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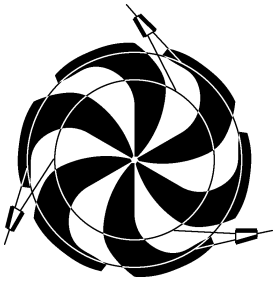

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FOR PARTICLE AND NUCLEAR PHYSICS

OPERATED AS A JOINT VENTURE

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UNDER A CONTRIBUTION FROM THE
NATIONAL RESEARCH COUNCIL OF CANADA

JULY 2000

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 Life Sciences program had eleven active projects during the past year as indicated by the reports below. Of these the PET program accounted for the bulk of the effort (LS0, LS3, LS4 and LS38). LS8 had two primary users: the Beryl group which uses the ^{7}Be that is produced on the TR13 for preparing targets for their cross section measurements at the University of Washington; the other major user continues to be the Botany Department at the University of British Columbia.

The PET program successfully renewed its Group grant with the Neurodegenerative Disorder Centre at UBC with a Medical Research Council award totalling $6.8 million for 6 projects and 3 cores over 5 years. Of the 6 projects, 4 use PET as their primary research tool. There is an additional MRC project with the Department of Psychiatry at UBC to study changes in serotonin receptors for patients with mood disorders.

The other Life Sciences projects related to the development of various PET tracers (LS2, LS35), the supply of FDG to local hospitals (LS29), and the characterization of a dual head camera that is operating in coincidence mode (LS33) to study breast and other cancers (LS24). Project LS10 is of particular interest since it ties together the tracer project capabilities at TRIUMF with molecular biology. If successful this project will open up a whole new field of tracer studies in biomedical science.

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

The TR13 cyclotron continues to conveniently and reliably supply isotopes throughout the day for radiochemical synthesis. Usage of the cyclotron dropped marginally (6% by delivered beam) this year over 1998, reflecting a lighter scanning schedule (LS3) and decreased demand for FDG (LS13, LS24) in the local community. The irradiation of lithium targets for the production of ^{7}Be (LS8) was up 50% over 1998 and beam delivered for target tests (LS4) was up 5 fold.

Downtime this year was minimal and was caused by an ion source filament failure. We changed the filament design to a dual filament assembly (compatible with other site ion sources) which provides a more consistent source output over the life of the filaments. We are in the process of learning how to determine when to change the filaments. In this case we observed a change in the required filament current to produce a particular beam current and interpreted this to be indicative of one broken filament. We naively assumed that we could continue to operate on the one remaining filament until the next maintenance day. We discovered, in this case, that the second filament failed rapidly.

We performed one extraction foil change and rebuilt the flouride water target and the flourine gas target.

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

- one ^{18}O-O_2 gas target
- one ^{18}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 10 hours at 50 μA. The TRIUMF licence was amended to include routine operation of this target up to the production of 1 Ci of ^{7}Be. A separate closed loop water cooling system was installed for this target after a small amount of ^{7}Be was released into the main site non-active cooling water.

Presentations were made this year at the 8th Workshop on Targetry and Target Chemistry (methane production, flouride production, and control systems) and at the Third International Conference on Isotopes (synthesis of the labelling precursor methyl bromide from methane).

ECAT tomograph

The ECAT has had a typical year with several detector block failures.

Siemens declined to renew our parts-only service contract this year due to 'unfavourable economics'. Fortunately Siemens Canada has agreed that we can buy parts directly from CTI and this has taken place.

The ^{68}Ge calibration line sources were replaced in June after being manufactured in-house. This procedure ended up taking much longer than anticipated due to procedural changes made by the technician hired to make these sources. Ultimately the rods were manufactured by the author.

Statistics

Table IX. TR13 run data.

Total runs conducted	749	
Total runs lost	1	
Integrated charge delivered	432875	μA-mins
Delivered to – LS2	915	
– LS3	227359	
– LS4	19302	
– LS8	149169	
– LS13	28253	
– LS24	7877	

Table X. ECAT scan data.

