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UNDER A CONTRIBUTION FROM THE
NATIONAL RESEARCH COUNCIL OF CANADA

OCTOBER 2001

The contributions on individual experiments in this report are outlines intended to demonstrate the extent of scientific activity at TRIUMF during the past year. The outlines are not publications and often contain preliminary results not intended, or not yet ready, for publication. Material from these reports should not be reproduced or quoted without permission from the authors.

LIFE SCIENCES

Introduction

The major news for the Life Sciences Program was the awarding of a $1,550,000 grant from the Canadian Foundation for Innovation to the PET group for the purchase of a new, high-resolution, research tomograph. It is anticipated that a companion grant application to the Provincial Government for $1,499,000 will also be successful. These grants, plus the $1,000,000 from the Canada Institutes of Health Research, will enable the PET group to install and characterize the scanner which has a resolution of 8 μl compared to the existing tomograph which has a resolution element of about 200 μl. In addition, the new tomograph will be about 3 times more sensitive than the existing camera.

As indicated in the various reports, collaboration with other groups continues to grow with projects involving the BC Cancer Agency and the University of Alberta as well as projects with Vancouver Hospital and Lions Gate Hospital. The project with Chemical Engineering at UBC appears to have exciting preliminary results which may have implications on the quantity of water needed for manufacturing paper.

The PET Target group also submitted a patent on the production of ultra high quantities of ^{18}F-fluoride from a gas target. A new water target fabricated from niobium was installed and yields are much more reliable than from the titanium one. These developments will be required in the new year as demands for more shipments of radioisotopes to Edmonton will increase.

Experiment LS0
PET facilities
(K.R. Buckley, TRIUMF)

The PET facilities comprise the TR13 13 MeV H$^-$ cyclotron, the ECAT 953B/31 tomograph, and ancillary equipment such as counting and data acquisition systems.

TR13 cyclotron

Usage of the TR13 cyclotron rose significantly (113% by delivered beam) this year over 1999, reflecting mostly an increased demand for irradiation of lithium targets for the production of ^{7}Be (LS8). Usage of beam rose almost fourfold for LS8, while most other projects remained constant with the addition of new LS projects making use of beam time (LS32, 35, 39).

Downtime this year was significant and was caused by a number of different systems, mostly due to age related wear. A small, and as yet undetermined, leak in the ion source has resulted in four filament changes this year. The cryopump system required extensive servicing and ultimately the cold head displacer assemblies and compressor were replaced with funds budgeted for this purpose from the PET-CIHR grant. The rf amplifier tube and ion source turbo pump were replaced this year. One vacuum valve required servicing *in situ* when it developed a leak and it was discovered that extensive dismantling of the injection line would be required to remove the valve.

Intermittent problems with a power supply in the rf amplifier led to significant downtime throughout the year. This supply was built by TRIUMF and would be very expensive (~$20k) to replace with a commercial unit. It has worked reliably until this year so it seems optimistic that the problem can be found and corrected. Downtime has been reduced since the fault was traced to this supply, but as yet the specific fault with the supply has not been found.

Ten extraction foil changes were required through the year, as well as one rebuild of the ^{18}O-O$_2$ gas target and four rebuilds of the CH_4 gas target. The titanium bodied water target for the production of fluoride was replaced this year when it developed a leak in a weld joint. The new target is constructed of niobium and is performing very well to date.

In addition to the above target work, an aluminum bodied water target was installed for the production of ^{13}N from natural water. This target allows us to supply the Botany group from the TR13, a capability which proved necessary during major repairs to the CP42 this fall (LS8).

One shipment of ^{18}F to Edmonton has occurred this year (LS32).

Presently there are six target locations occupied of the available eight. These consist of

- one ^{18}O-O$_2$ gas target
- one ^{18}O water target
- one ^{16}O water target
- one CH_4 gas target
- one experimental gas target
- one lithium metal target

The lithium target (LS8) has a reserved location and is installed and removed for each production. This target runs routinely for 15 hours at 50 μA.

ECAT tomograph

The ECAT too is showing signs of aging with new types of failures appearing.

While block detector failures continue, we have now repaired two blocks in house and are in the process of repairing more. We received information from CTI on how to access the voltage dividers in the block assembly and information on the specifications of components in use on the dividers. We are now able to repair detector faults at this level and reseal the blocks. At present only a simple visual balance of PMT outputs is performed on the bench, but we intend to assemble the necessary components to properly calibrate the blocks prior to returning them to the scanner.

Other faults with the scanner have been traced to failed programmable logic chips in the position/energy processors on the buckets. These chips are programmed off-line and are intended to hold their programming for up to 20 years. Fortunately this circuitry is repeated for each block detector in the scanner and we were able to obtain and program new ICs with the data from an IC in a functioning channel.

An intermittent problem with 2D reconstructions in the front end of the scanner implicates a fault in the VME processor which controls data acquisition. Since this processor is obsolete, CTI is the only source of a replacement. One has been obtained and we are in the process of trying to determine whether this will solve the intermittent troubles. We are hoping this processor will also solve other intermittent troubles with data archiving to tape which have cropped up over the last couple of months.

The ^{68}Ge calibration line sources were replaced this fall with commercial sources purchased from Sanders Medical. The uniform cylinder source was replaced also, purchased from Isotope Product Laboratories.

Statistics

Table XIV. TR13 run data.

