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CYCLOTRON PRODUCTION OF SHORT-LIVED  
RADIONUCLIDES AND LABELLED COMPOUNDS  
FOR USE IN BIOMEDICAL RESEARCH AND  
CLINICAL DIAGNOSIS

in  
TWO VOLUMES

**VOLUME II**

John Charles Clark

Submission for the Degree of DSc  
in the University of Durham

1994

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SHORT COMMUNICATION

## Tracing the fate of oxygen consumed during phagocytosis by human neutrophils with $^{15}\text{O}_2$

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(Received 3 March 1978; accepted 22 June 1978)

### Summary

1. The metabolism of oxygen by phagocytosing neutrophils was traced by using  $^{15}\text{O}_2$ .

2. The isotope did not exchange with the incubation medium or cells to an appreciable extent and unmetabolized oxygen was readily eluted by gassing the cell suspension.

3. The polarographic measurements of oxygen consumption closely paralleled the recovery of metabolized  $^{15}\text{O}_2$ .

4. Almost all the metabolized  $^{15}\text{O}_2$  was converted into water, both in the presence and absence of KCN, supporting the concept that the oxygen consumed by neutrophils is converted into  $\text{H}_2\text{O}_2$ . It is unlikely that an appreciable proportion of this oxygen is incorporated into the organic composition of the cell or of the ingested micro-organism.

Key words: granulocytes, hydrogen peroxide, leucocytes, oxygen, phagocytosis, radioactive gases.

### Introduction

The oxygen consumption of neutrophil polymorphonuclear leucocytes (neutrophils) increases with phagocytosis (Baldrige & Gerard, 1933). This is not due to mitochondrial respiration, as it is not inhibited by either cyanide (Sbarra & Karnovsky, 1959) or other inhibitors of mitochondrial cytochromes, and it is important for the bactericidal function of these cells (Mandell, 1974).

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Hydrogen peroxide, a possible substrate for the myeloperoxide-halide microbicidal system (Klebanoff, 1975) is produced as this oxygen is consumed, but (Iyer, Islam & Quastel, 1961; Homan-Müller, Weening & Roos, 1975) it is not known what proportion of the oxygen is reduced to  $\text{H}_2\text{O}_2$  and how much enters the organic composition of the ingested organism or of the cell itself. The methods for the measurement of  $\text{H}_2\text{O}_2$ , including  $^{14}\text{CO}_2$  release from [ $^{14}\text{C}$ ]formate and peroxidation of various substrates, are non-specific and may require high concentrations of cyanide or azide to prevent endogenous degradation of  $\text{H}_2\text{O}_2$ . The effect of these inhibitors on other cell functions and enzyme systems is unknown. Specific analysis of  $\text{H}_2\text{O}_2$  by measuring the  $\text{O}_2$  regenerated by an excess of catalase (EC 1.11.1.6) gives only a small percentage recovery of the oxygen, and the fate of most of the oxygen remains unexplained (Homan-Müller *et al.*, 1975; Zatti, Rossi & Patriarca, 1968). Aldehydes (Jacobs, Paul, Strauss & Sbarra, 1970) and oxidized lipids (Gutteridge, Lamport & Dormandy, 1976) have antibacterial activity, and the ingestion by neutrophils of particles containing linoleic acid generates malonaldehyde (Stossel, Mason & Smith, 1974). This suggests that the oxygen not otherwise accounted for might be incorporated into lipids in the cell, possibly producing a bactericidal agent, or into the ingested micro-organism.

We have studied the extent to which the metabolized oxygen is incorporated into the organic composition of the engulfing cell, or engulfed micro-organism, and how much eventually forms water, by using the short-lived isotope  $^{15}\text{O}_2$ .



### Materials and methods

Neutrophils were purified from human blood by the technique of Böyum (1968) and suspended in Hanks balanced salt solution ( $2.5 \times 10^8$ /ml). An aliquot of the cell suspension (1.0 ml) was stirred rapidly in the chamber of a platinum oxygen electrode (Rank) at 37°C and to this was added 1.0 ml of Hanks solution which had been gassed for 4 min with a mixture of  $^{15}\text{O}_2 + \text{N}_2$  (20:80, v/v; 1–2 mCi) (Clark & Buckingham, 1975). Phagocytosis was stimulated by the addition of  $1 \times 10^{10}$  latex particles coated with human IgG (0.81  $\mu\text{m}$  diameter) or  $1 \times 10^{10}$  serum opsonized, heat-killed staphylococci (Oxford strain) in 100  $\mu\text{l}$  of Hanks solution. Oxygen consumption was measured polarographically for 4–6 min, after which the cell suspension was removed, the incubation chamber washed with 1.0 ml of phosphate-buffered saline [sodium phosphate (8 mmol/l of sodium chloride solution (154 mmol/l), pH 7.2)] and the mixture was centrifuged at 8000 g for 30 s in an Eppendorf 3200 centrifuge. The pellets were washed twice with 1.5 ml of phosphate-buffered saline. The supernatants were pooled and gassed for 2 min with  $\text{N}_2$  to remove unmetabolized  $^{15}\text{O}_2$ . Radioactivity in the cell pellets and gassed supernatants was measured in a well-type gamma counter and compared with that in an aliquot of the  $^{15}\text{O}_2$ -gassed medium. Experiments were also performed with heat-killed cells (95°C for 4 min) and in the presence of KCN (5 mmol/l). Lactate dehydrogenase (EC 1.1.1.27) activity

was assayed (Davies, Page & Allison, 1974) in the cell pellet and supernatant medium of cells processed in an identical manner.

In four other experiments (four studies)  $5 \times 10^7$  neutrophils in 4 ml of Hanks solution were incubated at 37°C with latex particles and  $^{15}\text{O}_2$  for 2 min. The cell suspension was then gently heated under vacuum and a sample of distilled water was collected in a liquid nitrogen trap. Aliquots of the incubation mixture and distillate were weighed and the radioactivity was measured.

### Results

Unmetabolized  $^{15}\text{O}_2$  was completely removed from the medium by gassing it with  $\text{N}_2$ . The neutrophils demonstrated a burst of oxygen consumption after the addition of particles to the cell suspension, which was enhanced by the presence of KCN (Table 1). The proportion of the oxygen in the chamber that was consumed as measured by polarography was very similar to the proportion of radioactivity that was recovered. Almost all the radioactivity was in the aqueous medium and very little remained in the cells. In two control studies, 16 and 17% of the cellular lactate dehydrogenase, a marker of cell viability (Davies *et al.*, 1974), was released into the medium. The specific radioactivity of the water distilled from an incubation mixture of phagocytosing cells was 91.6, 101.4, 87.6 and 113.4% in four studies (mean value 98.5%).

TABLE 1. Oxygen consumption by human neutrophils and the subsequent distribution of  $^{15}\text{O}_2$  in the cells and incubation medium

Oxygen was consumed by neutrophils at a rate of  $0.05 \text{ fmol min}^{-1} \text{ cell}^{-1}$  before the addition of bacteria and latex particles, which did not consume oxygen by themselves. Results are shown for two studies in each category except for the single study on the cells that were killed by heating at 95°C for 4 min.

| Incubation conditions                           | Oxygen consumption (polarographic measurement)    |                         | $^{15}\text{O}_2$ radioactivity recovered (%) |             |       |
|-------------------------------------------------|---------------------------------------------------|-------------------------|-----------------------------------------------|-------------|-------|
|                                                 | Rate ( $\text{fmol min}^{-1} \text{ cell}^{-1}$ ) | Proportion consumed (%) | Cell pellet                                   | Supernatant | Total |
| Viable cells + latex particles                  | 0.59                                              | 54                      | 3.5                                           | 52.3        | 55.8  |
|                                                 | 0.51                                              | 51                      | 2.5                                           | 46.5        | 49.0  |
| Viable cells + latex particles + KCN (4 mmol/l) | 1.04                                              | 76                      | 1.3                                           | 78.0        | 79.3  |
|                                                 | 1.00                                              | 76                      | 4.1                                           | 68.4        | 72.5  |
| Viable cells + dead bacteria                    | 0.86                                              | 57                      | 3.5                                           | 51.1        | 54.6  |
|                                                 | 0.66                                              | 26                      | 1.7                                           | 25.8        | 27.5  |
| Dead cells + latex particles                    | 0                                                 | 0                       | 1.3                                           | 3.3         | 4.6   |

## Discussion

$^{15}\text{O}_2$  does not rapidly exchange with oxygen of water and could therefore be used to determine the fate of the oxygen in the striking burst of oxygen consumption that accompanies phagocytosis. Almost all the metabolized oxygen was found to enter the suspending medium. This was not due to damage to the cells as only a small proportion of the cytoplasmic marker enzyme lactate dehydrogenase (Segal & Peters, 1977) was released from the cells during the studies. The  $^{15}\text{O}_2$  was shown to enter the water itself, as opposed to being present as a soluble metabolite, as its specific radioactivity in water distilled from the incubation mixture was very similar to that in the original cell suspension. The  $^{15}\text{O}_2$  was not metabolized to water by mitochondrial respiration, as its consumption was not inhibited by KCN, nor was its exchange with the  $\text{O}_2$  simply accelerated by cellular components, as it did not occur in the presence of dead cells. Very little (less than 4%) if any, of the oxygen that is consumed becomes incorporated into the organic structure of neutrophils or ingested bacteria.

The current concept that all the oxygen consumed in association with phagocytosis forms  $\text{H}_2\text{O}_2$  (Klebanoff, 1975; Homan-Müller *et al.*, 1975; Iyer *et al.*, 1961) appears correct. The low recovery of  $\text{H}_2\text{O}_2$  previously observed, which accounted for less than 10% of the oxygen consumed, probably results from further metabolism of  $\text{H}_2\text{O}_2$  by myeloperoxidase (EC 1.11.1.17) as  $\text{H}_2\text{O}_2$  accumulates in myeloperoxidase-deficient neutrophils (Klebanoff & Pincus, 1971) and as the recovery of  $\text{H}_2\text{O}_2$  amounts to 50–70% of the oxygen consumed in the presence of cyanide and azide (Homan-Müller *et al.*, 1975), which inhibit this enzyme. Cyanide appears to enhance oxygen consumption under the conditions in which the present studies were conducted because it is basic and buffers the acid produced by phagocytosing cells (Sbarra & Karnovsky, 1959).

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# The Interpretation of Thallium-201 Cardiac Scintigrams

## Studies in Experimental Ischemic Heart Disease in Dogs

A.P. SELWYN, E. WELMAN, T.A. PRATT, J. CLARK, C. MCARTHUR, AND  
J.P. LAVENDER

**SUMMARY** Twenty-one anesthetized and thoracotomized dogs were studied. Scintigrams of the heart were recorded using a continuous infusion of krypton-81m into the aortic sinuses as well as intravenous injections of thallium-201. A gamma camera linked to a digital computer was used to record the myocardial distribution of these tracers. The distribution of  $^{201}\text{Tl}$  was similar to that of  $^{81\text{m}}\text{Kr}$  when myocardial blood flow was normal (seven dogs). In 14 dogs, the left anterior descending coronary artery (LAD) was narrowed to produce a regional decrease in myocardial blood flow. Blood flow changes were measured with an electromagnetic flowprobe. When the epicardial ECG was normal in seven dogs, the  $^{201}\text{Tl}$  scintigram showed no regional decreases in activity when the tracer was delivered after LAD narrowing. In contrast, a decrease in the activity of  $^{81\text{m}}\text{Kr}$  was observed in the region supplied by the LAD. When the decrease in blood flow was associated with ECG signs of ischemia in seven dogs, both  $^{81\text{m}}\text{Kr}$  and  $^{201}\text{Tl}$  scintigrams showed decreased activity in the ischemic area. The cardiac distribution of  $^{201}\text{Tl}$  was determined in five dogs while myocardial blood flow and metabolism were normal. LAD narrowing then produced 24 hours of severe myocardial ischemia. The distribution of creatine kinase activity in the left ventricle (U/mg DNA) was similar to the distribution of  $^{201}\text{Tl}$  (counts/mg DNA),  $r = 0.83$ ;  $P < 0.001$ . These studies suggest that  $^{201}\text{Tl}$  scintigraphy of the heart can demonstrate decreases in regional myocardial perfusion only when metabolism is disturbed.

THALLIUM-201 is being widely investigated and used to image the heart in the assessment of patients with coronary artery disease.<sup>1-3</sup> Following a peripheral venous injection, the regional distribution of this radionuclide in the heart has been used to obtain information about myocardial ischemia and infarction.<sup>4,5</sup>  $^{201}\text{Tl}$  cardiac scintigrams are reported as demonstrating abnormalities of regional myocardial perfusion.<sup>4</sup> Experimental research has suggested a close relationship between the distribution of  $^{201}\text{Tl}$  in the heart and regional myocardial blood flow.<sup>2</sup> These experiments have made certain assumptions about the energy-dependent cell membrane extraction of the indicator.<sup>4,6</sup> The purpose of this study was to test whether  $^{201}\text{Tl}$  scintigrams of the heart can provide an assessment of changes in regional myocardial perfusion. The relative importance of coronary blood flow and myocardial metabolic processes in the interpretation of  $^{201}\text{Tl}$  cardiac scintigrams is discussed.

### Methods

Twenty-one mongrel dogs weighing between 32 and 51 kg were anesthetized with intravenous sodium thiopental (Pentothal, 16 mg/kg). Anesthesia was maintained by the intermittent intravenous injection of pentobarbital

(Sagittal, 2 mg/kg). Respiration was maintained via a cuffed endotracheal tube with a mechanical ventilator. A left thoracotomy was performed and the heart supported in a pericardial cradle. A reversible snare was placed around the left anterior descending coronary artery (LAD) approximately 2 cm from where this vessel emerges from under the left atrial appendage. This snare consisted of 4 silk strands pulled through an 8-cm length of 3 french cardiac catheter tubing.

An electromagnetic flow probe (e.m.f.) of suitable size was positioned around the LAD immediately proximal to the snare. Most commonly a 3- to 5-mm probe was used. Pulsatile regional coronary flow was recorded by connecting the probe to a Systems Electronic for Medicine flowmeter (type 275). This has a carrier frequency of 285 Hz and the response is nominally flat (less than 3 dB down) to 80 Hz. Phasic and mean tracings were recorded on a multichannel instrument (Hewlett Packard 7785A). At the end of the experiments, calibration was carried out by tying off all the branches of the left coronary artery except the one of interest and perfusing the artery with the dog's own blood with a constant infusion pump. The accuracy of the probe and flowmeter was tested during each calibration by repeatedly measuring 20 values for flow between 10 and 150 ml/min. The probe showed a systematic and random error of less than 5% when compared to the pump and absolute measurement. The absolute measurements were made by taking timed collections into tared glass containers and weighing. The diastolic notch on an arterial pressure wave was used as the

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beginning of diastolic coronary flow. Planimetry was used to calculate areas under the phasic tracings and the calibration data were used to calculate mean and phasic regional coronary flow (ml/min).

Epicardial electrocardiographic (ECG) complexes were recorded, using a saline-soaked cotton wick electrode attached to the chest lead of a Hewlett Packard 7788A ECG amplifier (input impedance, 50 M  $\Omega$ ).<sup>7</sup> The standardization used throughout was 1 mm = 1 mV.

A 7 french cardiac catheter was inserted into the inferior vena cava via a right femoral venotomy. A specially designed no. 3 french catheter was inserted via a left femoral arteriotomy and seated in the aortic sinuses. <sup>81m</sup>Kr was continuously eluted in 5% dextrose from its cyclotron-produced parent compound rubidium-81 held in a portable generator.<sup>8</sup> <sup>81m</sup>Kr (7–10 mCi/min) was delivered into the aortic sinuses at a constant rate of between 5 and 10 ml/min of 5% dextrose.

Each dog was positioned so that the chest was within the field of a gamma camera (Toshiba GCA 202). A digital computer was used to record the total and regional activity of any gamma emissions from the heart as quantitative high spatial resolution images (Deltron-Nova 1220).

Throughout these experiments a high resolution collimator (parallel holes) was used (Toshiba RDH 606). The overall resolution of collimator and camera was 7 mm for <sup>81m</sup>Kr and 17 mm for <sup>201</sup>Tl. This was determined by calculating the full-width half-measure from a line source positioned within the left ventricular cavity of an asymptotic dog heart. The source was 5 cm from the camera face.

Seven dogs were studied while control regional coronary flow varied by less than  $\pm 5\%$  and the epicardial ECG showed no abnormalities. <sup>81m</sup>Kr cardiac scintigrams were recorded by collecting 250,000 counts in 30 seconds with the camera detection window at 190 keV  $\pm 20\%$ . The epicardial ECG complexes were recorded from eight positions within the area supplied by the LAD.<sup>7</sup> The camera detection window was changed to 80 keV  $\pm 20\%$ , and 1 mCi of thallium-201 (Duphar) was given as a single intravenous injection. After 10 minutes, quantitative <sup>201</sup>Tl scintigrams of the heart were recorded by the computer and Polaroid film by collecting 250,000 counts.

The LAD was then completely occluded in five of these seven dogs. The pericardium and chest were drained and closed and the anesthesia continued to 24 hours. The chest was opened and each heart surrounded with crushed ice. The hearts were excised and washed with sucrose solution (0.25 mol/liter). The right atrium and ventricle, left atrium, pulmonary artery, and aorta were removed by dissection. An incision along the junction between the interventricular septum and the posterior free wall of the left ventricle (LV) allowed the specimen to be laid flat on a cooled Perspex surface. The specimen was held in place by a metal grid. This divided the epicardial LV surface into 1 cm<sup>2</sup> regions. The outline of the LV specimen, the epicardial coronary vessels, the snare, electrode positions and the area of discoloration were marked on a Perspex sheet held over the grid and specimen.

The following procedures were carried out at 4°C. Up to 30 tissue samples (0.3 to 0.5 g) of the full thickness of

the specimen were taken from sites in the ventricular muscle supplied by the LAD, left circumflex coronary artery, and right coronary artery. The position of each sample site was noted with reference to the overlaid grid. The tissue samples were placed in tared tubes and weighed. Homogenization was then carried out in 10 volume sucrose (0.25 mol/liter) containing mercaptoethanol (10<sup>-3</sup> mol/liter) with a Polytron homogenizer by 3  $\times$  5 sec at setting 5. A sample (0.5 ml) of each homogenate was removed for determination of DNA. The remaining homogenate was centrifuged at 600 g for 10 minutes to sediment the nuclei, myofibrils, and membrane fragments. Samples of the post-nuclear supernatant extracts were taken for the assay of creatine kinase (CK) activity. These samples were first diluted 1 in 500 vol/vol with sucrose-mercaptoethanol solution.

DNA was measured by the method described by Patters;<sup>9</sup> CK activity was assayed in a Cecil spectrophotometer by the method of Oliver,<sup>10</sup> using the modifications described by Hearse.<sup>11</sup> The CK activity in units per milliliter was then calculated and expressed per milligram of DNA.

Samples (0.2 ml) of the post-nuclear supernatant extract from each homogenate were placed in counting tubes. The <sup>201</sup>Tl activity (80 keV gamma emissions) in each sample was measured in an automated well counter (Nuclear Enterprise). This result was then expressed per milligram of DNA and compared to the regional depiction of CK activity, using a linear regression analysis.

A constant infusion of <sup>81m</sup>Kr was delivered to the aortic sinuses in an additional seven dogs. Images of the myocardial distribution of this tracer were recorded by collecting 250,000 counts on Polaroid film and on the digital computer. During this time heart rate, blood pressure, and regional coronary flow were stable and the epicardial ECG showed no S-T segment or T wave changes. The LAD was then narrowed so as to reduce mean regional coronary flow by between 35% and 60%. The <sup>81m</sup>Kr cardiac scintigrams were recorded again and a continuous check was made that there were no changes in the epicardial ECG. After 7 minutes, the gamma camera detection window was changed to 80 keV  $\pm 20\%$  and the krypton infusion stopped. One mCi of <sup>201</sup>Tl was given intravenously and, after 10 minutes, images of the myocardial distribution of <sup>201</sup>Tl were recorded on Polaroid film and the digital computer by collecting 250,000 counts. After this, <sup>81m</sup>Kr scintigrams were recorded again.

Krypton-81m cardiac scintigrams were recorded in the seven remaining dogs while regional coronary flow, the epicardial ECG, heart rate, and blood pressure were stable. The LAD snare was tightened until mean coronary flow was reduced by between 35% and 60%. This was achieved by attaching the four strands of the snare to a screw clamp and turning slowly until mean regional coronary flow was diminished as required and the <sup>81m</sup>Kr scintigrams showed a regional defect. The screw clamp was held stable by attachment to the chest wall. Each experiment proceeded when the flowprobe and regional activity of <sup>81m</sup>Kr showed a stable decrease with variations of  $< \pm 7\%$ . In each dog of this group, the epicardial ECG complexes in the area supplied by the LAD showed

changes within 60 seconds. Krypton-81m cardiac scintigrams were recorded as described above and the energy detection window of the camera was changed to 80 keV  $\pm$  20%. Seven minutes after LAD narrowing, 1 mCi of  $^{201}\text{Tl}$  was given intravenously and images recorded after 10 minutes.

After each experiment the quantitative images of the heart obtained with  $^{81\text{m}}\text{Kr}$  were displayed on the computer oscilloscope visual display unit within a  $64 \times 64$  matrix of squares. The whole image was then enclosed within seven rectangular areas of interest. The activity (in counts per minute) in each area was expressed as a ratio of the total myocardial activity. The  $^{201}\text{Tl}$  cardiac scintigram from the same experiment was then displayed within the same areas of interest and the corresponding seven ratios calculated. Throughout each experiment care was taken that the orientation between the heart and the gamma camera did not change. The dogs were held in position throughout each experiment and the images on the visual display were checked to ensure that they had not moved within the  $64 \times 64$  matrix during the course of each experiment.

No computer processing, image enhancement, or background subtraction techniques were used.

At the end of each experiment the snared LAD was selectively injected with 5 ml of patent blue 5 dye, and the area of myocardium stained by the distribution of that vessel was dissected out and weighed.<sup>12</sup>

The experimental procedure for each group of dogs is summarized in Table 1. Paired *t*-tests were used to compare the regional myocardial distribution of  $^{81\text{m}}\text{Kr}$  and  $^{201}\text{Tl}$ . Linear regression analysis was used to compare the regional myocardial distribution of creatine kinase activity and  $^{201}\text{Tl}$ .

## Results

The regional myocardial distribution of  $^{81\text{m}}\text{Kr}$  was similar to that of  $^{201}\text{Tl}$  in the cardiac scintigrams from those

experiments with no LAD stenosis and normal epicardial ECG complexes (Fig. 1, a and c). The ratios demonstrated that there were no significant differences in the relative myocardial distribution of the two indicators (Fig. 1c). Paired *t*-tests showed  $P = > 0.50$ .

In all the experiments the percentage decreases in the regional myocardial activity of  $^{81\text{m}}\text{Kr}$  produced by LAD narrowing were closely related to the percentage decreases in regional coronary flow using the flow probe ( $r = 0.91$ ,  $P = < 0.001$ ,  $n = 21$  observations, linear regression analysis).

Twenty-four hours after LAD occlusion, the regional myocardial distribution of CK activity showed marked decreases in the area affected by the LAD occlusion. The activity in the affected areas was  $0.56 \pm 0.062$  U/mg DNA and in the unaffected areas it was  $1.30 \pm 0.13$  U/mg DNA (mean  $\pm$  SD). The regional myocardial activity of  $^{201}\text{Tl}$  in remote regions of the heart unaffected by the LAD snare was treated as 100% in each dog. The percentage decrease in the regional myocardial activity of  $^{201}\text{Tl}$  at each site was calculated from the measured activity within the region affected by the LAD snare. The relationship between the regional myocardial decrease in the activities of CK and  $^{201}\text{Tl}$  was  $Y = 0.918X + 8.45$ ,  $r = 0.93$ ,  $P = < 0.001$ ,  $n = 80$  myocardial samples.

In seven of the 21 dogs, LAD narrowing produced a fall in coronary blood flow of 37–56% (mean = 45%). The epicardial ECG showed no changes and the myocardial distribution of  $^{81\text{m}}\text{Kr}$  demonstrated regional decreases in activity in the area supplied by the snared vessel (Fig. 2a). The ratios in Figure 2b demonstrate the changes in the distribution of this isotope when regional coronary flow was diminished. The  $^{201}\text{Tl}$  cardiac scintigrams and the ratios shown in Figure 2, a and b, did not demonstrate any regional defect when this isotope was delivered while the LAD was narrowed in these seven dogs.

In seven dogs the LAD was narrowed until mean coronary flow decreased by 35–60% (mean = 51%). The

TABLE 1 Groups of Dogs and Experiments using Thallium-201 Scintigraphy

|                                | LAD flow*<br>(ml/min)† | $^{81\text{m}}\text{Kr}$ image | Epicardial<br>ECG                                 | Heart rate<br>(beats/min)‡ | BP<br>(mm Hg)‡ | Thallium-201<br>image  | CK to $^{201}\text{Tl}$<br>activity             |
|--------------------------------|------------------------|--------------------------------|---------------------------------------------------|----------------------------|----------------|------------------------|-------------------------------------------------|
| Group 1 ( $n = 7$ )<br>Control | $53 \pm 5.0$           | No regional<br>defect          | Isoelectric S-T<br>segment                        | $123 \pm 14.0$             | $93 \pm 7.0$   | No defects             |                                                 |
| 24 hr after LAD<br>narrowed    |                        |                                |                                                   |                            |                |                        | $Y = 0.918X + 8.45$<br>$r = 0.93$ , $P < 0.001$ |
| Group 2 ( $n = 7$ )<br>Control | $48 \pm 7.0$           | No regional<br>defect          | Isoelectric S-T<br>segment                        | $128 \pm 12.0$             | $95 \pm 5.0$   |                        |                                                 |
| After LAD<br>narrowed          | $24 \pm 9.0$           | Regional<br>defect             | Isoelectric S-T<br>segment                        | $121 \pm 17.0$             |                | No regional<br>defects |                                                 |
| Group 3 ( $n = 7$ )<br>Control | $52 \pm 8.0$           | No regional<br>defect          | Isoelectric S-T<br>segment                        | $118 \pm 15.0$             | $99 \pm 4.0$   |                        |                                                 |
| After LAD<br>narrowed          | $26 \pm 11.0$          | Regional<br>defect             | S-T elevation,<br>S-T depression<br>Both $> 2$ mm | $120 \pm 18.0$             |                | Regional<br>defect     |                                                 |

\* Measured by electromagnetic flowmeter.

† Mean  $\pm$  SD.

‡ Linear regression analysis: CK U/mg DNA and thallium-201 counts/mg DNA.

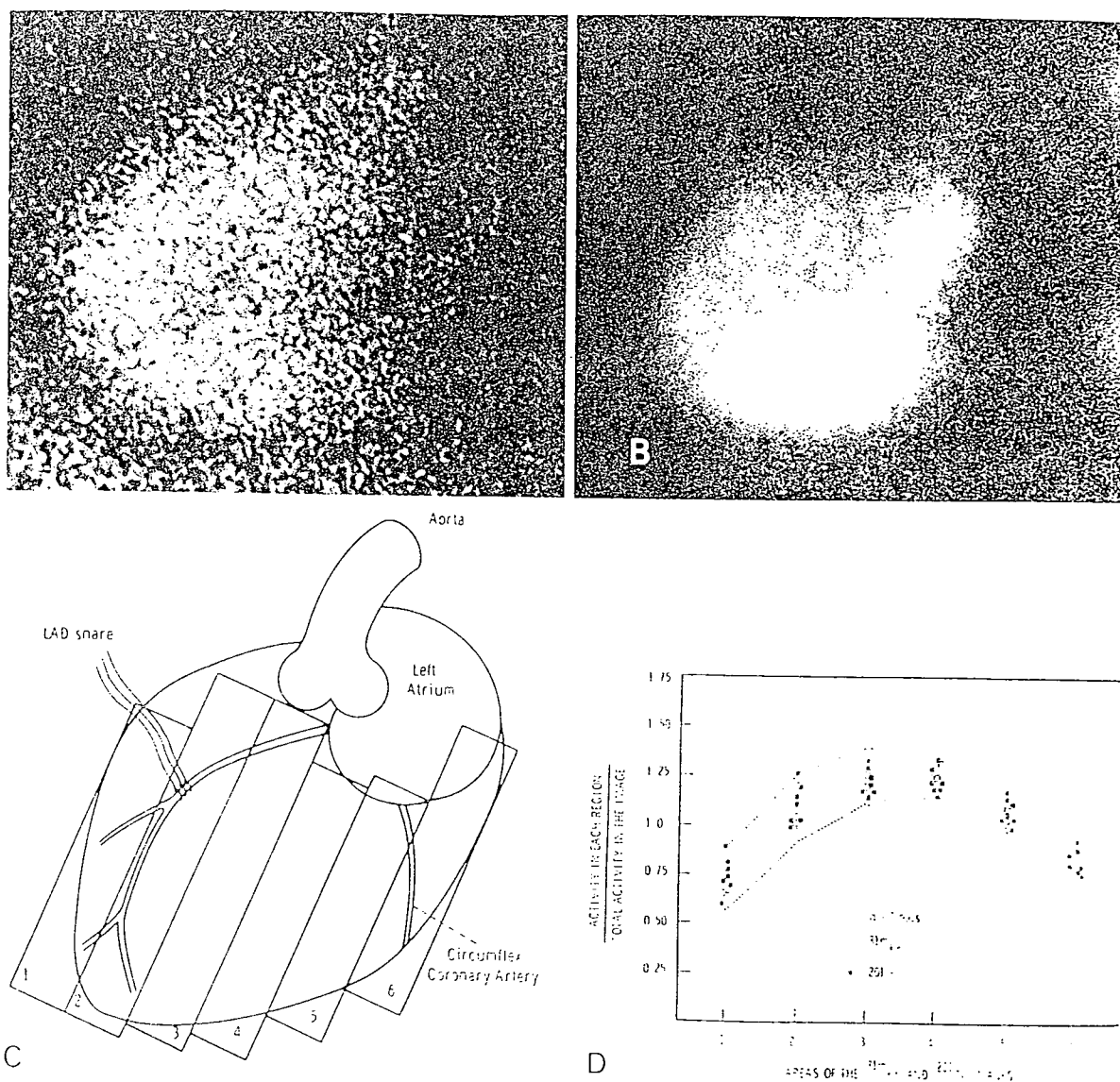


FIGURE 1. *a:* Scintigrams of the heart using  $^{81\text{m}}\text{Kr}$  and  $^{201}\text{Tl}$  are shown. The regional myocardial distribution of these tracers was similar when the coronary circulation was intact. The  $^{201}\text{Tl}$  image is shown in *a*; the  $^{81\text{m}}\text{Kr}$  image is shown in *b*. *c:* The  $^{81\text{m}}\text{Kr}$  and  $^{201}\text{Tl}$  scintigrams in each experiment were divided into six areas of interest on the visual display unit. The relationship between these areas and the cardiac anatomy is shown. *d:* In each experiment, the  $^{81\text{m}}\text{Kr}$  and  $^{201}\text{Tl}$  images were positioned within the same six areas of interest. The ratios of the regional to the total activity in each area shown, with imaging, that the regional myocardial distribution of the tracers was similar.

epicardial ECG showed planar S-T segment depression ( $< 2$  mm) and T wave inversion in two, and characteristic S-T segment elevation ( $< 2$  mm) in the other five. The  $^{81\text{m}}\text{Kr}$  cardiac scintigrams and the ratios of the myocardial distribution of  $^{81\text{m}}\text{Kr}$  showed a regional decrease in activity after LAD narrowing (Fig. 3, *a* and *b*). The  $^{201}\text{Tl}$  cardiac scintigrams and the ratios of activity in these dogs showed a corresponding regional decrease in activity (Fig. 3, *a* and *b*).