Total scans conducted	368
Total scans lost	31
Lost to – patient	7
– cyclotron	1
– chemistry	10
– scanner	1
– power outage	1
– staff absence	11

Experiment LS2
Synthesis of radiohalogenated carbohydrates for use as imaging agents in PET and SPECT

(M.J. Adam, TRIUMF)

2,2 dihalo-sugars

The use of 2-deoxy-2-[^{18}F]fluoro-D-glucose (2-FDG) to study glucose metabolism in the heart, tumours, and brain has seen extensive clinical application. 2-FDG/positron emission tomography (PET) has become the standard against which other functional imaging techniques are judged, because of the high resolution of PET and the well defined mechanism of 2-FDG accumulation in metabolically active tissues.

Recently we synthesized 2-deoxy-2-fluoro-2-iodo-D-hexoses (2-FIG and 2-FIM) as possible candidates for SPECT medical imaging analogues of FDG. In that project we carried out the chemistry, preliminary ^{123}I-labelling experiment, and enzyme kinetic study. We also have synthesized the 2-deoxy-2,2-[^{18}F]difluoro-glucose (DFDG) analogue with ^{18}F-acetylhypofluorite and carried out biodistribution and PET imaging studies.

Following radioiodination of FIM, the final product was stable *in vitro* for 24 hours. Injection in mice showed a rapid blood clearance and deiodination of the ^{123}I-FIM reflected by high stomach and thyroid uptake. Comparison with ^{18}F-FDG and ^{18}F-DFDG revealed a large discrepancy between the ^{18}F labelled sugars and the ^{123}I-FIM biological distribution. The iodinated product was not found as suitable as ^{18}F-FDG and ^{18}F-DFDG as a metabolic marker for *in vivo* studies. Testing still needs to be carried out on the other ^{123}I analogue FIG.

Glycosidase inhibitors

The mechanism of inhibition involves the formation (glycosylation step) and hydrolysis (deglycosylation step) of a glycosyl-enzyme intermediate via transition states with substantial oxocarbenium ion character. 2-deoxy-2-fluoroglycosides with good leaving groups at position 1 such as fluorine, have been shown to act as covalent mechanism based inhibitors of these enzymes by forming a relatively stable 2-deoxy-2-fluoroglycosyl-enzyme intermediate. Gaucher's disease, the most prevalent lysosomal storage disorder, is caused by an inherited deficiency in glucocerebrosidase, which results in accumulation of glucosyl ceramide principally in macrophages of the spleen, liver and bone marrow. Such an ^{18}F-labelled enzyme bound inhibitor may be a good imaging agent for diagnosing glycosidase activity and monitoring the effectiveness of enzyme replacement therapy.

Previously we have synthesized the mechanism-based glycosidase inhibitor 2-deoxy-2-[^{18}F]-fluoro-β-mannosyl fluoride and demonstrated its covalent binding to Agrobacterium-β-glucosidase *in vitro*. Turnover of the fluoro sugar-enzyme could be readily monitored *in vitro* by following release of radioactivity from the enzyme. However, it was shown that *in vivo* the glycosyl fluoride is hydrolyzed, giving rise to 2FDG. The 6-fluoro version of this molecule is also known to be a good inhibitor of glucosidase so it was a logical extension of this work to label this sugar in the 6 position only, with ^{18}F, to overcome the previous metabolic problem. The 6-fluoro sugar was labelled in a 20% radiochemical yield. It was then allowed to bind with glucocerebrosidase (Cerezyme) and the enzyme bound inhibitor complex purified by size exclusion chromatography. Optimization of the enzyme-inhibitor complex synthesis and its *in vivo* testing in mice deficient in glucocerebrosidase is under way.

