chemistry, B.S., 1992
PROGRESS REPORT AS REPORTED IN 1992

"Radiopharmaceuticals for Diagnosis and Treatment"

DOE DE-FG02-88ER60639

David E. Kuhl, M.D., Principal Investigator
Michael R. Kilbourn, Ph.D., Project Director

Summary

In this grant period we have continued our efforts in the areas of PET basic radiochemistry, radiopharmaceutical synthesis, and preclinical radiopharmaceutical evaluation.

1. Predictive abilities in nucleophilic aromatic substitution with [18F]fluoride. We have completed a study of the predictive abilities for [18F]fluorination of substituted aromatic rings. Although radiochemical yields can be predicted within a very similar group of compounds with similar leaving groups and substituent patterns, generalization to all nucleophilic substitutions with [18F]fluoride is not warranted. Kinetic studies indicate significantly different rates of reactions, depending on ring substituents.

2. Preclinical evaluation of new radiopharmaceuticals. We have synthesized and begun the preclinical evaluation of [11C]tetrabenazine, a new radioligand based on the vesicular monoamine transport system.

3. Our work on [18F]fluorination/decarbonylation reactions, structure-activity relationships in dopamine uptake inhibitors and effects of chronic drugs on radioligand binding have been published.

Details of this work are contained in the following sections. Portions of this work have been reported several meetings in the last year (Society of Nuclear Medicine, Ninth International Symposium on Radiopharmaceutical Chemistry). Manuscripts published, in press or submitted can be found in Appendix I.

PART 1. Predicting Yields in Nucleophilic Aromatic Substitution Reactions.

The correlation between the 13C-NMR chemical shift of the aromatic ring carbon bearing the leaving group and the yield of nucleophilic aromatic displacement with no-carrier-added [18F]fluoride ion was evaluated. In comparison of structurally analogous compounds (fluoro, nitro and trimethylammonium substituted benzaldehydes, benzophenones and benzonitriles), the 13C-NMR chemical shift of the reactive aryl ring carbon correlated quite well with the [18F]fluorination yield ($r^2 = 0.91$) for most but not all ring structures (Fig. 1). Compounds with trimethylammonium leaving groups or methyl ring substituents were found to not fit the proposed correlation. Kinetic studies indicated clearly different rates of reaction for these compounds, with much higher than expected reactivity for the compounds with the cationic leaving group. Competition experiments suggest that low reactivity of methyl-substituted rings may be due to conversion of [18F]fluoride to an unreactive form. Our results indicate that the correlation between [18F]fluorination yields for nucleophilic aromatic substitution reactions and the 13C NMR chemical shift of the aryl ring carbon bearing the leaving group is applicable to numerous structurally analogous compounds, but cannot be simply generalized to aromatic rings with different leaving groups or ring substituents.
Fig. 1. Correlation of radiochemical yields with carbon-13 NMR chemical shift values for various substituted benzaldehydes. Compounds 1-10 were used for the construction of the correlation line shown, with an r² value of 0.91.
PART 2 Preclinical evaluation of new radiopharmaceuticals: [11C]tetrabenazine

We have developed inhibitors of vesicular monoamine uptake as a new approach to the quantification of presynaptic neuron densities.

Chemistry

We have recently developed syntheses of [11C]tetrabenazine ([11C]TBZ) and [11C]methyl-dihydrotetrabenazine (TBZOMe), two high affinity radioligands for vesicular uptake sites. These radiotracers are easily prepared in one-step syntheses, in radiochemical yields of >30% and very high specific activities (>1000 Ci/mmole, end-of-synthesis).

In Vivo Pharmacology

We have begun the characterization of the pharmacokinetics and pharmacological specificity of these radioligands in rodents. Both [11C]TBZ and [11C]TBZOMe show rapid uptake and egress from brain tissues, with specific retention of the radioligands in the tissues most heavily innervated with monoaminergic neurons, striatum (STR) and hypothalamus (HYPO). From the kinetic curves it was determined that the best target/nontarget ratios, operationally defined as STR/CER and HYPO/CER (CER = cerebellum), were obtained at 10 and 15 minutes after i.v. injection for [11C]TBZ and [11C]TBZOMe, respectively. It was recognized that the cerebellum is not devoid of monoaminergic neurons (Slotkin et al, 1978), but from in vitro studies (Henry and Scherman, 1989) there is a much smaller fraction present relative to the other brain regions.

Our studies have shown some interesting characteristics of the binding of these radioligands, and are summarized below:

(a) Specific binding can be significantly reduced by pretreatment with reserpine; unexpectedly, complete blocking of striatal binding was not observed. We have repeated these studies, and each time find that reserpine pretreatment does not completely block [11C]TBZ or [11C]TBZOMe binding in the striatum, although it is completely effective in the hypothalamus. This raises interesting questions of the relative affinities of these two drugs for the purported two binding sites on the monoamine transporter. Studies with reserpine also point out the difficulties in simple analysis of tissue ratios when delivery of radioligand is uniformly disturbed, as can be the case with reserpine.
(b) Specific binding is completely blocked by co-injection of unlabeled TBZ. Even though our results and the literature suggest that TBZ is essentially completely out of the brain after a couple of hours, we find that in vivo TBZ binding is still reduced (about 20%) even 4.5 hours after injection of a large dose of cold TBZ.
(c) Specific binding is reduced by co-injection of ketanserin, a low affinity inhibitor of the TBZ binding site. The amount of TBZ blocking is dose dependent, but high doses of ketanserin were fatal to 50% of the test animals.
(d) Specific binding is not blocked by haldol, a D2 receptor antagonist. Our observation that haldol does not block [11C]TBZ binding, together with the loss of specific TBZ binding upon a presynaptic lesion (unilateral MPTP-treated monkey, see below), and 6-hydroxydopamine-treated rats (Masuo et al 1990) provides evidence that [11C]TBZ does not bind to dopamine receptors, even though it can be shown to have a low affinity (5 mM) in vitro (Login et al, 1982; Reches et al, 1983).
(e) Specific binding is unaffected by pretreatment with GBR 12935, the neuronal reuptake inhibitor.
(f) Specific binding is blocked by i.v. injection of possible metabolites of TBZ, including a-TBZOH, b-TBZOH, and 9-desmethylTBZ (OH-TBZ, the phenol).

Representative distributions of [11C]TBZ and [11C]TBZOMe in control animals are shown in Table 1. These preliminary results support the conclusion that in vivo [11C]TBZ and [11C]TBZOMe bind to the vesicular transport site for monoamines. Our preliminary experiments also demonstrate that drug treatments can alter radioligand delivery (as for reserpine, haldol and ketanserin by changes
in radiotracer levels in cerebellum) and such phenomena need to be understood and accounted for in any many vivo studies using radioligands.