Total runs conducted	808	
Total runs lost	42	
Integrated charge delivered	923229	μA-mins
Delivered to – LS2	4506	
– LS3	264122	
– LS4	11219	
– LS8	559218	
– LS13	46456	
– LS24	23598	
– LS32	1142	
– LS35	3167	
– LS39	9155	

Table XV. ECAT scan data.

Total scans conducted	387
Total scans lost	103
Lost to – patient	31
– cyclotron	42
– chemistry	6
– scanner	24

Experiment LS2
Synthesis of radiohalogenated carbohydrates for use as imaging agents in PET and SPECT
(M.J. Adam, TRIUMF)

A number of relatively commonly-occuring genetic diseases are the result of deficiencies in lysosomal enzymes (glycosidases) responsible for the hydrolysis of glycosides. The most common such ailment is Gaucher's disease, which is caused by an inherited deficiency in glucocerebrosidase. This results in the accumulation of glucocerebrosides, mainly in the cells of the mononuclear phagocyte system. Other lysosomal diseases include Tay-Sachs disease, which is due to a deficiency in hexosaminidase A, and Hurler's syndrome, which is due to a lack of iduronidase.

Gaucher's disease can now be treated by enzyme replacement therapy, and good progress has been made in the development of other such therapies. Both diagnosis of the presence and the severity of the disease, as well as the monitoring of treatment, would benefit from new non-invasive methods of quantitating and localizing enzymes *in vivo*. Selective covalent inhibitors of these glycosidases that can be detected by positron emission tomography (PET) would be valuable in this regard. Excellent candidates for this task are 2-deoxy-2-fluoro-α-D-glycopyranosyl fluoride. These compounds have been shown to function as specific mechanism-based inactivators of "retaining" α-glycosidases via formation of a relatively stable 2-deoxy-2-fluoro-α-glycopyranosyl-enzyme intermediate. Therefore, by use of a 2-deoxy-2-[^{18}F]-fluoroglycopyranosyl fluoride it should be possible to radiolabel all functional glycosidases of that class and then localize them with PET. In an earlier approach to this problem we synthesized 2-deoxy-2-[^{18}F]-fluoro-α-D-mannopyranosyl [^{18}F]-fluoride and showed that it covalently derivatized Agrobacterium sp. α-glucosidase. We further showed that the trapped species was catalytically competent since it was capable of "turnover" with release of 2-deoxy-2-fluoro-mannose and native enzyme. Unfortunately, tests of this derivative *in vivo* revealed that hydrolysis occurred with formation of 2-deoxy-2-fluoro-mannose.

The choice of the synthesized 2-deoxy-2-fluoro-α-D-mannopyranosyl fluoride was a consequence of the relative simplicity and speed of its synthesis in radiolabelled form via electrophilic addition of [^{18}F]-fluorine gas to D-glucal. A better, more specific, analogue would appear to be synthesized 2-deoxy-2-[^{18}F]-fluoro-α-D-glucopyranosyl fluoride. However, rapid synthesis of this derivative in the desired α-anomeric form is non-trivial. Further, should spontaneous hydrolysis occur, the release of 2-deoxy-2-fluoro-glucose would become rapidly phosphorylated and trapped in the usual manner, obliterating any imaging from labelled glycosidase. A possible solution to both problems was offered by replacement of the 6-hydroxyl group with fluorine which prohibits possible phosphorylation and offers a simpler means of radiolabelling using higher specific activity ^{18}F-fluoride. Earlier studies had demonstrated the tolerance of such enzymes for modifications of the substrate at the 6-position. Therefore, we have reported this year the synthesis and radiolabelling of 2,6-dideoxy-2,6-difluoro-α-D-glucopyranosyl fluoride (2,6FGF) 5 as a potential imaging agent for glucocerebrosidase, and the enzyme kinetic data obtained with Agrobacterium sp. α-glucosidase (Abg) and human glucocerebrosidase (GCase).

Experiment LS3
Synthesis of radiopharmaceuticals for positron emission tomography
(M.J. Adam, TRIUMF)

Daily routine production of up to 10 radiopharmaceuticals is currently performed with 3–4 radiophamaceuticals synthesized in one day. There was a total of 528 production syntheses carried out in 2000.

Out of these 10 radiopharmaceuticals, four (FDOPA, Raclopride, TBZ-OH, and MP) are used most heavily. The large increase in the number of Raclopride preparations this year is due to the "scatchard analysis" experiment that Doris Doudet is performing on monkeys. These experiments require 3–4 Raclopride preparations each day where one is at high specific activity (5–10 Ci/μmol) and the others have carrier added to give a range of specific activities of 2–35 Ci/mmol at time of injection. All preparations for these experiments are assayed by HPLC prior to injection to confirm the specific activity.

The demand for FDG for our group remains low. However, most of our FDG production is being used by Lions Gate and Vancouver Hospitals for studies in oncology and cardiology. We currently send FDG to these hospitals one day per week in amounts sufficient for imaging approximately three patients.

We have also started a pilot study into the feasibility of shipping ^{18}F-fluoride to Edmonton for tomograph calibrations as well as FDG production (see LS32). In our first shipment we produced 300 mCi for shipment and they received about 30 mCi in the lab several hours later. Nordion is handling the airline transport and Edmonton is paying all the transportation costs. More deliveries are planned.