The segments of myocardium supplied by the snared LAD weighed between 29 and 43 g.

The results are summarized in Table 1.

## Discussion

In these experiments regional coronary flow in the snared vessel was measured with the electromagnetic probe and changes in regional myocardial perfusion were observed by imaging the steady rate equilibrium of krypton-81m in the heart.<sup>13, 14</sup> During a continuous infusion of  $^{81\text{m}}\text{Kr}$  into the aortic sinuses, a proportion of this tracer reaches the coronary circulation. This inert and freely diffusing indicator has a half-life (13 sec, decay constant 3.2/min) that is faster than normal and reduced values for the turnover of myocardial flow per unit volume. The

steady state equilibrium of  $^{81m}\text{Kr}$  in the myocardial water space in these circumstances depends mostly on arrival of the tracer by blood flow and the constant radioactive decay. The  $^{81m}\text{Kr}$  myocardial signal will arise mostly from the extracellular fluid space and myocardial tissues. The short half-life will prevent washout of the tracer from being important.<sup>13,14</sup>

The results showed that when coronary flow was not obstructed, the regional myocardial distribution of  $^{81m}\text{Kr}$  and  $^{201}\text{Tl}$  were similar, using images recorded with a gamma camera. However, when regional perfusion was diminished as shown by the flow probe and  $^{81m}\text{Kr}$  scintigrams, the  $^{201}\text{Tl}$  images of the heart did not demonstrate the regional decrease in perfusion while the ECG was normal. When the LAD narrowing and decreases in myocardial perfusion were accompanied by epicardial ECG evidence of some cellular abnormality, the  $^{201}\text{Tl}$  scintigrams demonstrated regional decreases in activity corresponding to the defects seen in the  $^{81m}\text{Kr}$  images. The regional decreases in myocardial perfusion in these experiments may or may not be accompanied by manifestations of tissue ischemia. This will depend on the available collateral blood flow and metabolic demand ( $\text{MVO}_2$ ) at the time of LAD narrowing.

The regional myocardial depletion of CK activity has been used to assess ischemic damage after coronary occlusion.<sup>15</sup> These experiments showed that the distribution and severity of the myocardial depletion of  $^{201}\text{Tl}$  and CK activity 24 hours after LAD occlusion were similar. This suggested that in these circumstances  $^{201}\text{Tl}$  was acting as a tissue marker of ischemic damage.

The affected regions of myocardium were positioned on the edge of the images of  $^{81m}\text{Kr}$  and  $^{201}\text{Tl}$  activity. The segments of tissue affected by the coronary narrowing were dissected and weighed. The use of patent blue 5 dye overestimates the size of the ischemic area; however, this technique was used in an attempt to ensure that the areas of interest were big enough to resolve as defects using  $^{201}\text{Tl}$  and  $^{81m}\text{Kr}$ .

Strauss et al.<sup>2</sup> and others have used experimental models to show that the regional distribution of  $^{201}\text{Tl}$  in the heart is related to myocardial blood flow. Those experiments did not separate the energy-dependent cellular mechanisms that dominate the extraction of this tracer from the blood. Poe<sup>6</sup> has shown that the proportion of  $^{43}\text{K}$  and related compounds extracted by the myocardium is inversely related to blood flow. Buja et al.<sup>16</sup> have shown that ischemic myocardium has a limited ability to

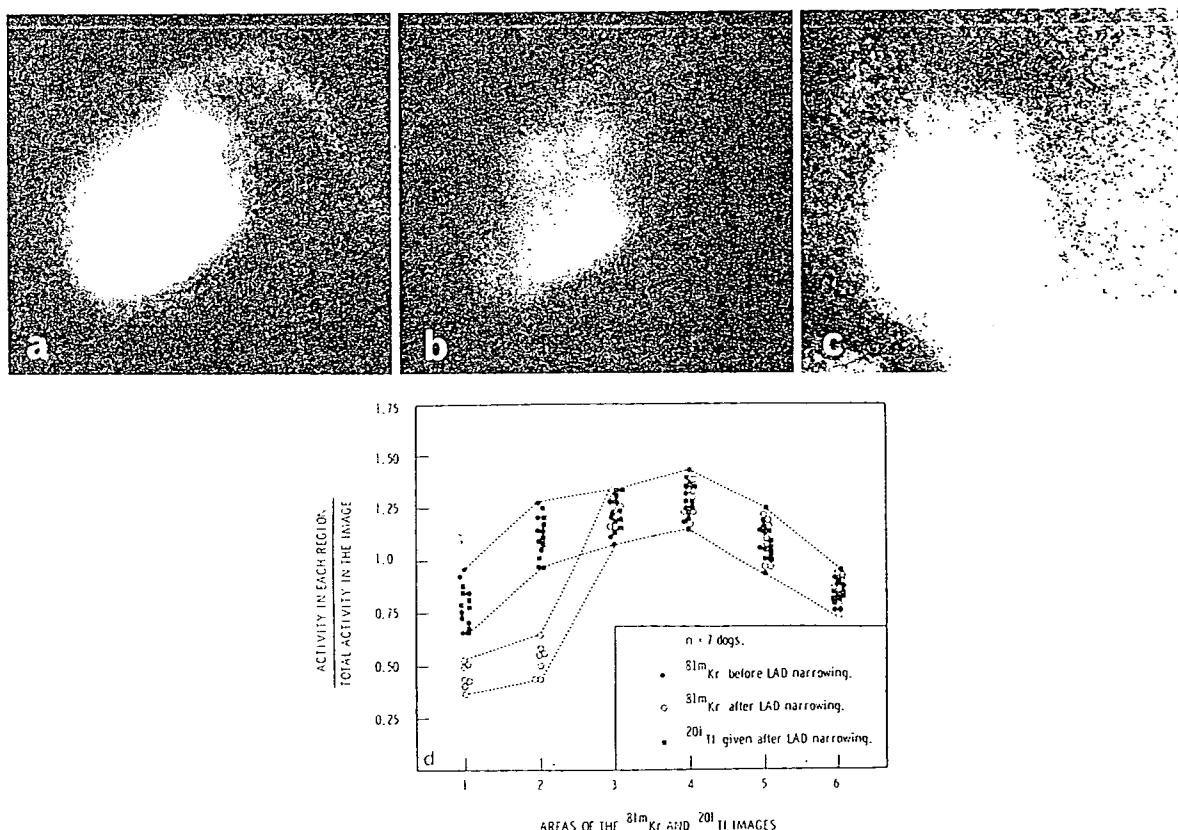


FIGURE 2 The myocardial distribution of  $^{81m}\text{Kr}$  is shown before (a) and after (b) partial occlusion of the LAD. The regional defect in activity that appears was not accompanied by epicardial ECG changes. The  $^{201}\text{Tl}$  scintigrams showed no such defect in activity (c). d: The ratios showing the regional activity of  $^{81m}\text{Kr}$  demonstrate the loss of activity in the area supplied by the LAD. The ratios for  $^{201}\text{Tl}$  show a normal distribution when the epicardial ECG was normal. Paired *t*-tests showed that the change in the regional myocardial distribution of  $^{81m}\text{Kr}$  were significant ( $P < 0.01$ ) and the changes in  $^{201}\text{Tl}$  were not ( $P < 0.20$ ) for areas 1 and 2.

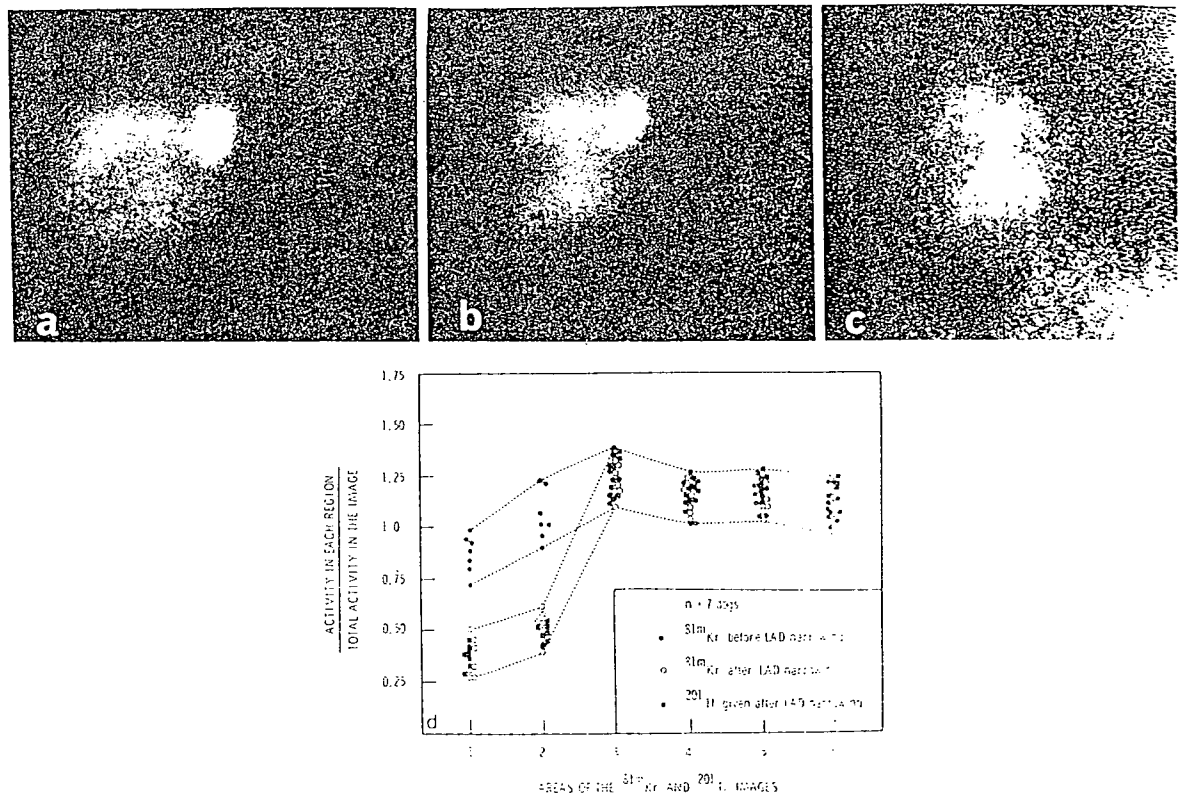


FIGURE 3 The myocardial distribution of  $^{81m}\text{Kr}$  is shown before (a) and after (b) partial occlusion of the LAD. The region in activity that appeared was accompanied by epicardial ECG changes in these dogs. The  $^{201}\text{Tl}$  cardiac scintigrams showed a defect in activity (c). d: The ratios showing the regional activity of  $^{81m}\text{Kr}$  demonstrates the loss of activity in the area supplied by the narrowed LAD. The ratios for  $^{201}\text{Tl}$  showed a similar regional loss in activity (compare to Figure 1b). Paired t-tests showed no changes in the regional myocardial distribution of  $^{81m}\text{Kr}$  and  $^{201}\text{Tl}$  were significant ( $P = < 0.01$ ) for areas 1 and 2.

take up  $^{201}\text{Tl}$ , and this is followed by a redistribution of the indicator resulting from rediffusion and recirculation and also collateral flow. Hamilton et al.<sup>17</sup> have demonstrated that ischemic but not infarcted myocardium can take up  $^{201}\text{Tl}$  during the complex kinetics of this tracer that follows the initial distribution of an intravenous injection.

The work of Adolph et al.<sup>18</sup> supports the view that reversibly hypoxic myocardium has a limited capacity for  $^{201}\text{Tl}$  uptake. Clinical research comparing  $^{201}\text{Tl}$  scintigraphy and the ECG testing during exercise does not always show  $^{201}\text{Tl}$  defects with regional ECG abnormalities.<sup>19</sup> This experimental model will tend to oversimplify the clinical situation in man, but the specific techniques of  $^{81m}\text{Kr}$  scintigraphy and graded coronary occlusion have been used to demonstrate a single principle in the use of  $^{201}\text{Tl}$ .

In patients with segmental rigid coronary artery stenosis and functional disturbances of regional myocardial perfusion,  $^{201}\text{Tl}$  scintigrams will demonstrate defects in activity on stress.<sup>4, 19</sup> However, the results of this study would suggest that  $^{201}\text{Tl}$  cardiac scintigraphy may not detect regional decreases in myocardial perfusion due to coronary artery disease until these have progressed sufficiently to disturb myocardial metabolism at rest or during stress.

In conclusion,  $^{201}\text{Tl}$  cardiac scintigrams could not detect experimental decreases in regional myocardial perfusion alone in these experiments. The myocardial distribution of this indicator did demonstrate decreases in regional myocardial perfusion when these were accompanied by disturbances of the epicardial electrocardiogram. These findings require further experiments that look at the cellular mechanisms of  $^{201}\text{Tl}$  uptake and may be important in the interpretation of cardiac scintigrams by using this tracer in man.

### Acknowledgments

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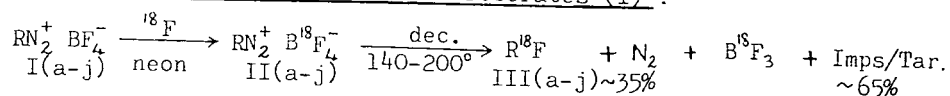
$^{18}\text{F}$ ]-L and DL-FLUOROARYL AMINO ACIDS. PRESENT STATUS.

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Although these compounds have been available since 1970 (1-4) they have not yet given successful results as pancreas imaging agents in man. On the other hand they have not been definitely rejected. The materials previously prepared could have contained labelled impurities and were originally made at relatively low specific radioactivities. Also the comments of ref (5) concerning the instability of derivatives of DOPA should be noted. The recent visualisation of the pancreas in man using  $^{14}\text{C}$ -valine(6) and the opportunities opened up by the  $\beta^+$ -camera encourage further work with the  $^{18}\text{F}$ -amino acids. We consider that as many as possible of the amino acids should be available for the user.

A re-evaluation of the preparation and use of these compounds has been undertaken. The key intermediates were the protected formamido, III(a-d) or acetamido, III(e-j), malonic esters(6). The protected  $^{18}\text{F}$ -labelled compounds (III) were obtained by thermal decomposition of the corresponding diazonium fluoroborates (II). Because of chemical and physical differences between the various compounds (III) a variety of methods of decomposition and subsequent de-protection(7) were needed.

 $^{18}\text{F}$ -labelling of the diazonium fluoroborates (I) :

The aryl diazonium fluoroborates (I) were labelled by heterogeneous exchange between  $^{18}\text{F}$ /neon(80 psi) and the organic solid (13mg) dispersed in a filter element, a 3.5cm diameter GFD disc (Fig 1) in the recirculatory target system(7,8). The effect of additives in the target gas was investigated. With pure neon, or neon containing oxygen (15%) or nitrogen (15%), about 42% of the fluorine-18 produced was trapped on the filter disc. When neon containing hydrogen (15%) was used, virtually all the fluorine-18 produced remained on the glass walls(8) of the target vessel. These results suggest that the species  $\text{NO}^{18}\text{F}$  or  $\text{O}_2^{18}\text{F}$  may be the carriers of fluorine-18 between the target vessel and the filter element, but this idea can be criticised.

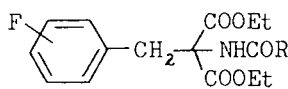
Decomposition of the  $^{18}\text{F}$ -diazonium fluoroborates (II) :

A variety of methods were investigated for this stage. Speed and ease of purification and of handling of the product (III) were desired. It was not possible to use a single method for all cases (a-j). Decomposition by heating alone ("dry") had the advantage that the crude product could be taken up in a volatile solvent for subsequent purification by preparative TLC, LC or GLC. The previously described inert solvent (tetralin) decomposition(9) suffered from several disadvantages and so was not used.

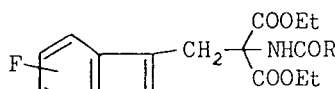
When the fluoro-ester was sufficiently volatile, III(a-d,i,j) it was feasible to purify (III) by vacuum sublimation or GLC. Vacuum sublimation did not prove to be useful because the sublimate still contained many impurities. On the other hand GLC using a 5ft  $\times$   $\frac{1}{4}$ in column of 3% OV1 or QF1 at 200°C gave a pure product (Fig 2) that was readily trapped at the chromatograph outlet. It was even possible to inject the  $^{18}\text{F}$ -diazonium fluoro

-borate in acetone directly into the injector of the chromatograph (glass column essential).

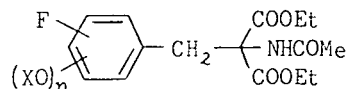
FLUORO-ESTERS III(a-j).



III(a-d)



III(e-h)



III(i,j)

(a)p-F (b)m-F, R = H.

(c)p-F (d)m-F, R = Me.

Protected forms of  
fluorophenylalanines

V(a p-F, b m-F).

(e)5-F (f)6-F, R = H.

(g)5-F (h)6-F, R = Me.

X = Ac. Protected forms  
of fluorotryptophans

V(e 5-F, f 6-F).

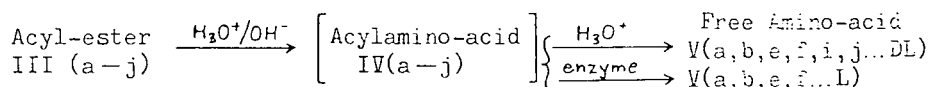
(i)3-F, X = 4-Me (n = 1)

(j)5-F, X = 3,4-Me<sub>2</sub> (n = 2)

Protected forms of  
fluoro-tyrosine V(i),  
fluoro-DOPA V(j).

Routine Procedure : The dry decomposition was always used. The GFD-disc with <sup>18</sup>F-diazonium fluoroborate was placed between two stainless steel discs (5 X 1cm) preheated to 150°C (170°C for indoles) and left for 7min. The crude products III(e-h) were extracted into chloroform and purified by preparative TLC on a 5 X 10cm "analytical" silica gel plate eluted with chloroform, ethyl acetate (5:1-vols), R<sub>F</sub> ~ 0.7 (Fig 3). The crude products III(a-d,i,j) were purified by preparative GLC as described above, t<sub>R</sub> ~ 10 min (Fig 2).

Hydrolysis of [<sup>18</sup>F]-Fluoro-esters (III) including enzyme resolution.



The acetyl esters III(c,d,g,h) gave the *N*-acetyl-DL-amino acids (IV) by mild hydrolysis (Method 1. In g,h X → H also). These intermediates were stereoselectively converted to the free L-amino acids V(a,b,e,f) using an amino acylase *ex Aspergillus Orizae*, kindly supplied by Dr I. Chibata (Method 2) (9,10). Cleavage of the acetyl esters III(c,d,i,j) with 47% HBr gave the DL-amino acids V(a,b,i,j)\* (Method 3). Compounds III(a,b,e,f) containing the labile *N*-formyl group are reported to give the DL-amino acids directly by Method 1 (2) but analysis of the products on the Biogel column indicated that this hydrolysis was incomplete, even negligible in 30min. [(i,j) X → H].

Method 1 did not work for compounds III(i,j) because of the great stability of the *O*-methyl group(s). A more labile protecting group is required. The *O*-isopropyl ethers would be suitable but the synthetic problems are great.

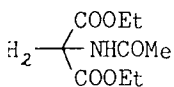
Routine Procedure : Method 1. The fluoro-ester III(a,b,e,f) was refluxed with 0.3N-NaOH (400μl) for 20min, and after the addition of 2N-HCl (75μl) refluxed a further 15min. The final solution was adjusted to pH 7.

Method 2. The enzyme (3mg, as supplied) was made up in water (200μl) with sodium acetate (7mg) and activated by the addition of Co<sup>2+</sup> (50μl of 0.0001M CoCl<sub>2</sub>). The enzyme solution was incubated with the acyl amino acid solution for 20min. The L-amino acid was isolated as below.

Method 3. The fluoro-ester III(c,d,i,j) was refluxed with 47%-HBr (200μl) for 45min. The resulting solution was diluted 5-fold with water and passed through a 7 X 1cm column of Amberlite IR4B(OH<sup>-</sup>).

Purification. The final solution (0.5-1.0ml) was passed through a 30 X 1 (10 X 1 for tryptophan) cm column of Biogel P2 (200-400mesh) eluted with de-ionised water at 0.3ml/min (amino acid t<sub>R</sub> ~ 30min, Fig 4).

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3,4-Me<sub>2</sub> (n = 2)  
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# FIGURES

Fig 1 : <sup>18</sup>F-exchange vessel used in recirculatory neon target system. (Ref 8 - complete system).

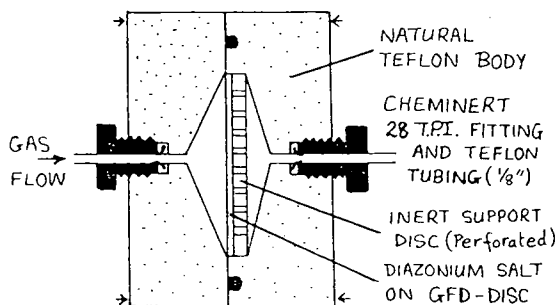


Fig 2 : Purification of fluoro-ester III(j) by preparative GLC. (See text).

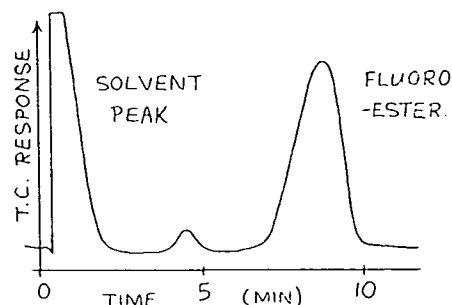


Fig 3 : Purification of fluoro-ester III(g) by preparative TLC. (See text).

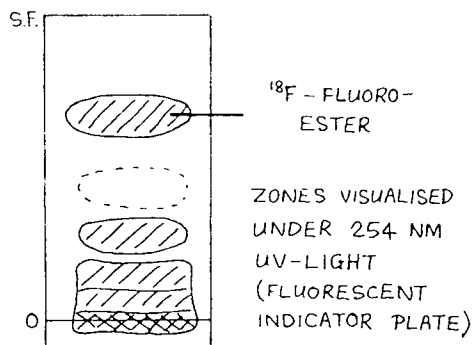
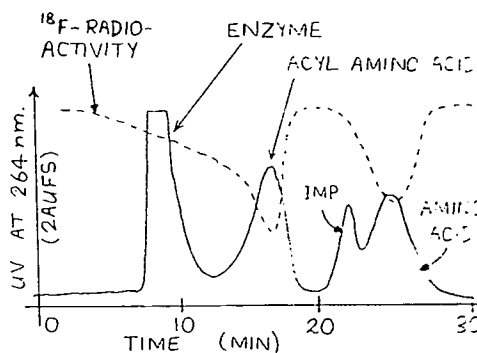


Fig 4 : Purification of fluoro-amino acid V (c) by preparative LC (after enzyme resolution - conditions in text).



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INCORPORATION OF FLUORINE-18 IN PERHALO COMPOUNDS USING THE  $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$  REACTION

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The recoil chemistry of fluorine-18 and simple organic substrates has been extensively studied, mainly at low radiation dose using the  $^{19}\text{F}(\gamma,\text{n})^{18}\text{F}$  and  $^{19}\text{F}(\text{n},2\text{n})^{18}\text{F}$  reactions (1). A variety of gaseous inorganic fluorinating agents have been labelled with high levels of radioactivity using the  $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$  reaction, where due to the high radiation doses encountered the effects of radiation chemical reactions would be expected to predominate (2-5). Sulphur hexafluoride and several halomethanes (I-V) have now been labelled in high activities by the bombardment of 0.3-2% mixtures of an appropriate substrate in neon with 14 MeV deuterons. This work was undertaken in order to make some of the compounds available for pharmacodynamic studies. In addition sulphur hexafluoride- $^{18}\text{F}$  is of interest for the study of regional pulmonary diffusion.

The target was a cylindrical aluminium vessel 45 cm long by 6 cm diameter with a 4 x 2 cm beam entry window of 0.050 mm stainless steel. A cylindrical copper liner electroplated internally with silver to a thickness of 0.1 mm was introduced via the detachable backplate. After evacuation to a pressure of  $10^{-3}$  mm Hg the target was filled with C.P. grade neon and the substrate (0.5 - 4 m moles) to a pressure of 3.5 Kgf.cm $^{-2}$ . Following irradiation the gaseous products were analysed by radio-gas chromatography on a 1.55 m x 6.25 mm column of 80-100 mesh Porapak-Q using a carrier gas flow rate of 20 ml min $^{-1}$ . The gaseous contents of the target were vented to a trap at -196° at a flow rate of 50-100 ml min $^{-1}$  and the extracted radioactivity measured.

The results obtained for sulphur hexafluoride and compounds in the series  $\text{CCl}_{4-n}\text{F}_n$  (n = 1 to 4) as substrates are given in Table 1. The radiochemical yields were calculated using previously reported data for the  $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$  reaction (6,7). With sulphur hexafluoride labelling of the substrate was the predominant reaction and the two major gaseous impurities were sulphuryl fluoride- $^{18}\text{F}$  and thionyl fluoride- $^{18}\text{F}$  (identified by glc - mass spectrometry) arising from the presence of traces of oxygen in C.P. neon. A cryogenic purification system for sulphur hexafluoride- $^{18}\text{F}$  has been developed.

Similarly, labelling of the substrate in high activity was also observed in the case of tetrafluoromethane (V). In this case traces of  $^{18}\text{F}$ -labelled hexafluoro ethane and octafluoropropane were detected (by glc-mass spectrometry) together with other unidentified impurities. With tetrachloromethane (I) and the chlorofluoromethanes (II, III, IV)  $^{18}\text{F}$  for Cl and  $^{18}\text{F}$  for F replacement reactions were observed, products other than the major one being other chlorofluoromethanes with minor amounts of unidentified material. Mass peaks on analytical glc were observed for all the identified labelled products. The silver plated target liner was essential in order to obtain the products in the yields reported here, indicating that chemical processes at the liner surface are important. Bombardments with these substrates result in chemical deposits on the

${}^3\text{He}(\text{d},\alpha){}^{18}\text{F}$

W12 OHS, UK.

ates has been  
 ${}^3\text{F}(\gamma, n){}^{18}\text{F}$   
 nic  
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 methanes (I-V)  
 of 0.3-2%  
 cons. This  
 ailable for  
 ${}^3\text{F}$  is of

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surface of the liner and it is likely that this unidentified material plays a part in the reactions. Neither the liner nor the target were chemically cleaned between bombardments.

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Of 60 questionnaires sent out to institutes known to be operating accelerators, replies were received relating to 55 individual accelerators all of which had a program for radionuclide production. The majority of the non-commercially operated accelerators were seen to be concentrating their efforts on producing radionuclides which were not generally available from the 9 commercially operated accelerators. Notable exceptions however were  $^{123}\text{I}$  and  $^{201}\text{Tl}$ . Some of the problems encountered in the use of these accelerators for large scale radionuclide production will be discussed.

[Accelerator, radionuclide production]

### Introduction

There are at least 55 accelerators engaged in radionuclide production at the present time.<sup>1</sup> Their energies range from the 6 MeV deuterons of the Allis-Chalmers classical cyclotrons in St. Louis and Boston to the 800 MeV protons of the linear accelerator at Los Alamos. However, the majority (19) of these accelerators are isochronous cyclotrons with a proton energy between 20 and 30 MeV. Table 2 gives a more detailed breakdown of the distribution. In order to get an up to date picture of the extent of radionuclide production programmes of these accelerators, two of my colleagues recently sent out a questionnaire to more than 60 institutes known to be operating accelerators. 48 replies were received relating to the 55 accelerators mentioned above. Unfortunately, no information was received from E. Europe, S. Africa, or S. America. The results are summarized in Table 1. Part A comprises the "non-commercial" accelerators (40 cyclotrons and 2 Linacs and lists for each of those radionuclides (a) which are currently being produced at least once a week, (b) those which are produced less frequently, and (c) those which are expected to be in production by 1980. Part B of the table lists the world's 9 commercially operated cyclotrons and their products.

In order to discuss some aspects of these accelerator operations that may be of interest the present gathering, it is useful to divide them into roughly four energy ranges namely 100-800 MeV, 50-100 MeV, 20-50 MeV, (including the large group at 20-30 MeV), and those up to 20 MeV proton energies. I will avoid any reference to cost effectiveness and dwell only on some of the practicalities of radionuclide production with each group, where possible mentioning some unique aspects.

#### Accelerators With Proton Energies of 100-800 MeV

Five accelerators with a large scale radionuclide production potential exist in the Western World. Two are linear accelerators, the 200 MeV injector for the alternating gradient synchrotron at Brookhaven<sup>2</sup> and the 800 MeV giant at the Los Alamos meson physics facility.<sup>3-4</sup> The remaining 3 are cyclotrons and include the very large isochronous cyclotron at Vancouver<sup>5</sup> at 500 MeV, and two separated sector cyclotrons at Bloomington, Indiana<sup>6</sup> (200 MeV) and Villigen, Switzerland<sup>7</sup>, (590 MeV). All these accelerators rely heavily on spallation reactions for radionuclide production. This is a field which is probably much more familiar to some of our participants than myself so I hope my interpretation of the problems stand up to their criticism. The term spallation seems to cover a complex series of nuclear events when incident nucleons with energies in excess of

100 MeV interact with a target, including knock-on cascade, evaporation, fragmentation and for sufficiently high Z materials, fission processes. In addition, secondary nuclear reactions can occur between emitted particles and other target nuclei. Thus one can expect the formation, to a greater or lesser extent, of every element from Z + 2 or 3 above the target to Z = 1. At 800 MeV three distinct regions can be seen if the formation cross sections are plotted against mass number. In the case of a bismuth target a peak A = 180 to 200 can be identified due to spallation products near the target mass, a fission product peak between A = 60 and 140, and a fragmentation continuum below A = 40.

To the radionuclide production chemist this can only mean formidable radiochemical recovery and decontamination problems. However, it must be fairly self-evident that several radionuclides of that Z will be present in the product. For example,  $^{82}\text{Sr}$  ( $t_{1/2} = 25$  day) is of interest in the medical field as a source of radiogenic  $^{82}\text{Rb}$  ( $t_{1/2} = 1.25$  min), a  $\beta^+$  emitter of potential value in emission tomographic studies of heart and kidney function. The most effective means of production is via spallation of a molybdenum target using 800 MeV protons at LAMPF.<sup>8</sup> All samples of  $^{82}\text{Sr}$  however, contain significant amounts of  $^{84}\text{Sr}$  ( $t_{1/2} = 65$  d) so that great care must be exercised when designing and operating a radionuclide generator<sup>9-10-11</sup> for the in vivo use of  $^{82}\text{Rb}$  as a strontium leakage could result in unacceptable  $^{84}\text{Sr}$  burdens in the bone. Fortunately, systems have been perfected for this use so that high levels of  $^{82}\text{Rb}$  can be available due to this fortunate radiogenic relationship.

The production of high purity  $^{123}\text{I}$  ( $t_{1/2} = 13$  h) and  $^{201}\text{Tl}$  ( $t_{1/2} = 73$  h) are further examples of radiogenic purification and will be referred to below.

#### Accelerators With Proton Energies of 50-100 MeV

There are around 10 accelerators, all cyclotrons, in this group and almost all originally dedicated to nuclear physics work. However, they have now turned some of their attentions to the production of medically useful radionuclides and are recovering some of their operating costs by doing so.