Table 1. Regional mouse brain distributions of $[^{11}C]$TBZ (10 min post inj) and $[^{11}C]$TBZMe (15 min post inj). Data are given as mean ± S.D., for N = 4 animals.

<table>
<thead>
<tr>
<th>region</th>
<th>$[^{11}C]$TBZ</th>
<th>$[^{11}C]$TBZMe</th>
</tr>
</thead>
<tbody>
<tr>
<td>striatum</td>
<td>6.02 ±1.24</td>
<td>6.09 ± 0.69</td>
</tr>
<tr>
<td>cortex</td>
<td>1.96 ±0.31</td>
<td>2.36 ± 0.25</td>
</tr>
<tr>
<td>hippocampus</td>
<td>2.13 ±0.30</td>
<td>2.37 ± 0.18</td>
</tr>
<tr>
<td>hypothal</td>
<td>3.14 ± 0.51</td>
<td>3.82 ± 0.32</td>
</tr>
<tr>
<td>thalamus</td>
<td>2.02 ±0.37</td>
<td>---</td>
</tr>
<tr>
<td>cerebellum</td>
<td>1.77 ±0.26</td>
<td>1.77 ± 0.24</td>
</tr>
<tr>
<td>blood</td>
<td>1.47 ± 0.16</td>
<td>1.71 ± 0.31</td>
</tr>
<tr>
<td>STR/CER</td>
<td>3.39 ±0.30</td>
<td>3.48 ± 0.37</td>
</tr>
<tr>
<td>HYPO/CER</td>
<td>1.78 ±0.11</td>
<td>2.18 ± 0.22</td>
</tr>
</tbody>
</table>

**In Vivo Imaging**

We have completed the first successful in vivo imaging of monoamine vesicular transporters in living primate brain is described, using $[^{11}C]$tetrabenazine ($[^{11}C]TBZ$) and Positron Emission Tomography (PET). Radioligand uptake into brain is rapid, and at short time periods (10-20 minutes) the higher uptake and retention of the radiotracer in the more densely dopaminergic innervated striatum is clearly visualized. Ratios of striatum to nontarget tissues were around 2; however, it should be recognized that there is not a good non-innervated region to use as a "blank", particularly in a small monkey brain. Specific binding in striatum can be entirely blocked with co-administration of a pharmacological dose (1 mg/kg i.v.) of tetrabenazine. In a unilaterally MPTP-lesioned monkey, specific binding of radioligand was absent in the striatum on the affected side, with no effect on radiotracer distribution in the contralateral striatum.

At the time of writing of this application, we had received permissions (Radioactive Drug Research Committee and Institutional Review Board) for human studies of the biodistribution of $[^{11}C]$tetrabenazine in normal human subjects.

**Kinetic modeling**

In preliminary experiments, we have applied a 2-compartment model to the mouse brain distribution of $[^{11}C]TBZ$. This analysis gave distribution values ranging from 1.7 (cerebellum) to 4.36 (striatum), and these values correlate well ($r = 0.97$) with the in vitro distribution of $[^{3}H]$TBZOH binding sites (Henry and Scherman, 1989).
Fig. Correlation between in vitro and in vivo binding of radioligands for vesicular transport sites.

PART 3 Development of Radioactive Gases Containment System

Radioactive Gas containment system. In response to concerns about radioactive gas emissions from our Cyclotron/PET facility, we have undertaken the design, construction and implementation of a unique radioactive gas containment system. The design is a proactive system to remove, compress and store all air passing through our shielded hot cells during a radiochemical synthesis. Compressed gases are stored in high pressure cylinders located in our cyclotron vault, and kept there for an appropriate length of time until they can be released to the environment (complete decay of radionuclides). The system was built with sufficient capacity that a new synthesis, using up to 2.5 Curies of carbon-11, can be initiated at every 40 minute period. The stack exhaust is now permanently monitored, and no radioactive gases now escape out of our facility through the hot cell exhaust stacks. The entire system is self-controlled, using a Programmable Logic Controller with computer access for monitoring/troubleshooting.

This system exceeds all requirements meeting regulatory requirements (federal or state regulatory bodies). The costs of this system were largely met through University funds. Installation of this system did, however, result in 12 weeks of down-time for the cyclotron and radiochemistry laboratories.
"Radiopharmaceuticals for Diagnosis and Treatment"

DOE DE-FG02-88ER60639

David E. Kuhl, M.D., Principal Investigator
Michael R. Kilbourn, Ph.D., Project Director

Summary

In this grant period we have continued our efforts in the areas of PET basic radiochemistry, radiopharmaceutical synthesis, and preclinical radiopharmaceutical evaluation.

1. Development of new methods for fluorine-18 labeling. A new synthetic sequence, consisting of no-carrier-added fluorine-18 labeling of substituted benzaldehydes followed by reductive decarboxylation, has been developed. This new methodology can be applied to the fluorine-18 labeling of a wide variety of drugs not previously accessible through existing fluorine-18 labeling methods.

2. Predictive abilities in nucleophilic aromatic substitution with [18F]fluoride. Following up on a literature report that the ability to radiolabel aromatic rings can be predicted by 13C-NMR chemical shifts, we have examined the generality of this correlation in aromatic rings bearing a variety of substituents. Although the original correlation holds for nitro substituted anisaldehydes, it cannot be extended to other rings substitution patterns.

3. Quantitative Structure-Activity relationships for in vivo radioligands. We have examined the relationship of in vivo localization of various fluorine-18 labeled dopamine uptake inhibitors to their in vitro binding affinities and lipophilicities. We have found that remarkably small decreases in binding affinity result in dramatic losses of in vivo binding to the desired high affinity binding sites.

4. Study of in vivo pharmacology. In order to probe the effects of endogenous neurotransmitter on the in vivo binding of radiolabeled dopamine uptake inhibitors, we have examined the in vivo regional localization of [18F]GBR 13119 after acute and chronic drug treatments which alter the endogenous levels of dopamine. We have found that acute changes in dopamine levels do not affect the binding of this radioligand, but chronic depletion of neurotransmitter results in down-regulation of the in vivo binding sites.

Details of this work are contained in the following sections. Portions of this work have been reported at two Department of Energy co-sponsored symposia, In Vivo Imaging of Neurotransmitter Functions in Brain, Heart and Tumors, (Montreal, CN, August 1990) and Research in PET: International and Institutional Perspectives (Washington D.C., Oct. 1991).

PART I. New Methods for Fluorine-18 Labeling.