Experiment LS3
Synthesis of radiopharmaceuticals for positron emission tomography

(M.J. Adam, TRIUMF)

PET radiopharmaceutical production for 1999 totalled 474 deliveries. The total number of radiopharmaceuticals went up to 9 this year and they include: FDG, FDOPA, setoperone, MDL 100907, raclopride, methylphenidate, dihydrotetrabenazine, SCH23390 and PK11195. The 474 deliveries included 41 shipments of FDG or fluoride to Vancouver Hospital and Lions Gate Hospital for cardiology and breast

cancer research and 77 shipments of N-13 to the Department of Botany at UBC for plant studies. All of the other production runs were used by the Neurodegenerative Disorders Program at UBC to study movement disorders such as Parkinson's disease.

One major chemistry project that continued this year was the development of methods to reduce the amount of precursor used in the labelling reactions and to improve the radiochemical yields of existing syntheses. We have now developed a new method based on using tetrabutylammonium fluoride as the base in reactions using C-11 methyl iodide as the labelling reagent. The results are very encouraging with a 10–50% improvement in yields with precursor amounts as low as 100 μg.

Both C-11 labelled PK11195 and MDL100907 are new to the routine production lineup this year. The PK11195 compound is a peripheral benzodiazapine receptor drug that has shown promise as an anti-inflammatory drug. This new agent will be used by our group as a possible imaging agent for arthritis and for the study of Parkinson's disease. The MDL100907 is a highly selective serotonin receptor antagonist that is expected to be a better agent than the currently used setoperone. This agent is used mainly by the Psychiatry Group under the direction of Dr. Lakshmi Yatham at UBC and will also be used in ECT studies carried out by Dr. Doudet.

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

This past year the three target systems that continued under development were the $^{11}CH_4$, ^{124}I and the ^{18}F-fluoride from ^{18}O-O_2 targets.

Developments in the production of ^{11}C methyl iodide, ^{11}C-CH_3I, via a gas phase reaction reported at the 6th International Symposium on Radiopharmaceutical Chemistry and Workshop on Targetry and Target Chemistry meetings prompted us to look at the production of methane, CH_4, from the irradiation of a gas mixture of N_2 and H_2. We felt the direct production of ^{11}C-CH_4 in target was the most elegant implementation of the gas phase reaction for routine use of ^{11}C-CH_3I. However, unexpectedly poor yields of ^{11}C-CH_4 from our small volume high pressure gas targets led us to further investigate the conditions required for efficient production of methane. We routinely produced several hundred millicuries of ^{11}C-CO_2 in an aluminum 9 ml STP target, open to a 20 ml expansion volume, operated with an initial pressure of 400 psi at 15 μA, 13 MeV protons on our TR13 cyclotron. Similar operating conditions with a gas mixture of N_2/5% H_2 yielded only tens of millicuries of ^{11}C-CH_4.

Investigation of other targets reported in the literature used to produce methane indicated that they tended to be large volume targets (flow through design in some cases) operated at comparatively low pressures with 1 to 5% hydrogen content. It has been reported that on a flow through design employing 5% H_2, it had been noted that 1% H_2 resulted in 50% of the methane production compared to 5% H_2, and that at least 0.1 eV/molecule was necessary for the decomposition of formed products to CH_4.

The present target has a volume of 23 ml STP, with fill and empty valves located at the end of a 20 ml 1/8 in. SS tube. We have tested this target with 5% H_2 and at a range of pressures, currents, and run duration for 10% H_2 concentrations. In fact, the most dramatic improvement in yield came from the change to 10% H_2 concentrations. Single test runs at 15% and 20% H_2 concentrations were not enticing enough to follow through on.

Gas chromatography was performed on the irradiated target gas to look for any other radioactive species. The target gas contains only $^{11}CH_4$ as the radioactive species in any significant quantity.

Mechanisms proposed in the literature for the reactions of recoil ^{11}C postulate the primary molecule to be CN*. Subsequent reactions are to HCN and CH_4 via hydrogen abstraction from H_2 via radiolysis. Perhaps, due to a quirk of our target geometry, these or other intermediate species are irreversibly bound to the target wall. Lamb *et al.* report HCN stuck to their target wall until they tried heated quartz liners. Possibly a longer path length for the recoil ^{11}C and intermediate species is necessary for the efficient formation of $^{11}CH_4$.