$^{123}\text{I}$  seems to be by far the most popular radionuclide to produce, and several ingenious and highly effective target systems have been devised and perfected to produce it via the  $^{127}\text{I}(p,n)^{123}\text{Xe}$   $t_{1/2} = 2.1$  h  $\rightarrow$   $^{123}\text{I}$  reaction. The relevant nuclear data have recently been reviewed by STOCKLIN AND QAIM.<sup>12</sup> The various chemical

forms of iodine used include  $I_2$ ,<sup>6-13</sup>  $CH_2I_2$ ,<sup>14</sup> mixtures thereof,<sup>15</sup> and NaI.<sup>16</sup> In addition the use of liquid Cs and the  $^{133}Cs(p,2p9n)^{123}Xe$  reaction has been proposed when protons of variable energy are being dumped.<sup>14</sup> At the Crocker cyclotron, (UC Davis) an elegant target system centred around a stainless steel vessel containing molten NaI is maintained at 650°C by a combination of beam power and a controlled helium flow.<sup>18</sup> The  $^{123}Xe$  is removed from the target by the helium and transported to a remote laboratory where it is recovered and purified cryogenically prior to allowing its decay to  $^{123}I$ . Several important factors such as target thickness and NaI density during irradiation had to be carefully studied before the routine target was perfected. In the use of methylene iodide,  $CH_2I_2$ , at the variable energy cyclotron at Harwell, U.K., different problems arose.<sup>15</sup> Here the aim was to circulate the organic liquid through a titanium target cell and recover the  $^{123}Xe$  using a gas/liquid scrubber. Early experience showed that extensive radiolytic polymerisation of the  $CH_2I_2$  to a thick oil occurred at high beam currents making it impossible to pump it around the circuit. However, the application of a classical radiation chemist's radical scavenger, molecular iodine  $I_2$ , not only solved the polymerisation problem but also increased the iodine content of the target mixture. The target system has now been in routine use for about 3 years satisfying some of the demand for  $^{123}I$  in the U.K.

#### Accelerators With Proton Energies of 20-50 MeV

This group includes all the commercially operated cyclotrons and perhaps mention of some of the problems encountered here would be of interest. The major logistic problem is that the accelerator produced product must be available on a routine scheduled basis. Thus a commercial operator would expect his accelerator to be dedicated to radionuclide production and be ready for action at all times. He may even have more than one accelerator! He will also be keen to make the very best use of the beam available from his particular accelerator.

When a charged particle beam interacts with a target the principle method of energy loss is by ionisation; only about one particle in a thousand produce a nuclear reaction and the rest of the energy appears as heat and here lies the major problem for the high current target designer. The internal beam of the cyclotron, where the highest intensities are usually available, has a radial distribution that is approximately triangular with the high intensity towards the centre of the machine. If a target plate is placed at a tangent to the beam the resultant beam pattern will be spread over a larger area. If the plate is then either angled to the tangent or bent to a radius slightly larger than the beam radius a still larger spread can be obtained. The more the beam can be spread out the lower the power density ( $Wcm^{-2}$ ). The power density in the unspread beam of a typical production cyclotron compares well with those found in the arc welding field, being typically 15-50  $KWcm^{-2}$ ; thermal damage to targets is therefore an ever present hazard. The beam dimensions of the flat field or classical cyclotron, few of which remain in the radionuclide production field, have internal beams of between 15mm X 5mm (Hammersmith 45")<sup>19</sup> and 70mm X 6mm (ORNL 86").<sup>20</sup> A typical isochronous cyclotron might have a beam of 4mm X 1.5mm.<sup>21</sup> Thus beam power densities in excess of 50  $KWcm^{-2}$  are easily achieved. The peak power density that can be handled by normal water cooling is about 3  $KWcm^{-2}$  so that it can be seen that if the full output potential of the more recently introduced commercial isochronous cyclotron is to be achieved, beam spreading techniques are of prime importance.

One manufacturer offers a beam interactive spreader that uses RF driven deflector plates above and below the circulating beam close to the final radius.<sup>22</sup> In other cases the users have found that rotating<sup>23-24</sup> or oscillating the target has helped provide the necessary power density reduction. The use of thin targets in this field is not usually practicable. However, the use of enriched isotopic targets can often give rise to a higher intrinsic yield together with a reduced interfering impurity. The production of  $^{201}Tl$  with protons of up to 28 MeV being a typical example.<sup>25</sup> Of course the use of highly enriched target materials is expensive, so high efficiency recovery procedures have to be devised. Often radionuclides of the target material are also made making target recovery and refabrication more difficult.

Target fabrication includes all the normal metallurgical techniques. A typical target would consist of copper or other high thermal conductivity backing of the shape most suited to the beam spreading criteria outlined above, with a thin layer (typically 0.050 - 0.1mm) of the desired target material deposited and securely fixed in a thermal sense onto its surface. Techniques such as electrodeposition, flame spraying, chemical vapour deposition, vacuum evaporation, soldering, brazing, casting and spot welding finding application, depending on the properties of the specific target material.

Many of the non commercial accelerators in this group do not have provision for internal targets and rely on the extracted or external beam for radionuclide production. Although somewhat less efficient the external arrangement does allow much more scope for target thickness selection and thus the opportunity to optimise a particular nuclear reaction to minimize impurity. The direct production of  $^{123}I$  via the  $^{124}Te(p,2n)^{123}I$  reaction is a good example. Several groups have applied themselves to this problem and have found that by using a thin highly enriched (92%)  $^{124}Te$  target and carefully selected proton energy the  $^{124}I$  impurity due to  $^{124}Te(p,n)^{124}I$  can be suppressed to below 1% at end of bombardment (EOB).<sup>26-27-28</sup> However, as the half lives of  $^{123}I$  and  $^{124}I$  are 13.3h and 4.3d respectively the  $^{124}I$  level increases quite rapidly with time.

One further advantage of the external beam is that gaseous and liquid targets can be irradiated. These are usually considered impracticable for internal beam irradiation due to the severe space restrictions imposed in the typical modern cyclotron's accelerating region.

The production of  $^{81}Rb$  ( $t_{1/2}$  4.5h) has achieved commercial status using an external beam and is used in the increasingly popular  $^{81}Kr^m$  ( $t_{1/2}$  13s) radionuclide generator. Krypton-81m has found widespread use in nuclear medicine organ flow studies.<sup>29-30</sup> Once again due to the radiogenic nature of the desired product,  $^{81}Kr^m$ , the presence of other Rb radionuclides in the generator is in practice of no importance. Thus the most favourable route to large scale production of  $^{81}Rb$  is via the  $Kr(p,xn)^{81}Rb$  reactions.<sup>31-32-33</sup> Protons of 20 MeV upwards have been found to be useful here, however the use of enriched  $^{82}Kr$  is essential for the lower energies.<sup>32</sup> Several approaches have been described for the recovery of the Rb from the target walls after irradiation but the most popular seems to be the use of a rotating target liner which can conveniently be washed with water.<sup>31</sup> The Rb is then simply bound to a small column of cation exchanger.  $^{81}Kr^m$  may be recovered continuously during clinical use in the gas phase, for lung ventilation assessment, by passing humidified air through the column or alternatively, in a solution suitable for infusion for blood flow investigations, by passing isotonic dextrose through the column.<sup>29-30</sup>



Here external beams and gas targets seem to be the order of day and the most commonly produced radionuclides are  $^{13}\text{N}$  ( $t_{1/2} = 10$  min),  $^{11}\text{C}$  ( $t_{1/2} = 20$  min),  $^{15}\text{O}$  ( $t_{1/2} = 2$  min) and  $^{18}\text{F}$  ( $t_{1/2} = 110$  min). They are almost invariably destined for incorporation into biomolecules or varying complexity for use in emission tomographic studies, description of which is beyond the scope of this presentation. However, there are several features of the production of these radionuclides in a chemical form directly applicable to these syntheses that may be of interest.  $^{13}\text{N}$  may readily be produced as  $^{13}\text{NO}_3^-$  by the  $^{16}\text{O}$  (p,  $\alpha$ )  $^{13}\text{N}$  reaction using a water target,<sup>34</sup> a simple reduction reaction yielding high specific activity  $^{13}\text{NH}_3$ .<sup>35</sup> In recent studies  $^{11}\text{C}$  produced via the  $^{14}\text{N}$  (p,  $\alpha$ )  $^{11}\text{C}$  reactions has been incorporated into brain receptor selective drugs.<sup>36</sup> Here the major aim is to exclude  $^{12}\text{C}$  from the target system so that the synthesis should result in very high specific activity biomolecules typically 0.5-1 Ci/ $\mu\text{M}$  or better. This is quite a challenging problem and has led workers into designing gas targets to standards usually only found in the ultra high vacuum field. The target gas has also received careful attention to remove traces of  $\text{CO}_2$  and hydrocarbons. One group in the field has observed an increasing reluctance of the  $^{11}\text{CO}_2$  to escape from the target<sup>37</sup> possibly due to selective adsorption sites trapping the desired product rather than being saturated with the undesired  $^{12}\text{CO}_2$ . This is probably a gross over-simplification of events and much work still seems to be necessary before an approach to the theoretical specific activity of  $^{11}\text{C}$  is achieved.

In the case of  $^{15}\text{O}$  specific activity has not yet received much attention but the chemical form in which  $^{15}\text{O}$  may be recovered, after the  $^{14}\text{N}$ (d, n)  $^{15}\text{O}$  reaction in target gases of different compositions, has been superficially studied.<sup>38-39</sup> The  $^{15}\text{O}$  atom produced here is a highly reactive species and has been seen to react with a variety of substrates including the target gas  $\text{N}_2$ . Radiation chemical effects can also be seen which alter the initial product composition as evidenced by studies at different beam currents (beam distribution remaining sensibly constant).

Turning to  $^{18}\text{F}$  we find that both the reactivity of the nucleogenic atom and specific activity are the problems confronting the target chemist. If recovery of  $^{18}\text{F}$  as a molecular  $\text{F}_2$  is desired for some synthetic purpose, the addition of carrier  $\text{F}_2$  to the Ne target gas ( $^{20}\text{Ne}$  (d,  $\alpha$ )  $^{18}\text{F}$ ) is essential as is careful attention to target vessel construction materials and techniques.<sup>40</sup> If recovery as  $\text{H}^{18}\text{F}$  is desired the addition of  $\text{H}_2$  to the Ne target gas can under favourable circumstances yield a high recovery of  $^{18}\text{F}$  as  $\text{HF}$  without the addition of carrier<sup>41</sup> which is of value as in the  $^{11}\text{C}$  case for the synthesis of receptors specific radiolabelled drugs.

#### The Place Of Nuclear Data In This Field

It must be obvious that for the generation of any radionuclide adequate nuclear data for the prediction of yields of both the desired product and any impurities are essential. Most of the accelerator groups engaged in this field have when necessary attempted an extension or improvement of the existing cross section data to suit their requirements. Often, however, it is not the nuclear physics that is the limiting factor. Problems of designing a target that will work reliably at the desired beam intensity and produce a recoverable product often seem to make the fine detail sometimes quoted in nuclear data of little practical consequence.

Perhaps to put this into better perspective consider a gas  $\text{N}_2$  target for the production of  $^{11}\text{C}$ . A calcula-

ted yield from the excellent cross section data available is seen in practice only to hold if carrier  $^{12}\text{C}$  is present and irradiations are carried out at low beam currents. As the current is increased the  $^{11}\text{C}$  production versus beam current becomes grossly non-linear due to a variety of effects which are as yet little understood.<sup>37,40-42</sup>

Factors involved may be simply thermal or induced plasma effects in the beam path both reducing the number of target nuclei in the beam path and also perhaps increasing the mean free path of the product nucleus, enabling it to react with the target walls (e.g.  $^{18}\text{F}$  and high specific activity  $^{11}\text{C}$ ). Further study is going to be essential before problems such as these are to be resolved.

However, the production of radionuclides with an accelerator is a challenging interdisciplinary and multidisciplinary field and the contributions of the workers in the cross section field are gratefully acknowledged.

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TABLE 2

| Proton Energy MeV | No of Accelerators |
|-------------------|--------------------|
| up to 10          | 4                  |
| 10 - 20           | 6                  |
| 20 - 30           | 19                 |
| 30 - 40           | 3                  |
| 40 - 50           | 6                  |
| 50 - 60           | 4                  |
| 60 - 70           | 3                  |
| 70 - 80           | 3                  |
| 80 - 100          | 0                  |
| 100 - 200         | 2                  |
| 200 - 800         | 3                  |

TABLE 1B

ACCELERATORS FOR RADIONUCLIDE PRODUCTION

B. COMMERCIALY OPERATED CYCLOTRONS

|                              |              |
|------------------------------|--------------|
| Firm                         | Machine Type |
| Location                     |              |
| Person Supplying Information |              |
| Radionuclides Produced       |              |

JAPAN

|                                           |           |
|-------------------------------------------|-----------|
| Nihon Medi-Physics                        |           |
| Takarazuka, Hyogo                         | TCC CS-30 |
| Dr. Masaaki Hazue                         |           |
| F-18, Ga-67, Rb-81, In-111, I-123, Tl-201 |           |

NETHERLANDS

|                                |                  |
|--------------------------------|------------------|
| Mallinckrodt (formerly Duphar) | Philips          |
| Petten, N. Holland             | (28 MeV protons) |
| No Information Given           |                  |

UNITED KINGDOM

|                                                                                                  |                  |
|--------------------------------------------------------------------------------------------------|------------------|
| The Radiochemical Centre                                                                         | Philips          |
| Amersham                                                                                         | (27 MeV protons) |
| Dr. M. Finlan                                                                                    |                  |
| Ga-67, In-111, Co-57, As-73, 74, Au-195, Be-7, Bi-206, 207, Co-56, Fe-55, Mn-52, 54, Ti-44, V-48 |                  |

UNITED STATES

|                                     |                    |
|-------------------------------------|--------------------|
| Medi-Physics                        |                    |
| Dr. A. Fleischer                    | Scanditronix MC-40 |
| (i) Arlington Heights, Ill          |                    |
| Rb-81, I-123, Tl-201                |                    |
| (ii) Emeryville, Calif.             | TCC CS-22          |
| Ga-67, Rb-81, In-111, I-123, Tl-201 |                    |
| (iii) South Plainfield, N.J.        | TCC CS-22          |
| Ga-67, Rb-81, I-123                 |                    |
| New England Nuclear                 | (i) TCC CS-22      |
| North Billerica, Mass.              | (ii) TCC CS-30     |
| Dr. J. Need                         | (iii) TCC CS-30    |
| (i) Co-57, Ga-67, Cd-109, In-111    |                    |
| (ii) Ge-68, Au-195, Tl-201          |                    |
| (iii) Ge-68, Au-195, Tl-201         |                    |

TABLE 1A ACCELERATORS FOR RADIONUCLIDE PRODUCTION:

DECEMBER 1978

## A. NON-COMMERCIAL ACCELERATORS

Location Machine Type  
 Institute Time Spent on Radio-  
 Person Supplying Information nuclide Production

\*Radionuclides Produced

BELGIUM

Ghent University OGR-MeV 520  
 Institute for Nuclear Studies  
 Dr. C. Vandecasteele 8 hr/wk

Rb/Kr-81, N-13, (C-11)

Liege University OGR-MeV 520  
 Cyclotron Research Centre  
 Dr. M.A. Guillaume 25 hr/wk

C-11, N-13, O-15, F-18, K-43, Rb/Kr-81, Se-73  
 Ga-67, In-111

Louvain-la-Neuve University OGR-MeV 930  
 Nuclear Chemistry Laboratory  
 Dr. M. Cogneau 15 hr/wk

Fe-52, I-123, Mg-28, At-211, (Tl-201)

CANADA

Vancouver, B. C. TOC CP-42  
 TRIUMF and  
 Dr. B.D. Pate TRIUMF 500-MeV

I-123, (C-11, N-13, O-15, F-18, Fe-52, Se-77m,  
 Ga-67, Ge-68, Cd-109, In-111, Xe-127, Tl-201)

Winnipeg (50 MeV protons)  
 University of Manitoba  
 Dr. M.I. Gusdal

I-123, Rb/Kr-81, Rb-84

FINLAND

Turku Technobexport, USSR  
 Abo Academy (20 MeV protons)  
 Dr. M. Brenner 16 hr/wk

C-11, F-18, Rb/Kr-81, O-15, K-43, Fe-52, Br-77,  
 Tl-197-202, Re-181, Bi-206, (I-123)

FRANCE

Orsay  
 Service Hospitalier Fred. OGR-MeV 520  
 Joliot  
 Dr. D. Comar 30 hr/wk

C-11, N-13, O-15, F-18, K-43, Ru-97, Ge-68, Bi-206,  
 (Rb/Kr-81)

GERMAN FEDERAL REPUBLIC

Essen TOC CV-28  
 University Clinic  
 Dr. H.J. Machulla (50%)

C-11, F-18, I-123, (N-13, O-15)

Heidelberg AEG-Compact  
 Inst. F. Nuklearmedizin, DKFZ  
 Dr. F. Helus

N-13, F-18, Rb/Kr-81, I-121, I-123, Hg-197m, O-15,  
 Fe-52, Cu-61, Zn-62, Ru-97, Tl-201, (C-11)

Julich (1) TOC CV-28  
 Inst. f. Chemie 1, KFA  
 Dr. G. Stocklin 24 hr/wk

C-11, F-18, P-30, Cr-48, Br-77, I-123, Cl-34m,  
 Tl-201, Pb-203, (more F-18, and Cr-48)

(2) "Julic"

(45 MeV protons)  
 12 hr/wk

Mg-28, I-123, -every two weeks, Br-76,77

Karlsruhe Isochronous  
 Kernforschungszentrum (26 MeV protons)  
 Dr. H. Schweickert 5 hr/wk

I-123 (500mCi/wk), (double I-123, Rb/Kr-81)

INDIA

Calcutta V.E.C.  
 (60 MeV protons)

Dr. R.S. Mani (Bhabha  
 Atomic Res. Centre, Trombay)

(K-43, Fe-52, Ga-67, In-111, I-123, Tl-201)

ITALY

Milan University A.V.F.  
 Cyclotron Laboratory (45 MeV protons)  
 Dr. C. Birattari 10-15 hr/wk

Rb/Kr-81, I-123, Tl-201, V-48, As-71-74, Cd-107-  
 109, Hg-197, Pb-203, Bi-205-206

JAPAN

Chiba OGR-MeV 930  
 Natl. Inst. of Radiological  
 Sciences

Dr. T. Hiramoto 12 hr/wk

C-11, N-13, O-15, F-18, Ti-45, Fe-52, Br-77, I-123

Saitama  
 Inst. Phys. Chem. Research (18 MeV protons)  
 Sciences

Dr. T. Nozaki 5 hr/wk

F-18, Mg-28, K-43, Fe-52, Br-77

Sendai OGR-MeV 680

Tohoku University  
 Dr. S. Morita 30-40 hr/wk (planned)

(F-18, Br-77)

Tokyo University TOC CS-30

Inst. of Medical Science  
 Dr. Akira Ito 2 hr/wk

F-18, Ti-44, Co-56, I-123

NETHERLANDS

Eindhoven AVF  
 University of Technology (30 MeV protons)  
 Dr. R.L.P. van den Bosch 8 hr/wk

Br-77, I-123, Fe-55, Sr-87m, Cd-109, (Fe-52,  
 Rb/Kr-81)

# NETHERLANDS Cont.

Groningen University AVF  
Inst. of Nuclear Physics (65 MeV protons)  
Dr. W. Vaalburg 7 hr/wk  
C-11, N-13, Co-55, O-15, Fe-52, Rb/Kr-81, I-123,  
Hg-197

# SWITZERLAND

Villigen VEC (72 MeV protons)  
Natl. Inst. for Nuclear Research and  
Dr. I. Huszar Ring Accelerator  
(590 MeV protons)  
3-4 hr/wk

In-111, I-123, (Xe-127)

# UNITED KINGDOM

Birmingham University Fixed-frequency  
Dept. of Physics (10 MeV protons)  
Prof. J. H. Fremlin 20 hr/wk  
Rb/Kr-81, I-123, Na-22, Po-208, (daily Rb/Kr-81)

Edinburgh TCC CS-30  
M.R.C. Western General  
Hospital  
Mr. T. Saxton

(O-15, N-13, C-11, Rb/Kr-81)

Harwell VEC  
Chem. Division AERE (58 MeV protons)  
Mr. J. G. Cuninghame 12 hr/wk

I-123, S-38, Nb-92m, Rh-99m, Pu-236, Pu-237,  
(Tl-201)

London Classical 45"  
M.R.C. Hammersmith Hospital (8 MeV protons)  
Mr. I.A. Watson 37 hr/wk

C-11, N-13, O-15, F-18, K-43, Rb/Kr-81, Fe-52,  
I-123, Cu-61, Zn-62, Br-77, Ru-97, Cs-129, Pb-203,  
(K-38)

# UNITED STATES OF AMERICA

Bloomington Separated sector VEC  
Indiana Univ. Cyclotron (200 MeV protons)  
Facility (under 1%)  
Dr. D. L. Friesel

I-123

Boston Allis-Chalmers  
Mass. General Hospital (6 MeV deuterons)  
Dr. G. L. Brownell 40 hr/wk

C-11, N-13, F-18, O-15

Brookhaven National Lab. Isochronous 60"  
Upton Long Island, N.Y. (36 MeV protons)  
Dr. A.P. Wolf 40-50 hr/wk

C-11, N-13, O-15, F-18, K-38, Mn-51, Mn-52,  
Rb/Kr-81, I-123, Ar-211, Ne-19, Cl-34m, Fe-55,  
Br-76, Br-77, Se-97m, Tc-92, Os-191, Au-195m,  
Pb-203

Brookhaven National Lab. ELIP (Lin. Acc.)  
Upton Long Island, N.Y. (200 MeV protons)  
Dr. Louis Stang 145 hr/week

Mg-28, Fe/Mn-52, Ga-67, Rb/Kr-81, Ru-97, I-123,  
Ge-68, Cd-109, Sn-113, Te-118, Xe/I-122, Xe-127,  
W-178

Chicago University TCC CS-15  
Franklin McLean Mem. Res.  
Institute 10 hr/wk  
Dr. P.V. Harper

C-11, N-13, O-15, F-18, Mn-51

Cleveland, Ohio VEC  
NASA (45 MeV protons)  
Dr. J.W. Blue 3 hr/wk

C-11, Tl-201

Davis, California VEC  
Crocker Nuclear Lab. (66 MeV protons)  
Dr. M.C. Lagunas-Solar 20-35 hr/wk

I-123, Tl-201, N-13, Co-55, Rh-101m

Los Alamos, New Mexico LAMPF (Lin. Acc.)  
C.P. Anderson Meson Phys. (800 MeV protons)  
Facility 168 hr/wk  
Dr. H. A. O'Brien

Fe/Mn-52, Br/Se-77, Sr/Rb-82, Hf/Lu-172, (Cu-67,  
Ge-68, Se-72, I-123, Xe-127)

Los Angeles, California TCC CS-22  
U.C.L.A. School of Medicine  
Dr. N.S. MacDonald 50 hr/wk

C-11, N-13, F-18, Zn/Cu-62, Cu-64, Rb/Kr-81, O-15,  
Cd-107, In-111, I-123

Miami Beach, Florida TCC CS-30  
Mount Sinai Medical Center (27 MeV protons)  
Mr. J.E. Beaver 100 hr/wk

Rb/Kr-81, Ga-67, In-111, Tl-201, C-11, O-15, (N-13)

Michigan  
State University Cyclotron VEC  
Laboratory (50 MeV protons)  
Dr. P.S. Miller av. 6 hr/wk

C-11, N-13

New York, N.Y. TCC CS-15  
Sloan Kettering Institute  
Dr. R.S. Tilbury 20 hr/wk

C-11, N-13, O-15, F-18, K-38, Fe-52, Se-73

Oak Ridge, Tennessee Isochronous Cyclotron  
O.R.N.L. (55 MeV protons)  
Dr. S. W. Mosko 2%

No Data Given

St. Louis, Missouri (1) Allis-Chalmers  
Washington University (6 MeV deuterons)  
Dr. M.J. Welch

C-11, N-13, O-15, F-18

do

(2) 52" Cyclotron  
(30 MeV <sup>4</sup>He)  
12 hr/wk

Br-77, Kr-77, Rb/Kr-81

do

(3) TCC CS-15

(C-11, N-13, F-18)

Washington, D.C. Sector-Focusing Cycl.  
Naval Research Laboratory (65 MeV protons)  
Dr. R. O. Bondelid

C-11, F-18, I-123

\* Radionuclides produced Routinely at least once a  
week; underlined.  
Irregularly or in future; in parenthesis.

AMERICAN ACADEMY OF NEUROLOGY  
Scientific Program Abstract Form

46

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| Type of presentation:                                           | Projection needs:                                |
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Title of paper: Measurement of Regional Cerebral Blood Flow (rCBF) by Positron Emission Tomography Using  $^{15}\text{O}$ -Water -- Validation of an In Vivo Autoradiographic Paradigm.

Presenter's name and mailing address: Myron D. Ginsberg, M.D.  
Department of Neurology (D4-5)  
University of Miami School of Medicine  
P.O. Box 016960  
Miami, Florida 33101

Co-authors' names, cities, and states: Myron D. Ginsberg, Alan H. Lockwood, Raul Busto,  
Ronald D. Finn, and John C. Clark. Miami and  
Miami Beach, Florida.

Type 200-word, double-spaced abstract here:

Water labelled with the positron-emitting radionuclide  $^{15}\text{O}$  ( $T_{1/2}$  123 sec.) is ideal for positron-emission tomographic (PET) studies of rCBF in man, owing to its ease of synthesis and low patient radiation exposure.  $^{15}\text{O}_2$  was prepared by the (p,pn) nuclear reaction on  $^{16}\text{O}_2$  and was reacted with  $\text{H}_2$  on a platinum catalyst to yield  $^{15}\text{O}$ -water. In physiologically monitored Wistar rats anesthetized with nitrous oxide, we first employed indicator fractionation to compare rCBF using  $^{15}\text{O}$ -water and simultaneously administered  $^{14}\text{C}$ -iodoantipyrine (IAP). Agreement between tracers was excellent (hemispherical rCBF  $1.96 \pm 0.17$  ml/gm/min with  $^{15}\text{O}$ -water;  $1.89 \pm 0.16$  with  $^{14}\text{C}$ -IAP; correlation coefficient 0.999; range of  $\text{pCO}_2$  44-73 torr). In other experiments simulating human PET studies, cranial radioactivity was recorded from rats with a pair of collimated bismuth germanate crystals in a coincidence circuit during a 60-sec. modified ramp intravenous infusion of  $^{15}\text{O}$ -water. An operational equation was derived from which CBF was readily calculated by an iterative method from the integrated cranial activity. CBF values computed by this in vivo strategy agreed closely with values obtained by direct assay of brain activity (in vivo method,  $1.65 \pm 0.19$  ml/gm/min; direct method,  $1.76 \pm 0.23$ ). Integrated cerebral activity followed CBF well even at high flow rates. This method promises ease and exactitude in human measurements of rCBF by emission tomography, and image resolution may be optimized inasmuch as the method does not require repeated, discrete measurements of cranial clearance.

## Measurements of regional ventilation using nitrogen-13 and krypton-81m in mechanically ventilated dogs

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Departments of Medical Physics and Anaesthetics and the Medical Research Council Cyclotron Unit, Hammersmith Hospital, London W12 0HS, England

Received 23 February 1981, in final form 28 July 1981

**Abstract.**  $^{13}\text{N}_2$  and  $^{81}\text{Kr}^m$  were used to measure changes in upper/lower (U/L) ratios of regional ventilation in supine anaesthetised dogs mechanically ventilated at different tidal volumes and the results compared. Analysis of the slopes of the  $^{13}\text{N}_2$  washout curves indicated that U/L specific ventilation was less than unity at low tidal volumes and decreased as tidal volume increased. U/L total ventilation, measured using the specific ventilation results and estimations of relative lung volume using  $^{13}\text{N}_2$ , also decreased with increase in tidal volume. Preferential distribution of ventilation to dependent lung regions thus increased with tidal volume despite the opposing hydrostatic forces from the abdomen. Regional count density distributions in  $^{81}\text{Kr}^m$  images of the lungs were analysed. At high specific ventilation rates, correction of  $^{81}\text{Kr}^m$  count density is required in order to obtain correct regional ventilation information. The specific ventilation rates in this series were such that the uncorrected U/L count ratios of  $^{81}\text{Kr}^m$  increased as tidal volume increased in contrast to the decrease in U/L total ventilation recorded by the  $^{13}\text{N}_2$  method. When corrections using the  $^{13}\text{N}_2$  specific ventilation results were applied, the changes in U/L  $^{81}\text{Kr}^m$  count ratios with tidal volume showed a similar trend to the changes in total ventilation measured with  $^{13}\text{N}_2$ . Sources of error in the two techniques are briefly discussed.

### 1. Introduction

The use of a gamma camera to obtain  $^{13}\text{N}_2$  washout curves in the whole lung has been found to give washout rates in good agreement with those obtained using expired air concentration curves, and in fair relationship with those obtained using measured lung volumes and tidal volumes (Arnot *et al* 1978). Since the shapes of washout curves are not affected by different gamma camera sensitivities to radioactivity in different regions, relative regional washout rates and changes in distribution may be expected to be measured correctly with  $^{13}\text{N}_2$ , and if measurements of the relative volumes in the regions under consideration are also made, then values of the relative regional total ventilation may also be obtained. In this study, changes in relative regional washout rate, volume and total ventilation due to variation in tidal volume were measured with  $^{13}\text{N}_2$  and a gamma camera in immobile anaesthetised dogs mechanically ventilated under reproducible conditions.

Krypton-81m was administered to the dogs under the same ventilatory conditions and the results of analysing regional count densities in gamma camera images were compared with the  $^{13}\text{N}_2$  total ventilation results. When the gas  $^{81}\text{Kr}^m$  (half-life 13 s) is breathed continuously, radioactive equilibrium in the lungs is obtained, the amount of

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radioactivity present in a lung region (and the count density recorded from that region) depending on the relationship between the concentration inhaled, the ventilation to the region and the  $^{81}\text{Kr}^m$  decay constant. In humans, the relative count densities can be used to detect underventilated regions of lung (Fazio and Jones 1975). It is a convenient technique, the gas being obtained by continuous elution of a generator and images being accumulated during tidal breathing so that respiratory manoeuvres such as breath holding are unnecessary. Gamma camera detection of its radiation is efficient and its short half-life minimises radiation dose and disposal problems. Means of quantifying regional ventilation using  $^{81}\text{Kr}^m$  were suggested by Jones (1978), a correction to count densities being required at high ventilation rates. In this study the possibility of obtaining useful quantitative or qualitative information from  $^{81}\text{Kr}^m$  images without such a correction was investigated. Corrections to count densities were also applied so that estimations of regional ventilation could be compared with the  $^{13}\text{N}_2$  results to test the validity of the theory underlying the  $^{81}\text{Kr}^m$  technique.

## 2. Methods

The studies were performed on five dogs of varying breeds weighing 21–33 kg. The animals were anaesthetised with thiopentone 20–30 mg kg<sup>-1</sup> and anaesthesia was maintained with pentobarbitone given in divided doses of 60–120 mg throughout the experiment. A 10 mm cuffed endotracheal tube was passed under direct vision and ventilation controlled mechanically. Apnoea was maintained with 6–8 mg increments of pancuronium bromide. The animals lay in the supine position in a shallow trough which supported the spine but did not prevent thoracic expansion, and their forelegs were held gently extended so that the gamma camera could be placed close to the right lateral thoracic wall. 500–1000 ml of Ringer's lactate solution was infused during the 6–8 h of the experiment and arterial pressure was monitored from a line in the aorta.