Aromatic nucleophilic substitution has emerged as the best method for high specific activity fluorine-18 labeling of PET radiopharmaceuticals. This reaction requires an electron-activating substituent on the aromatic ring; this substituent must subsequently form a part of the intended radiopharmaceutical, be altered to a more desirable substituent, or be completely removed. As part of this research we have previously developed methods for changing such activating groups to phenols, and have reported methods for synthesis of NCA fluorine-18 labeled fluorophenols and fluorocatechols. In the last year, we have developed a new method which allows the removal of aldehyde activating groups, yielding simple fluorine-18 substituted ring systems. The reaction sequence uses decarboxylation with Pd-C to convert intermediate [18F]fluorobenzaldehydes to the corresponding aryl [18F]fluorides:
This has been applied to the synthesis of 3-[18F]fluoro-4-methylphenol and 3-[18F]fluorophenol. This work is reported in publications 8 and 12.

PART 2. Predicting Yields in Nucleophilic Aromatic Substitution Reactions.

Following a published report showing a correlation between 13C-NMR chemical shift and radiochemical yields in nucleophilic aromatic substitution reactions with [18F]fluoride, we have examined the general applicability of this correlation, particularly in regards to different ring substituents and different leaving groups on the aromatic ring. A number of appropriately substituted benzaldehydes and benzonitriles were either purchased or synthesized, and subjected to reaction with no carrier added [18F]fluoride ion. Yields were determined under controlled reaction conditions and reaction times. These yields were then correlated with 13C-NMR chemical shifts, using latter values either measured in our laboratories, reported in the literature, or calculated de novo. We have found that there is a good correlation between 13C-NMR chemical shift for a leaving groups and the radiochemical yield within a group of methoxy or dimethoxy substituted benzaldehydes. However, two types of compounds clearly did not fit the correlation line: a trimethylammonium substituted benzaldehyde, and two methyl-substituted benzaldehydes. We have preliminary evidence that the reasons for this is that the kinetics of [18F]fluorinationof these derivatives is completely different: the trimethylammonium substituent is displaced far more rapidly the nitro or fluoro leaving groups, and the methyl groups are slowing down the nucleophilic aromatic substitution reaction. The faster kinetics of the trimethylammonium group is preceded in the literature, but the explanation for the deleterious effects of the methyl group is not clear. These studies have import for researchers trying to predict the yields obtainable in fluorine-18 labeling of complex drug structures.

A manuscript on this work is currently being prepared for submission.
As part of our development of radiopharmaceuticals for imaging the dopamine reuptake system in man, we have synthesized a number of fluorine-18 and radiiodine-labeled derivatives of the parent compound, [18F]GBR 13119. For the fluorine-18 labeled derivatives, the changes in the structure were intended to reduce the lipophilicity of the drug; the effects of such changes in the structure had been previously examined in in vitro binding assays. The two new derivatives, lacking either one or two methylene groups, were synthesized using the methodology developed for preparation of [18F]GBR 12909 and [18F]GBR 13119. Regional brain distribution was determined in mice, according to previously developed protocols. Surprisingly, both new derivatives showed essentially no localization in the striatum, and hence no selective binding to the dopamine uptake carrier. This was despite reasonably good in vitro binding affinities to be expected for these compounds. It is thus clear
PART 4. In Vivo Pharmacology

Recently, it has become evident that endogenous neurotransmitter levels may affect the in vivo biodistribution of radioligands, and their binding to high affinity sites. As part of this project we have introduce [18F]GBR 12909, a dopamine uptake inhibitor, into clinical trials for brain imaging. The effects of endogenous transmitter on in vivo binding have not been previously examined. We have therefor studied, in mice, the effects of acute and chronic treatment with dopaminergic drugs which should alter endogenous levels of dopamine. Treatment with acute or chronic pargyline, a monoamine oxidase (MAO) inhibitor, did not affect in vivo binding of [18F]GBR 13119, despite the known effects of this drug to raise extracellular dopamine levels. Acute amphetamine did decrease the in vivo binding of [18F]GBR 13119, but it is not possible to distinguish between the ability of amphetamine to release dopamine and its ability to block dopamine reuptake. Reserpine, a drug known to acutely and chronically deplete tissue levels of dopamine, had interesting effects on [18F]GBR 13119 binding. Acute reserpine had no effect on the binding of this radioligand, but chronically reserpinized animals showed a statistically significant decrease in [18F]GBR 13119 binding sites. This would be consistent with down-regulation of the dopamine uptake system, as a compensatory mechanism for the lower tissue dopamine levels. This has been previously seen in the norepinephrine and serotonin uptake systems. Down-regulation of dopamine uptake sites would have important ramifications for the interpretation of PET scans using dopamine reuptake inhibitors: whereas they have previously been considered as possible markers of neuronal loss, the in vivo decreases should now be considered as resulting from either decreased dopamine uptake density per neuron, loss of neurons, or both. This work has been reported in publications 10,11 and 13.

TABLE 1

Effects of chronic reserpine (2 mg/kg i.p., once daily for 3 days, last injection 24 h prior to injection of radiotracer) on the regional mouse brain accumulation of [18F]GBR 13119. Radiotracer was injected i.v. and animals sacrificed at one hour after injection. Values are mean ± S.D. (n = 3 to 6).

<table>
<thead>
<tr>
<th>tissue</th>
<th>control</th>
<th>chronic reserpine</th>
</tr>
</thead>
<tbody>
<tr>
<td>striatum</td>
<td>2.33 ± .63</td>
<td>1.77 ± .19 (-25%)*</td>
</tr>
<tr>
<td>cortex</td>
<td>0.64 ± .13</td>
<td>0.91 ± .14 (+42%)*</td>
</tr>
<tr>
<td>cerebellum</td>
<td>0.58 ± .13</td>
<td>0.75 ± .16 (+29%)*</td>
</tr>
<tr>
<td>blood</td>
<td>1.03 ± .11</td>
<td>1.55 ± .27 (+50%)*</td>
</tr>
<tr>
<td>STR - CER</td>
<td>1.66 ± 6</td>
<td>1.05 ± .39 (-58%)*</td>
</tr>
<tr>
<td>STR/CER</td>
<td>3.66 ± .71</td>
<td>2.25 ± .39 (-39%)*</td>
</tr>
</tbody>
</table>

* p < 0.05 vs. control.
PROGRESS REPORT AS SUBMITTED IN JUNE 1990

During this grant period, 1 Jan 1988-31 Dec 1990, we have successfully developed a number of new approaches to fluorine-18 labeled compounds, prepared several new radiotracers for both animal studies and eventual clinical trials, and explored the utility of a high-quality industrial robot in radiopharmaceutical applications. The progress during the last grant period is summarized briefly in the following sections. Publications arising from this research are listed below and can be found in Appendix I.