The synthesis of ^{11}C-PMP was undertaken this year. PMP is an acetylcholinesterase inhibitor that will be used by Dr. Chong to study dementia in Parkinson's disease and diffuse Lewy body disease. Previous studies in postmortem brains have shown that choline acetyltransferase activity, but not muscarinic cholinergic receptors, was markedly reduced in PD, diffuse Lewy body disease and Alzheimer's disease and that neocortical cholinergic deficits correlate with severity of dementia in PD, diffuse Lewy body disease and Alzheimer's disease. As reduced cholinergic activity in the neocortex correlates with marked neuronal loss in the nucleus basalis of Meynert, these observations suggest that loss of cholinergic neurons in the basal forebrain may play a significant role in the pathogenesis of dementia in PD and diffuse Lewy body disease as well as in Alzheimer's disease. Recently, tracers for cholinergic neurons have become available for PET studies in human subjects. By using PET to explore cholinergic factors that contribute to the development of dementia in both PD and diffuse Lewy body disease, we will cultivate a better understanding of the pathogenetic mechanisms underlying dementia in PD and diffuse Lewy body disease. This should ultimately lead to better strategies for the pharmacological management of PD and diffuse Lewy body disease. The first human scans will start in February, 2001.

We continue to use TBAF in most of the ^{11}C methyl iodide reactions to enhance and stabilize the yields. We are fortunate to still obtain the nitro precursor of Setoperone as a gift from Jannsen Pharmaceuticals. We are also grateful to ASTRAZENECA AB for the gift of Raclopride Tartrate, used as carrier for the scatchard experiments, and Dihydroxy Raclopride as the precursor for labelling. However, for high specific activity preparations (SA = 5000–10,000 Ci/mmol) we use our own precursor since the Astra precursor contains significant amounts of carrier (maximum SA = 500–1,000 Ci/mmol). The Astra material is suitable for the carrier added runs. All other precursors are synthesized in house which requires a major time commitment from Dr. Lu.

Several new computers have been purchased and installed on the chemistry systems this year. Lookout software still needs to be implemented in some of the processes. A new QC HPLC has been purchased and installed as well as other new equipment for the lab to replace some of the 20-year-old items such as a rotary evaporator and fridge. We also anticipate a new hot cell being installed sometime early in the new year which will be located in place of the fume hood that now contains the FDG and FDOPA systems. These systems are about 15 years old now and will need to be redesigned and upgraded so that they can be housed in the new hot cell. The upgrade to the FDG system is a priority because of rapidly increasing demands for this agent by outside users such as VGH, LGH and St. Paul's Hospital. Fluoro-dopa is still heavily used by our own Movement Disorder group.

Experiment LS4
Targets for PET radioisotope production
(T.J. Ruth, TRIUMF)

New gas targets for the high yield production of ^{18}F-fluoride

There is growing demand throughout the world for a large production capacity of clinical doses of FDG. Thus there is a real need to develop a superior high yield target system.

Therefore we investigated a possible route to a higher yield target system for ^{18}F-fluoride production. Both approaches rely on gas target designs since TRIUMF has developed the technology to build gas targets capable of operating at proton beam currents greater than 100 μA.

The fluoride target will use the ^{18}O-O_2 gas target concept in an analogous manner to the F_2 double shoot system. The first irradiation would generate the ^{18}F as fluoride that sticks to the walls. But instead of performing a second irradiation, the target is washed out with water in a similar fashion to that used for ^{123}I production by MDS Nordion. Depending on the energy of bombardment, extremely high yields may be possible. For example, the saturation yields at 13 MeV are approximately 200 mCi/μA and 250 mCi/μA at 18 MeV. Thus for a 2 hour run at 100 μA one can produce 8 Ci and 10 Ci respectively, even if one has an 80% recovery from the target wash (50% saturation $\times$ 0.8 = 0.4 $\times$ saturation yield). At these rates one can imagine very long shipments. The advantages include straightforward and efficient recovery of the target material, and higher beam currents than is possible for water targets (or liquids in general). Disadvantages include having to wash and dry the target between runs and finding the best material for this process.

For the new target systems, we investigated the various steps involved in preparing ^{18}F as fluoride.

- Operation of gas target at 100 μA.
- Windows, cooling, pressure/chamber size, etc.
- Recovery of target gas.
- Efficient washing of target.
- Selection of target chamber material.
- Drying target.
- Elution of ^{18}F radioactivity.
- Recovery of ^{18}F fluoride from large volume of water.

Target materials tested thus far:

- Ni plated Al – (66.4% + 7.4%) = 73.8%, n = 4
  - H_2O Temp = 80°C
  - H_2O T60 = 50.4%
  - H_2O T20 = 38.4%
  - Each wash = 10 mL
- SS – (80.6% + 12.6%) = 93.2%
- Al – (5.6% + 1.8%) = 7.5%
- Glassy Carbon = 98.3% (single wash)

Next we spiked 30 ml of water with fluoride and passed the resulting solution through our standard QMA ion exchange resin. The ^{18}F-fluoride was quantitatively extracted onto the resin. Elution with potassium carbonate Kryptofix solution resulted in more than 90% recovery.

We performed a series of proof of principle experiments using a modified stainless steel cooling jacket to serve as a target. The irradiations were performed at 25 μA for between 15 and 30 minutes using a 5% ^{18}O-O_2 mixture. Water heated to 80°C was passed through the target and over an ion retardation column (QMA) and into a waste vessel. The column was then removed and washed with a bicarbonate solution to recover the ^{18}F-fluoride. The results of these experiments are as follows.