### 2.1. The ventilator

The ventilator consisted of a powerful electric motor which drove a camshaft via a variable speed gearbox. The camshaft was fitted with cams which could be positioned under a spring-loaded cam follower. The latter drove one end of a rocker arm, the other end of which was attached to the positive-pressure bellows. The tidal volume was adjusted by altering the position of the pivot on the rocker arm and the geometry was so designed that the bellows emptied completely at each stroke regardless of the tidal volume. The cams were cut to produce a constant inspiratory flow pattern. The bellows were connected to a pressure-operated non-rebreathing valve (Sykes 1969) with a 90 cm length of 11 mm ID plastic tubing. Expired gas volumes were measured either with a dry gas meter (accuracy  $\pm 2\%$  of the reading) or a dry bellows spirometer which recorded tidal volumes with an accuracy of  $\pm 20$  ml. The ventilator circuit was appropriately screened to prevent stray radiation from affecting the gamma camera.

### 2.2. The $^{13}\text{N}_2$ circuit

$^{13}\text{N}_2$  was generated in the MRC Cyclotron Unit (Clark and Buckingham 1975) and piped to the experimental laboratory where it was dispensed. The ventilator circuit was arranged as shown in figure 1. The ventilator controls were first adjusted whilst the dog was being ventilated with the non-rebreathing circuit and measurements of expired

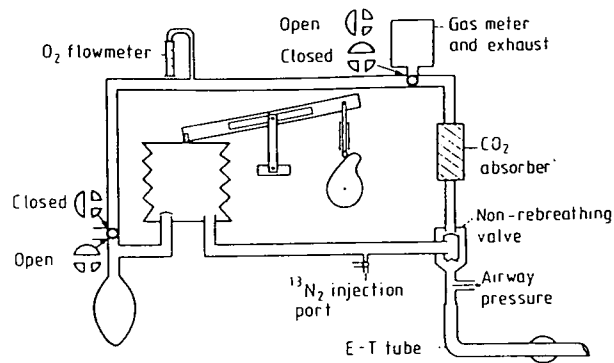


**Figure 1.** Ventilation circuit for  $^{13}\text{N}_2$  measurements. The enlarged sections of the three-way taps indicate the closed circuit (rebreathing) and open circuit (washout) positions.

volume and airway pressure were made. The circuit was then closed by turning both taps during inspiration and the flow of oxygen into the circuit was adjusted to match the dog's oxygen consumption. When conditions were stable, 520–740 MBq (14–20 mCi) of  $^{13}\text{N}_2$  contained in 4–6 ml of gas were injected into the circuit and allowed to equilibrate throughout the lungs and ventilator circuit for 4 min. The ventilator circuit was then switched to non-rebreathing by turning both taps during an inspiration and the nitrogen washout followed for a period of ten minutes.

### 2.3. The $^{81}\text{Kr}^m$ circuit

$^{81}\text{Kr}^m$  (half-life 13 s) is produced continuously by passing air through a cation exchange column to which the parent radionuclide  $^{81}\text{Rb}$  is bound (Clark, Horlock and Watson 1976). The  $^{81}\text{Rb}$  is produced by bombarding sodium bromide with alpha particles in the MRC Cyclotron at Hammersmith Hospital and has a half-life of 4.6 h.

One of the problems in using the  $^{81}\text{Kr}^m$  technique is that variations in inspired radioactivity concentration may result from differences in transit times through the ventilator circuit. To minimise errors from this source, the circuit shown in figure 2 was developed. The  $^{81}\text{Kr}^m$  was eluted from the krypton generator by a stream of humidified air and mixed with an additional supply of air to create a total gas flow of 12–13 l min<sup>-1</sup>. This flow was directed into a reservoir bag, situated close to the non-rebreathing valve.

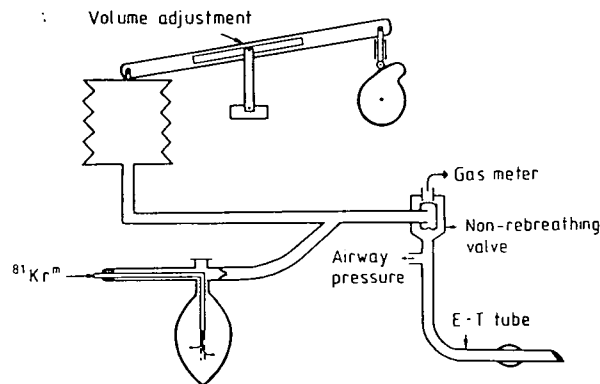


**Figure 2.** Ventilation circuit for  $^{81}\text{Kr}^m$  measurements.



aspirated into the bellows via the short, 11 mm bore inspiratory tube and then driven directly through the non-rebreathing valve to the lungs. The resulting inspired concentration measured just before the non-rebreathing valve was constant to within  $\pm 5\%$ . The radioactive gas flow rate to the reservoir bag was adjusted to match the highest minute volume used, and at lesser tidal volumes the excess gas was exhausted to the outside of the building from a blow-off valve situated on the bag mount.

#### 2.4. Recording of radioactivity distribution

The gamma camera was fitted with a high-energy collimator and aligned so that it viewed the whole of the lateral aspect of the right lung, the face of the collimator being positioned 2–5 cm from the chest wall at its nearest point. Images obtained using the 190 keV gamma radiation of  $^{81}\text{Kr}^m$  and the 511 keV annihilation radiation of  $^{13}\text{N}_2$  were recorded on magnetic tape for computer processing (Vernon and Glass 1971). For  $^{13}\text{N}_2$  measurements, data were recorded during the last 30 s of rebreathing to provide a measurement of regional lung volume, and during 10 min of washout. Recording of  $^{81}\text{Kr}^m$  gamma camera data began after radioactive equilibrium in the lungs was reached and was continued until a total of 200 000 counts from the whole lung had been accumulated. The maximum count rate in all studies was kept below  $7000 \text{ counts s}^{-1}$  in order to avoid the need to correct for gamma camera dead time.

#### 2.5. Analysis of data

Corrections for non-uniformity of camera response were made and regions were selected for analysis as shown in figure 3.  $^{13}\text{N}_2$  equilibrium count rates and washout

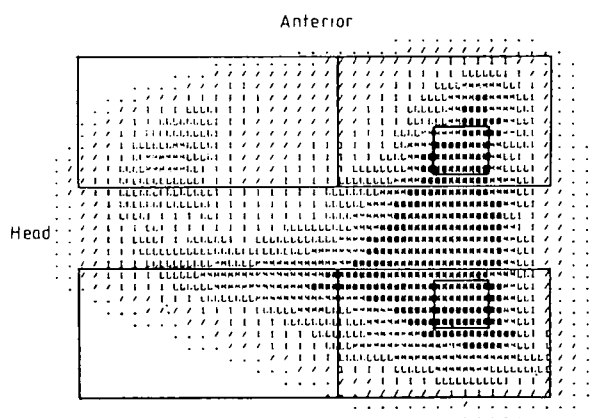


**Figure 3.** Computer print-out showing distribution of radioactivity over right lateral aspect of dog's thorax. The dog was supine with the head on the left. The rectangles used for analysis are outlined.

curves and  $^{81}\text{Kr}^m$  accumulated counts were derived from the two horizontal slices which embraced the upper and lower zones of the lung field. Further analyses of  $^{81}\text{Kr}^m$  counts were performed on the caudal sections of these rectangles and on smaller areas of high count rates centrally placed in each zone, but no significant differences were found between the results derived from these areas and those derived from the large rectangles. For each animal, identical areas were used for all analyses. The areas were

chosen using the radioactivity pattern obtained at the highest tidal volume in order to allow for lung movement and so to ensure as far as possible that the same lung tissue was contained within each area at all stages of the respiratory cycle at all tidal volumes.

Regional  $^{13}\text{N}_2$  washout curves were analysed into two exponential components using a computer programme (Berman, Shahn and Weiss 1962) and the average washout rate ( $\bar{\alpha} \text{ s}^{-1}$ ) was derived from the intercepts ( $A_1$  and  $A_2$ ) and slopes ( $\alpha_1$  and  $\alpha_2$ ) of the fast and slow components:

$$\bar{\alpha} = \frac{A_1 \cdot \alpha_1}{A_1 + A_2} + \frac{A_2 \cdot \alpha_2}{A_1 + A_2}.$$

Specific ventilation ( $\dot{V}/V$ ) was calculated for each region using the expression  $\dot{V}/V = 60\bar{\alpha} \text{ min}^{-1}$ . The  $^{13}\text{N}_2$  regional count rates at equilibrium were used to calculate relative regional lung volumes ( $V$ ). Relative regional ventilation ( $\dot{V}$ ) was calculated by multiplying relative regional specific ventilation ( $\dot{V}/V$ ) by relative regional volume.

All results for  $^{13}\text{N}_2$  and for  $^{81}\text{Kr}^m$  counts were expressed as the ratio of the value in the upper zone to that in the lower zone, normalised to the value obtained at the minimum tidal volume.

The upper/lower  $^{81}\text{Kr}^m$  count ratios were used to obtain an estimation of relative regional ventilation ratios using the lung model described by Jones (1978). This assumes a continuous passage of  $^{81}\text{Kr}^m$  through the lungs such that at radioactive equilibrium, in each region,

Rate of inflow of radioactivity = Rate of outflow of radioactivity

$$\dot{V}_c = A(\dot{V}/V + \lambda)$$

where  $\dot{V}$  = ventilation to region,  $c$  = concentration inspired, assumed the same to all regions,  $A$  = radioactivity present in region,  $\dot{V}/V$  = fractional rate at which radioactivity is lost from region due to ventilation,  $\lambda$  = fractional rate at which radioactivity is lost due to  $^{81}\text{Kr}^m$  radioactive decay.

Thus

$$A = \dot{V} \times \frac{c}{(\dot{V}/V) + \lambda}. \quad (1)$$

The ratio of radioactivity present in two regions 1 and 2 is given by

$$\frac{A_1}{A_2} = \frac{\dot{V}_1}{\dot{V}_2} \times \frac{(\dot{V}/V)_2 + \lambda}{(\dot{V}/V)_1 + \lambda} \quad (2)$$

and the ratio of counts detected in the two regions in a given time is given by

$$\frac{C_1}{C_2} = \frac{S_1 \dot{V}_1}{S_2 \dot{V}_2} \times \frac{(\dot{V}/V)_2 + \lambda}{(\dot{V}/V)_1 + \lambda} \quad (3)$$

where  $S_1$  and  $S_2$  are instrument sensitivity factors for regions 1 and 2. When ratios in the same regions are normalised to the value obtained under one physiological condition, the sensitivity factor  $S_1/S_2$  cancels out. Thus, normalised values for  $\dot{V}/V$  are obtainable from normalised count ratios if the absolute values of  $\dot{V}/V$  are known or if these are all so small in comparison with  $\lambda$  that the last fraction of equation (3) reduces to unity. In the latter situation,  $^{81}\text{Kr}^m$  activity present at equilibrium is proportional to lung ventilation, which is the basis for the widespread clinical use of  $^{81}\text{Kr}^m$  (Fazio and Jones 1975). In this study,  $\dot{V}/V$  was not small in comparison with  $\lambda$  and the values of

$\dot{V}/V$  obtained using  $^{13}\text{N}_2$  were used to calculate normalised values of upper/lower  $\dot{V}$  ratios from the normalised upper/lower  $^{81}\text{Kr}^m$  count ratios, using equation (3) rearranged.

## 2.6. Protocols

**2.6.1. Effect of tidal volume.** The regional distribution of ventilation was measured at a number of tidal volumes from 300–1300 ml whilst the inspiratory:expiratory time ratio was maintained at 1:2 for four dogs and at 1:4 for one dog, at a frequency of 15 breaths per minute.

**2.6.2. Additional  $^{81}\text{Kr}^m$  experiments.** Some experiments were carried out to investigate the effect of the inhalation of  $^{81}\text{Kr}^m$  of non-constant concentration on relative regional count rates.

(i) *Tracheal sampling.* In one of the dogs, a continuous sample of gas was obtained from a catheter placed with its tip at the carina. The gas was sampled by a thin bore tube at a rate of 500 ml min<sup>-1</sup> and passed through a coil of the same tubing in a well counter, whose output was fed to a multiscaler for graphing by computer.

(ii) In another dog, images of the distribution of boluses of radioactivity delivered to the trachea at two different times in the inspiratory phase were obtained. A 50 ml syringe, driven by compressed CO<sub>2</sub> and a solenoid valve that was activated by switching controlled by the ventilator cam, was used to elute the generator and drive a highly concentrated bolus of  $^{81}\text{Kr}^m$  to the tip of a tube situated in the trachea. The bolus was delivered in 0.2 s at either the beginning or in the middle of the inspiratory phase, during every tenth breath. The cam switching also controlled recording of data from the gamma camera, data being recorded only while boluses were being delivered during every tenth inspiration. Thus only the initial distribution of each bolus was recorded since clearance by decay and ventilation took place between recordings.

## 3. Results

Figure 4 shows the variation in upper/lower zone relative regional lung volume ( $V$ ) derived from  $^{13}\text{N}_2$  equilibrium count rates, relative regional specific ventilation ( $\dot{V}/V$ ) derived from the  $^{13}\text{N}_2$  average washout rate  $\bar{\alpha}$ , and relative regional ventilation ( $\dot{V}$ ) obtained by multiplying relative regional specific ventilation by relative regional volume. Three of the dogs showed some decrease in relative regional lung volume with increase in tidal volume, while in two dogs relative volume remained constant (1 and 3). Two of the dogs (2 and 4) showed a progressive and marked decrease in upper/lower (U/L) zone ratio of regional specific and total ventilation as tidal volume was increased. In the other three dogs there were variable changes up to a tidal volume of about 700 ml, but above this tidal volume there was a decrease in the U/L ratio of regional specific and total ventilation. The absolute value of U/L specific ventilation was always less than unity. Values of specific ventilation increased with tidal volume from about 2 min<sup>-1</sup> to about 7 min<sup>-1</sup> in the lower region and from about 1.5 to 4.5 min<sup>-1</sup> in the upper region, the greater increase in the lower region producing the decrease in upper/lower ratio.

The  $^{81}\text{Kr}^m$  U/L count ratios are shown in figure 5. In four out of the five dogs the U/L ratio increased with tidal volume whilst there was little change in the remaining animal. Thus a decrease in U/L ventilation ratio of 10–30% as demonstrated by  $^{13}\text{N}_2$  measure-

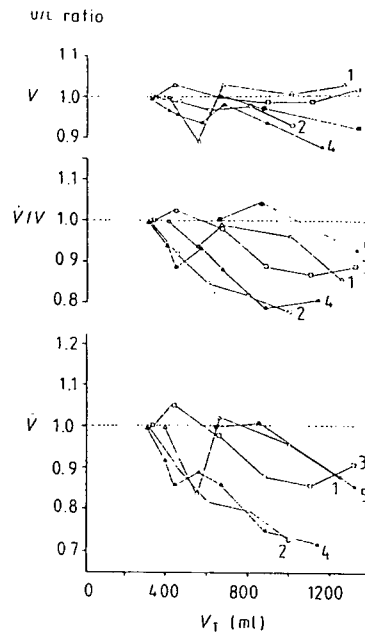


**Figure 4.**  $^{13}\text{N}_2$  measurements of upper/lower regional volume ( $V$ ), specific ventilation ( $\dot{V}/V$ ) and total ventilation ( $\dot{V}$ ) at various tidal volumes ( $V_T$ ). The values are normalised to the ratios obtained at the lowest tidal volume.

ments was associated with an increase in  $U/L$   $^{81}\text{Kr}^m$  count ratio of up to 25%. When the normalised  $^{81}\text{Kr}^m$   $U/L$  count ratios were corrected using  $^{13}\text{N}_2$  specific ventilation values, the resultant normalised  $U/L$  ventilation ratios showed similar trends to those obtained using  $^{13}\text{N}_2$  alone except in the case of Dog 1 whose very high count ratios are under-corrected (figure 6). The corrected  $^{81}\text{Kr}^m$  results were ( $7 \pm \text{SD } 6$ )% greater than the  $^{13}\text{N}_2$  results ( $n = 21$ ).

Figure 7 shows the variation with time of the concentration of  $^{81}\text{Kr}^m$  in the trachea of a dog ventilated at an inspiratory:expiratory ratio of 1:4 at different tidal volumes. At high tidal volume (figure 7(c)), the  $^{81}\text{Kr}^m$  concentration in the dead space gas was much

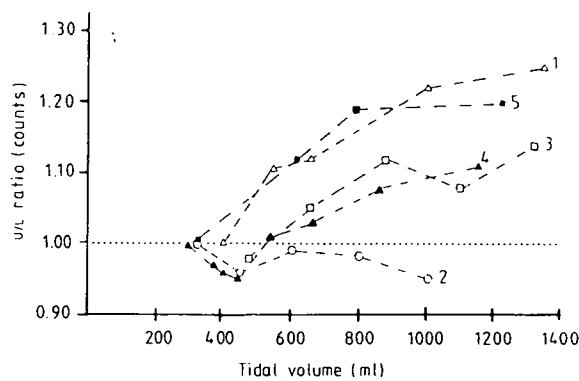


**Figure 5.** Upper/lower ratios of regional  $^{81}\text{Kr}^m$  counts at various tidal volumes. The values are normalised to the ratio obtained at the lowest tidal volume.

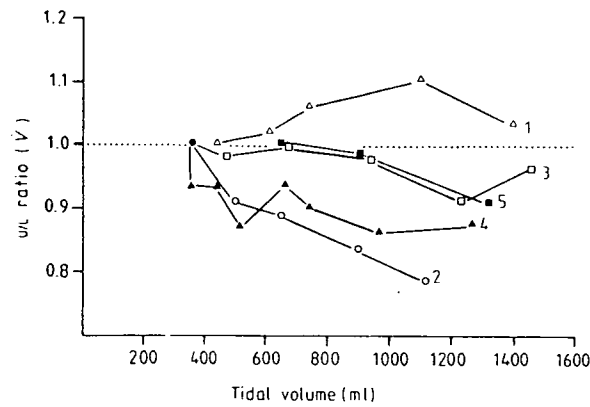


**Figure 6.** Upper/lower ratios of regional total ventilation  $\dot{V}$  obtained from  $^{81}\text{Kr}^m$  U/L count ratios and  $^{13}\text{N}_2$  specific ventilation measurements. The values are normalised to the ratio obtained at the lowest tidal volume.

higher than at low or medium tidal volumes (figures 7(a) and (b)), but the dead space gas constituted a smaller fraction of the total volume inspired since the increase in anatomical dead space is not linearly related to the increase in tidal volume (Shepard *et al* 1957). The effect of the relative concentration and volume of the dead space gas on the distribution of radioactivity depends on whether there is preferential distribution of the dead space gas to certain areas of the lungs and whether the distribution varies with tidal volume. The results of investigating these factors are as follows.

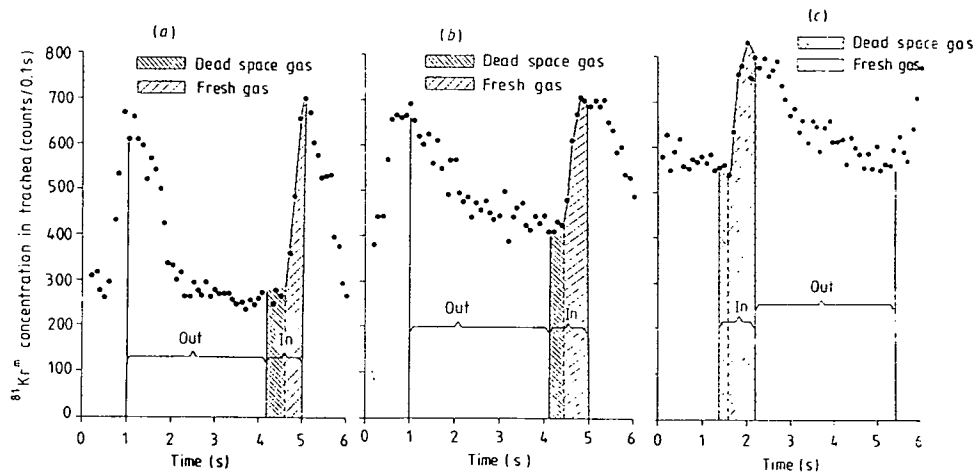


**Figure 7.** Radioactivity of  $^{81}\text{Kr}^m$  in gas sampled from trachea at three tidal volumes: (a) low, (b) medium, (c) high. The proportions of dead space and alveolar gas were estimated from separate measurements of anatomical dead space made by the single breath Fowler technique.

Table 1 shows the results obtained on the dog to which boluses of  $^{81}\text{Kr}^m$  were administered to the trachea during the initial and middle part of the inspiratory phase. The U/L distribution of the initial boluses did not vary significantly with tidal volume in either the thirds of the lungs or in the smaller caudal regions. The U/L ratio of distribution of the middle boluses was lower than that of the initial boluses. The distributions of the initial and middle boluses are representative of the distributions of

**Table 1.** Upper/lower ratios of distribution of  $^{81}\text{Kr}^m$  boluses administered to trachea during initial and middle 0.2 s of inspiratory phase. The regions examined were the non-dependent and dependent large rectangles shown in figure 3 and the caudal areas of these rectangles.

| Tidal volume | 1/3 lungs       |                | Caudal regions  |                |
|--------------|-----------------|----------------|-----------------|----------------|
|              | Initial boluses | Middle boluses | Initial boluses | Middle boluses |
| 300          | 0.84            | 0.48           | 0.75            | 0.46           |
| 600          | 0.82            | 0.73           | 0.73            | 0.66           |
| 900          | 0.82            | 0.60           | 0.76            | 0.55           |
| 1200         | 0.84            | 0.58           | 0.77            | 0.51           |

dead space gas and gas entering at the mouth respectively. These results indicate that the fraction of the dead space gas distributed to the upper regions was greater than the fraction of the middle part of the breath distributed to those regions, but the dead space distribution did not vary with tidal volume. On the other hand, the distribution of the middle part of the breath did change with tidal volume, in keeping with the finding of variations in ventilation distribution in the dogs when using  $^{13}\text{N}_2$ .

#### 4. Discussion

All five dogs showed a reduction in U/L ratio of ventilation at high tidal volumes though results were variable in three of the dogs at intermediate tidal volumes. In two of the dogs the decrease in U/L ratio was due solely to a reduction in the U/L ratio of  $\dot{V}/V$  but in the remaining three animals there was also a reduction in the U/L ratio of  $V$ .

Kaneko *et al* (1966) and Milic-Emili *et al* (1966) showed that at functional residual capacity (FRC) the volume of the non-dependent alveoli is greater than those in the dependent zones. This variation in regional lung volume results from the vertical gradient of pleural pressure which results in a greater transpulmonary pressure difference in non-dependent zones. Since the volume-pressure curve of the lung is non-linear, this gradient of pleural pressure also results in regional differences in ventilation during spontaneous breathing, the lower alveoli situated on the steeper part of the volume-pressure curve receiving more ventilation than the upper alveoli during inspiration from FRC.

During mechanical ventilation the respiratory muscles are paralysed so that the distribution of ventilation is modified by external pressures acting on the chest wall. In the supine subject the abdominal contents create a hydrostatic pressure gradient which acts maximally on the dependent portions of the diaphragm. This reduces regional lung volume in the dependent portions of the lung and also causes ventilation to be redistributed away from the dependent lung zones. When tidal volume is increased from 300 ml to 1200 ml the peak inflation pressure increases from approximately 8 cm  $\text{H}_2\text{O}$  to 25–30 cm  $\text{H}_2\text{O}$ . The resulting increase in end-inspiratory transpulmonary pressure probably shifts the non-dependent alveoli onto the flatter portion of the volume-pressure curve, and so tends to increase the proportion of ventilation distributed to the dependent zones (Froese and Bryan 1974).

In the present experiments an increase in tidal volume resulted in a small relative increase in dependent zone regional lung volume in three of the dogs. The simplest

explanation for these changes is that FRC increased progressively with tidal volume. Because of the shape of the volume-pressure curve this would have resulted in a relatively greater increase in the volume of dependent alveoli. Closed circuit helium FRC measurements made on these animals earlier in the day revealed that FRC increased by 100–300 ml when tidal volumes increased from 300–1200 ml. It seems likely that this change was due to inadequate time for the lung to empty at the high tidal volumes since subsequent experiments have demonstrated that the increase is not observed when the closed circuit helium apparatus is connected to the endotracheal tube 15 s after the latter has been opened to atmosphere.

#### 4.1. Nitrogen-13: error considerations

$^{13}\text{N}_2$  has been used in studies of both normal and abnormal lungs using collimated scintillation counters (Rosenzweig *et al* 1970) and gamma cameras (Ronchetti *et al* 1975b, Ewan *et al* 1975, Nosil *et al* 1976). Some sources of error in the measurement technique may be considered in relation to their possible effect on the measurement of change in regional ventilation. The advantage of  $^{13}\text{N}_2$  over other inert gases such as  $^{133}\text{Xe}$  is its lower solubility in blood and tissue: this reduces the magnitude of two sources of error, namely washout of radioactivity from tissue into the lungs, and the presence of radioactivity in the chest wall (Matthews and Dollery 1965, Ronchetti *et al* 1975a). Although small, both sources of error warrant further consideration. During washout, radioactivity which has accumulated in the tissues and blood passes from the blood into the alveoli and so slows the washout curve. Examination of data obtained on dogs has shown that this error slows the washout rate by 1–2% (Rhodes *et al* 1980). Radioactivity in the chest wall is detected with high efficiency by the gamma camera since the chest wall is close to the crystal. The radioactivity remaining during the last part of the washout recording was regarded as tissue background and subtracted from the recorded count rates, but any previous local tissue components would have been included in the data recorded and would have caused an under-estimation of the true washout rate. Comparison of gamma camera results with results obtained from simultaneous expired gas concentration curves indicated that a chest wall correction of 5% is probably applicable to washout rates recorded with a gamma camera (Arnot *et al* 1978). However, both these factors are of negligible importance when the results are expressed in terms of normalised U/L ratios since both regions of lung are equally affected.

A third factor which may lead to an error is the determination of specific ventilation by equating it directly to the washout rates defined by smooth curves fitted to ventilation data. Ventilation is a discontinuous process and this should be taken into account when calculating specific ventilation from the parameters of smooth washout curves (Briscoe and Cournand 1959). The effects of the different methods of calculating specific ventilation have been investigated. The differences are less than 5% at low washout rates (such as are found in resting humans), but increase with increasing washout rates. However, the effect on the measurement of changes in relative regional ventilation is small. In this series, changes in U/L ratios, calculated by taking the discontinuous nature of ventilation into account, differed by a maximum of 8% from the values determined by direct use of  $^{13}\text{N}_2$  washout rates. This difference occurred at the highest tidal volumes used, the difference usually being only 1–3%. Calculation by this method did not affect the direction of change of U/L ratio in any dog at any tidal volume.

A fourth source of error, regional variations in the distribution of the dead space gas, may also affect regional washout curves because the proportions of dead space gas (i.e. alveolar gas) and fresh gas entering the alveoli may vary. The experiments on the distribution of dead space gas were carried out only once, and can only relate to supine anaesthetised dogs, but the relevance of such information to ventilation measurements is worth consideration.

At the end of expiration, the dead space is filled with gas from the alveoli. The composition of this gas varies throughout the lung depending on regions  $\dot{V}/V$ , but in the major airways the gas contains mixed contributions from all lung regions. During inspiration, this gas may be distributed in a non-uniform fashion, so altering the fraction of radioactivity re-inhaled by each region of the lung. This would distort the relationship between regional washout curves. For example, if the whole of the dead space gas were delivered to one particular region while the rest of the lungs received only fresh inactive gas during inspiration, then the washout from that region would be slowed down. However, it can be argued that if all the dead space gas (high  $\text{PCO}_2$ , low  $\text{PO}_2$  gas) is redistributed to a particular region, then the effective ventilation to that region is decreased, and it is proper for this to be reflected in a slower washout measurement. Thus the effect of the inspiration of radioactive dead space gas on  $^{13}\text{N}_2$  measurements is in the correct direction though it cannot be assessed quantitatively. The effect is likely to be greater in the dog than the human since the anatomical dead space after intubation is about  $1 \text{ ml kg}^{-1}$  in the human and  $4 \text{ ml kg}^{-1}$  in the dog (Severinghaus and Stupfel 1955). Any variation in dead space distribution with tidal volume could therefore affect the values of changes measured as well as of individual clearance rates. In our experiment on the distribution of different parts of the breath, the labelled gas in the dead space was distributed more to the upper part of the lungs than labelled gas entering half way through the inspiratory period, but the distribution did not alter with tidal volume. If the distribution was unchanged in the other dogs in the series, the effect of the distribution of dead space radioactivity on the measured changes in relative regional ventilation must have been minimal.

#### 4.2. Krypton-81m: assessment of technique and error considerations

As may be seen from equation (1), when  $^{81}\text{Kr}^m$  of a constant concentration  $c$  is breathed continuously until radioactive equilibrium is reached, the amount of  $^{81}\text{Kr}^m$  present in a lung region ( $A$ ) is proportional to the ventilation in the region ( $\dot{V}$ ) provided specific ventilation ( $\dot{V}/V$ ) is small in comparison with the  $^{81}\text{Kr}^m$  decay constant  $\lambda$  (Fazio and Jones 1975). The value of  $\lambda$  is  $3.2 \text{ min}^{-1}$  and figure 8, in which  $\dot{V}/[(\dot{V}/V) + 3.2]$  is plotted against  $\dot{V}$ , shows how  $^{81}\text{Kr}^m$  equilibrium radioactivity varies with ventilation for human and dog lungs of volumes 2.5 l and 1.0 l respectively. It is seen that the relationship between equilibrium radioactivity and ventilation is closely linear for values of  $\dot{V}/V$  up to the normal adult human value of  $1.5 \text{ min}^{-1}$  ( $\dot{V} = 3.75 \text{ l min}^{-1}$  in humans,  $1.5 \text{ l min}^{-1}$  in dogs). At higher values of  $\dot{V}/V$ ,  $A$  increases more slowly with  $\dot{V}$  until it reaches a maximum value which reflects lung volume alone. The effect on results obtained when comparing count densities in regions with increasing  $\dot{V}/V$  can be seen by reference to figure 8. At points P and Q, the ratio of ventilation is  $2.5/5 = 0.5$ , and the ratio of activity is  $0.5 \times 1.44 = 0.72$  (from equation (2);  $\dot{V}/V = \dot{V}$  since  $V = 1$  on this curve). At points R and S, the ratio of ventilation is still 0.5 (6/12) but the ratio of activity is  $0.5 \times 1.65 = 0.83$ . Thus each single activity ratio (or count ratio if sensitivity factors are similar; equation (3)) gives, in this case, a correct qualitative indication of



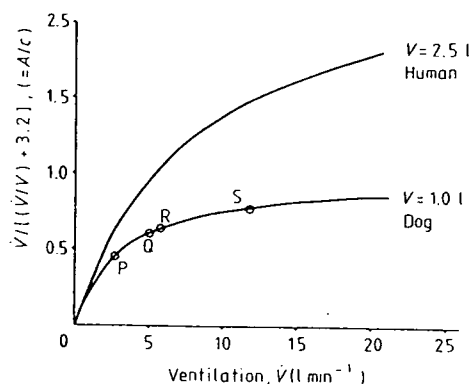


**Figure 8.** Theoretical curves showing how  $^{81}\text{Kr}^m$  equilibrium radioactivity varies with ventilation for an adult human (FRC 2.5 litres) and 25 kg dog (FRC 1.0 litres). When  $\dot{V}/V$  is less than  $1.5 \text{ min}^{-1}$  the relationship is relatively linear. At higher values of  $\dot{V}/V$  the method becomes less sensitive and the relationship non-linear.

relative ventilation (e.g.  $\dot{V}_P < \dot{V}_O$ ), but comparison of the two activity ratios gives an incorrect indication of the relation between the two ventilation ratios owing to the differing absolute values of  $\dot{V}/V$ . The regional  $\dot{V}/V$  values found in this series were such that while the U/L ventilation ratios decreased, the normalised U/L count ratios remained constant or increased. The difference between activity ratios and ventilation ratios is not inconsiderable even at clearance rates found in human lungs. It may be calculated that in assessing changes in regional distribution of ventilation when specific ventilation ranges from well below normal to normal, the difference between changes in activity ratios and changes in ventilation ratios can be of the order of 10%, while if specific ventilation ranges from normal values to those found after exercise then such differences can be more than 25%. The use of  $^{81}\text{Kr}^m$  alone for accurate measurements is therefore very limited.