I. Summary of accomplishments.

Project 1. New Methods of $^{18}F$ Fluorination.

(1) Production of $^{18}F$ fluoride ion. When this grant period began, high specific activity $^{18}F$ fluoride ion was not available at this institution. We have constructed and fully tested a small volume (1 ml), high pressure water target for the routine production of $^{18}F$ fluoride ion using the $^{18}O(p, n)^{18}F$ nuclear reaction on enriched water. This target has proven exceptionally successful, and can be used to prepare large quantities of radionuclide (700 mCi) in short times (20 min). This target design is used in our routine production target, used daily for preparation of $^{18}F$FDG and $^{18}F$GBR 12909 for clinical studies. To maximize reliability and allow for periodic cleaning (without concommitant downtime for activation products to decay) we have actually constructed two identical targets which are rotated on a periodic basis. The target is more fully described in publication 17.

(2) New precursors for nucleophilic aromatic $^{18}F$ fluorination. We have prepared and fully examined a series of aryl trimethylammonium trifluoromethanesulfonates as easy to prepare, stable, and highly reactive precursors for the synthesis of $^{18}F$ aryl fluorides. The use of the cationic precursors allows both the use of lower reaction temperatures and the simple isolation of neutral, $^{18}F$-labeled products (easily separated from charged precursor). We also examined aryl dimethylsulfonium trifluoromethanesulfonates as precursors, but these proved unsuitable for high yield synthesis of aryl $^{18}F$ fluorides.

This method of fluorination proved important in the development of $^{18}F$GBR 13119 and $^{18}F$GBR 12909, new radiopharmaceuticals we have used in animal and human studies of the dopamine reuptake system. Furthermore, the synthesis and application of these cationic precursors has been adopted and duplicated by at least two other research groups worldwide.

This work is reported in publications 2 and 16.

(3) Synthesis of $^{18}F$fluorobenzyl bromide. We have examined the synthesis of 4-$^{18}F$fluorobenzyl bromide as a precursor for radiopharmaceutical syntheses. The benzyl groups is an important functional group in a number of drugs, including dexetimide (muscarinic antagonist). We have examined three routes to fluorobenzyl bromide. Route (a) utilized the nitrile precursor, which was then reduced to the corresponding aldehyde and brominated. Route (b) again utilized the nitrile precursor, but in this case the nitrile was reduced all the way to the hydrocarbon, which was then brominated. Finally, route (c) utilized the aldehyde as the precursor, which was in the second step brominated; this was the shortest route to the desired product. All of these synthetic routes have worked in low yield on both the carrier and carrier-free scales.

(4) Ring-opening reactions. The opening of cyclic ethers using dimethylboron bromide has been reported to yield the corresponding bromoalcohols, by the nucleophilic attack of bromide ion on the oxygen-Lewis acid complex. We have examined the possibility of interception of the incipient activated ether by $^{18}F$fluoride ion, to yield the corresponding $^{18}F$fluorohydrin. Although the reagent was successful in the ring-opening of cyclohexene oxide to the corresponding bromohydrin, in reactions where $^{18}F$fluoride was added we could not detect the presence of the $^{18}F$fluorohydrin. We are continuing our efforts in this novel approach to fluorination of alkanes.

5. Fluorination of thiophene rings. We have extended the nucleophilic aromatic substitution reactions with $^{18}F$fluoride ion to include preparation of fluorothiophenes. The reaction of $^{18}F$fluoride with three thiophene-2-carboxaldehydes was examined. Substitution of the 5-bromo compound gave reasonable yields, but the 4-bromo derivative proved unreactive, and the nitro substituted thiophene...
gave small amounts of the $[^{18}\text{F}]$fluorothiophene but mostly decomposition products. These results are consistent with the literature on nucleophilic substitution in thiophene rings. This work is reported in paper 5.

6. Preparation of $[^{18}\text{F}]$trifluoromethyl groups. A new and potentially powerful method for the synthesis of no-carrier-added $[^{18}\text{F}]$trifluoromethyl groups has been devised, in collaboration with scientists from Parke-Davis Co. Reaction of nca $[^{18}\text{F}]$fluoride ion with bromodifluoromethyl groups yields the $[^{18}\text{F}]$trifluoromethyl groups in good radiochemical yield. This reaction does not require high temperatures or Lewis acid catalysts. This is potentially a method for labeling a wide variety of pharmaceuticals which contain trifluoromethyl substituents. This work is reported in paper 9.

7. Synthesis of $[^{18}\text{F}]$fluoro ethers. In an unusual reaction, we have examined the isotopic substitution of 1,1-difluoro-2,2-dichloroethyl ethers by $[^{18}\text{F}]$fluoride ion to yield the corresponding carrier-added, fluorine-18 labeled ethers. This reaction is an isotopic substitution; reaction in the ring was ruled out by successful synthesis of the benzene analog, and the product obtained was identical by all chromatographic methods with authentic starting material. This substitution by nucleophiles is unprecedented in the literature of perfluoroalkyl ethers. This work is reported in papers 10 and 29.

8. New approach to fluorocatechols. As part of our interests in the synthesis of fluorine-18 labeled catecholamines, such as dopamine, DOPA, and norepinephrine, we have examined a new synthetic route to no carrier added fluorocatechols. This approach utilizes a substituted o-salicylaldehyde as the synthetic precursor, which is first substituted with $[^{18}\text{F}]$fluoride ion and then oxidized to the catechol. By this route we have prepared NCA $[^{18}\text{F}]$fluorocatechol. This work is reported in paper 28.

9. $[^{18}\text{F}]$Fluorination of pyridazines. We have also examined the reaction of $[^{18}\text{F}]$fluoride ion with chloropyridazine, nitrogen heterocycles which may be substitutes for benzene rings in certain drugs. We have successfully prepared several $[^{18}\text{F}]$fluoropyridazines by this approach, including one which does not have activation by an electron-withdrawing group. This work is reported in publication 30.