- Approximately 70% of theoretical yield recovered from target (n = 4). Target washes were of 100 ml volume.
- Distribution – 65% on QMA, 3% passed through column, 3% drained from target.
- Unable to measure what was retained in target (we have no way of determining whether the radioactivity was stuck on the target walls or simply not produced).
- >95% of the $^{18}F^-$ extracted by QMA was eluted with 2 $\times$ 2 ml washes of bicarbonate solution.

These results were accepted for publication in the spring.

MDS Nordion routinely operates its Xe gas target at over 100 μA. Therefore, in principle, it is possible to design a low energy gas target to operate under these conditions. In order to operate at the 50–100 μA beam current, the target cooling system must be optimized. The key aspects of the He cooling window design include flow rate, pressure, the number of ports (inlet and outlet), and the direction of the ports. Preliminary results indicate that He pressure is one of the most important parameters, as well as inlet port direction.

Experiment LS8
Radiotracers for the physical and biosciences
(T.J. Ruth, TRIUMF)

The following projects are designed to take advantage of TRIUMF's unique accelerator facilities.

The production of ^{7}Be will be done in collaboration with the Beryl group at TRIUMF as part of their effort to measure the cross section for the $^7\mathrm{Be}(p,\gamma)^8\mathrm{B}$ reaction to address anomalies in the international solar neutrino measurements. Routine irradiations are under way (see Expt. LS0, this Annual Report).

^{13}N tracers have been used extensively at UBC for more than 10 years to study the kinetics of nitrogen incorporation by plants. The program has grown and looks forward to continued collaboration with TRIUMF in these studies.

Based on results from the nitrogen studies, the researchers in Dr. Glass' lab wish to explore the kinetics of potassium uptake in plants as a function of nitrogen concentration. In order to perform these experiments they will need access to ^{42}K.

Researchers in the Physics Department need a long-lived isotope of silver for the nuclear orientation experiments. ^{110m}Ag is the candidate of choice. Target and chemistry developments have been completed for producing ^{110m}Ag by irradiating ^{nat}Pd with low energy protons.

Details of botany program

Rice research (International Rice Research Institute (IRRI) of the Philippines)

We have demonstrated that the half-life of NH_4^+ turnover in roots of rice appears to be independent of the concentration of NH_4^+ provided to plants. This constancy, which involves the integration of influx, efflux, fluxes to vacuolar and assimilation, extends to short term perturbations of influx associated with elevating or reducing external [NH_4^+]. Despite a brief perturbation of influx and efflux, the half-life of $^{13}NH_4^+$ exchange is restored within minutes to its original value. The implications for overall control of all reactions leading to NH_4^+ accumulation within the cytosol of root cells is profound.

Nitrogen uptake in trees. A collaboration with BCRI

This project is funded by NSERC and BCRI for 3 years. The goal is to express the high-affinity NO_3^- transporter gene (AtNrt2.1), that we cloned from Arabidopsis thaliana [Zhuo *et al.* (1999)], in hybrid spruce and in poplar seedlings. Since spruce is notoriously inefficient at absorbing NO_3^- [Kronzucker *et al.* (1997)] our goal is to increase the seedling vigour of these plants for reforestation programs. We used tobacco as a model system to evaluate the methodology, and have successfully produced transgenic tobacco plants and obtained a limited number of lines that over-express the high-affinity transporter gene. We are awaiting sufficient numbers of transgenic plants to measure their $^{13}NO_3^-$ uptake rates. In order to assess the relative effects of over-expression of this gene, it has been necessary to characterize $^{13}NO_3^-$ influx in wild-type tobacco, spruce and poplar. We have completed the characterization of NO_3^- transport systems in all three species during 1999–2000. In the near future we will be evaluating $^{13}NO_3^-$ transport in transgenic plants. A preliminary report of this work was presented by Dr. Simon in Bonn in September.

NH_4^+ compartmentation in plant tissues

Dev Britto has now completed work on the compartmentation of N in plant tissues and analyses of $^{13}NH_4^+$ fluxes in leaves and roots of various crop plant and wild species. His thesis has been approved by his research committee and will be sent out to the external examiner before the end of term. Analyses reveal that excessive NH_4^+ uptake in species susceptible to NH_4^+ toxicity leads to substantial (∼40%) increases in respiration that are not involved in NH_4^+ assimilation *per se*, but rather in the energy-dependent pumping out of NH_4^+ in a futile attempt to reduce cytosolic [NH_4^+].

$^{13}NH_4^+$ uptake by roots of Arabidopsis

Dr. Rawat has isolated insertional mutants, in which the gene encoding the high-affinity NH_4^+ transporter (AMT1.1) in A. thaliana is disrupted. The point of this methodology is to critically test the hypothesis that the gene in question is actually crucial for NH_4^+ transport. Since this gene was cloned in 1994, four related high-affinity genes have been identified from this species. To what extent do these other genes participate in NH_4^+ transport? By rendering the AMT1.1 inactive it is possible to evaluate the potential role of the other genes. Dr. Brent Kaiser has continued this work and fully characterized the molecular biology aspects of this mutant and has recently begun to characterize NH_4^+ transport in these mutants. Our first experiments appear to indicate little change in the rates of NH_4^+ influx, indicating that the other genes are able to compensate for the disruption of AMT1.1.