Based on these studies we feel that there are two major factors affecting the yields from the various gas targets. The $^{11}CO_2$ results can be interpreted as a measure of the production capacity of the targets which varies with configuration while the yields of methane reflect the likelihood of recoil hot atoms interacting with the target walls. For geometries that allow multiple collisions before encountering the wall the methane yield approaches that of CO_2. However, in situations where the target chamber approximates the beam shape there is a high probability of a portion of the generated hot-atom C-11 species sticking to the walls as seen for the conical targets. Thus the optimum design of a gas target should include efficient cooling to minimize density reduction and sufficient range in the target volume to allow the *in situ* hot atom processes to reach their equilibrium.

We are investigating a possible route to higher yield target systems for ^{18}F-fluoride production. This approach relies on gas target designs since TRIUMF has developed the technology to build gas targets capable

of operating at proton beam currents >100 μA.

This approach will make use of 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 as 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 you produce 8 Ci and 10 Ci respectively, even if you have an 80% recovery from the target wash (50% saturation * 0.8 = 0.4 saturation yield). At these rates one can imagine very long shipments to remote locations. The advantages include: recovery of the target material is straightforward and efficient, and higher beam currents are possible than for water targets (liquids in general). Disadvantages include having to wash and dry the target between runs and finding the best material for this process.

We tested a number of materials for the target chamber and stainless steel appears to be the best material that was readily available. We then built a proof-of-principle target system and verified that each step in the process was feasible. The next step is to build a prototype target that can operate at high beam current.

We have also been investigating the production of ^{124}I from a TeO_2 target. We have designed and built a target that can operate in the 20 to 30 MeV range on either of the MDS Nordion cyclotrons. We will next determine the maximum beam current at which the target can be safely operated using natural tellurium. Once these studies are complete then an enriched ^{125}Te target will be constructed.

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

We investigated the synergism between NO_3^- and NH_4^+ that leads to enhanced N uptake and translocation of N to the shoot, using $^{13}NH_4^+$ and $^{13}NO_3^-$. This synergism in turn may account for the widely documented improved plant growth associated with supplying both forms of nitrogen (NO_3^- and NH_4^+) to growing plants.

The mechanism whereby ammonium inhibits nitrate uptake has been controversial for some years. We employed compartmental analysis to measure both the influx and efflux terms of nitrate uptake in barley roots and effectively resolved this controversy. We were able to demonstrate that the major NH_4^+ effect was a reduction of NO_3^- influx; however, there was also a small but significant increase of NO_3^- efflux.

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* [Zhuo *et al.* (1999)], in hybrid spruce 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. While this part of the project is strictly molecular biology, the assessment of efficacy in NO_3^- uptake and assimilation will require the use of $^{13}NO_3^-$. Because it takes so long to regenerate spruce seedlings after genetic transformation, we will use tobacco and poplar seedlings as model systems in order to get rapid feedback on the potential offered by this methodology.

Large quantities of NH_4^+ are generated by photochemical reactions in chloroplasts and by phenylpropanoid biosynthesis via the shikimic acid pathway. In addition, NH_4^+ may be transported from roots to shoots. We have been focusing our energies on characterizing the NH_4^+ fluxes into leaf cells, an area of study that has largely been neglected in favour of root studies.

We have been examining the expression of the *AMT1* gene which codes for the high-affinity NH_4^+ transporter in *Arabidopsis* while undertaking correlated studies of $^{13}NH_4^+$ uptake. It appears that the gene is only expressed when N supply is limited. At high levels of available N, another (low-affinity) transport system is adequate to absorb sufficient N. We have demonstrated by the use of parallel $^{13}NH_4^+$ flux studies and Northern blot analysis of *AMT1* expression that the gene is regulated by glutamine accumulation and not by NH_4^+ itself.