Thus to obtain quantitative information on ventilation from  $^{81}\text{Kr}^m$  images at high specific ventilation rates, additional information on washout rates is required (Jones 1978). The correlation that was found in this series between  $^{81}\text{Kr}^m$  measurements and  $^{13}\text{N}_2$  measurements showed that agreement between theory and practice is obtained using a gamma camera and data analysis as described when changes in relative regional ventilation are measured. Comparison of this  $^{81}\text{Kr}^m$  theory based on a continuous flow model of the lungs with that obtained on a discrete breath model has been carried out by the authors and is shortly to be reported. Using data from this study, the differences between the results calculated by the two methods were small. Several sources of error in the technique may be briefly mentioned.

The first is the effect of redistribution of dead space gas. The radioactivity in dead space gas closely parallels that expired from the alveoli connected to the dead space, but the ratio between inspired and alveolar activity is dependent on alveolar  $\dot{V}/V$ . Thus at low tidal volumes (figure 7(a)) the concentration in dead space gas is about 40% of the inspired concentration but at high tidal volumes (figure 7(c)) it is 70% of the inspired concentration. Regional radioactivity will therefore depend on regional  $\dot{V}/V$  and on the proportion of dead space gas in the alveolar inspirate. Similar to the effect of dead space inhalation on  $^{13}\text{N}_2$  measurements, a high proportion of dead space low concentration  $^{81}\text{Kr}^m$  distributed to a region reflects less effective ventilation in the region, so that the effect is again in the correct direction but difficult to assess. The experiment with boluses showed that the dead space gas appears to constitute a greater proportion

of the ventilation to the upper lobes than to the lower lobes, but since the U/L distribution does not vary with tidal volume, there is likely to be little effect on the measured change in count ratios. Other factors which could affect count ratios are the inclusion of the tracheal gas volume in the lower of the two rectangles subjected to analysis, movement of lung tissue between the computation areas caused by expansion of the thorax, variation in the radioactivity concentration of the inspired gas and accumulation of counts before equilibrium is fully established. Errors in the  $^{81}\text{Kr}^m$  technique have been considered in more detail for a future report, and it is apparent that their overall effect would be to cause a small increase in the measured normalised count ratios.

## 5. Conclusion

Inherent errors in the  $^{13}\text{N}_2$  technique are unlikely to cause significant errors when measuring changes in relative regional ventilation. When corrected for regional specific ventilation,  $^{81}\text{Kr}^m$  equilibrium count ratios indicate similar changes in relative regional ventilation to those measured by the  $^{13}\text{N}_2$  technique. This supports the theoretical analysis of the  $^{81}\text{Kr}^m$  technique. Without correction,  $^{81}\text{Kr}^m$  count ratios can give a qualitative indication of the relative regional ventilation in a single image if geometrical differences between the regions are small, but when comparing different images, changes in relative regional ventilation may be accompanied by no change in the  $^{81}\text{Kr}^m$  count ratios or by a change in the opposite direction, depending on the values of specific ventilation involved. Similarly, changes in  $^{81}\text{Kr}^m$  count ratios may occur when there is no change in ventilation ratio. The value of  $^{81}\text{Kr}^m$  lies in those situations where it can be used alone, when specific ventilation always remains low in comparison with the radioactive decay rate of  $^{81}\text{Kr}^m$ .

It was found that increasing tidal volume in mechanically ventilated supine dogs results in a decrease in the upper/lower ratio of specific ventilation, regional lung volume and regional total ventilation.

## Acknowledgments

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## Résumé

Mesure de la ventilation régionale chez le chien ventilé artificiellement à l'aide d'azote  $^{13}\text{N}$  et de krypton  $^{81}\text{m}$ .

Nous avons utilisé, pour mesurer les modifications des rapports haut/bas (H/B) de ventilation régionale chez des chiens anesthésiés, en décubitus dorsal, ventilés artificiellement à différents volumes inspiratoires, le  $^{13}\text{N}_2$  et le  $^{81}\text{Kr}^m$ , et nous avons comparé les résultats obtenus. L'analyse des courbes de washout du  $^{13}\text{N}_2$  indique que la ventilation spécifique H/B est inférieure à 1 à de faibles volumes inspiratoires et diminue quand le volume inspiratoire augmente. La distribution préférentielle de la ventilation aux différentes régions du poumon augmente avec le volume inspiratoire malgré les forces hydrostatiques opposées provenant de l'abdomen. Nous avons analysé la distribution de densité de comptage régionale sur les images pulmonaires obtenues avec le  $^{81}\text{Kr}^m$ . A de hauts taux de ventilation spécifique, il est nécessaire d'effectuer une correction

de la densité de comptage pour obtenir des informations correctes sur la ventilation régionale. Les taux de ventilation spécifique dans cette série sont tels que l'on observe une augmentation des rapports de comptage H/B non corrigés pour le  $^{81}\text{Kr}^m$  avec le volume inspiratoire, au lieu d'une diminution des rapports de ventilation totale H/B enregistrés avec le  $^{13}\text{N}_2$ . Quand les corrections utilisant la ventilation spécifique du  $^{13}\text{N}_2$  sont appliquées, les modifications des rapports de comptage H/B avec le  $^{81}\text{Kr}^m$  en fonction du volume inspiratoire montrent une évolution similaire à celles de la ventilation totale mesurées par le  $^{13}\text{N}_2$ . Nous discutons brièvement les sources d'erreur des deux méthodes.

### Zusammenfassung

Messungen der regionalen Ventilation mit Stickstoff-13 und Krypton-81m bei mechanisch beatmeten Hunden.

Um Änderungen der Verhältnisse zwischen oberer und unterer regionaler Ventilation (O/U-Verhältnisse) mit Hilfe von  $^{13}\text{N}$  und  $^{81}\text{Kr}^m$  messen zu können, wurden narkotisierte Hunde bei verschiedenen Atemvolumina mechanisch beatmet und die Ergebnisse verglichen. Die Analyse der  $^{13}\text{N}_2$ -Auswaschkurven zeigt, daß die O/U-spezifische Ventilation bei wachsendem Atemvolumen sinkt. Die O/U-totale Ventilation, die man erhält aus den Ergebnissen der spezifischen Ventilation und aus Abschätzungen des relativen Lungenvolumens mit  $^{13}\text{N}_2$ , sinkt ebenfalls mit wachsendem Atemvolumen. Die Verteilung der Ventilation in verschiedenen Lungenabschnitten wächst mit dem Atemvolumen, trotz der entgegengesetzten hydrostatischen Kräfte des Abdomens. Regionale Dichteverteilungen in  $^{81}\text{Kr}^m$ -Bildern der Lunge wurden analysiert. Bei hohen spezifischen Ventilationsraten ist eine Korrektur der  $^{81}\text{Kr}^m$  Zählratendichte erforderlich, um richtige Informationen über die regionale Ventilation zu erhalten. Die spezifischen Ventilationsraten waren in dieser Serie so, daß die nicht korrigierten O/U-Verhältnisse von  $^{81}\text{Kr}^m$  mit wachsendem Atemvolumen ebenfalls wuchsen, ganz im Gegensatz zum Abfall bei der O/U-totalen Ventilation, aufgenommen mit der  $^{13}\text{N}_2$ -Methode. Korrigiert man die Zählraten mit Hilfe der Ergebnisse der  $^{13}\text{N}_2$ -spezifischen Ventilation, so zeigen die Änderungen der O/U-Zählratenverhältnisse mit dem Atemvolumen bei  $^{81}\text{Kr}^m$  einen ähnlichen Trend wie die Änderungen bei der totalen Ventilation mit  $^{13}\text{N}_2$ . Die Fehlerquellen beider Techniken werden kurz diskutiert.

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## THE PREPARATION OF A RUBIDIUM-82 RADIONUCLIDE GENERATOR

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$^{82}\text{Sr}$  has now been produced by the spallation of Molybdenum by protons of up to 800 MeV. The radiochemical recovery of strontium is described together with a description of the analytical techniques used to estimate recovered yields of the various radionuclides generated. A radionuclide generator is described for the rapid recovery of  $^{82}\text{Rb}$ , the 1.25 min half-life decay product of  $^{82}\text{Sr}$ . An outline is given of the quality control procedure adopted to ensure that the  $^{82}\text{Rb}$  is suitable for clinical use.

### Introduction

The preparation of radiopharmaceuticals from radionuclide generator systems is well established in nuclear medicine practice. For example  $^{99\text{m}}\text{Tc}$  ( $T = 6\text{ h}$ ), separated from its parent  $^{99}\text{Mo}$  ( $T = 66\text{ h}$ ), using an alumina column chromatographic technique, is now used in the majority of routine nuclear medicine diagnostic procedures. The attraction of generator produced radionuclides in medicine is the ability to have materials of short half-life readily available at sites remote from the production facility. The use of short half-life tracers usually leads to a significantly reduced radiation dose to the subject without compromising the statistical quality of the clinical data acquired.

Recently an increasing emphasis has been placed on the use of positron emitting short lived radionuclides as a result of the development of the technique of positron emission tomography,<sup>1</sup> a radionuclide counterpart of the x-ray transmission tomographic technique developed by EMI.<sup>2</sup> The latter provides information on tissue density whilst the radionuclide technique has the potential to provide information on tissue function. Many of the short lived positron emitting radionuclides for example  $^{15}\text{O}$  ( $T = 2.03\text{ m}$ ),  $^{13}\text{N}$  ( $T = 9.96\text{ m}$ ), and  $^{11}\text{C}$  ( $T = 20.3\text{ m}$ ) have to be made by a cyclotron at, or very close to the site of use. However there are

some that are in principle available from radionuclide generator systems. These are  $^{68}\text{Ge}$  ( $T = 287\text{ d}$ )/ $^{68}\text{Ga}$  ( $T = 68\text{ m}$ )<sup>3</sup>,  $^{62}\text{Zn}$  ( $T = 9.13\text{ h}$ )/ $^{62}\text{Cu}$  ( $T = 9.76\text{ m}$ )<sup>4</sup> and  $^{82}\text{Sr}$  ( $T = 25\text{ d}$ )/ $^{82}\text{Rb}$  ( $T = 75\text{ s}$ )<sup>5</sup> and is the chemical development of the last that is the subject of this paper. Earlier attempts at producing a medically acceptable  $^{82}\text{Sr}/^{82}\text{Rb}$  generator used a single column containing an organic ion exchange material e.g. Bio-Rex-70 or Chelex 100.<sup>5-7</sup> At tracer levels these materials performed reasonably satisfactorily; however, when applied to a more practical situation where much larger amounts of parent radionuclide were being loaded onto the generator, typically 100–200 mCi, these organic materials proved to be unsuitable as they were unstable with respect to the high radiation dose that they received during the six to eight weeks usable life of a generator. Discolouration of these materials occurred overnight when loaded with only 20–30 mCi of parent, and retention of the parent, a very important factor in medical applications, was reduced.

Recent work by YANO et al. at Berkeley,<sup>8,9</sup> suggested that basic alumina, an inorganic ion exchange material could be used. The long term radio-resistance of alumina is borne out by its use in medicine for  $^{99}\text{Tc}^{\text{m}}$  generators and the separation of fission products.

### Materials and methods

#### *Production and purification of $^{82}\text{Sr}$*

$^{82}\text{Sr}$  is produced in Curie quantities at the Los Alamos Meson Physics Facility (LAMPF) by irradiating a thick Mo target with 300–600  $\mu\text{A}$  of up to 800 MeV protons for a duration period of 1–2 weeks.<sup>10</sup> Spallation of the Mo nuclei results not only in the desired  $^{82}\text{Sr}$  product, but also in a wide variety of primarily neutron deficient isotopes spanning the elemental range from Tc to H. A post-bombardment radiochemical separation procedure must therefore isolate radiostrontium ( $^*\text{Sr}$ ) from numerous other species, and must also do so reasonably quantitatively. Because of the intense radiation levels of LAMPF-irradiated targets (typically  $\sim 10^7\text{ R/hr}$  at 1 cm), the radiochemistry must be performed remotely in a suitable hot cell.

There are currently two related procedures for the recovery of  $^{82}\text{Sr}$  that are implemented in LASL's radiochemistry hot cells. Because of the non-specificity of spallation as an isotope production mechanism, other useful nuclides (such as  $^{77}\text{Br}$ ,  $^{88}\text{Zr}$  and  $^{88}\text{Y}$ ) are also produced in a Mo target, and procedures for their isolation have been developed as well. The choice of the particular  $^{82}\text{Sr}$  separation scheme is dependent upon the need for these attendant isotopes. The schemes are shown in Figs 1 and 2.

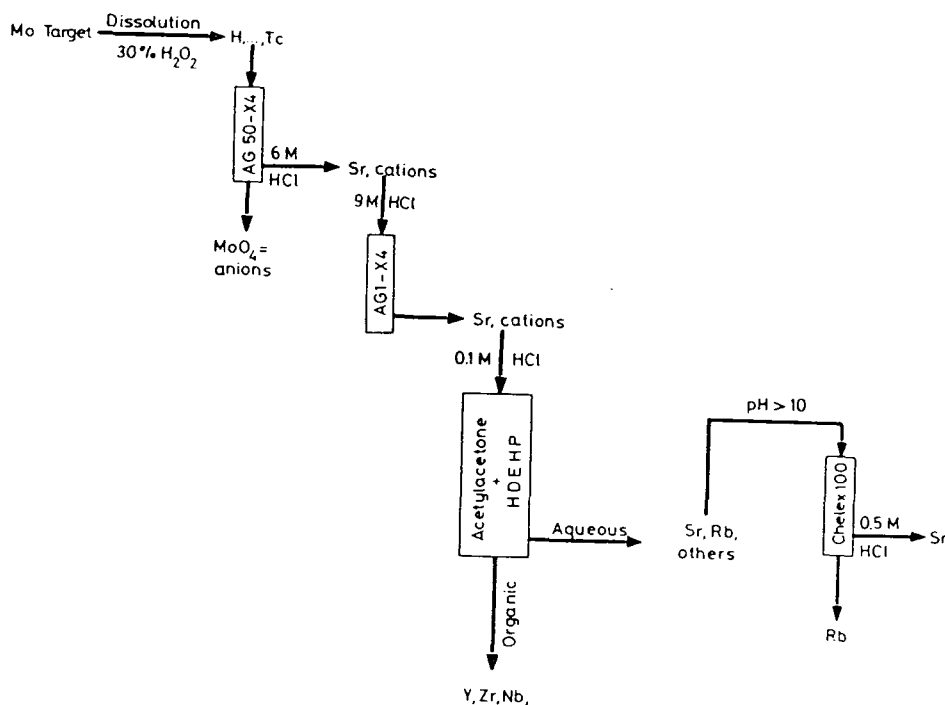


Fig. 1. LASL Mo-<sup>82</sup>Sr hot cell separation

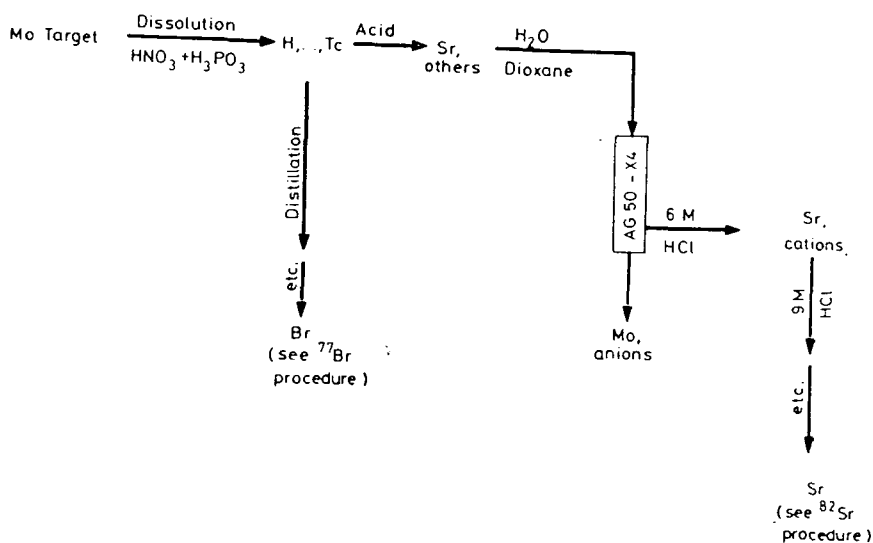


Fig. 2. LASL combined Mo-<sup>82</sup>Sr, <sup>77</sup>Br hot cell procedure

If <sup>88</sup>Zr and/or <sup>88</sup>Y are desired as nuclear physics targets or for safeguards experiments, the irradiated Mo target (typically 30–60 g in weight) is dissolved with unstabilized 30% H<sub>2</sub>O<sub>2</sub>. The resulting molybdic acid solution is passed through a strong acid cation exchange column (Bio-Rad AG50-X4), where cationic species

are adsorbed while bulk peroxymolybdates and other anions are not. The cations are then eluted with 6M HCl, the medium adjusted to 9M HCl, and the solution passed through a strong base anion exchange column (Bio-Rad AG1-X4). Anionic chloro-complexes of  $^{*}\text{Co}$ ,  $^{*}\text{Fe}$ ,  $^{*}\text{Zn}$  and other contaminants are retained on this resin, and  $^{*}\text{Sr}$  and other cations of interest remain in the eluate. This solution is then evaporated to near-dryness and the pH adjusted to 0–1 by the addition of 0.1M HCl. Solvent extraction with a mixture of HDEHP [bis-(2-ethylhexyl) orthophosphoric acid], acetylacetone and toluene follows. The organic phase extracts any  $^{*}\text{Y}$ ,  $^{*}\text{Zr}$ ,  $^{*}\text{Nb}$  and Al (from target cladding materials) present, while  $^{*}\text{Sr}$  and  $^{*}\text{Rb}$  reside in the aqueous phase. The aqueous solution is adjusted to a pH > 10 by the addition of 50% NaOH, and the fluid is passed through a chelating ion exchange column (Bio-Rad Chelex-100). The  $^{*}\text{Sr}$  is adsorbed by the resin, while  $^{*}\text{Rb}$  is found in the effluent and subsequent wash. Lastly, the purified  $^{*}\text{Sr}$  is eluted from this column with 0.5M HCl. This final solution contains primarily  $^{82}\text{Sr}$  and  $^{85}\text{Sr}$  (as an isotopic impurity), with small quantities of  $^{*}\text{Co}$  and  $^{*}\text{Mn}$  often observable as well.

If on the other hand,  $^{77}\text{Br}$  is desired for radiopharmaceutical research, in the interests of speed and volume reduction the Mo target is dissolved in a mixture of conc.  $\text{HNO}_3$  and  $\text{H}_3\text{PO}_4$ . The  $^{77}\text{Br}$  is isolated via subsequent distillation, pre-precipitation, and ion exchange technique, and  $^{*}\text{Sr}$  remains in the strongly acidic dissolution medium along with other product and target species. This solution is then diluted with equal volumes of water and 2,4-dioxane and passed through the strongly acidic cation column to adsorb  $^{*}\text{Sr}$  and other cations. Following elution with 6M HCl, the  $^{*}\text{Sr}$  is processed as described above in the  $\text{H}_2\text{O}_2$  dissolution procedure.

#### *Mass separator studies of the radionuclidic purity of $^{82}\text{Sr}$*

The NPL mass separator was used to investigate the levels of possible strontium radionuclide impurities in  $^{82}\text{Sr}$  (containing high levels of  $^{85}\text{Sr}$ .) Of particular interest were the long-lived pure  $\beta$ -emitters  $^{89}\text{Sr}$  and  $^{90}\text{Sr}$  which could not be assayed by  $\gamma$ -ray spectrometry.

The NPL mass separator has a linear separation of 15 mm between successive mass numbers at mass number 80 and an enrichment factor of better than 2000.  $^{82}\text{Sr}/^{82}\text{Rb}$  solution was loaded into a graphite crucible together with strontium chloride carrier (20 mg) and the solution was evaporated to dryness. The crucible was placed in the mass separator ion source and heated to a temperature of 1000 °K. Positive ions were extracted at 50 kV, focussed, and mass separation was achieved in a 90° sector magnet with a magnetic flux of 0.6 tesla. Mass num-

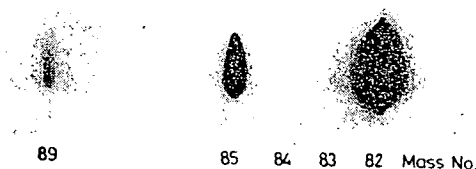


Fig. 3. The low intensity lines at 83 and 84 are due to  $^{83}\text{Rb}$  and  $^{84}\text{Rb}$ , respectively

bers 80 to 90 were collected on a strip of thin aluminium foil. An autoradiograph of this foil is shown in Fig. 3. The foil was cut into sections corresponding to mass numbers 82, 85, 89 and 90. The activities of the strontium-82 and strontium-85 were assayed using a calibrated intrinsic germanium detector by counting, respectively, annihilation quanta and 514 keV  $\gamma$ -rays. The strontium-89 and strontium-90 were assayed in a low background anti-coincidence shielded  $4\pi\beta$  proportional counter. The radionuclidic purity of all samples was checked by  $\gamma$ -ray spectrometry.

#### *Preparation and loading of the radionuclide generator*

In an attempt to make use of the better features of the previously reported generator systems based on basic  $\text{Al}_2\text{O}_3$  or Chelex-100 we chose to test a dual column system. The primary generator column is a 5 cm x 1 cm diameter Perspex tube filled with Whatman chromatographic grade basic alumina supported by sintered polyethylene (Fisons, Loughborough) discs at top and bottom, the alumina being washed exhaustively prior to assembly to remove fines. The second column is a 2 cm x 1 cm diameter Perspex tube of the same design containing Bio-Rad Chelex-100 (200–400 mesh preconditioned with 1.8% NaCl at pH 10), and performs a final chemical purification on the primary column eluate, Fig. 4. Both columns are provided with threaded ports that can be connected using 1/16" o.d. 1/32" i.d. flanged PTFE tube and 1/4" x 28 TPI screws (Altex fittings, Anachem, Luton).

The  $^{82}\text{Sr}$  solution in 0.5M HCl is adjusted to pH 9–10 using 3M NaOH and loaded onto the  $\text{Al}_2\text{O}_3$  column using a peristaltic pump at  $1\text{ ml min}^{-1}$ . The column is then washed with 200 ml of 1.8% NaCl at pH 9–10 to remove any poorly adsorbed activities. Typically more than 99% of the  $^{82}\text{Sr}$  and  $^{85}\text{Sr}$  is retained on the column. A sample of eluate is taken to assess the  $^{82}\text{Sr}$  breakthrough or leakage from the primary column using a calibrated Ge/Li spectrometer, the Chelex column is then connected and further samples are taken on flow to assess  $^{82}\text{Sr}$  breakthrough for the complete assembly at flow rates of up to  $18\text{ ml min}^{-1}$ . Elution efficiency measurements on a  $\sim 5\text{ }\mu\text{Ci}$  test generator were made by direct observation of the change in the intensity of the 777 keV line of  $^{82}\text{Rb}$



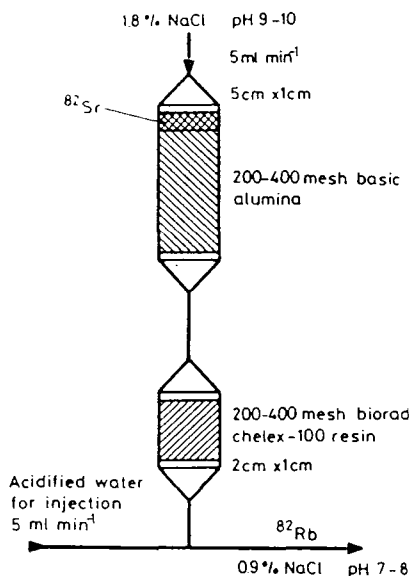


Fig. 4. <sup>82</sup>Sr-<sup>82</sup>Rb generator

from the equilibrium value to its steady state value during elution at 4.4, 12 and 18 ml · min<sup>-1</sup>. A low geometry Ge/Li spectrometer was used to view the generator columns whilst the effluent was transferred to a well shielded position.

To make the column effluent suitable for human administration it is necessary to adjust the pH 6.5-7.5 and reduce the NaCl concentration to 0.9% which is isotonic with blood. This is achieved by mixing the column output flow with acidified water for injection/HCl pH 2-4 at an equal flow rate. The mixed solution is then passed through a sterilizing filter having a pore size of 0.22 μm and low dead vol (~0.3 ml) (Millipore, SLG0250S) to remove any bacterial contamination. In vitro tests for pyrogens are carried out using the Limulus test (Pyrogen-Mallincrodt). Additional in vivo pyrogen tests are carried out on random batches of eluate using rabbits.

## Results

Combined data from Ge/Li spectroscopy and beta and gamma counting of a mass separated sample are shown in Table 1. The radiochemical impurities, <sup>58</sup>Co, <sup>84</sup>Rb and <sup>87</sup>Y present the starting material as shipped from LASL are eliminated during the Al<sub>2</sub>O<sub>3</sub> column loading procedure. The <sup>82/85</sup>Sr breakthrough has never been found to exceed 250 pCi · mF<sup>-1</sup> and Fig. 5 shows values measured

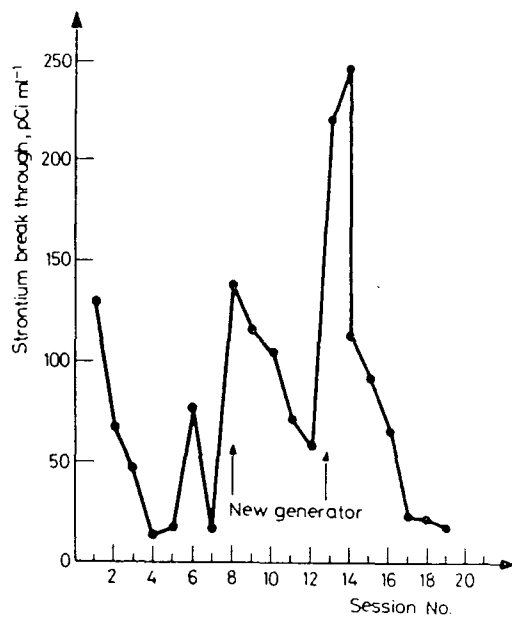


Fig. 5. Generator stability

Table 1  
Example assay of <sup>82</sup>Sr batch shipped 19th December, 1979.  
Reference date 18th December, 1979.

| Nuclide          | Half-life | Activity, mCi |
|------------------|-----------|---------------|
| <sup>82</sup> Sr | 25 d      | 130           |
| <sup>85</sup> Sr | 65 d      | 36            |
| <sup>89</sup> Sr | 50.5 d    | 0.66          |
| <sup>90</sup> Sr | 28.5 y    | 0.01          |
| <sup>87</sup> Y  | 80 h      | 0.47          |
| <sup>58</sup> Co | 71 d      | 0.33          |
| <sup>84</sup> Rb | 33 d      | 0.14          |

prior to clinical use of three recently prepared generators. The elution efficiency was found to be 66, 49, 14 percent at 18, 12, 4.4 ml min<sup>-1</sup> respectively, these flow rates being found convenient in our clinical situation.

### Discussion

Material suitable for  $^{82}\text{Sr}$  radionuclide generator preparation can be separated from the spallogenic mixture of products found in the proton irradiated molybdenum target. The resulting generator described here represents a significant improvement in  $^{82/85}\text{Sr}$  retention whilst some elution efficiency is sacrificed. However, we have chosen to use the generator for steady state infusion studies where the flow rate is of necessity much lower than reported by other workers. As strontium radionuclides are well known to concentrate in the bone it is imperative that the generator is carefully monitored before each clinical use. The calculated radiation doses to the skeleton of a 70 kg man from 1 microcurie of  $^{82}\text{Sr}/^{82}\text{Rb}$ ,  $^{85}\text{Sr}$  and  $^{90}\text{Sr}/^{90}\text{Y}$  are 71.1, 7.6, 5900 mrad,<sup>11</sup> thus the search for pure beta emitting radionuclidic impurities using the cumbersome technique of mass separation is amply justified. The assay of  $^{85}\text{Sr}$  and  $^{82}\text{Sr}$  by Ge/Li spectrometry leaves much to be desired as the 514 keV line of  $^{85}\text{Sr}$  is not fully resolved from the 511 keV annihilation line. In addition values for the abundance of the 777 keV line of  $^{82}\text{Rb}$  show wide variations in the literature (9–13%)<sup>12,13</sup> making the proportional subtraction of the 511 from the combined 511 + 514 peak imprecise. This problem is accentuated by the poor statistics encountered with assay breakthrough samples containing typically 2 nCi. Thus in the present work  $^{82/85}\text{Sr}$  breakthrough values are consistently overestimated as no allowance is made for the presence of both  $^{82}\text{Rb}$  annihilation photons in the 514/511 combined peak. Attempts are being made to improve this situation using mass separated  $^{82}\text{Sr}$  samples.

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We are indebted to the United States Department of Energy for their support in this collaborative programme. The enthusiasm from our clinical colleagues Drs. A. P. SELWYN and R. M. ALLAN has been a great encouragement. We would like to thank M. J. KENSETT for his contribution in carrying out the routine gamma-ray spectrometry

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# Radiation-Induced Defects as Illustrated by the $^{81}\text{Rb}$ – $^{81m}\text{Kr}$ Target System

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The preparation of cyclotron-produced radionuclides for incorporation into clinically useful diagnostic agents requires special attention to quality control determinations. Of particular concern is the paucity of data on the toxicity of those chemical contaminants produced within the target during the bombardment and subsequent radiation cooling as well as those arising from materials used in the handling and construction of targets. At Mount Sinai Cyclotron Facility, a krypton–rubidium targetry system has been in use for five years. Consequently we shall use this targetry and generator system as an illustration of the problems created when such unexpected chemical moieties are encountered. The chemical species apparently formed by radiolytic processes as well as their effects on the chemical processing of the diagnostic agent are presented.

## Introduction

THROUGHOUT the past decade there has been a significant increase in the utilization of short-lived radioactive gases having clinical applications. One such gas which has been of particular interest to the Nuclear Medicine Department of Mount Sinai Medical Center is the generator-produced krypton-81m ( $t_{1/2} = 13$  s) used to study pulmonary ventilation functions. Furthermore, the parent radionuclide of krypton-81m, rubidium-81 ( $t_{1/2} = 4.58$  h), having metabolic similarity to potassium, is also expected to show diagnostic potential.