A preliminary report of this work was presented at the annual meeting of the German Soc. Plant Nutrition held in Giessen in September.

$^{13}NO_3$ influx in the fungus Aspergillus nidulans. A collaboration with St. Andrews, Scotland

The first eukaryotic NO_3^- transporter genes (crnA) were cloned from Aspergillus nidulans by Dr. Unkles and co-workers in 1991. The fungal gene served as a model system for higher plant studies, enabling the corresponding genes (highly conserved through evolution) to be isolated from roots of barley and Arabidopsis. We have cloned a second crnA homologue and also generated strains of the fungus which are mutated in each of these genes as well as a strain that is mutated in both genes. We have been able to characterize the contributions of each of the fungal gene products by means of $^{13}NO_3^-$ flux analysis. This work is in preparation for publication.

The double mutant, lacking any capacity for $^{13}NO_3^-$ transport, will be used to characterize seven different homologues of the fungal gene that we have been able to clone from A.thaliana. The strategy will be to transform the Aspergillus double mutant with the plant genes (one at a time) and evaluate their capacity to transport $^{13}NO_3{}^-$.

The pathway of $^{13}NH_4{}^+$ assimilation in GDH mutants of tobacco. A collaboration with the University of Illinois in Carbondale

The normal pathway of $NH_4{}^+$ assimilation in crop species may be blocked by the herbicide (inhibitor) phosphinothricin. Dr. Lightfoot generated an alternate pathway of $NH_4{}^+$ assimilation in transgenic tobacco by over-expressing a bacterial enzyme glutamate dehydrogenase (GDH). His transgenic plants were immune to the herbicide by virtue of having an alternative pathway to assimilate $NH_4{}^+$. Proof was needed to demonstrate that the alternative pathway was indeed operating in this transgenic plant. Using $^{13}NH_4{}^+$ it was possible to show that $^{13}NH_4{}^+$ assimilation proceeded normally in the transgenic plants (by virtue of the alternative pathway) while non-transgenic plants incorporated virtually no $NH_4{}^+$. The accumulation of elevated $NH_4{}^+$ proves to be toxic. This work has been written up for publication.

Sabbatical visitors

The availability of ^{13}N in a context that allows for good biochemical and physiological experiments has resulted in a string of foreign and local visitors who have made use of the availability of ^{13}N. Dr. Unkles, from St. Andrews, visited from November to December. A postdoctoral fellow from Dr. Lightfoot's lab in Carbondale, IL was to have undertaken the work on the GDH transgenics but was unable to visit UBC. Together with a research associate we were able to complete the project earlier (May/June).

Production of ^{110m}Ag

A stack of two ^{nat}Pd foils was bombarded in February. The chemical processing involved dissolving the palladium target, removing excess acid through evaporation, followed by taking up the Pd in 0.01 M HCl. The Pd and Ag are separated on an alumina column with the Pd fraction eluting with the 0.01 M HCl. All indications are that the Ag fraction remains at the top of the column. Once all of the Pd has been removed, as evidenced by colour change of the column, the Ag fraction can be eluted with 0.1 M HCl. The Ag is eluted in less than 10 ml.

Aliquots of both fractions were assayed for radioactive species. The Pd fraction does not have a strong γ-ray for identification. We will have to develop a technique to determine the relative radiochemical purity, although from the colour change on the column we are certain that it is greater than 90% separation.

The silver fraction contained radioisotopes which could be identified as $^{105,\,106m,\,110m}$Ag. We performed a γ-ray spectroscopic analysis on the unseparated foil to estimate production rates. The estimated EOB yields are as follows:

- ^{105}Ag – combined foils – 26 mCi/μA at saturation;
- ^{106m}Ag – combined foils – 2.3 mCi/μA at saturation;
- ^{110m}Ag – combined foils – 0.75 mCi/μA at saturation.

At this rate it would require about 1250 μAh to achieve the required 100 μCi. However, the coproduction of ^{105,106m}Ag would be about 15 and 2.2 mCi, respectively. The exact mix would depend upon the beam current and length of bombardment.

In order to proceed we have had to modify a target so that we can irradiate with a beam of about 50 μA. At this intensity it will require about 25 hours of beam that could be spread over several days. Following irradiation, the foil would be left in place for at least 40 days to allow the decay of ^{105}Ag (50% remaining) and ^{106m}Ag (0.04 remaining). Even at this level there would be about 4.80 mCi of ^{105}Ag left. We could of course allow for a longer decay period, if needed, for the physics experiment.

Experiment LS10
Aptamer imaging agents
(H. Dougan, TRIUMF)

This work has been concerned with a DNA aptamer which has the potential to bind thrombin and image thrombi. A model thrombus in the rabbit jugular vein was used to test the radiolabelled aptamer *in vivo*. Primary goals were to measure the extent of aptamer binding, and to test if binding was specific for thrombin. Secondary goals were to relate *in vivo* results to previous *in vitro* biochemistry diffusion experiments, and to provide an *in vivo* model where the intactness of DNA could be readily evaluated. Several approaches were evaluated: (i) probe with labelled clot, (ii) imaging with labelled clot, (iii) imaging with labelled clot and heparin, (iv) imaging with applied label and clear veins, (v) fluoroscopy with applied label, double label. Without anticoagulant, the thrombus model was prone to occlude, trapping aptamer and giving the false impression of aptamer binding. On the other hand, anticoagulant would invalidate quantitative binding studies with thrombin. We turned to fluoroscopy, where bloodflow could be verified with iothalmate injections. Ovalbumin was the neutral control. The amount of aptamer binding was low, 0.1% to 1% on a single pass. We had previously shown that the lifetime of the aptamer in circulation is short, permitting only a single passage through the thrombus. The low uptake would make it difficult to detect images, and it restricts biochemical applications of the assay. More encouraging results were obtained to a second question, whether the bound aptamer indicated thrombin, and not merely non-specific diffusion. Aptamer (ODN 1 or 2) and ovalbumin labelled the clot equally, when applied externally. A positive aspect was that after 1 hour, the ratio was 3:1 aptamer (ODN 2) to ovalbumin. This appears consistent with aptamer retention. Alternative explanations must still be ruled out. A preliminary manuscript is available.