10. New radiotracer development. This research grant has supported in part the development of four specific types of radiotracers, dopamine reuptake inhibitors, calcium channel blockers, GABA reuptake inhibitors, and norepinephrine reuptake blockers.

a. Dopamine reuptake blockers. We have prepared, fully characterized, and extensively examined in animal models the new radioligands $[^{18}\text{F}]$GBR 13119 and $[^{18}\text{F}]$GBR 12909. These are selective, high affinity ligands for the dopamine reuptake site. These radiotracers could be successfully utilized to image the DA uptake sites in animals, and $[^{18}\text{F}]$GBR 12909 was further developed and brought into clinical trials. In attempts to improve the kinetic behaviour of this compound, a limited QSAR study was done, showing that some alterations (substitution of a thiophene ring) are allowed but other changes (shortening of alkyl chain) are not. The results in this area are in publications 1,2,3,4,5,7,8,12,13,15,19,20,22,23,25, and 26.

b. Norepinephrine reuptake blockers. We have prepared and examined in mice a specific high affinity ligand for the norepinephrine reuptake site, $[^{11}\text{C}]$nisoxetine. Although this radiotracer showed regional specificity and pharmacological specificity expected of a NE reuptake inhibitor, this compound shows high non-specific binding which may limit its usefulness in vivo using PET. This data is reported in publications 6,14 and 15.

c. GABA reuptake inhibitors. In a collaboration with Parke-Davis company we have prepared several GABA reuptake compounds as potential mapping agents for this site. These compounds utilized the chemical synthesis methods developed for the GBR class of compounds. Animal work with these new compounds is underway. The chemical results are described in publications 9,24 and 27.

d. Calcium channel blockers. With an interest in the diacylpeperazine class of calcium channel blockers, due to their similarity in structure to the dopamine reuptake inhibitors, we have prepared fluorine-18 labeled cinnarazine and flunarizine. Initial animal studies did not show regional binding to the DA uptake site. This work is reported in publications 11 and 21.
Project 2. Applications of Robotics in Radiopharmaceutical Synthesis.

Our initial goals were to incorporate robotics into the synthesis of PET radotracers. However, continued successful chemical work (funded through DOE grant DE-FG02-87ER60561, D.E. Kuhl, P.I.) has eliminated the need for a robot in most of our syntheses. We have therefore evaluated a robot in three other applications.

(1) Material handling. We have utilized the PUMA robot for the insertion and sealing of product vials into the "rabbits" used in our pneumatic delivery system. This was a high dose, manual operation which defeated the goals of reduction of radiation dose to personnel. The robot proved quite adept at this operation. This need was eliminated, however, by redesign of the rabbits and the pneumatic tube sending station.

(2) Metabolite analysis. A routine, repetitive task in PET studies is the analysis of blood samples for radotracer metabolites. This is an excellent applications point for robotics. To this end, we have modified standard laboratory equipment (syringes, centrifuges, vortex mixers) to operate in tandem with the PUMA robot. The robot is thus capable of performing metabolite analyses using standard SEP-PAK technology for separations.

In this work we have showed the feasibility of a robotic approach to this task. It was clear that a simpler, less expensive robot (i.e., fewer degrees of freedom in the robot arm) would function just as well.

(3) Preparation of Copper-62 generators. Our most recent application of the robot is in the preparation of generators for copper-62. The chemistry behind the production of the parent nuclide, zinc-62, and assembly of a resin column used for the generator have been reproduced from the literature (our cyclotron, at 30 MeV, can prepare zinc-62 readily). We have assembled a compact robot cage (Figure 1) which fits inside a standard-sized chemical hood. The robot operates in the inverted position, as this provides the greatest envelope of working space. Specialized hands for this robot have been constructed in our facility. The robot system is currently undergoing evaluation as a safe, automated method for the production of copper-62 for PET studies in our institution.

Project 3. Review of fluorine-18 chemistry.

This grant has also supported the work by the Project Leader Michael R. Kilbourn in the research for and writing of a comprehensive review of fluorine-18 radiopharmaceutical chemistry, which is in press (to be published by National Academy of Sciences). This is publication 31.
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FIGURE 1
PROGRESS REPORT AS REPORTED IN 1987

Research Accomplishments: Period 1985-1986

1. Radiopharmaceutical Development For The Heart, Brain, Pituitary and Adrenal Cortex (Donald Wieland, Ph.D.):

   a) The vasopressor agent metaraminol (MR) has been shown in our laboratory to closely mimic norepinephrine in its ability to map peripheral adrenergic nerves. Not only is MR more specific for noradrenergic nerves than the benzylguanidines, unlike norepinephrine, it is neither a substrate for catechol-O-methyltransferase nor monoamine oxidase. Metaraminol holds two major advantages over norepinephrine: (1) because of its resistance to metabolism, MR should be easier to develop a quantitative tracer kinetic model for; (2) greater in vitro chemical stability of MR should make it easier to F-18 label. Considerable effort over the past 11 months has been aimed at F-18-labeling MR for use as neuronal imaging agent for the heart. Our initial efforts with F-18-acetylhypofluorite have been reported in abstract form (33). More recent efforts have been more fruitful; MR has been successfully mercurated in the 6-position and experiments are now in progress to regiospecifically incorporate F-18 in the 6-position by reaction of the 6-mercuro-N-t-BOC derivative of MR with activated F-18-N-fluorosulfonamides or the more traditional F-18-acetylhypofluorite.

   b) Meta-iodobenzylguanidine (MIBG) cannot be used to map brain catecholamine stores because it fails to penetrate the blood-brain-barrier. Work from 1983 to 1986 supported by this grant showed that certain lipophilic protracer forms of MIBG, such as I-125-trifluoroacetyl-MIBG, enter the brain and apparently undergo hydrolysis to parent MIBG (29,34). This promising effort was funded (August 1, 1986). Beginning in 1987, this phase of the protracer work will no longer be funded by D.O.E., but it must be stressed that D.O.E. support was critical in establishing the initial feasibility of the overall protracer approach. Basic design principles that we have learned from this work will be profitably applied to developing other brain imaging agents.
c) Various I-125-N-phenalkyl analogs of spiperone have been synthesized as possible dopamine D-2 receptor binding agents for imaging the brain and pituitary. Although high striatum-to-cerebellum ratios were obtained with one of these compounds, the absolute brain concentration was low (32). Attempts are now being made to lower the lipophilicity of this agent to decrease blood protein binding and hopefully increase brain extraction. In vitro D-2 receptor binding affinities of all compounds will be determined by Dr. Anne Young in the Neurosciences Department.

d) Three I-125 labeled analogs of the 11-beta hydroxylase inhibitor etomidate have been tested in rats and dogs. One compound showed favorable adrenal cortex-to-nontarget ratios but none of the compounds warranted further testing due to high hepatobiliary uptake. Two other analogs (compounds 5 and 6 in reference 30) are slated for evaluation before the study is complete.

2. Radiolabeled Antibodies (Richard Wahl, M.D.):

a) Radioimmunotherapy and Diagnosis of Glioblastoma Using Monoclonal Antibodies: Richard L. Wahl, M.D., Monica Liebert, Ph.D., James Taren, M.D., Paul McKeever, M.D.