Rubidium-81 was prepared by utilizing several nuclear reactions upon a variety of targets.<sup>(1–9)</sup> The  $^{82}\text{Kr}(p, 2n)^{81}\text{Rb}$  reaction on natural krypton gas has been utilized by the Mount Sinai Cyclotron Facility for several years.<sup>(10)</sup> This study illustrates that although the chemical effects resulting from the nuclear transformation are of particular interest, one must also appreciate the synthetic potential of the cyclotron beam as a powerful tool capable of catalyzing reactions and thus inducing preparation of a variety of reactive species.

The currently used rubidium–krypton targetry system dramatically emphasizes the necessity of anticipating the formation of such chemical contaminants and the importance of devising appropriate quality control procedures to assess the purity of the short-lived radiopharmaceuticals produced.

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## Nuclear Reactions for the Preparation of Rubidium-81

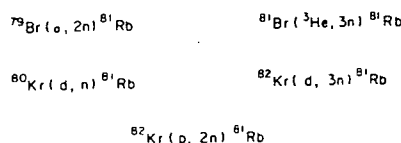


FIG. 1.

## Materials and Methods

### Rubidium target and processing

The rubidium-81 radionuclide is prepared on the Mount Sinai Medical Center Cyclotron (Cyclotron Corp. CS-30) by using a thick-target containing research-grade krypton gas (Matheson, East Rutherford, New Jersey). The external target assembly is a modification of a fluorine-18 target system<sup>(11)</sup> which facilitates the remote recovery of the rubidium radionuclides. Employing a mean 15- $\mu\text{A}$  beam current, the measured yield of rubidium-81 is 4.4 mCi/ $\mu\text{Ah}$ . Apart from rubidium-81 other concurrently produced radioisotopes of rubidium do not interfere with the clinical usefulness of the generator as none decay to radio-krypton. The target system is diagrammed in Fig. 1. Following bombardment of the natural krypton with 26 MeV protons, the rubidium moieties deposited on the walls of a stainless steel, electrically rotated liner are dissolved in distilled water which is introduced onto the liner by means of a remotely operated pneumatic syringe. After rotation of the liner to effect dis-

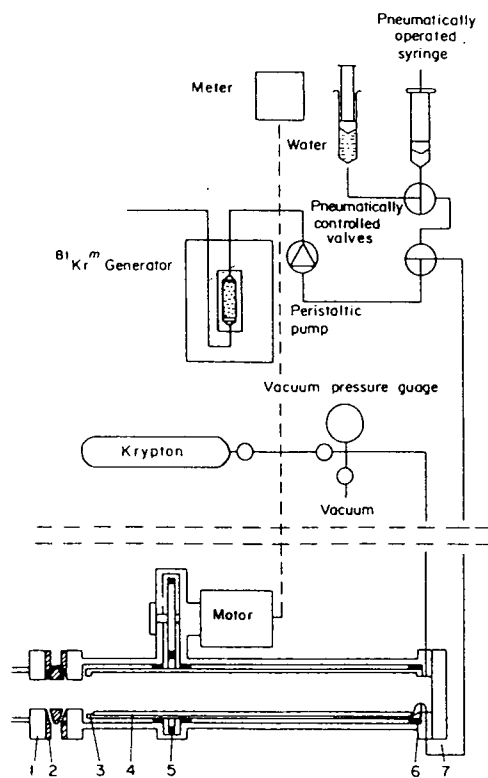


FIG. 2. Target and recovery system for the preparation of krypton-81m generators using the  $^{82}\text{Kr}(p, 2n)^{81}\text{Rb}$  reaction. (1) Aluminum beam mounting flange; (2) EN58B stainless steel beam entry windows (0.001 in. in thickness) and insulator; (3) Stainless steel liner; (4) Borosilicate glass rod; (5) Rotational drive assembly; (6) Stainless steel water collection tube; (7) Aluminum target back plate.

solution of the rubidium species, the aqueous solution is forced from the target through a cation exchange resin (AG50X8, 100–200 mesh,  $\text{H}^+$ ). Typically, 40 mCi of rubidium-81 are loaded onto the generator and an elution efficiency of 90% of krypton-81m achieved using humidified air. The target gas is retained within the target and removed every two months for routine target maintenance. During the course of this work, target gas analyses for nitrogen and oxygen were performed by conventional gas chromatography and mass spectrometry.

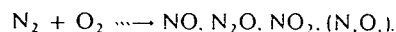
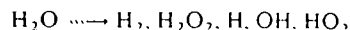
#### Gases and protons

Historically, the noble gases have been used as moderators in gas phase hot-atom chemical investigations in an attempt to differentiate hot reactions from thermal and secondary radiolytic effects.<sup>(13–16)</sup> However, the assumption that noble gases are chemically unreactive may not be completely valid<sup>(17)</sup> when considering these gases in an environment of energetic charged particles. Minute concentrations of impurities, e.g. nitrogen and oxygen, have been found to profoundly alter the ionization properties of noble gases.<sup>(18)</sup>

The effects of radiation on both water and air are well documented.<sup>(19,20,21)</sup> Radiation induced reac-

tions caused by charged particles are dependent upon the average energy expended per ion pair formed. For the noble gases the value of energy ion pair is dependent upon the concentration of impurities.<sup>(18,22)</sup>

Radiolysis of mixtures of nitrogen, oxygen and water vapor produces active species such as ions, atoms and excited molecules which react to produce such species as NO,  $\text{NO}_2$ ,  $\text{N}_2\text{O}$ ,  $\text{N}_2\text{O}_2$  and  $\text{O}_3$  as final products.<sup>(19,20,23,24,25)</sup>



#### Chemical contaminants

When considering the fabrication of a cyclotron target, the stability of various components toward their surroundings; temperature considerations; and resistance to radiation damage without chemical or structural alteration are of prime importance. Deterioration and loss of material due to chemical erosion may be termed corrosion, which generally occurs through the interaction of the processes of dissolution and oxidation. In operation the rubidium-krypton target system described in this work possesses all the necessary conditions for galvanic-induced corrosion. Electrical contact between dissimilar materials and the presence of oxygen and other ions may be expected to greatly accelerate the corrosion effects. Stress points and composition galvanic cells are a consequence of this target's fabrication. The necessity for rotation of the liner coupled with the machining of parts from dissimilar metals results in internally stressed components. The presence of such stressed areas often significantly accelerates the chemical deterioration of the component.

#### Results and Discussion

Although the targetry-processing system was designed as a closed-loop operation, the introduction of water to dissolve the rubidium moieties from the walls of the stainless steel liner allows gaseous impurities (primarily oxygen and nitrogen) to accumulate in the target gas. The use of dissimilar metals such as the casement of aluminum, stainless steel inner liner and copper guide ring in the construction of the target, coupled with the water solvent containing ionic species in the target leads to galvanic corrosion. Both aluminum and iron serve as the anode as indicated in the target photographs.

HPLC analysis of the solution which remains within the target until routine maintenance is performed, has indicated the presence of nitrate and nitrite ions. Determinations of nitrate and nitrite ion concentrations have been performed by high pressure liquid chromatography using a Partisil SAX column.<sup>(12)</sup> Furthermore, wet chemical analysis using diphenylamine has been employed for confirmation of nitrate ions.<sup>(26)</sup> The maximum concentration of these

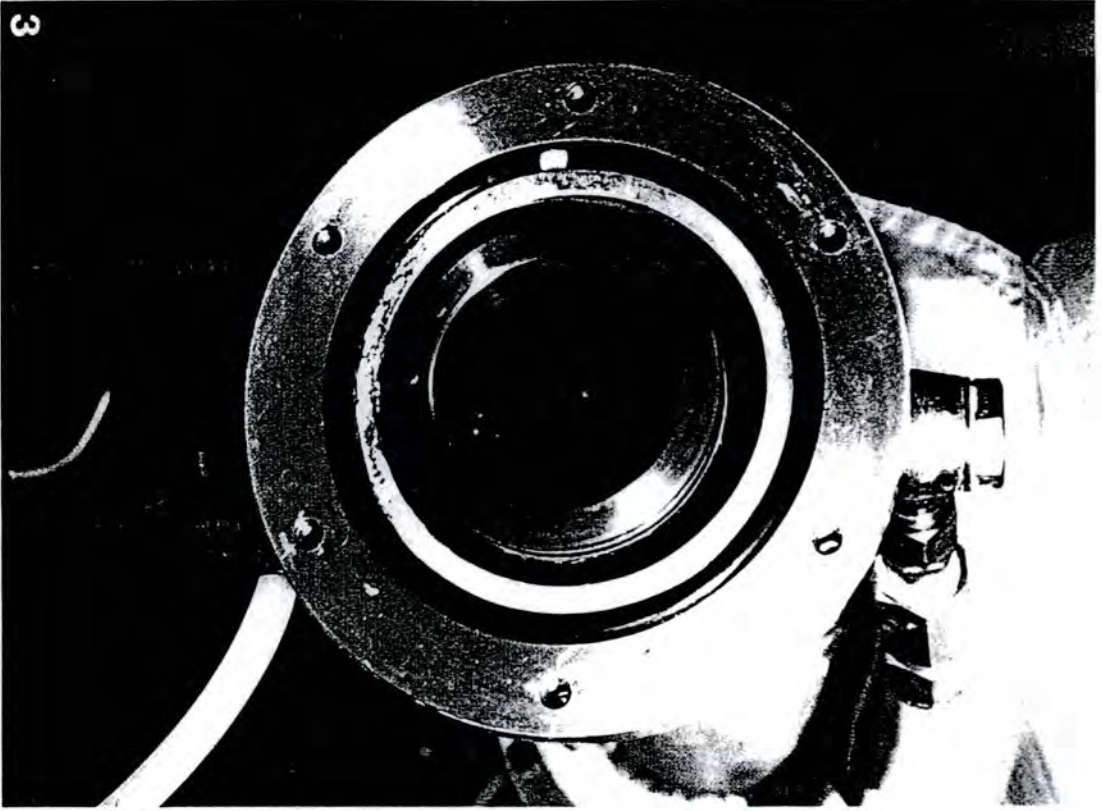


FIG. 3



FIG. 4

ions has been recorded at 1.2 mg/ml and 0.06 mg/ml respectively, although variability in this determination is dependent upon target operating conditions. It should be noted that the efficiency of absorption of the rubidium radionuclides on the generator resin support has been shown to be a function of the pH of the target water. Data obtained in experiments studying this efficiency of absorption as a function of time of target operation were too variable to allow quantitation of the nitrate concentration. However, with continued operation it was noted that the pH of the aqueous phase residing within the target became strongly acidic and the concentration of nitrate ions increased to a maximum with time. The combined oxygen and nitrogen content of the target gas was determined to be nearly 17% immediately prior to maintenance. In addition a slight turbidity was noted in the residual aqueous solution with time elapse. Filtration of the aqueous solution prior to the loading of the generator column caused slight adsorption of rubidium moieties. Chemical testing confirmed the presence of aluminium. Possible chemical forms for this precipitate include  $\text{AlO}(\text{OH})$  and  $\text{Al}(\text{OH})_3$ .

No attempt has been made to diagnose all the possible reaction schemes that could be occurring in this system. However, if one considers the anodic nature and amphoteric character of aluminium, the possibility of formation of oxides of nitrogen; the presence of water vapor; and the generation of heat from the bombardment of the target gas with energetic protons; products such as nitric acid,  $\text{Al}(\text{OH})_3$  or  $\text{AlO}(\text{OH})$  are readily rationalized.

### Conclusions

A cyclotron adjunct to a large medical center can be an extremely useful tool in the armamentarium for basic biomedical research and clinical applications. However, intrinsic complications related to the cyclotron beam and targetry can arise. The impact of energetic charged particles upon any target system usually leads to a complex mixture of chemical species. These excited molecules and ions formed by the radiation are in most systems chemically unstable and are rapidly converted to neutral molecules. The radiation and hot-atom chemical results coupled with the lack of toxicity data on such chemical contaminants produced or introduced into the cyclotron targetry processing system must be anticipated and if the specific has a toxic potential, removed. As evidenced by the continued operation of the krypton-rubidium target

system, the inert nature of the fabrication materials can be misleading. Products formed can contribute to target deterioration and chemical processing difficulties such as generator breakthrough or parent radionuclide adsorption on precipitated galvanic products with and/or within the target itself.

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## Relation Between Regional Myocardial Uptake of Rubidium-82 and Perfusion: Absolute Reduction of Cation Uptake in Ischemia

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Experiments were undertaken using rubidium-82 and positron tomography to examine the relation between myocardial perfusion and cation uptake during acute ischemia. Rubidium-82 was repeatedly eluted from a strontium-82-rubidium-82 generator. In six dogs emission tomograms were used to measure the delivered arterial and myocardial concentrations at rest and after coronary stenosis, stress and ischemia. There was a poor overall relation between regional myocardial uptake and flow measured by microspheres and a large individual variability. Extraction of rubidium-82 was inversely related to flow. Significant regional reduction of cation uptake was detected in the tomograms when regional flow decreased by more than 35 percent. This reduction was significantly greater when ischemia was present. A small but significant decrease ( $33.0 \pm 9.1$  percent, mean  $\pm$  standard deviation) in the myocardial uptake of rubidium-82 was detected only when flow was increased by more than 120 percent in relation to a control area after administration of dipyridamole.

The technique using rubidium-82 and tomography was applied in five volunteers and five patients with angina pectoris and coronary artery disease. Myocardial tomograms recorded at rest and after exercise in the volunteers showed homogeneous uptake of cation in reproducible and repeatable scans. In contrast, the patients with coronary artery disease showed an absolute mean decrease of  $36 \pm 14$  percent in regional myocardial uptake of rubidium-82 after exercise. These abnormalities persisted in serial tomograms for more than 20 minutes after the symptoms and electrocardiographic signs of ischemia.

Radioactive cations are widely used to image the myocardium.<sup>1</sup> The major problems include the inability to measure the absolute regional myocardial uptake in relation to the injected dose or arterial concentration. In addition, the variable tissue attenuation and inefficient detection of low or high energy emissions hinder quantitation. The long half-life of thallium-201 makes serial studies difficult, and not enough is known about the relative importance of the effects of flow, extraction and mass within the myocardium.<sup>2</sup>

This paper describes the use of a strontium-82-rubidium-82 generator for the intermittent elution of rubidium-82 in saline solution. The physical and biologic properties of this tracer (half-life [ $t_{1/2}$ ] = 78 seconds; 511 keV coincidence photons) with positron tomography largely overcome variable attenuation and permit measurement of regional myocardial uptake of the cation. The short half-life is exploited in serial tomograms permitting each subject to be used as his own control. A reference measure of flow has been used in experiments to explore the relation between regional myocardial cation uptake and perfusion, with and without ischemia. The clinical application of this method is demonstrated in patients.

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## Methods

**Preparation of the generator:** The parent, strontium-82 is produced at the Clinton P. Anderson Meson Physics facility at the Los Alamos Scientific Laboratory, New Mexico. The parent material contains as a production contaminant strontium-85 (half-life 65 days) in significant quantities. The strontium isotopes are bound to an alumina column, which has a high affinity for strontium at high pH (9 to 10), but a low affinity for rubidium and sodium. Rubidium-82 can be eluted continuously from the generator by 1.8 percent sodium chloride at pH 10 at flow rates up to 30 ml/min.<sup>1,2</sup> An equal flow of acidified water for injection is added to adjust the infusate to 0.9 percent sodium chloride at pH 7 to 8. The infusate was sterilized by terminal filtration (Millipore SLG02505, 0.22  $\mu\text{m}$ ). The product has been shown to be pyrogen-free by *in vivo* rabbit testing (Consultox, London). The elution of strontium isotopes is low and an upper limit of 1  $\mu\text{Ci}$  per liter<sup>-1</sup> of strontium-82 and strontium-85 has been set. The calculated radiation dose to the skeleton of a 70 kg man from 1  $\mu\text{Ci}$  of strontium-85 and strontium-82-rubidium-82 is 7.6 and 71.1 mrad, respectively.

The output of the strontium-82-rubidium-82 generator (10 ml/min of normal saline solution) was pumped over a calibrated detector (Bicron 1.5M2/2P, ORTEC). The activity stabilized within 90 seconds and varied by  $\pm 5$  percent for 40 minutes of observation. The breakthrough of strontium-82 and strontium-85 was less than 0.30  $\mu\text{Ci}$ /liter.

**Theoretical considerations:** Rubidium-82 has a half-life of 78 seconds. During a continuous intravenous infusion of constant activity a radioactive equilibrium is achieved in the arterial blood owing to rapid decay of the isotope in the systemic blood pool. The arterial concentration of rubidium-82 at equilibrium represents the input concentration to the coronary circulation and myocardium. The activity of rubidium-82 in the myocardium will also reach an equilibrium because of the constant input and rapid decay of rubidium-82. The relation between the arterial and myocardial concentrations is determined by the conservation of energy (that is, radioactivity) of rubidium-82:

Input = output + decay of rubidium-82:

$$E \times F \times \text{Ca} = F \times \text{CV} + \lambda \text{AT}, \quad (1)$$

where  $E$  = extraction of rubidium-82 from blood into tissue,  $F$  = blood flow to the myocardium,  $\text{Ca}$  = arterial activity of rubidium-82,  $\text{CV}$  = venous activity of rubidium-82,  $\lambda$  = decay constant for rubidium-82 and  $\text{At}$  = activity of rubidium-82 in the tissues. If we divide this expression by volume ( $V$ ) then,

$$E \cdot \frac{E}{V} \cdot \text{Ca} = p \cdot \frac{F}{V} \cdot \text{Ct} + \frac{\lambda \text{At}}{V}, \quad (2)$$

where  $\text{Ct}$  now represents the tissue activity of rubidium-82 and  $p$  = the partition coefficient for rubidium-82 and, hence,

$$p\text{Ct} = \text{CV}.$$

This equation can be rearranged:

$$\text{Ct} = \left( E \cdot \frac{F}{V} \cdot \text{Ca} \right) / p \cdot \frac{F}{V} + \lambda \quad (3)$$

Once rubidium-82, like potassium, is taken up into myocardial cells the turnover determining release (40 minutes and hours) is slow in relation to the rapid decay (78 seconds). Therefore, insignificant quantities of rubidium-82 will be washed out of the myocardium in the active form and therefore  $p(F/V)$  is small in relation to  $\lambda$ .

Therefore, the expression is:

$$\text{Ct} = \frac{E \cdot \frac{F}{V} \cdot \text{Ca}}{\lambda} \quad (4)$$

$\text{Ca}$  can be measured from the tomographic equilibrium scan.  $\text{Ct}$  can be measured from the tomographic washout scan.  $\lambda$  is constant and therefore the above expression can be used to calculate  $E(F/V)$  in each region of the myocardium:

$$\lambda \frac{\text{Ct}}{\text{Ca}} = E \cdot \frac{F}{V} \quad (5)$$

## Animal Studies

**Experimental preparation:** Six mongrel dogs (weight 20 to 35 kg) were anesthetized using intravenous thiopentone sodium (12 mg/kg body weight). Anesthesia was maintained using pentobarbitone (2 mg/kg) intermittently and respiration was maintained with a cuffed endotracheal tube and Harvard ventilator. Portex tubing and three-way taps were filled with heparinized saline solution (1,000 IU/liter) and inserted by way of the femoral artery and femoral vein into the thoracic aorta and inferior vena cava. In four dogs the arterial blood was constantly withdrawn from the femoral artery, pumped over a calibrated detector (Bicron 1.5M2/2P, ORTEC) and then returned to the femoral vein. The level of activity measured by the detector was recorded on a strip chart before, during and after infusion of rubidium-82 into a peripheral vein for 10 minutes.

A left thoracotomy was performed and the heart supported in a pericardial cradle. A plastic cuff and wedge device was positioned around the mid portion of the left anterior descending coronary artery in order to produce a graded decrease in luminal diameter. Proximal to this a 3 or 4 mm diameter flow probe (Systems Electronic, SE) was positioned around the left anterior descending coronary artery. Pulsatile and mean coronary flow were recorded using a flowmeter (SE 273). The linear response of the probe was checked and mechanical occlusions of the left anterior descending coronary artery were made to ensure a less than 5 percent drift in zero level throughout the study. The probe was calibrated *in-situ* at the end of the experiments. Flow in ml/min was calculated using the mean flow tracing and calibration data.

A 7 French catheter was inserted through a purse-string suture into the left atrium and pacing wires were stitched to the left atrial appendage. Aortic and left atrial pressures (in mm Hg) were measured using P 23 dB Statham transducers and devices amplifiers. The epicardial electrocardiogram was recorded using a saline-soaked cotton electrode (1 mm = 1 mV). Pressures, electrocardiographic and flow data were recorded on a multichannel strip chart recorder (Devices).

Arterial blood samples were taken intermittently for measurement of partial pressure of carbon dioxide and oxygen pH, hemoglobin and acid-base balance.

**Positron emission transaxial tomography:** Each dog was positioned within a hexagonal ring of sodium iodide crystals organized for detection of coincidence radiation (ECAT produced by ORTEC). A mid left ventricular position was selected for serial transaxial emission tomography. The position was fixed in relation to the detectors by a laser beam.

An external ring source of germanium-68 was positioned between the detectors and the anatomic position chosen for transaxial tomography. Transmission scans were collected for later attenuation correction of emission data.<sup>4</sup> Rubidium-82 (15 to 18 mCi/min) was eluted in 10 ml/min of 0.9 percent sodium chloride solution and infused intravenously.<sup>3</sup> Serial arterial blood samples were collected and counts/ml measured in a calibrated well counter. At equilibrium of activity in the

arterial blood, the ECAT recorded coincidence events for 120 seconds (equilibrium scans). The infusion was stopped and tomographic data were again recorded at between 30 and 150 seconds after the rubidium-82 infusion (myocardial scans). Emission data were corrected for attenuation and reconstructed by computer into images within a 100 by 100 matrix of pixels.

The instrument has a resolution of 1.4 cm and a slice thickness of 1.6 cm. Weekly calibrations using phantoms and known activity are used to ensure linearity and stable sensitivity. A weekly calibration factor is calculated in order to relate ECAT counts to well counter counts/ml per s.

### Protocol of the Experiments

**Phase 1:** Equilibrium and myocardial scans, electromagnetic coronary flow, epicardial electrocardiogram, left atrial pressure and aortic pressure were all recorded during a control period without interventions. Three arterial blood samples were drawn during the equilibrium scans and three to eight samples were drawn during the equilibrium scans and three to eight samples were drawn during the myocardial scans, and activity (counts/ml per s) in each sample was measured in a well counter. Two to five million microspheres (7 to 15  $\mu$ ), labeled with 30  $\mu\text{Ci}$  of iodine-125 (3M Company), were suspended in normal saline solution, mixed, agitated and injected into the left atrium. A timed arterial sample was collected for the later calculation of myocardial blood flow.

**Phase 2:** The screw device was used to decrease the diameter of the left anterior descending coronary artery. This was done until resting flow was decreased by 10 to 15 percent and reactive hyperemia abolished. Care was then taken that no fluctuations or decreases in flow greater than 15 percent occurred. Observations were recorded to be sure that the left atrial pressure did not increase progressively or significantly above the control values ( $5.0 \pm 3.0$  mm Hg before stenosis,  $5.5 \pm 2.2$  after stenosis). The regional epicardial electrocardiograms were recorded to ensure that no significant changes occurred in the S-T segment (depression or elevation greater than 1 mm), in the QRS width or the rhythm. This evidence was used to ensure that the stenosis alone did not cause regional myocardial ischemia. Left atrial pacing was used to increase the heart rate by 20 to 25 percent for 15 minutes. Equilibrium and myocardial scans plus all the observations listed in phase 1 were recorded. Cerium-141-labeled microspheres were used.

**Phase 3:** After 30 minutes of recovery after phase 2, the electrocardiogram and left atrial pressure were used to detect evidence of myocardial ischemia. If no ischemia was found the left anterior descending arterial stenosis was checked and the heart rate increased by 70 to 80 percent for 10 to 14 minutes. Equilibrium and myocardial scans plus all the observations listed in phase 1 were recorded. Strontium-85-labeled microspheres were used.

**Phase 4:** After 30 minutes of recovery after pacing, a check was again made for evidence of regional myocardial ischemia. If none was found, the critical left anterior descending arterial stenosis was checked and then 0.3 mg/kg per min of dipyridamole was infused intravenously for 10 minutes. This was used to produce a 5 to 10 percent decrease in mean aortic pressure. This intervention was used in an attempt to produce coronary vasodilation, increases in perfusion to areas unaffected by the coronary stenosis and therefore regional differences in flow in the left ventricle. The effects of the drug on flow were measured with the labeled microspheres. Equilibrium and myocardial scans plus the observations listed for phase 1 were repeated. Scandium-46-labeled microspheres were used.

**Well counting of blood samples:** All blood samples were drawn into tared tubes and weighed. The time from withdrawal to completion of well counting was recorded and used for decay correction. The number of counts/s per ml was calculated using a calibrated detector.

**Postmortem dissection of the heart:** At the end of each experiment the laser light was used to mark the track of the transaxial slice on the heart seen by the detectors. The orientation of the heart and slice within the ring was recorded in relation to the field of view, laser, ventricular anatomy and epicardial coronary arteries. This procedure allowed later comparison of eight regions of ventricular myocardium with eight regions in the emission tomographic scans. Calculation of regional myocardial flow for each area (ml/g per min) was made by multiple biopsy, well counting with separation of energy levels emitted by the four microsphere labels and the reference arterial withdrawal.<sup>5</sup>

### Calculations Made From Positron Tomography

Tomographic images were recorded on 35 mm film and inspected by three observers. The tomographic distribution of activity is recorded within a 100 by 100 matrix of pixels, and a printout of this image provides a matrix display of 20 by 20 numbers, each representing a mean of 25 pixels. The dimension (in mm) for each pixel is given for each scan. Two independent observers calculated the data. The peak count in the matrix was identified and all counts of 50 percent or more of the peak value were enclosed, thus identifying the tomogram of the myocardium. The favorable myocardium to background ratio allowed eight regions to be identified in the tomogram with the spatial orientation recorded as described earlier, thus allowing regional comparison with microsphere data.

**Myocardial scans:** The mean of four peak counts in each of the eight areas of interest was calculated. Counts/s per g of tissue were calculated from the calibration factor relating ECAT counts to well counter equivalents. Corrections for the partial volume effect were made using measurements of wall thickness taken from the dissected slice of myocardium. The wall thickness (in mm) in each region was used to select a systematic correction factor derived from published phantom studies. Because wall thickness is under the resolution of the detector this factor, with calibration, allows full count rate recovery from the tissue. The activity in each area was corrected for decay back to the end of the rubidium-82 infusion.

**Equilibrium scans:** The peak counts over the left ventricle were used to calculate counts/s per ml. These values were compared with the counts/s per ml obtained from the corresponding arterial blood samples taken simultaneously.

The arterial and myocardial concentrations were related as shown:

$$\frac{\text{Regional myocardial (counts/s per g)}}{\text{Arterial activity (counts/s per ml)}} \times 0.693/78 \times 60/1,$$

where 0.693/78 represents the turnover of rubidium-82 due to decay. This calculation provides a fraction representing the uptake of rubidium-82 in each region of the myocardium in relation to the delivered arterial activity presented to the heart, that is, a fraction for extraction  $\times$  flow/mg per min.

**Statistical analysis:** The six dogs studied provided the following comparisons between regional myocardial flow and cation uptake: (1) Control, six comparisons; (2) stenosis plus pacing, six comparisons; (3) stenosis plus rapid pacing, four comparisons; and (4) stenosis plus dipyridamole, four comparisons. Paired *t* tests and linear regression analysis were used to compare the following measurements: (1) arterial activity (counts/s per g) at equilibrium measured from ECAT

TABLE I

Hemodynamic Changes in Each Stage of the Dog Experiments

|                                                                    | Phase 1<br>Control  | Phase 2<br>Pacing 1                      | Phase 3<br>Pacing 2   | Phase 4<br>Dipyridamole                  |
|--------------------------------------------------------------------|---------------------|------------------------------------------|-----------------------|------------------------------------------|
| Heart rate<br>(beats/min)                                          | 120 $\pm$ 15.1      | 150 $\pm$ 10.0                           | 195 $\pm$ 24.0        | 124 $\pm$ 17.0                           |
| Mean aortic<br>pressure (mm Hg)                                    | 101 $\pm$ 7.0       | 97 $\pm$ 10.0                            | 93 $\pm$ 11.4         | 94 $\pm$ 12.3                            |
| Mean left atrial<br>pressure (mm Hg)                               | 5.5 $\pm$ 2.2       | 4.5 $\pm$ 2.6                            | 10.5 $\pm$ 6.0        | 7.6 $\pm$ 4.1                            |
| Electrocardiogram*                                                 | Normal in<br>6 dogs | Normal in<br>5 dogs and<br>positive in 1 | Positive in<br>4 dogs | Normal in<br>3 dogs and<br>positive in 1 |
| Number of<br>comparisons<br>between rubidium-82<br>uptake and flow | 6                   | 6                                        | 4                     | 4                                        |

\* A positive electrocardiographic response was S-T depression greater than 1 mm below the T-P segment at 20 ms after the end of the QRS complex.

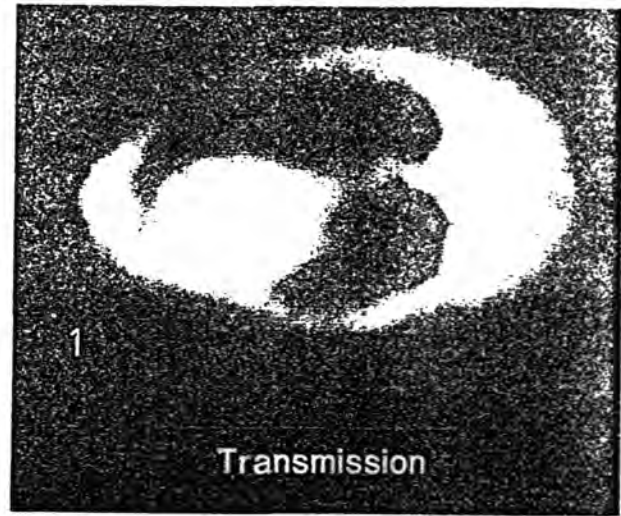
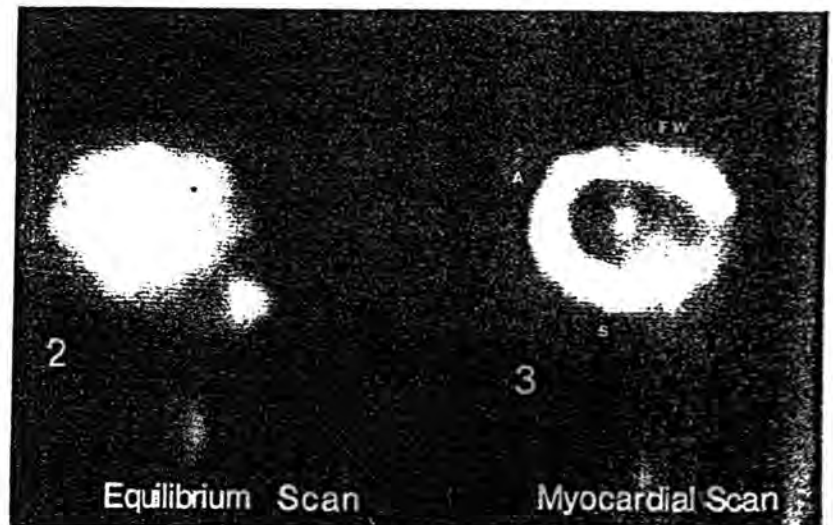


FIGURE 1. The transmission scan (1) shows the heart, lungs and chest within a transaxial slice of a dog. This is used to correct emission scans for tissue attenuation. The equilibrium scan (2) is recorded during rubidium-82 infusion and shows the activity in the ventricles and aorta which is used to calculate delivered concentration. The myocardial scan (3) records tissue activity in the free wall (FW) of the left ventricle and the interventricular septum(s). This tomogram was recorded at 30 to 150 seconds after cessation of the intravenous infusion of rubidium-82 and shows the free wall and apex (A) of the left ventricle and the interventricular septum. The earlier arterial concentration plus the myocardial concentration allows measurements of regional myocardial uptake of the cation.



and from the blood samples, and (2) the regional myocardial uptake of rubidium-82 and regional myocardial flow in each of the eight regions of interest in each phase of the experiments.