Experiment LS29
Production and distribution of FDG for clinical studies
(T.J. Ruth, TRIUMF)

The Canadian Society of Nuclear Medicine has petitioned the regulatory authorities in Canada to strike a committee to establish the environment in which PET radiopharmaceuticals would be regulated. The committee would comprise the stakeholders who produce, use and regulate the radiopharmaceuticals labelled with positron emitting isotopes.

Experiment LS32
^{18}F-H$_2$O to the University of Alberta
(S. McQuarrie, Alberta)

Due to a recent success in obtaining major support from the Canadian Foundation for Innovation (CFI), the University of Alberta in collaboration with the Cross Cancer Institute is now proceeding with the design and construction of a new building that will house the cyclotron and chemistry labs necessary for the production of research radiopharmaceuticals. The proposed schedule will see the completion of a building by September, 2001 with the cyclotron installed and running before the end of 2001. We have recently obtained our first shipments of ^{18}F as fluoride from TRIUMF and we wish to have these shipments continue on a routine basis through 2001 up to the commissioning of our own machine.

The ^{18}F shipments are required in order to gain expertise in the labelling and handling of fluorinated compounds having applications in oncology. In particular, we would like first to acquire skills required to prepare and use ^{18}F-fluorodeoxyglucose (^{18}F-FDG). The value of ^{18}F-FDG in cancer diagnosis and in the assessment of treatment outcomes has been well documented. A second project will establish a synthesis of ^{18}F-fluoroestradiol. This product will be used in a research project to identify and determine treatment outcomes of breast cancer patients who show positive estrogen receptor status in their disease. As we gain expertise with fluorine chemistry we will also attempt other syntheses such as ^{18}F-misonidazole or the ^{18}F-nucleoside analogue ^{18}F-FIAZA developed at the University of Alberta. The recent acquisition of an ADAC C-PET camera will permit a limited clinical research investigation on lung cancer patients using ^{18}F-FDG and breast cancer patients with ^{18}F-FES. We have purchased a Coincidence Technologies synthesis box which will be used in the preparation of ^{18}F-FDG. We have applied for funding from the Alberta Breast Cancer Foundation, which will assist in the development of an automated synthesis unit for ^{18}F-fluoroestradiol. The development and preliminary investigations with these agents will require a routine supply of ^{18}F-fluoride.

An application has also been submitted to the Alberta Heritage Foundation for Medical Research to purchase an animal PET camera such as the microPET camera from Concord Microsystems Ltd. A variety of researchers from the Cross Cancer Institute and the University of Alberta have submitted proposals for the use of this system with ^{18}F products and some of this work will be initiated with the availability of ^{18}F-FDG and other ^{18}F products.

Experiment LS33
Evaluation and improvement of a dual head coincidence camera
(V. Sossi, TRIUMF)

The ADAC molecular coincidence dual head camera was installed at Lions Gate Hospital in North Vancouver in August/September, 1997. The attenuation correction hardware and software was implemented in September, 1999.

Studies accomplished this year

1. The effect of radioactivity, outside the field of view shielding, on image contrast was defined.
2. A patient shield has been built that reduces the scatter by 9%.
3. Experiments to determine the new NEMA NU2-2000 performance standards have been completed.
4. Monte Carlo investigation of scatter is under way as part of a Ph.D. thesis.

Experiment LS35
Development of ^{18}F labelled nitroimidazole PET imaging agents for tissue hypoxia
(M.J. Adam, TRIUMF)

Hypoxia in cells and tissues is an important component of various pathological states (e.g. ischemia and stroke). Hypoxic tumour cells are extremely important within cancer treatment because they are more likely to survive radiation and chemotherapy, leading to an increase in tumour resistance to treatment. More recent evidence suggests that hypoxia is related to the aggressiveness of disease. Such studies employed a microelectrode, used in many centres, but were limited because of invasiveness and requirement for an accessible tumour. Derivatives of 2-nitroimidazole are used extensively as hypoxia markers. The 2-nitroimidazoles are not metabolized in oxygenated tissues, but bind to macromolecular proteins after reduction in hypoxic cells. This permits detection by a variety of techniques. For example, the products of such binding for the (pentafluoropropyl)acetamide (EF5) and (trifluoropropyl)acetamide (EF3) derivatives of 2-nitroimidazole can be detected by specific fluorescent antibodies. Earlier studies used 2-nitroimidazoles labelled with radioactive isotopes such as ^{3}H and ^{14}C, followed by autoradiographic methods. These compounds provided proof, in principle, for detection of tissue hypoxia. However, the high level of radioactivity, toxicity and price limit their application. Furthermore, this is also an invasive approach.