Specific Aims: To better diagnose and ultimately treat brain tumors using radiolabeled monoclonal antibody technology.

Progress: In the past year, several monoclonal antibodies reactive with brain tumors have been produced using the somatic cell fusion technique and murine parental myelomas. We have not yet produced a totally tumor-specific antibody, but one of our antibodies reactive with an extracellular matrix component shows promise (45). We are expanding our efforts on evaluating this agent in vitro for tissue reactivity and as regards radiolabeling. Also, in collaboration with Dr. Paul McKeever, we have evaluated the behavior of several antibodies related to aggressiveness of brain tumors (15).

We have also conducted extensive studies in animals on the rate of clearance of radiolabeled antibodies administered intracranially as compared to intravenously and have evaluated their localization following direct i.c. injection. In brief, there is a major delivery advantage to brain following i.c. injection, and it persists for days. In addition, there is diffusion of non-specific antibody from the injection site which is adequate to reach brain tumors. A draft of a manuscript ready for submission to the J. of Neurosurgery (28) clearly illustrates this delivery advantage. In addition, in preliminary experiments, there appears to be an even greater delivery advantage for fragments of antibodies given i.c. as compared to i.v., This is likely due to more rapid renal clearance of the antibody via the kidney.
Our current efforts are now centered on establishing human brain tumors intracranially in the nude rat and at evaluating a new monoclonal antibody reactive with the GD2 ganglioside (provided by Dr. Cheung) present in high concentrations in human brain tumors (and to a much lesser extent in normal brain). We hope to be able to evaluate both non-specific and specific monoclonal antibodies within brain tumors in an animal model in the coming year, as well as expand our growing repertoire of monoclonal agents reactive with brain tumors.

b) Intralymphatic Delivery of Antibodies for Lymphoscintigraphy: Richard Wahl, M.D.

Preliminary data were reported on this area in our initial submission. In brief, the intralymphatic route of antibody delivery has been capable of delivering large amounts of antibody to tumor foci in nodes. The delivery has been far-superior to that seen by i.v. delivery in many cases (25). This work is being extended to human studies supported by an NIH grant, Monoclonal Antibody Lymphoscintigraphy in Melanoma, with Dr. Wahl as P.I. This work is showing promise (46).

Work in the past year on lymphoscintigraphy has been directed at evaluating the kinetics of monoclonal antibodies and their fragments (27). We also have evaluated the kinetics of TcHSA, Tc antimony colloid, and TcMDP following subcutaneous injection (46). Clearly antimony colloid is highly accumulated, but TcMDP and TcHSA are also heavily retained. The progressive accumulation of Tc antimony colloid is in contrast to the uptake of TcHSA, antibodies, and TcMDP. Of particular interest was the fact that TcMDP, apparently free of colloid, could be injected sq and then reach lymph nodes, possibly indicating that colloidal status was not essential for nodal uptake (26). A manuscript is submitted regarding this work (26), which was also presented at the Society of Nuclear Medicine meeting in Washington, D.C. (47).

In the coming year, we plan to carefully examine, both by node counting and by autoradiography, the localization in nodes of antibodies given sq and directly into the dermis as relates to dose/charge/isotype etc. We also plan to see if non-specific nodal uptake can be diminished.

c) Monoclonal Antibodies to Small Cell Carcinoma of the Lung: Ralph Stevens, Ph.D., Monica Liebert, Ph.D. and Richard Wahl.

During the past year we have presented data indicating that we could successfully image in vivo in the nude mouse model, small cell carcinoma of the lung (48) with each of three monoclonal antibodies we have produced reactive with small cell carcinoma (49). This work is in press in a monograph from the National Cancer Institute (14). Unfortunately, detailed characterization of these reagents has shown that they have reacted with antigens that: 1) are not stable with paraffin determinants, 2) are not totally specific to small cell cancer and 3) the antibodies studied are not stable to standard labeling.

We currently are evaluating a more promising reagent (6G10) reactive with small cell carcinoma. This agent is now undergoing extensive in vitro testing by immunoperoxidase techniques. We hope, that if it maintains its current specificity, that it may be useful in diagnosing and treating
small cell carcinoma using radiolabeled antibody techniques. We also have done careful studies determining the toxicity of varying doses of radiolabeled antibodies given i.p. or i.v. as a prelude to rational therapy (50).


Previously we reported that radiolabeled monoclonal antibodies reactive with T-cell subsets could be useful in the diagnosis of transplant rejection (9). This observation was in a model system that mimicked a non-vascularized skin graft in some respect, and a vascularized graft in others. The key determination is whether there is any utility of this technique in the diagnosis of transplant rejection in rejecting vascularized grafts. To this end we evaluated the possibility of radiolabeled anti-rat T cells localizing to rejecting allografts of heart transplants and to normal hearts. In brief, two anti-rat T-cell antibodies show some potential in the detection of transplant rejection, both OX-8 and W3/25 have this potential (51). We are currently evaluating ways to optimize labeling of these agents and other antibodies that may be more specific for the activated T-cells responsible for graft rejection.

e) Imaging Tumor Heterogeneity with a Slit Aperture Tomographic Device:

Therapy with radionuclides is dependent upon adequate target localization of the therapeutic agent, low non-target uptake, and radiosensitivity of the target tumor. Agents with radiotherapeutic potential are being developed, yet dosimetric estimates still are based on the assumption of uniform tumor uptake. This assumption if untrue, as is often the case, can foil dosimetry attempts. To this end we have preliminarily evaluated the macro-heterogeneity of human tumor xenograft uptake (In 111 56G.4 uptake into ovarian carcinoma xenografts in nude mice) by SPRINT scanning. The SPRINT tomograph, developed by Dr. Les Rogers, is capable of high-resolution imaging of tumors and other structures. Through this work we have preliminary data showing tumor heterogeneity by imaging. This work will be expanded, with definitive autoradiographic correlation in the coming year, and will be correlated with standard gamma imaging using pinhole collimation. The influence of the heterogenous uptake will also be determined on eventual dosimetric estimates. This work lays a groundwork for the construction of imaging devices with even more capability in terms of measuring tumor uptake accurately, and thus in predicting dose to portions of a tumor.