### Patient Studies

**Normal volunteers:** Five volunteers with no detectable evidence of heart disease were positioned in the ECAT camera and an 18 gauge needle was placed in an antecubital vein. The strontium-82-rubidium-82 generator and tubing were prepared using aseptic technique and the infusate was filtered (Millex, 0.22  $\mu$ , Millex SA). Transmission scans were recorded for later attenuation correction of emission data. Equilibrium scans were recorded (120 seconds) when one bank of detectors showed constant activity on a linear digital rate meter. Myocardial scans were recorded between 30 and 150 seconds after cessation of the infusion. Equilibrium and myocardial scans were performed with each subject at rest and again at 0, 10 and 20 minutes after a symptom-limited maximal exercise test performed supine using a bicycle ergometer.

**Patients with coronary artery disease:** Five patients (four male and one female, aged 36 to 65 years) were selected for study. These patients all had exertional angina at least

once daily (class II, New York Heart Association). All five patients had significant S-T segment depression and chest pain during a positive exercise test and the work load achieved ranged from 11,000 to 21,000 joules during the test. Four of the five patients had three vessel coronary artery disease and one had two vessel disease on angiocardiology. Each patient was positioned in the ECAT camera. Equilibrium and myocardial scans were recorded at rest as described for the healthy volunteers. Each patient then performed a symptom-limited graded exercise test with use of a bicycle ergometer and continuous electrocardiographic monitoring. Equilibrium and myocardial scans were performed at 0, 10 and 20 minutes after exercise.

**M mode echocardiography** was used to measure the thickness of the interventricular septum and the free wall of the left ventricle. These measures were used for partial volume correction of emission data.<sup>4</sup>

### Results

#### Animal Studies

During the six experiments the partial pressure of oxygen ranged between 90 and 110 mm Hg, the partial pressure of carbon dioxide between 30 and 46 mm Hg and the pH between 7.38 and 7.40. Table I shows the changes in heart rate, blood pressure, left atrial pressure and electrocardiogram during the four phases of the experiments in six dogs.

**Arterial blood radioactivity:** The activity detected in 10 ml/min of normal saline solution continuously eluted from the strontium-82-rubidium-82 generator showed that within 2.0 minutes an equilibrium of activity was achieved (at 5 to 15 mCi/min) and was maintained for 7 minutes (within  $\pm 3.0$  percent). Similarly, the activity detected in 30 ml/min of arterial blood during a peripheral intravenous infusion reached equilibrium within 4 minutes. During the next 6 minutes of infusion there was  $\pm 4.0$  percent variability and a small systematic decrease in activity ranging from 5 to 6 percent. When the peripheral intravenous infusion of rubidium-82 was stopped, the arterial activity began to decrease within 10 seconds and decreased below 25 percent of peak activity within 30 seconds in all the experiments.

**Equilibrium scans:** Figure 1 shows an example of the tomograms recorded during the constant infusion of rubidium-82. The heart to background ratio of activity was  $4.0 \pm 1.3:1$  (mean  $\pm$  deviation). The peak activity in the scan was calculated (counts/ml per s) and then used as the arterial concentration of rubidium-82 at equilibrium. There was a significant relation between the arterial activity measured by the camera and the simultaneous measurement of activity made using blood samples and well counting ( $Y = X0.85 + 828.0$ , correlation coefficient  $[r] = 0.90$ , probability  $[p] = <0.001$ ,  $n = 19$ ). This measure was made without partial volume effect because of the size of the cavity and of the heart.

**Myocardial scans:** Figure 2 shows an example of the myocardial scans recorded during control conditions as well as a sequence of images recorded from a single experiment showing the regional myocardial distribution of rubidium-82 in tomograms during the control period

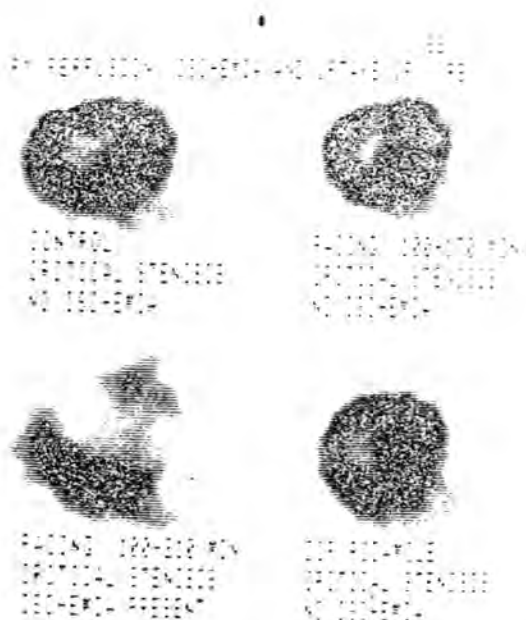
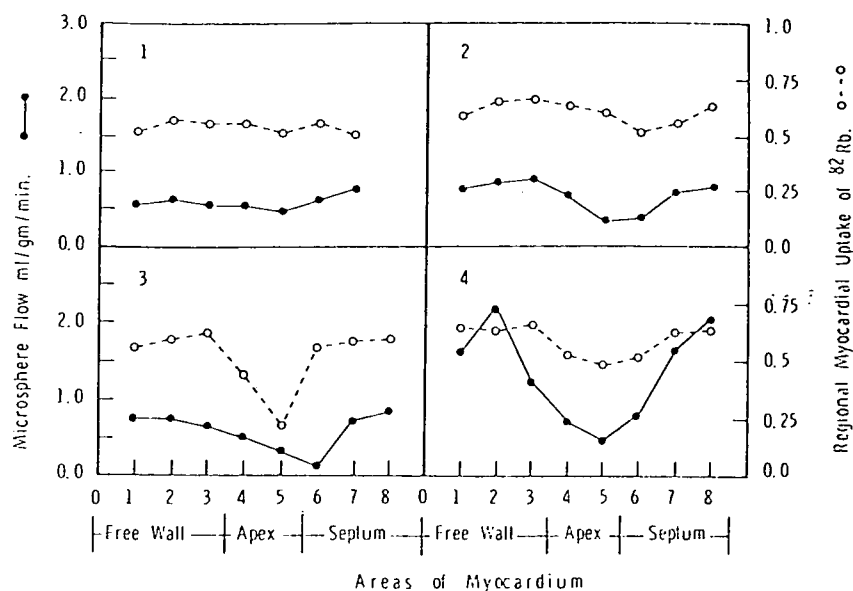


FIGURE 2. These serial tomograms were recorded during four phases of the same dog experiment shown in Figure 1. There was a uniform regional myocardial uptake of rubidium-82 during the control period with critical coronary stenosis but no stress (phase 1). Atrial pacing (116 to 150 beats/min) with no ischemia (phase 2) produced insignificant differences in the regional myocardial uptake of rubidium-82. Rapid atrial pacing (120 to 190 beats/min) with evidence of ischemia (phase 3) produced a decrease in regional myocardial uptake of rubidium-82 and microsphere flow. Although dipyridamole (phase 4) produced a 2.2:1 difference in regional myocardial blood flow between post-stenotic and remote areas there were no ischemia and no obvious regional differences in uptake of rubidium-82.

FIGURE 3. With critical stenosis and no stress or ischemia (phase 1) the regional myocardial distribution of rubidium-82 (upper graph) is similar to that of flow (lower graph). Relation between regional myocardial perfusion and uptake of rubidium-82. Critical stenosis with pacing but no evidence of ischemia (phase 2) produced regional inequalities of blood flow but no significant inequalities of regional myocardial uptake of rubidium-82. Critical stenosis with rapid pacing and ischemia (phase 3) produced significant changes in both regional myocardial uptake of rubidium-82 and flow. Critical stenosis and dipyridamole (no evidence of ischemia) (phase 4) produced large differences in regional myocardial blood flow with only a minor difference in the regional myocardial uptake of rubidium-82.



(with critical stenosis without stress), during critical stenosis and pacing, during critical stenosis with rapid pacing and during stenosis with dipyridamole. Figure 3 shows the regional myocardial uptake of rubidium-82 in each of the eight areas of interest at each stage of one experiment. This figure also shows the measurements of regional myocardial perfusion in the eight areas during each phase of the same experiment. Table II shows the changes in regional myocardial uptake of rubidium-82 and blood flow for all the experiments taken from a normal area (three segments selected independently by each observer as unaffected) and an affected area (two segments selected independently by each observer as being changed by the stenosis of the left anterior descending coronary artery and stress).

Figure 4 shows the relation between the regional myocardial uptake of rubidium-82 and regional myocardial blood flow in the normal and affected areas in the six dogs. This significant relation shows that in each experiment any given change in regional myocardial perfusion is accompanied by a smaller change in the regional myocardial uptake of rubidium-82.

#### Human Studies

Figure 5 shows examples of equilibrium and myocardial scans recorded from a healthy subject. The heart to background ratio of activity was  $3.5 \pm 0.4:1$ . Figure 6 shows a sequence of scans recorded before and after supine exercise in a healthy volunteer. This shows a homogeneous distribution of cation uptake throughout. During exercise the heart rate increased from a mean of 72/mm to 170/mm and the volunteers achieved a work load of 24,000 to 32,000 joules. There was homogeneous regional myocardial uptake of rubidium-82 before and after exercise in the five healthy subjects. The areas of interest were selected independently by the

observers as described for the experimental section. An analysis of variance showed a significant  $16 \pm 4.0$  percent increase in rubidium-82 uptake with no significant differences between regions (coefficient of variance =  $5.2 \pm 0.90$  percent). During exercise in the patients with coronary artery disease the mean heart rate increased

TABLE II

Values for the Regional Myocardial Uptake of Rubidium-82 and Flow With Critical Stenosis, Pacing, Fast Pacing and After Dipyridamole

|                                              | Phase 1<br>(control)          | Phase 2<br>(pacing 1)         | Phase 3<br>(pacing 2)           | Phase 4<br>(dipyridamole)       |
|----------------------------------------------|-------------------------------|-------------------------------|---------------------------------|---------------------------------|
| <b>Normal Area</b>                           |                               |                               |                                 |                                 |
| Tomogram                                     |                               |                               |                                 |                                 |
| Regional myocardial uptake of rubidium-82    | 0.54<br>$\pm 0.08$<br>(n = 6) | 0.62<br>$\pm 0.10$<br>(n = 4) | 0.64<br>$\pm 0.14$<br>(n = 4)   | 0.72<br>$\pm 0.12^*$<br>(n = 4) |
| Microspheres                                 |                               |                               |                                 |                                 |
| Regional myocardial perfusion (ml/g per min) | 0.89<br>$\pm 0.30$<br>(n = 6) | 1.20<br>$\pm 0.40$<br>(n = 4) | 0.99<br>$\pm 0.20$<br>(n = 4)   | 1.92<br>$\pm 0.46^*$<br>(n = 4) |
| <b>Affected Area</b>                         |                               |                               |                                 |                                 |
| Tomogram                                     |                               |                               |                                 |                                 |
| Regional myocardial uptake of rubidium-82    | 0.59<br>$\pm 0.10$<br>(n = 6) | 0.52<br>$\pm 0.07$<br>(n = 4) | 0.37<br>$\pm 0.11^*$<br>(n = 4) | 0.66<br>$\pm 0.21$<br>(n = 4)   |
| Microspheres                                 |                               |                               |                                 |                                 |
| Regional myocardial perfusion (ml/g per min) | 0.88<br>$\pm 0.32$<br>(n = 6) | 0.85<br>$\pm 0.31$<br>(n = 4) | 0.39<br>$\pm 0.13^*$<br>(n = 4) | 0.75<br>$\pm 0.30$<br>(n = 4)   |

\* Value significantly different ( $p < 0.01$ ) from the value recorded during phase 1 (control).

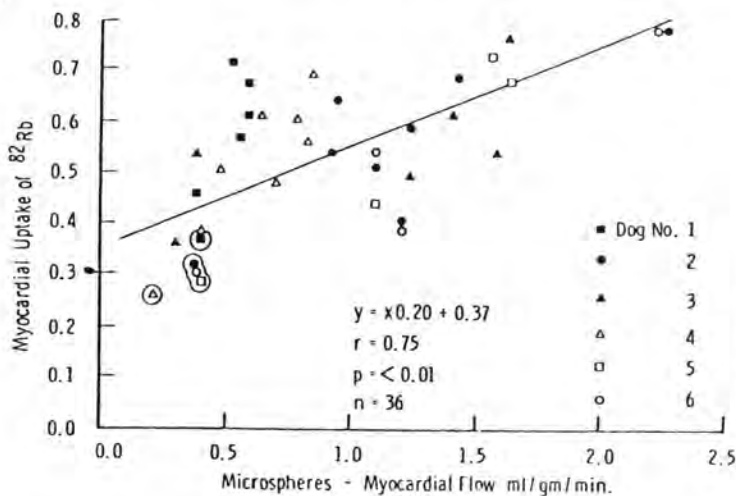


FIGURE 4. In six dogs the relation between the regional myocardial uptake of rubidium-82 and flow shows a limited smaller change in tracer uptake for any change in flow with wide individual variability. The significant reductions in regional myocardial uptake of rubidium with defects in tomograms occurred only when a regional myocardial reduction of flow was accompanied by evidence of ischemia (symbols enclosed by circles).

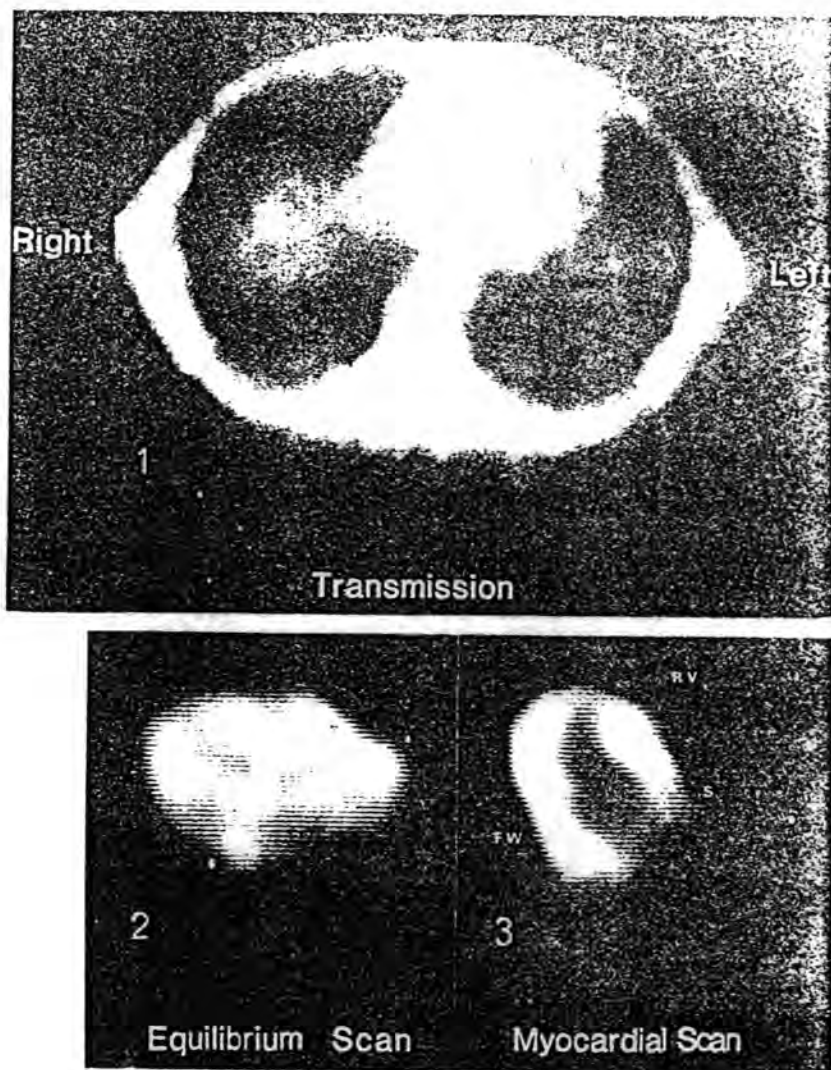


FIGURE 5. Patient studies. The transmission scan (1) shows the heart, lungs and chest wall in a mid left ventricular and transthoracic slice. The equilibrium scan (2) during rubidium-82 infusion provides the arterial concentration. At 30 to 150 seconds after infusion the myocardial scan (3) shows the free wall (FW) of left ventricle and septum (S). The free wall of the right ventricle (RV) is faint but can be seen.



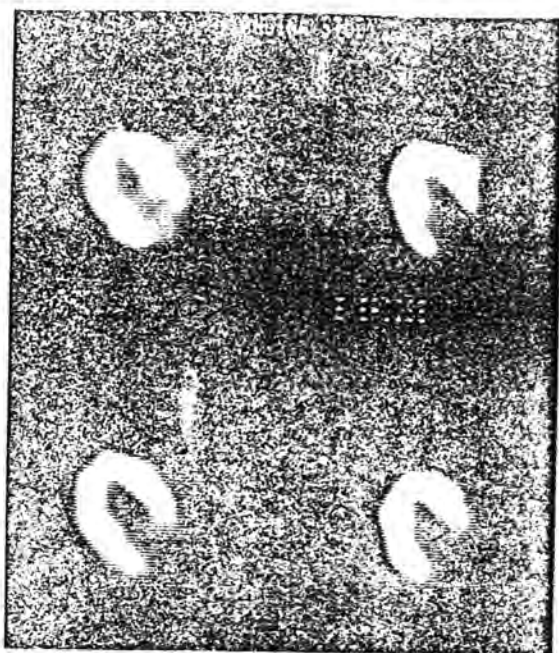


FIGURE 6. Serial tomograms were recorded before (upper left) and after (upper right) exercise in a healthy volunteer. Exercise was associated with a small ( $16 \pm 4.0$  percent) but significant increase in the regional myocardial uptake of rubidium-82. No change in distribution of rubidium-82 occurred except for the region of the mitral orifice which is particularly affected by any changes in geometry. The lower scans (post) were delayed scans after exercise.

from 78 to 141 beats/min and all five patients complained of angina pectoris, which limited the exercise. They achieved a work load of 11,000 to 21,000 joules during the test. All the patients showed an obvious regional abnormality of rubidium-82 uptake in the tomograms recorded after exercise.

Figure 7 shows a sequence of scans recorded from a representative patient. The tomograms recorded after exercise showed an obvious decrease in regional myocardial uptake of rubidium-82. The regional myocardial uptake of rubidium-82 in the affected segment decreased from  $0.56 \pm 0.07$  to  $0.32 \pm 0.10$  fractional uptake of rubidium-82. This regional decrease in uptake of rubidium-82 lessened; however, at 20 minutes after exercise there was still a significant regional decrease at  $0.47 \pm 0.04$  ( $p < 0.01$ ). The calculated radiation dose to each patient was less than 1 rad and no complications were encountered.

#### Discussion

Radionuclide techniques using cations (thallium-201, for example) have found clinical application in detection of transient regional myocardial ischemia and coronary artery disease. However, the inability of these techniques to quantify events in each region of the myocardium and ambiguities about the effects of flow and metabolism have hindered interpretation of the results.<sup>1,2,6</sup> Our experiments show that the delivered ar-

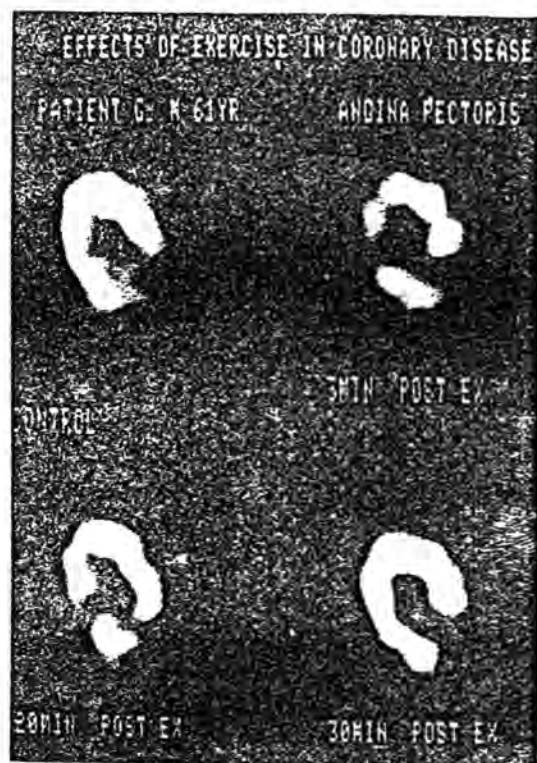


FIGURE 7. Serial tomograms recorded from a patient with angina pectoris. Recovery from exercise (3, 28 and 38 min post exercise [EX]) was associated with a severe regional defect in uptake of rubidium-82 compared to control. The defect in this cation scan occurred not because of increased uptake in unaffected areas but because of an absolute decrease in cation uptake in the affected area.

terial concentration of rubidium-82 can be measured in tomograms during intravenous infusion. Thirty to 150 seconds after infusion it is possible to record tomograms of the regional myocardial concentration of rubidium-82. These tomograms are of adequate physical quality even though the arterial concentration is approximately 5 to 20 percent of peak activity at this time. The blood pool represents 5 to 15 percent of the volume of the myocardium within the tomogram. The contamination of the myocardial activity by arterial activity (less than 20 percent of peak activity in 5 to 15 percent of the tomogram) is not enough to interfere with the adequate physical quality of the data. This is supported by the high myocardium to background ratio of activity.

**Relation between myocardial flow and uptake and extraction of rubidium-82:** Our experiments have confirmed in vivo the inverse relation between myocardial flow and extraction of rubidium-82. Although the initial distribution of the cation occurs through flow, the camera detects the mass of intramyocardial tracer. Therefore flow, residence time, extracted fraction, fractional escape and metabolism are all important variables that affect the information.<sup>7-12</sup> Any alterations in flow were accompanied by much smaller changes in



uptake of rubidium-82. The relations among flow, extraction and uptake also showed that changes in uptake of rubidium-82 will not clearly indicate increases in flow unless these are extreme (more than 120 percent). Increases in flow were produced by different mechanisms using atrial pacing and dipyridamole. The different physiologic effects may in themselves affect cation uptake; however, in these experiments the removal of pacing or dipyridamole data does not change the relation between flow and uptake. Similarly, decreases in myocardial perfusion between 35 and 50 percent (without evidence of ischemia) are accompanied by only small decreases in uptake of rubidium-82. When decreases in regional myocardial perfusion were accompanied by evidence of ischemia the uptake of rubidium-82 was severely and absolutely decreased below the control level. Although the relation between uptake of rubidium-82 and flow is weak and variable the technique appears to respond and consistently indicates the presence of acute transient regional myocardial ischemia. The steady-state administration of trace quantities of rubidium-82 produces no isotope effect and the uptake into the myocardium is governed by the same physiology that determines uptake after a bolus injection (that is, flow, extraction, myocardial pool).

**Tomographic detection of rubidium-82:** The use of rubidium-82 carries the advantages of the 511 keV coincidence photons, tomographic detection, delivered arterial concentration and an absolute measure of regional myocardial uptake of the cation. The short half-life ( $t_{1/2} = 78$  seconds) allows serial studies in the same subject. All of these features permit measurement and interpretation of directional changes in events in each region of the myocardium.

The signal is a mean of events over 2 minutes and requires expensive equipment. In all forms of external nuclear imaging the detector loses sensitivity as the object decreases in size (the partial volume effect).<sup>4</sup> The importance of object size in recovery of activity from the heart is shown in these experiments. The measurement of wall thickness in the dog experiments and echocardiographic studies in the patients are both imperfect but necessary attempts to correct for partial volume effects when trying to quantitate events in the myocardium.

**Patients with angina pectoris:** The technique in patients has provided information about the regional myocardial uptake of rubidium-82 in tomograms of the heart. Repeatable studies using each patient as his or her own control were possible because of the short half-life of the radionuclide. Spatial resolution, reproducibility and homogeneous cation uptake are shown in the normal subjects. The work performed during exercise probably produced marked increases in myocardial perfusion. However, these were accompanied by

a small ( $16.0 \pm 4.0$  percent) increase in uptake of rubidium-82. This result is similar to and supports the experimental findings. The patients all had angina pectoris, a positive electrocardiographic exercise test and coronary artery disease; they all showed regional myocardial defects in the tomograms after exercise. Measurements of regional myocardial uptake of tracer showed that these defects did not occur because of an increase in tracer uptake in normal areas. All the tomograms after exercise showed one region with an absolute decrease in cation uptake. These abnormal changes recovered slowly and returned to control level only after 20 minutes in four of the patients. The dog experiments suggest that these absolute decreases in regional myocardial cation uptake are likely to be due to a significant reduction in regional myocardial perfusion and ischemia. Nevertheless, the scans record the regional myocardial uptake of the cation and cannot dissect out the separate effects of flow and metabolism. The serial scans showed that the regional myocardial disturbance lasted longer than the symptoms and electrocardiographic signs of ischemia.

**Clinical implications:** Patients with coronary artery disease are thought to have transient disturbances of regional myocardial perfusion and metabolism. The resultant regional ischemia is probably an important cause of morbidity and mortality. The detection of transient ischemia and its causes is an important goal, and a variety of techniques are available.<sup>13,14</sup>

The strontium-82 rubidium-82 generator is portable and provides safe quantities of rubidium-82 for peripheral intravenous infusion. With positron tomography the arterial and myocardial activities can be measured for calculation of absolute regional myocardial uptake of rubidium-82 in tomograms of adequate physical quality. The short half-life of the tracer permits serial studies to assess changing pathophysiology. Although the relation between myocardial uptake of rubidium-82 and flow is weak the technique is suitable for detection of acute transient regional myocardial ischemia. The strontium-82-rubidium-82 generator can be used without a cyclotron. This means that the advantages of positron tomography can be used clinically without the expense of an on-site cyclotron.

Our preliminary results in patients with angina pectoris have shown that abnormalities (that is, defects) appear in cation scans not because of increased tracer uptake in normal areas but because of an absolute decrease in uptake in an affected area of myocardium. These ischemic lesions persist for much longer than pain or the electrocardiogram suggests. This information may help to characterize and elucidate acute myocardial ischemia in patients with angina and coronary artery disease.

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## THERMAL CHARACTERISTICS OF THE RELEASE OF FLUORINE-18 FROM AN INCONEL-600 GAS TARGET

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Several publications dealing with no carrier added syntheses of radiopharmaceuticals containing fluorine have made only brief mention of the methods by which the "anhydrous no-carrier-added HF-18" was prepared(1,2). A patent(3) refers to the recovery of F-18 in an unspecified chemical form after deuteron irradiation of a neon-filled nickel target is followed by a hydrogen purge whilst heating to 900 °C. Inconel(4), Monel(5) and copper(6) have also been proposed as constructional materials for neon targets to be operated in the anhydrous no-carrier-added mode. The target (internal dimensions 162x50mm) used in this study was fabricated from Inconel-600 using the tungsten-arc inert gas welding process (T.I.G.) and Inconel filler metal 82(7). The walls were polished using silicon carbide paper and water as a lubricant. Heating was achieved using a 1.25KW ceramic knuckle band heater on the target barrel and a 0.35KW annular ring heater on the window flange. With the ceramic fibre lagging (as shown in Fig.1) conditions of minimal temperature gradients were demonstrated on the inner surface of the target by thermocouple scanning. A 25mm diameter 0.050mm thick Inconel-625 entry window was sealed to the target vessel using a 3mm square section soft copper ring. Before filling with target gas mixtures the target was treated in two alternative ways.

a) Target heated to 500 °C under a neon flow of 100ml/min and allowed to cool isobarically.

b) Target heated to 500 °C under a hydrogen flow of 100ml/min and allowed to cool isobarically.

Hydrogen concentrations were varied between 10 and 20 volumes % in neon and target filling pressures were typically 200psig. Irradiations were carried out with 16MeV deuterons for approximately 1μamp.h. at 2 to 3μamp. After irradiation the target was removed from the cyclotron to a hot cell where the target gas was released through a potassium carbonate trap with no observed loss of F-18. Valve V4 Fig.1 was removed and a 1/8 inch PTFE tube together with a PTFE coil trap were connected to the target outlet. Dry oxygen-free hydrogen was then passed through the target at a flow rate of 250ml/min whilst the target was heated to a preselected maximum temperature of 700 °C. The PTFE outlet connection was held at below 150 °C by forced air cooling and the PTFE coil cooled to -78 °C. As shown in Fig.2 the release of F-18 starts between 80 °C and 140 °C when the target was pretreated with hydrogen and between 200 °C and 400 °C when the target was pretreated with neon. The recovery profile also strongly depends on the neon to hydrogen ratio in the irradiated mixture; the starting temperature decreases with increasing amounts of hydrogen. This is possibly due to direct formation of HF-18 during the irradiation. The decrease in the recovery temperature observed after hydrogen pretreatment is probably the result of HF-18 formation at the hydrogen-saturated Inconel wall. Another important observation, which may lead to a better understanding of the chemical processes and kinetics of F-18 removal from a target wall, refers to the situation of low hydrogen concentration in the target gas and the pretreatment with neon (run 5 Fig.2b). Here temperatures in excess of 500 °C were reached before any F-18 was recovered. Thus the temperature must be related to the activation temperature for the thermal desorption of the fluorine atoms from the target wall; these reactive fluorine atoms then combine with the hydrogen purge gas to form HF-18.