The preparation of hypoxia markers carrying ^{18}F allows for the use of the PET technique. The first and most investigated compound of this type is [^{18}F]fluoromisonidazole, which was developed by the Seattle group. Although widely used in research, this compound is not ideal as it shows some hypoxia-independent tissue retention. Recently, the synthesis of another fluorinated nitroimidazole, [^{18}F]fluoroetanidiazole, was reported, but it is not clear if it has the right redox and pharmacokinetics.

The synthesis of ^{18}F-EF5 has now been developed by Dolbier *et al.*, and was achieved by preparing the allyl precursor and fluorinating it with ^{18}F elemental fluorine in trifluoroacetic acid. The radiochemical yield is 17% after HPLC purification. During this year the chemistry to synthesize ^{18}F-EF5, and the subsequent purification step, was established in the TRIUMF laboratory, and several syntheses have been performed for cell and animal studies. Initially we carried out cell studies to determine the effects of hypoxic and aerobic conditions on the uptake of EF5. This experiment indicates that about 10-fold more hot EF5 is concentrated in cells under hypoxic conditions as compared to aerobic conditions, and that the addition of cold EF5 has little effect. The gamma counting results were basically the same as those obtained by using cold EF5 and flow cytometry. These cell experiments were followed by rat experiments; rats with 9L tumours were injected with ^{18}F-EF5 and imaged on our PET tomograph, followed by dissection to determine biodistribution.

An image of the brain tumour in the rat was obtained. Although the image quality is very poor, it indicates a significant uptake on the brain tumour bearing rat as compared to the image of a normal rat.

The next stage of the research is to proceed to human studies. Two proposals have been submitted for funding by research groups at the BC Cancer Research Agency. One study will focus on the use of EF5 in lung cancer, and the other on breast cancer.

Experiment LS37
^{125}Xe implantation as a source for ^{125}I brachytherapy
(J.S. Vincent, TRIUMF)

The objective was to determine whether radioxenon implanted 20–50 Å beneath common metal surfaces is stable and could provide a source of radioiodine for brachytherapy devices.

Two shifts of beam time were used to investigate the implantation of radioxenon into steel, titanium and gold using TISOL, the on-line test isotope separator. Steel foils have been analyzed to date. Implanted at 12 and 22 kV, ^{127}Xe seems to be stable within the statistics of measurement ($\sim$10%) up to 600 hr after bombardment at room temperature. ^{125}I from ^{125}Xe decay *in situ* is quantitative within better than 10%. Measurements of electron emission from the iodine-implanted surface have been made in high vacuum using an electron multiplier with a threshold of 100 eV. There appear to be several electrons per 35 keV photon emitted from one 12 kV implanted foil. Several foils have been soaked in normal saline at room temperature and 55°C for periods up to 3 days. The saline has been dried at low temperature and the residue counted for trace ^{125}I. No statistically significant quantity of ^{125}I has been detected in the saline wash.

Experiment LS38
Dopaminergic tracers kinetic modelling with minimally invasive scanning procedures
(V. Sossi, TRIUMF)

The development of a new method for the determination of dopamine turnover has been completed. The method was fully validated on non-human primate data and is being applied to human data.

These studies complete the main scope of this research proposal regarding the study of kinetic modelling with minimally invasive scanning procedures. Cluster analysis that was initially part of this research proposal is going to be investigated as part of a new research proposal, "Modelling of the dopaminergic system in more severely affected PD patients".

Summary of the animal model results

Changes in dopamine turnover due to disease states such as Parkinson's disease may be reflected in corresponding changes in the kinetics of the positron emission tomographic tracer ^{18}F-fluorodopa. We had previously refined the conventional irreversible-tracer graphical approach to determine both the uptake rate constant K_i and the rate constant k_{loss} that describes the slow loss of the trapped kinetic component. Because these parameters change in the opposite sense with disease, their ratios may be more powerfully discriminating than either one alone. The ratio k_{loss}/K_i is indicative of effective dopamine turnover (EDT). Its inverse K_i/k_{loss} can be interpreted as the effective distribution volume (EDV) of the specific uptake compartment referred to FD concentration in plasma. Here we present a new approach to the estimation of EDV based on reversible-tracer graphical methods. When implemented with a plasma input function, the method evaluates EDV directly. When implemented with a tissue input function, the outcome is proportional to the ratio of the distribution volumes of the specific uptake and precursor compartments. Comparison of the new and previous approaches strongly validates this alternative approach to the study of effective dopamine turnover.

Summary of the results from the human subjects data

Objectives:

There are two objectives to this study.

1. To test a new method to determine the effective dopamine distribution volume (EDV), the inverse of the effective dopamine turnover, in normal volunteers (N) and Parkinson's disease (PD) patients.
2. To compare the abilities of four data analysis methods used in the early detection of disease: plasma input uptake rate constant K_i, tissue input uptake rate constant K_{occ}, EDV, and tissue input effective distribution volume ratio (EDVR).

Method:

Twelve subjects [5 N and 7 early stage PD (UPDRS III = 16/10)] underwent a 4-hour dynamic ^{18}F-dopa (FD) PET scan. 23 blood samples were taken and selected ones analyzed for metabolites. A new method based on the reversible process analysis was used to determine the EDV and EDVR. This method had been previously developed and validated with non-human primate data and here for the first time applied to human data. Data from 90–240 min after injection were used to determine the EDV and EDVR, while data from 20–90 min were used to determine K_i and K_{occ} using the Patlak analysis. The parameter values averaged over the putamen were used to compare the N to the PD group.