Another project sought to clone and characterize the high-affinity NO_3^- transporters of barley roots. Molecular evidence suggests that there may be as many as 8 genes encoding high-affinity NO_3^- uptake in roots of barley. We cloned two of these genes (two were cloned in England) and combined molecular studies of gene expression with physiological studies of high-affinity NO_3^- transport, using $^{13}NO_3^-$. We also undertook amino acid analyses of the root tissue in order to correlate amino acid concentration with gene expression and $^{13}NO_3^-$ influx. Our evidence suggests that glutamine regulates expression of the nitrate transporter gene.

Another project seeks to investigate molecular and physiological aspects of the regulation of ammonium absorption by roots of rice plants. Using the *arabidopsis* model system (see paragraph 4) we have strong evidence that glutamine regulates expression of the gene encoding the high-affinity NH_4^+ transporter. In rice we anticipate a more complex system possibly involv-

ing a larger number of genes. We have already undertaken flux analysis using $^{13}NH_4^+$ as well as hplc analysis of the changes of amino acid pools during down-regulation of NH_4^+influx. The next stage will be to clone the rice gene and investigate its regulation.

We are also exploring the relationship between the ectomycorrhizal fungi that infect roots of tree species and the trees themselves. These fungi actually contribute nutrients (drawn from some distance from the tree species) to the tree. In particular we want to explore the uptake of NO_3^- and NH_4^+ by these fungi and the interaction between fungus and tree at the gene level. In some studies of phosphate uptake it has been shown that the activity of the fungus suppresses gene expression on the part of the plant because the nutrients delivered to the plant via the fungal symbiont make it unnecessary for the plant to express high affinity transporter genes.

The other major user of radiotracers is the Beryl Group at TRIUMF which is preparing ^{7}Be targets for its astrophysics experiments. The production statistics from the TR13 cyclotron can be found in LS0.

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

Research is progressing with radiolabelled ^{123}I short DNA molecules (aptamers) with an affinity for blood clot components. An *in vitro* biochemical examination of aptamer specificity has been completed, written up, and is about to be submitted to the journal. The exosite 1 class of aptamers binds thrombin *in vitro*, but access is blocked by fibrin (the major protein in clots) *in vivo*. The exosite 2 class of aptamers binds thrombin *in vitro* and *in vivo*. The latter aptamers are suited for *in vivo* imaging studies.

This year we have extended the *in vitro* results to the *in vivo* case. An experimental system was mastered for testing the ^{123}I aptamers in jugular vein thrombi in living rabbits. For several months artifacts were experienced with unwanted coagulation surrounding the main experimental thrombus; this artifact trapped aptamers. The clots were labelled with ^{123}I aptamer and allowed to slowly release the aptamer into the bloodstream by diffusion. This type of experiment can reveal the affinities for various clot proteins which retain the aptamer in the thrombus. We had anticipated this experiment would show the specific interaction of the exosite 2 aptamer with thrombin. Instead, indications are emerging of several types of interactions between the aptamer and the thrombus. These alternative types of interaction are still under study. We have also begun to label clots by aptamer injected into the bloodstream.

A new publication (in press) addresses a central problem, rapid clearance in 1–2 minutes of short DNA fragments from the blood. The short lifetime impedes natural DNA from exerting pharmaceutical or radiotracer functions in the blood. It was shown that bioconjugate complexes with a protein have improved lifetimes in circulating blood, and even more extended lifetimes when trapped in stationary targets (such as thrombi). Experimental work this year appears to have extended the aptamer lifetime still further in circulating blood, based on an improved bioconjugate design.

Oral and poster presentations based on the two manuscripts were given at the 11th International Symposium on Radiopharmacology, St Louis.

Experiment LS24
PET scanning for detection, staging, and monitoring of therapy response in breast and ovarian cancer using a coincidence gamma camera
(P. Cohen, Lions Gate Hospital)

In the past year, funds have been raised to install attenuation hardware and software to improve the performance of the ADAC coincidence PET system (molecular coincidence detection or MCD) gamma camera at Lions Gate Hospital.