3. Pharmacology of and therapy with radiolabeled meta-iodobenzylguanidine (James Sisson, M.D.).

MIBG acts as an analog of the natural adrenergic hormone and neurotransmitter, norepinephrine (NE). As such MIBG is concentrated in adrenergic tissues and this property has enabled scintigraphic portrayal of tumors such as pheochromocytomas and neuroblastomas. MIBG is also sequestered by adrenergic neurons and its uptake may reflect nerve integrity and density, and its rate of disappearance may be an index of nerve function. Thus, we propose hypothesis that MIBG will determine the presence and activity of adrenergic neurons within organs, particularly the heart.

a) Experiments in rats demonstrated that the $^{123}$I-MIBG taken up by the heart resides largely in intra-neuronal sites. Rates of disappearance of $^{125}$I-MIBG from the heart correlated with neuronal activity. However, only 50%
of $^{125}\text{I}-\text{MIBG}$ in salivary glands appeared to be in intra-neuronal locations and disturbances of nerve function within these glands must be more substantial than in the heart before they are detectable as changes in MIBG uptake or loss.

b) The results of experiments using $^{125}\text{I}-\text{MIBG}$ in rats were, for the most part, duplicated in man with $^{123}\text{I}-\text{MIBG}$. Generalized autonomic neuropathy was associated with little or no uptake of $^{123}\text{I}-\text{MIBG}$ in the heart. Adrenergic nerves in salivary glands appeared more resistant to the injury of generalized neuropathies, but the regional denervation of Horner's Syndrome was detected by differences in uptake of $^{123}\text{I}-\text{MIBG}$ between the two parotid glands of the patient.

c) Twenty-eight different patients with malignant pheochromocytoma have received one-to-six doses of $^{131}\text{I}-\text{MIBG}$, 100-220 mCi/dose. Partial remissions have been observed in about a fourth of the patients, and untoward effects have been minor and transient. Follow-up evaluations, including radiation dosimetry of tumor and body, in these patients will help to determine factors that may predict a favorable response.

d) Twelve patients with neuroblastoma have received treatments similar to those given to patients with malignant pheochromocytomas. Responses have been less impressive, and the smaller size of these patients (children) caused a greater radiation dose to bone marrow so that a decrease in circulating platelets was the rule, and leucopenia was a frequent consequence. Evaluations of dosimetry should lead to a better strategy of $^{131}\text{I}-\text{MIBG}$ therapy wherein the radiopharmaceutical may be used sequentially with chemotherapy to attack cells via multiple avenues.
Research Accomplishments: Period 1986-1987

A. Radiopharmaceutical Development For the Heart (Donald M. Wieland, Ph.D.):

Work continues on the development of a $^{18}$F-labeled analog of metaraminol (MR), a compound that acts as a norepinephrine storage analog. MR has been mercurated selectively in the 6 position. Fluorodemercuration of 6-HgOAc-MR-t-BOC with $^{18}$F-acetylhypofluorite (ref. 30) has provided after Sep-Pak purification and acid treatment $^{18}$F-6-fluorometaraminol ($^{18}$F-MR). $^{18}$F-6-FMR is a highly selective marker for peripheral noradrenergic nerves as shown by pharmacological studies with the norepinephrine uptake blocker desmethylinipramine and sympathectomy studies with the neurotoxin 6-hydroxy-dopamine. Pretreatment of rats with either of these agents lowers the uptake of $^{18}$F-6-FMR in the heart by > 85%. PET imaging of dogs administered $^{18}$F-6-FMR provides excellent tomographic images of the heart. Dogs with neuronal defects induced by regional application of phenol on the heart are presently under study. The synthesis of $^{18}$F-4-FMR has also been accomplished; it will undergo biological evaluation in the coming months. The 4-isomer is expected to be metabolized to a lesser extent than the 6-isomer by liver hydroxylase.

B. Pharmacology and Therapy With Radiolabeled Meta-Iodobenzylguanidine (James C. Sisson, M.D.):

This project has been based on the concept that MIBG acts as an analog of the neurotransmitter and hormone, norepinephrine. Therefore, experiments further established the concept of analogy between MIBG and norepinephrine. We also tested the hypotheses that diagnostic doses of MIBG would enable development of indices of the integrity and function of adrenergic neurons, and that large doses of MIBG would impart therapeutic radiation of adrenergic tumors, particularly neuroblastomas.

1) Experiments in animals have established the similarities and differences between MIBG and norepinephrine in adrenergic neurons, particularly in the heart and salivary glands. These results have been published or accepted for publication (see list of publications).
Initial experiments have been aimed at demonstrating that a larger portion of MIBG than norepinephrine resides in the cytosol of neurons, a concept that would explain the more rapid loss of MIBG from neurons.

Also preliminary experiments are aimed at establishing an animal model where adrenergic neuron function can be decreased and increased. Clonidine, an alpha-2 agonist, will be useful in this model.

The regional localization of MIBG and norepinephrine in the rat heart is being demonstrated by autoradiography of $^{125}$I-MIBG, $^{123}$I-MIBG and $^3$H-norepinephrine.

2) The experiments in animals have been duplicated in man. The results of these experiments have been accepted for publication in the Journal of Nuclear Medicine (ref. 14).

3) Using large doses of $^{131}$I-MIBG, we have treated two additional patients with neuroblastoma. The toxicity of $^{131}$I-MIBG in six patients will be described in a paper presented at the Annual Meeting of the Society of Nuclear Medicine on June 4, 1987 (ref. 27). The effects of these therapies on the neuroblastoma and the subsequent course of patients will be sent for publication upon completion of radiation dosimetry.

C. Development of New Radiolabeled Muscarinic Cholinergic Antagonists (G. Keith Mulholland, Ph.D. and Charolotte A. Otto, Ph.D.):

We are evaluating a group of antimuscarinic compounds as potential PET ligands for the central muscarinic receptor. These compounds can readily be labeled in high specific activity using $^{11}$CH$_3$I or $^{18}$F fluoride. The binding abilities of the cold compounds to central receptors are being evaluated in mice by *in vitro* as well as *ex vivo* methods. The *in vitro* tests involve incubation of brain homogenates with varying concentrations of cold drug followed by $^3$H-quinuclidinyl benzilate (QNB) to determine drug Ki values. In the *ex vivo* tests varying amounts of cold drugs are injected into mice. After a certain time period the animals are sacrificed, brain homogenates are prepared and the level of unbound muscarinic receptors is determined with $[3^H]$QNB. The *ex vivo* approach can detect differences in pharmacokinetics and metabolism between the various drugs which are not apparent from *in vitro* studies. These differences are important factors in deciding on the best candidate drug for PET labeling.

D. Radiolabeled Antibodies (Richard L. Wahl, M.D.):

1) Radioimmunotherapy and Diagnosis of Glioblastoma Using Monoclonal Antibodies: Richard L. Wahl, M.D., Monica Liebert, Ph.D., James Taren, M.D., Paul McKeever, M.D.