Results:

The new method provided a reasonable value for the EDV and EDVR in all cases, which was not possible with a previously existing graphical method. Discriminant analysis applied to the data determined the EDV to be the best single classifier of the two groups. EDV alone correctly classified the N group at a 100% level and the PD group at 71.4%, with the misidentified PD patients having the lowest EDV value in the algorithm defined N group. A quadratic discriminant analysis using EDV and K_i combined classified both groups correctly at 100%.

Conclusion:

A new method to determine the EDV was validated with human data. The EDV was found to be the most sensitive classifier in early PD, indicating that changes in the effective dopamine turnover can be measured earlier with PET than changes in the uptake rate constant, which is related to dopamine synthesis and storage. This analysis also suggests that EDV and K_i provide some independent information on disease state.

Modelling of the dopaminergic system in more severely affected PD patients

Parkinson's disease is characterized by progressive loss of dopaminergic terminals and by a reduced neurotransmitter dopamine level in the striatum. The radiotracer ^{18}F-fluorodopa (FD) is often used to quantify the uptake rate constant of the neurotransmitter dopamine (DA) into the pre-synaptic vesicles. Traditionally the FD kinetics are described by an irreversible model approach reflecting the DA trapping in the vesicles. The plasma input or the tissue input Patlak graphical approach is applied to the tracer time activity distribution to obtain the uptake rate constants K_i or K_{occ}, respectively. We have, however, recently observed that in the case of moderately severely lesioned patients the data do not satisfy the requirements of the Patlak graphical approach, indicating that the observed process is no longer irreversible. This fact can be most likely explained by a loss of DA vesicular storage ability. The Patlak approach applied to such data introduces a significant bias into the results, thus yielding inaccurate quantification of disease progression. These observations prompted the following studies.

1. Identification of an analysis method that can correctly quantify disease progression.
2. Development of a method that can distinguish regions with different tracer behaviour. Cluster analysis is being explored for this purpose. Comparison of the location and extent of the regions with the two different tracer behaviours in repeated studies of the same subject could provide an objective tool to measure the topographical pattern of disease progression.

The results of these studies will provide a better quantitative assessment and an objective topographical pattern of disease progression.

Experiment LS39
PET (profiling) for pulp and paper
(M. Martinez, UBC)

Two everyday examples of multiphase fluids are bubbly liquids and dusty gases. The term "multiphase flow" denotes a wide variety of phenomena in which particles, drops, or bubbles are present in a flowing fluid, such as air or water. The presence of a dispersed phase often significantly affects the flow as a whole. These inter-phase effects have to be accounted for to the lowest order of approximation.

For single-phase flows of Newtonian gases and liquids, experimental, mathematical, and numerical methods have, with some exceptions, reached a state of reasonable maturity. For the broader classes of phenomena that appear in multiphase flows, this is certainly not so – the experimental measurements are notoriously difficult and usually limited to fairly dilute conditions. As for the mathematical and numerical methods encountered in multiphase flows, one often experiences problems where the existence of a solution is by no means guaranteed beforehand.

Multiphase flows are very common in industrial applications. One such example is in the pulp and paper industry where a suspension of wood pulp fibres is filtered and consolidated, at very high speeds, to form a sheet of paper. Papermaking suspensions are typically composed of a mixture of fibres with a wide distribution in both length and diameter. Each class of fibre behaves differently during the filtration process depending on the hydrodynamic force applied and its degree of mobility in the formed network. Ascertaining these effects is difficult as these suspensions are opaque and difficult to visualize at reasonable penetration depths. Clearly, there is a need to visualize the motion of the individual classes of components in a fibre suspension in order to better understand the development of the physical properties of paper.

As an initial step, we have recently conducted a gravity-settling experiment at TRIUMF to test the feasibility of using PET as a novel visualization method. In this work, a selected class of "long fibres" was radioactively labelled and allowed to settle in an untreated fibre suspension (with a distribution of fibre lengths). The suspension was agitated and allowed to settle under the action of gravity in a cylindrical jar. The radioactive fibres were moving from right to left and four different axial cross sections were observed at different times.

From this initial study it is clear that this visualization technique has the potential to help clarify the behaviour of mixtures of fibres with differing lengths during papermaking. However, further development work needs to be conducted. A collaborative project is proposed to further develop this technique. A graduate student in chemical engineering, funded by Mark Martinez, will conduct the work.

Experiment LS42
Configuration modelling and image reconstruction studies on a depth encoding research tomograph
(V. Sossi, TRIUMF)

Simulations of the interaction of 511 keV γ-rays in LSO and GSO have been accomplished using GEANT. GEANT was interfaced with DETECT, previously developed by C. Moisan, which simulates the light transport in the crystal and the crystal-photomultiplier tube (PMT) interface. The programs were modified to include two layers of different material. An algorithm that decodes the crystal position as a function of light distribution in the four PMTs has been developed.

Spatial resolution estimates as a function of γ-ray incidence angle are being obtained by simulating two detectors placed opposite to each other at varying angles.

Future plans

The following issues are going to be addressed.

1. Block testing.
2. Data reconstruction techniques.
3. Data transfer and storage schemes.

In addition to the above listed studies, patient motion correction techniques will be investigated.