We have imaged 27 patients with suspected cancers, including 5 patients with the new attenuation correction upgrade, as this is the only PET system capable of imaging tumours with FDG in Western Canada, and it is the only means of getting clinical experience with FDG-PET. While breast cancer patients have comprised the largest group of patients enrolled to date, we have enrolled other patients referred from various oncology services in Vancouver while waiting to begin our experimental work on treatment response to advanced breast cancers.

Our data confirms the work of other centres, which shows that in carefully selected oncology patients, FDG-PET is extremely useful at detecting tumours. In our case, our patients were primarily selected for PET scan on the basis of strong clinical suspicion of recurrent primary tumour (lymphoma, breast, colo-rectal) and/or rising serum tumour markers suggesting recurrent disease. In this situation, about 75% of PET scans undertaken were positive for recurrence, even when other modalities were negative or equivocal.

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

In addition to the PET tracers that are used in the UBC neurological and psychiatric studies, the PET Chemistry group has been supplying FDG to the Nuclear Medicine Departments at Lions Gate Hospital (LGH) and Vancouver General Hospital (VGH). The

LGH program has been focusing on the use of their dual head coincidence camera (LS33) to study breast cancer (LS24). The VGH program is using a high energy collimator on a SPECT camera to study cardiac patients who are being considered for heart transplants (LS28). The progress associated with these projects is reported elsewhere in this section.

Experiment LS33
Lions Gate Hospital dual head coincidence PET scanning
(V. Sossi, UBC/TRIUMF)

The ADAC molecular coincidence dual head camera was installed at Lions Gate Hospital (LGH) in North Vancouver in August/September, 1997.

During the last year, approximately 20 patients were scanned on the camera, mostly with breast, lung and bowel cancer. The results of the scans had an impact on patient treatment in approximately 75% of the cases.

In September, 1999 the attenuation correction hardware and software were implemented on the camera which greatly improved the visual image quality of the camera.

LGH had recently been approached by the Hospital of New Westminster to perform a PET scan on twenty lung cancer patients. The Cancer Agency has also expressed interest in collaborating with the LGH Nuclear Medicine Department to test the new hypoxic PET agent EF5 on a group of lung cancer patients, so that a much larger number of patient studies is anticipated for this coming year.

The physics aspect of the research performed during the second year was mostly focused towards determining lesion detectability as well as towards the design of an efficient patient shield to reduce the effect of radioactivity outside the field of view. To accomplish this we have:

- Performed and analyzed a set of experiments to determine lesion detectability in accordance with the proposed draft of the NEMA-NU2 standards.
- Determined the distribution of detected events originating from radioactivity outside the FOV as a function of source extent and location.
- Determined the effect that a lead shield may have on noise equivalent counts.
- Completed a patient shield design.

We are presently investigating the effect of radioactivity outside the field of view shielding on image contrast. Camera characteristics will be redefined using the NEMA-NU2 standards. Preliminary simulation studies are also under way.

During the course of the coming years we are planning to address the reconstruction and quantitative aspects of dual head imaging.

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 aggressiveness of disease. Such studies employed a microelectrode, used in many centres, but 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 macromolecule 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 florescent 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 obvious route to fluorination of EF5, ^{18}F-fluoride displacement of a tosylate or halide, has more or less failed due to elimination or cyclization. Only the monofluorinated EF1 has been labelled in a few per cent with $^{18}F^{-}$. Due to this and the fact that high specific activity is not required for these compounds, success has been achieved by our collaborators at the University of Pennsylvania based on the ^{18}F-F_2 addition to an alkene. The ^{18}F labelled EF5 has been injected

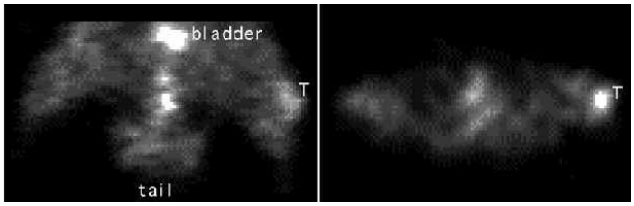

Fig. 98. The left image is a coronal looking from the tail end of the mouse. The image on the right is a transverse section at the level of the tumour showing the hypoxic leg tumour. Photo courtesy of Dr. Cameron Koch, University of Pennsylvania.

into hypoxic tumour-bearing mice and the images in Fig. 98 show good tumour uptake.