Specific Aims: To better diagnose and ultimately treat brain tumors using radiolabeled monoclonal antibody technology.

Progress: The safety and efficacy of direct intracranial installation of monoclonal antibodies has been established in normal mice. The manuscript describing this work will be submitted to the Journal of Neurosurgery. Several promising monoclonal antibodies to brain tumors have been produced. The Fab fragment is not interesting as far as this
mode of delivery is concerned. It is probable that separate funding will be solicited for the continuation of this work, most probably from the NCI.

2) Intralymphatic Delivery of Antibodies for Lymphoscintigraphy: Richard Wahl, M.D.

The preliminary data from this work allowed Dr. Wahl to secure an NIH grant for clinical studies in patients with melanoma. More basic investigations using Tc99m MDP, various classes and fragments of monoclonal antibodies, and albumin, antimony and other antibody reagents were conducted as a part of the DOE supported work. Of interest is that IgM antibodies are taken up more to nodes than IgG's, a finding previously unrecognized.

3) Monoclonal Antibodies to Small Cell Carcinoma of the Lung: Richard Wahl, M.D.

This project has been largely terminated due to the difficulty in producing a sufficiently tumor-specific reagent. The 6G10 monoclonal antibody, though reactive with virtually all small cell carcinomas, also reacts with more normal tissues than expected, and may not be useful in this endeavor. At present, work on this project has been suspended.

4) Detection of Transplant Rejections Using Radiolabeled Antibodies: Richard Wahl, M.D.

This work has progressed in that a very specific monoclonal antibody reactive with the T-cell growth factor receptor (expressed on activated lymphocytes) has been labeled and used to scintigraphically detect cardiac transplant rejection in the rat allograft model. This monoclonal antibody, ART-18, appears more promising in terms of target/background ratios than did the OX-8 and W3/13 reagents. This preliminary work is of sufficient promise that an application for NIH funding will be prepared.
MAJOR ACCOMPLISHMENTS DURING THE LAST FIVE YEARS

Radiopharmaceutical Development Research: (Direct DOE support)

Introduction of $^{131}$I-meta-iodobenzylguanidine (MIBG), a tracer agent that has shown diagnostic localization in pheochromocytoma, neuroblastoma, and carcinoid tumors. (Wieland).

Established the value of $^{123}$MIBG in mapping the peripheral adrenergic nervous system, especially that of the heart. (Wieland and Sisson)

Established the basis for developing selective positron-emitter labeled enzyme inhibitors of neuronal specific enzymes in the brain. (Wieland).

Positron Emission Tomography Research: (Associated with DOE supported research)

Introduction of a new PET reconstruction technique which reduces variance in image pixel values without significantly degrading resolution. (Hutchins)

Development of a new tracer kinetic model which should make possible the in vivo determination of regional muscarinic acetylcholine receptors in humans using PET. (Frey and Agranoff)

Development of a new test/retest method of cerebral blood flow determinations using PET and $^{18}$F-fluoromethane. (Koeppe)

Development of a new SPECT camera (SPRINT II) designed to improve resolution and quantification. (Rogers).

Discovery that abnormal patterns of glutamate and flunitrazepam receptor binding are characteristic in Alzheimer's Disease and in Huntington's Disease and may be demonstrable by PET. (Young and Penney)

Demonstration with PET-FDG that basal ganglia metabolism is highly correlated with overall functional capacity of patients with Huntington's Disease. (Young)

Discovery that caudate hypometabolism can be found using PET-FDG in mentally retarded patients with Lesch-Nyhan Syndrome, an inherited metabolic disease. (Pallela and Kelley)

Principal Investigator, David E. Kuhl, M.D. (Direct DOE support at UCLA prior to joining Michigan faculty in 1986).

Introduction and validation of the use of $^{123}$I-IMP for quantitative mapping of local cerebral blood flow using SPECT.

First demonstration of a characteristic, abnormal, cerebral metabolic pattern determined by PET in an inherited disorder, Huntington's Disease. First demonstration that this metabolic abnormality was detectable in some at-risk persons before the appearance of symptoms.

First demonstration in patients with partial epilepsy that the site of seizure onset is hypometabolic and may be detected by FDG-PET to aid localization in planning for surgical treatment.

Demonstration that accentuated parietal hypometabolism determined by PET-FDG is an early finding in Alzheimer's Disease and is also found in demented patients with Parkinson's Disease.
Papers published:


Papers in press:

1. DaSilva JN, Kilbourn MR, Domino EF: In vivo imaging of monoaminergic nerve terminals in normal and MPTP-lesioned primate brain using positron emission tomography (PET) and $^{11}$C-tetrabenazine. Synapse (in press).

Abstracts published:


PAPERS PUBLISHED AS REPORTED IN 1992


Abstracts :


PAPERS PUBLISHED AS REPORTED in 1991


Papers in Press:


Abstracts:

Manuscripts


Abstracts


Books

III. Postdoctoral scholars trained 1988-1990

Although this DOE grant has not provided funds for the training of postdoctoral chemists, the grant has been used to support the laboratory work of a number of postdoctoral fellows. The following list of individuals were involved in this work during the grant period.

Dr. G. Leonard Watkins  
Director of PET Chemistry  
University of Iowa

Dr. Michael S. Haka  
Research Investigator  
Division of Nuclear Medicine  
University of Michigan  
Ann Arbor, MI

Dr. Pulak Chakraborty  
Research Fellow  
Division of Nuclear Medicine  
University of Michigan  
Ann Arbor, MI

Dr. Alaa Mourad  
Research Fellow  
Division of Nuclear Medicine  
University of Michigan  
Ann Arbor, MI

Dr. Raghu Subramanian  
Midwest Research Institute  
Kansas City, MO
PAPERS PUBLISHED AS REPORTED IN JULY 1987.

BIBLIOGRAPHY, WITH TITLES OF PUBLICATIONS ASSOCIATED WITH THIS CONTRACT

Publications from 1969 - 1984, 15 year period of funding by D.O.E.


151. Shapiro B, Copp J, Sisson J, Eyre P, Beierwaltes WH: The efficacy of 131-I-metaiodobenzylguanidine (MIBG) scintigraphy for location of phenochromocytomas; experience in 400 cases. Accepted for presentation at the 7th International Congress of Endocrinology, Quebec City, Canada. July 1-7, 1984 (abstract).


172. Wahl RL, Philpott GW, Parker CW: Successful preparation of F(ab')2 fragments from murine monoclonal antibody. (For submission to J Immun Methods).

173. Wahl RL, McClenann BL, Rodey GA: HLA-typing of reactors to parenterally administered radiographic contrast media.