We now have the precursor alkene at TRIUMF for labelling with ^{18}F and we initally propose to do some cell studies with the ^{18}F compound followed by a study similar to the mouse imaging study which was carried out at the University of Pennsylvania. This work will be performed as a collaboration with Kirsten Skov at the BC Cancer Research Agency.

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

The feasibility and accuracy of bloodless methods for the tracers currently used in our centre to study the dopaminergic system have been determined.

Reversible tracers

The tissue input Logan method [Logan *et al.*, JCBF **16**, 834 (1996)] and the reference tissue method (RTM) [Lammertsma and Hume, Neuroimage **4**, 153 (1996)] have been implemented and compared for four dopaminergic tracers: ^{11}C-methylphenidate (MP), ^{11}C-dihydrotetrabenazine (DTBZ), ^{11}C-raclopride (RAC) and ^{11}C-Schering 23390 (SCH). The methods have been shown to perform comparably in spite of the different assumptions that each method imposes on tracer kinetics. The binding potential (BP) estimates obtained by each method had similar numerical values, reproducibility and reliability. The additional parameters R1 and k2 yielded by the RTM method (ratio between the target and reference influx rate constants k1 and k1′ and the target efflux rate constant, respectively), were also investigated. R1 showed little variability amongst subjects, while the k2 estimates were found to be in general highly variable. Data have been presented at the 1999 Brainpet conference in Copenhagen [Sossi *et al.* (1999)], accepted for publication by the Journal of Blood Flow and Metabolism [Sossi *et al.*, in press], and submitted for a book chapter [Sossi *et al.*, in *Molecular and Pharmacological Brain Imaging*, eds. Gjedde *et al.* (Academic, in press)].

Irreversible tracers

The initial approach used to analyze the effective dopamine turnover [Holden *et al.*, J. Nucl. Med. **38**, 1568 (1997); Doudet *et al.*, Neuropharmacology **36**, 363 (1997)] proved not to be successful on human data. A new method to determine the effective dopamine turnover using ^{18}F-dopa is presently under development. The approach is based on the observation that often, at later times, a zero slope was observed for the Patlak slope [Patlak *et al.*, JCBF **3**, 1 (1983)], implying the absence of a trapping compartment. Consequently the *Logan analysis* for reversible tracers was applied to the data at times larger than a selected $t_0(1)$. A plasma and a tissue input Logan analysis is currently being tested both on human and on monkey data and a paper describing the theory and the initial results is currently being prepared. An abstract has been submitted to the Neuroreceptor Mapping Meeting in New York, June, 2000 [Sossi *et al.*].

New tracers

We have scanned Parkinson's disease (PD) patients using the ligand ^{11}C-PK11195 (PK). This ligand is known to bind to peripheral benzodiazepine binding sites on activated microglia and macrophages in regions of active pathology in the human brain. PK was used in six PD subjects to explore if tissue inflammation might play a role in the aetiology of Parkinson's disease. There is not a commonly accepted way to analyze the PK data and the most promising method seems to be cluster analysis. Cluster analysis is a data-led technique that can be used to partition the pixel-time activity curves obtained from a dynamic study into a smaller number of clusters, each described by a multinormal distribution about a mean [Ashburner *et al.*, in *Quantification of Brain Function Using PET*, eds. Myers *et al.* (Academic, San Diego, 1996)]. Each cluster thus represents a particular shape of a time-activity curve. The advantage of this method is that no a priori knowledge about the tracer distribution or kinetics is required when analyzing the data and is consequently well suited to an exploratory study. The potential problems associated with the method are patient motion that changes the shape of the time activity curves on a pixel level, the non-stationary aspect of the image noise, and the need to determine a priori the number of clusters desired. We are presently implementing cluster analysis for the PK data and plan to explore its potential for the other dopaminergic tracers.