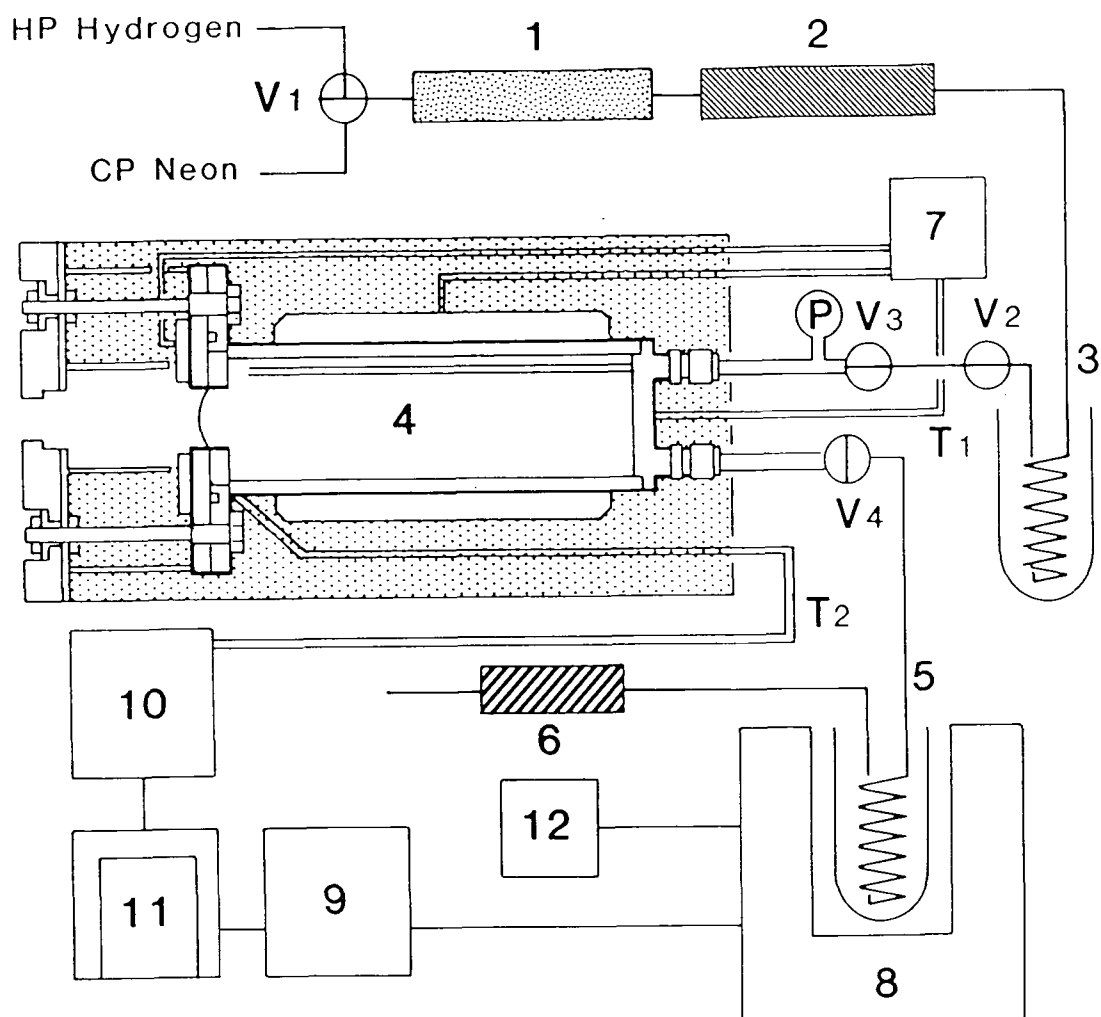


Figure 1

(1) Palladium catalyst (Engelhard "Deoxo Model-D") 150x25mm. (2) Magnesium perchlorate 150x25mm. (3) Copper trap -196°C. (4) Inconel target vessel with heaters and thermocouples T1 and T2 for temperature control and monitoring. (5) PTFE trap 400x3mm OD coil -78°C. (6) Potassium carbonate trap (for checks on the efficiency of trap 5). (7) Temperature controller. (8) Shielded re-entrant ionisation chamber. (9) DC Amplifier. (10) Thermocouple conditioning amplifier. (11) Dual channel recorder. (12) Ionisation chamber polarising supply.

These preliminary results from extraction studies of F-18 from the target system described could be interpreted in terms of model calculations for heterogenous fluorine atom reactions on metal surfaces(8). Further problems relating to the kinetics of adsorption and desorption processes of fluorine atoms highly diluted in an inert medium are presently under investigation at The German Cancer Research Center using a model based on the Langmuir-Rideal mechanism(9). This model agrees very well with our observations on the removal of F-18 from the target walls. We suggest that the following reactions give rise to observations described here.

- (I)  $F-18 + HH = HF-18 + H$ .  
 (II) ADSORBED F-18 + DISSOLVED HH = DESORBED HF-18.  
 (III) ADSORBED F-18 + (T>400 C) = DESORBED F-18 + HH = HF-18 + H.

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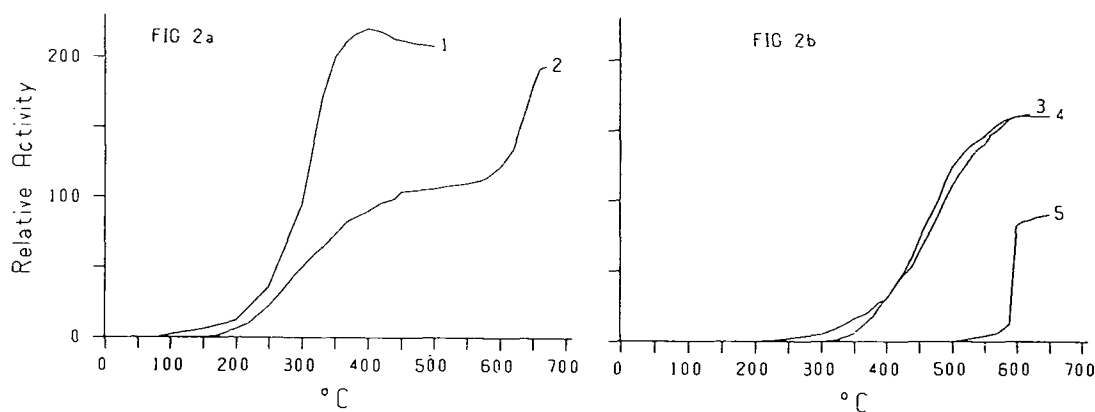


Figure 2a. Target pretreated with hydrogen at 500°C. Runs 1 and 2 15 volumes % hydrogen in neon.

Figure 2b. Target pretreated with neon at 500°C. Runs 3 and 4 15 volumes % hydrogen in neon. Run 5 <10 volumes % hydrogen in neon.

# Rate of uptake of carbon monoxide at different inspired concentrations in humans

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JONES, HAZEL A., J. C. CLARK, E. E. DAVIES, R. E. FORSTER, AND J. M. B. HUGHES. *Rate of uptake of carbon monoxide at different inspired concentrations in humans.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 52(1): 109–113, 1982.—The rate of uptake of carbon monoxide (CO) in the lungs of normal subjects was measured at inspired concentrations of <1, 300, and 3,000 ppm (<0.0001–0.3%) using radioactive CO ( $^{11}\text{CO}$ ). In nine subjects the rate of uptake was monitored at the mouth during rebreathing. At inspired CO concentrations of approximately 1, 300, and 3,000 ppm and a mean alveolar  $\text{O}_2$  fraction of 0.15, the mean lung diffusing capacity was 25.8, 26.4, and 25.3  $\text{ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1}$ , respectively. In seven subjects the measurements were repeated after a period of  $\text{O}_2$  breathing, giving a mean alveolar  $\text{O}_2$  fraction of 0.78. The calculated membrane diffusing capacity was 31.9, 33.7, and 32.0  $\text{ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1}$  at <1, 300, and 3,000 ppm inspired CO. We conclude that there is no difference in the rate of uptake of CO over the range of concentrations studied in these experiments. No evidence for the presence of a facilitated transport system for CO in the normal human lung was found.

lung diffusing capacity for CO; membrane diffusion for CO; facilitated transport; radioactive CO; rebreathing techniques

CARBON MONOXIDE (CO) is generally considered to diffuse across the pulmonary membrane between alveolar gas and capillary blood and across the placental membrane between maternal and fetal capillary blood at a rate proportional to the difference in partial pressure of CO. However, variations in the proportionality constant (the diffusing capacity) have led various authors to propose facilitated transport of CO in the placenta of sheep and dogs (1, 5, 7). Following Longmuir et al. (9), Gurtner and Burns (6) have suggested that cytochrome  $\text{P}_{450}$  may act as a carrier for  $\text{O}_2$  and CO in the placenta.

A similar mechanism has been proposed for the lung (2, 12) and evidence for the presence of such a system in dogs and sheep has been put forward on the basis of a differential rate of uptake of CO with varying inspired concentrations. If the transfer of CO is purely passive, the rate of uptake will be independent of initial concentration, but if the transfer is facilitated by any means, for example by a cytochrome carrier, the rate of uptake will be higher at low concentrations than at concentrations high enough to saturate the carrier mechanism (9).

Recently, confirmation of the claim that pulmonary

diffusing capacity varies systematically with the inspired concentration of CO has been sought using isotopic techniques (8, 13). Meyer et al. (13) used the stable isotopes,  $^{12}\text{C}^{18}\text{O}$  and  $^{13}\text{C}^{18}\text{O}$  and a mass spectrometer, and we used the radioactive isotopes,  $^{11}\text{C}^{16}\text{O}$  and  $^{12}\text{C}^{15}\text{O}$ . In neither study was any dependence on inspired concentration found. In this paper, we report a further series of measurements in which the membrane diffusing capacity for CO has been measured in addition to the pulmonary diffusing capacity, over a 3,000-fold range of CO concentration.

## METHODS

The rate of uptake of CO was studied by a rebreathing method in nine normal male subjects, whose anthropometric data are shown in Table 1. All gave informed consent; the maximum radiation dose to each subject (7 mrad) had been approved by the Health Services Division of the Department of Health and Social Security. We were able to make measurements at very low concentrations, less than 1 ppm by the use of  $^{11}\text{C}$ -labeled CO a positron ( $\beta^+$ ) emitting isotope of CO with a half-life of 20 min. The  $^{11}\text{CO}$  was produced in the Medical Research Council cyclotron, London, using an  $\text{N}_2$  target (3). The  $^{11}\text{CO}$  was free from stable CO and had no radioactive contaminants. The  $^{11}\text{CO}$  was dispensed into a syringe in stable  $\text{N}_2$ . The concentration at this low level was measured as less than 1 ppm using an Ecolyser CO analyzer (Energetics Science). Monitoring was carried out on a gas radiochromatograph to ensure that no radioactive contaminants were present.

The  $\beta^+$  emissions from this isotope were detected using the apparatus shown in Fig. 1. This detector consists of a plastic scintillator  $2 \times 2 \times 0.4 \text{ cm}^3$  in a thin light-tight stainless steel envelope that projects into the rebreathing apparatus at the same point as the tip of a sampling line from a mass spectrometer (Centronics MGA 200). The plastic scintillator emits light when bombarded by positrons. The light is converted into an electronic signal and amplified in a photomultiplier tube. Counts proportional to the amount of  $^{11}\text{CO}$  present are relayed to a computer (Digico  $\mu 16$ ) simultaneously with signals of flow and volume. The response of the detector was linear well beyond ranges of count rates achieved. These signals are all delayed to synchronize with signals coming from the

mass spectrometer, which lag the other signals due to transit time down the sample line. The rebreathing apparatus is shown in Fig. 2. This consists of a 1-liter bag in a bottle connected via a pneumotachograph to a wet spirometer for the measurement of flow and volume. The test gas consisted of 10% He, 10% SF<sub>6</sub>, and 30% O<sub>2</sub> in Ar with either no CO, 0.03% CO, or 0.3% CO. The <sup>11</sup>CO at a concentration of <1 ppm was added to all these mixtures to give total CO concentrations of <1, 300, and 3,000 ppm. The bag was filled with 0.65 liter of one of the test gas mixtures. The subject was switched into the rebreathing system at end expiration and asked to empty and fill the bag completely 15 times at a rate of 1 breath/s, set by a metronome. During the rebreathing maneuver the concentrations of all gases, N<sub>2</sub>, O<sub>2</sub>, Ar, CO<sub>2</sub>, He, and SF<sub>6</sub> measured in the mass spectrometer and <sup>11</sup>CO monitored by the β<sup>+</sup>-detector were sampled 50 times/s together with signals of flow and volume. The computer printed out the time and volume of each breath, followed by the flow-weighted mean concentrations of all the gases for each inspiration. Paired measurements were made at inspired CO concentrations of <1, 300, and 3,000 ppm in random order. In seven subjects three additional measurements, one at each inspired concentration, were made after an initial period of breathing 100% O<sub>2</sub> for 5 min.

TABLE 1. Anthropometric data for the subjects

| Subj | Age, yr | Ht, m | Wt, kg | VC, liters (BTPS) | FEV <sub>1</sub> , liters (BTPS) | Smoking History |
|------|---------|-------|--------|-------------------|----------------------------------|-----------------|
| SC   | 33      | 1.68  | 74     | 4.3               | 3.7                              | Exsmoker        |
| MH   | 44      | 1.69  | 63.5   | 5.0               | 3.9                              | Nonsmoker       |
| AS   | 30      | 1.85  | 82     | 5.6               | 5.0                              | Smoker          |
| RC   | 23      | 1.83  | 68     | 5.5               | 4.3                              | Nonsmoker       |
| MT   | 30      | 1.81  | 75     | 5.8               | 4.6                              | Nonsmoker       |
| LN   | 29      | 1.63  | 57.6   | 3.9               | 3.4                              | Pipe smoker     |
| ED   | 44      | 1.77  | 85     | 4.6               | 3.3                              | Pipe smoker     |
| EE   | 40      | 1.85  | 75     | 6.3               | 5.4                              | Nonsmoker       |
| RA   | 31      | 1.73  | 71     | 4.6               | 3.5                              | Nonsmoker       |

VC, vital capacity; FEV<sub>1</sub>, forced expired volume at 1 s.

The rate of uptake of CO ( $K_{CO}$ ) was calculated as the exponential slope of the fall in radioactivity in the gas sampled at the mouth once the initial mixing phase had passed, as shown by the equilibration of the He and SF<sub>6</sub>. The diffusing capacity ( $DL_{CO}$ ) in ml·min<sup>-1</sup>·Torr<sup>-1</sup> was calculated as  $(K_{CO} \times V_s \times 60 \times 0.826)/713$ , where  $K_{CO}$  is the rate constant for CO uptake (s<sup>-1</sup>),  $V_s$  the system volume, i.e., the end-expiratory volume plus the bag volume in ml (BTPS) calculated from insoluble gas dilu-

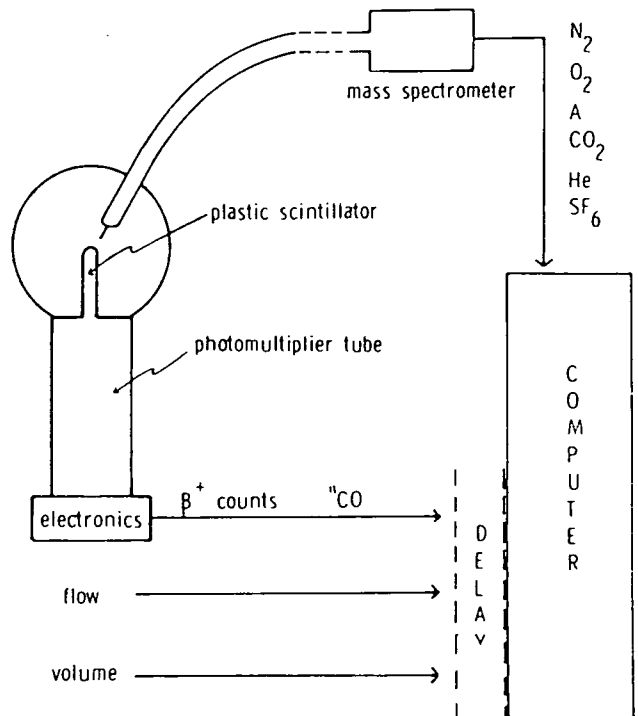


FIG. 1. Diagram of detector used for monitoring <sup>11</sup>CO, showing its position relative to other detection systems and computer. Circle represents cross section of tube (2.5 cm ID) connecting mouthpiece to rebreathing bag.

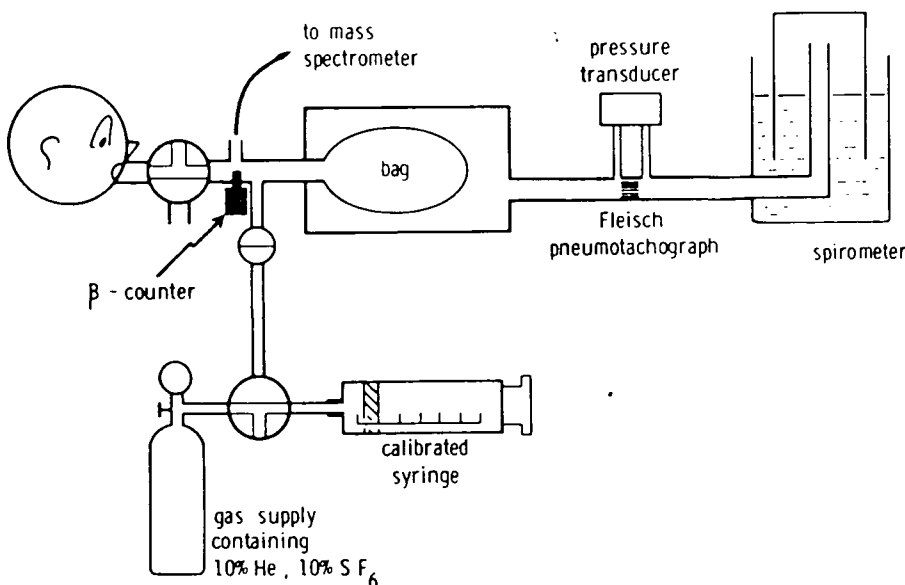


FIG. 2. Diagram of whole rebreathing apparatus.

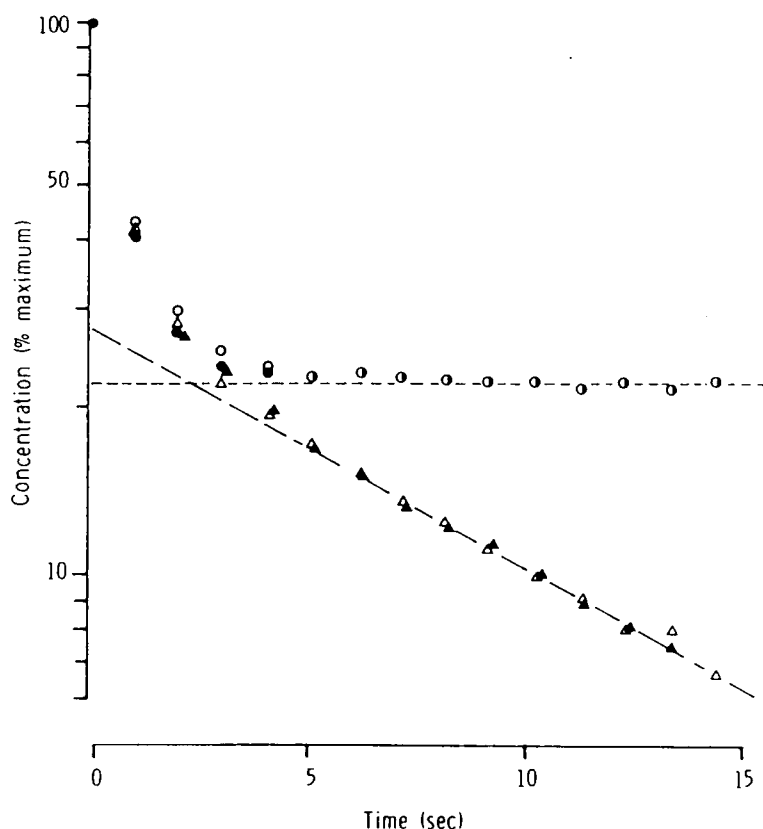


FIG. 3. Semilogarithmic plot of breath-by-breath He (●), SF<sub>6</sub> (○), and <sup>11</sup>CO (Δ) concentrations (as percent of maximum) against time, in subject SC, rebreathing a high (3,000 ppm) CO mixture. <sup>11</sup>CO values at low concentration (▲) have been added for comparison. Insoluble gas dilution was identical for both measurements.

tion, 60 the conversion from s to min, 0.826 the conversion from BTPS to STPD, and 713 the barometric pressure minus water vapor pressure.

The membrane diffusing capacity (D<sub>m</sub>) and the capillary blood volume (V<sub>c</sub>) were calculated graphically from the relationship described by Roughton and Forster (14)

$$\frac{1}{DL} = \frac{1}{D_m} + \frac{1}{\theta V_c}$$

$\theta$ , the rate of reaction of CO with hemoglobin, was calculated from the mean alveolar O<sub>2</sub> concentration during the post-mixing part of the rebreath, measured by the mass spectrometer.

$$\frac{1}{\theta} = (\alpha + \beta \cdot P\bar{c}_{O_2}) / [Hb] \cdot (1 - S\bar{c}_{CO})$$

We followed Cotes (4) in using values of 0.34 and 0.006 for the coefficients  $\alpha$  and  $\beta$ .  $P\bar{c}_{O_2}$  is the mean partial pressure of O<sub>2</sub> in the plasma of the alveolar capillaries, [Hb] is the hemoglobin concentration as a fraction of normal, and  $S\bar{c}_{CO}$  the mean fractional saturation of hemoglobin with CO (11).

The backpressure of <sup>11</sup>CO was measured by repeating the rebreathing maneuver with a bag filled with air immediately after the final measurement.

## RESULTS

Figure 3 shows data obtained in one subject (SC) rebreathing high and low concentrations of CO after air breathing. After five breaths mixing has been established

TABLE 2. *V<sub>s</sub>, K<sub>CO</sub>, and DL<sub>CO</sub> for all subjects at high, medium, and low inspired CO concentrations*

| Subj | Approximate Inspired Concentration, ppm |      |      |                                     |       |       |                                                             |       |       |
|------|-----------------------------------------|------|------|-------------------------------------|-------|-------|-------------------------------------------------------------|-------|-------|
|      | 3,000                                   | 300  | <1   | 3,000                               | 300   | <1    | 3,000                                                       | 300   | <1    |
|      | V <sub>s</sub> (BTPS), liters           |      |      | K <sub>CO</sub> , %·s <sup>-1</sup> |       |       | DL <sub>CO</sub> , ml·min <sup>-1</sup> ·Torr <sup>-1</sup> |       |       |
| SC   | 2.99                                    | 2.94 | 2.99 | 10.12                               | 10.05 | 10.18 | 20.92                                                       | 20.53 | 21.13 |
| MH   | 3.95                                    | 4.10 | 4.19 | 8.94                                | 8.67  | 8.76  | 24.52                                                       | 24.75 | 25.13 |
| AS   | 4.64                                    | 4.85 | 4.92 | 8.42                                | 8.72  | 8.52  | 27.21                                                       | 29.41 | 29.11 |
| RC   | 4.26                                    | 4.14 | 4.36 | 10.77                               | 11.88 | 11.65 | 31.88                                                       | 35.10 | 35.07 |
| MT   | 4.43                                    | 4.47 | 4.45 | 9.91                                | 11.05 | 8.61  | 30.47                                                       | 34.20 | 26.55 |
| LN   | 3.64                                    | 3.68 | 3.41 | 7.43                                | 7.21  | 7.49  | 18.66                                                       | 18.28 | 17.77 |
| ED   | 4.11                                    | 3.55 | 3.82 | 8.07                                | 8.66  | 7.04  | 23.07                                                       | 21.40 | 18.28 |
| EE   | 5.68                                    | 6.39 | 6.23 | 7.69                                | 6.73  | 6.84  | 30.29                                                       | 29.77 | 29.55 |
| RA   | 3.31                                    | 3.61 | 3.49 | 11.02                               | 9.49  | 10.03 | 25.18                                                       | 23.80 | 24.17 |
| Mean | 4.11                                    | 4.20 | 4.21 | 9.15                                | 9.16  | 8.79  | 25.80                                                       | 26.36 | 25.22 |

Values are means of 2 measurements. Mean alveolar O<sub>2</sub> fraction is 0.15. V<sub>s</sub>, system volume i.e., bag plus end-expiratory lung volume; K<sub>CO</sub>, rate constant for CO uptake; DL<sub>CO</sub>, pulmonary diffusing capacity.

as shown by the constant He and SF<sub>6</sub> concentrations; the uptake of CO is monoexponential after this point. For all subjects the regression coefficients of the 75 calculated slopes were never less than 0.687 with a mean of 0.959 ± 0.062 (SD). The accumulated data for all the studies is shown in Table 2. Each number is the mean of two measurements made at each inspired concentration. A two-tailed paired *t* test of significance was carried out on the individual values between the high and medium concentrations and the high and low concentrations, yielding *P* values of 0.51 and 0.55, respectively. For all



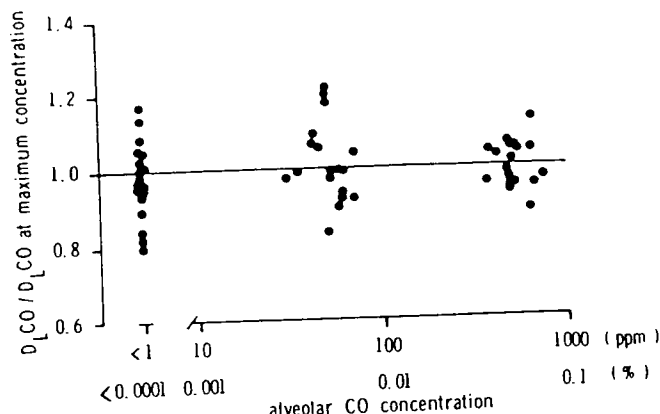


FIG. 4. Lung diffusing capacity for CO ( $DL_{CO}$ ) at different alveolar concentrations. Each point represents a single measurement, and all are plotted as fraction of mean of two measurements at highest inspired concentration. No concentration-linked difference is apparent.

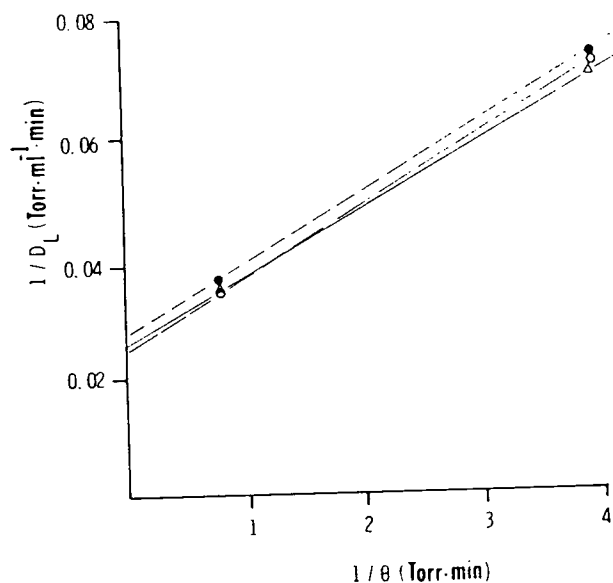


FIG. 5. Graphic analysis of  $D_m$  and  $V_c$ .  $1/D_L$  is plotted against  $1/\theta$  for high (●), medium (○), and low (Δ) inspired concentrations. High values for  $1/\theta$  were obtained by  $O_2$  breathing. Intercept on y-axis represents  $1/D_m$  and slope of lines  $1/V_c$ .

subjects the individual values for  $DL_{CO}$  are plotted against the calculated initial alveolar concentration for CO (Fig. 4). The points are all shown as a fraction of the mean  $DL_{CO}$  at the highest concentration. Thus values greater than 1.0 would indicate an increased CO uptake. Despite the expanded scale there appears to be no such increase at the intermediate or low levels.

Figure 5 shows the graphical analysis for  $D_m$  and  $V_c$  on one subject (AS);  $1/D_m$  obtained from the intercepts on the y-axis did not vary with concentration, nor did  $1/V_c$  obtained from the slopes of the lines. The results for  $D_m$  and  $V_c$  are shown in Table 3. A two-tailed paired  $t$  test of significance gave  $P$  values of 0.25 for  $D_m$  between high and medium and 0.95 between high and low concentrations. The individual results for  $D_m$  at differing alveolar CO concentrations are shown in Fig. 6, each point

TABLE 3.  $DL_{CO}$ ,  $D_m$ , and  $V_c$  for 7 subjects at high, medium, and low CO concentrations

| Subj | Approximate Inspired Concentrations, ppm             |      |      |                                                  |      |      |            |     |     |
|------|------------------------------------------------------|------|------|--------------------------------------------------|------|------|------------|-----|-----|
|      | 3,000                                                |      |      | 300                                              |      |      | <1         |     |     |
|      | $DL_{CO}$ , ml·min <sup>-1</sup> ·Torr <sup>-1</sup> |      |      | $D_m$ , ml·min <sup>-1</sup> ·Torr <sup>-1</sup> |      |      | $V_c$ , ml |     |     |
| SC   | 12.2                                                 | 12.2 | 12.1 | 25.8                                             | 24.6 | 21.7 | 85         | 98  | 98  |
| MH   | 13.2                                                 | 13.0 | 11.9 | 30.7                                             | 32.2 | 34.8 | 90         | 83  | 72  |
| AS   | 13.7                                                 | 14.0 | 14.4 | 37.1                                             | 40.3 | 39.1 | 87         | 88  | 91  |
| RC   | 14.0                                                 | 17.2 | 18.6 | 45.2                                             | 44.6 | 44.6 | 80         | 111 | 125 |
| MT   | 17.9                                                 | 16.6 | 18.2 | 35.9                                             | 45.2 | 40.3 | 148        | 103 | 140 |
| LN   | 10.1                                                 | 8.5  | 9.4  | 22.9                                             | 25.5 | 22.9 | 74         | 49  | 61  |
| ED   | 16.2                                                 | 15.6 | 14.2 | 25.8                                             | 23.8 | 20.6 | 171        | 174 | 170 |
| Mean | 13.9                                                 | 13.9 | 14.1 | 31.9                                             | 33.7 | 32.0 | 105        | 101 | 109 |

Mean alveolar  $O_2$  fraction is 0.78.  $DL_{CO}$ , pulmonary diffusing capacity;  $D_m$ , membrane diffusing capacity;  $V_c$ , capillary blood volume.

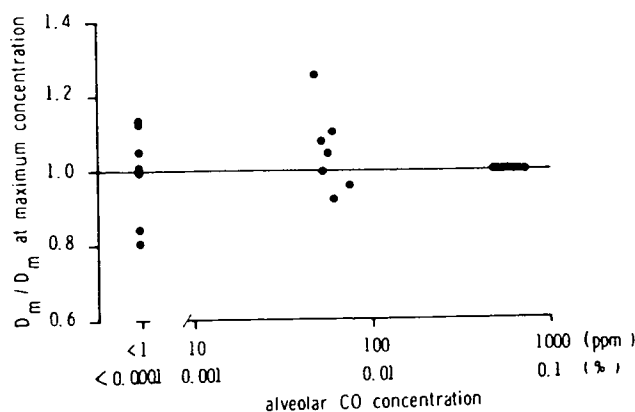


FIG. 6. Membrane diffusing capacity ( $D_m$ ) for CO at different alveolar concentrations. Each point represents a single measurement as fraction of that at highest inspired concentration.

being plotted as the fraction of the value at the highest CO concentration. There appears to be no influence of CO on the membrane diffusion as all points at the medium and low concentrations lie around the line of unity.

## DISCUSSION

The use of radiolabeled  $^{11}CO$  enabled us to measure CO uptake accurately at very low concentrations. The CO backpressure poses a significant problem in experiments not using tracers of CO, but no backpressure of  $^{11}CO$  was detected at any time.

The rate of uptake of CO is influenced by the state of inflation of the lung (10). The variation in the lung volumes at which our measurements were made, shown in Table 2, was not large enough nor in the appropriate range of the vital capacity to affect the results.

The rate of uptake during air breathing is also dependent on the capillary blood volume. The studies carried out after  $O_2$  breathing were designed specifically to study the properties of the alveolar membrane in which the proposed carrier resides. At high  $O_2$  levels the  $\theta V_c$  term is reduced fourfold and the  $D_m$  term dominates. Under these conditions CO uptake was again not dependent on inspired concentration.

It is possible that there may be a carrier present in

human lungs whose  $O_2$  half saturation pressure ( $P_{50}$ ) is at a CO concentration that we did not study. Even were this so, we would have expected to find a slightly increased uptake for at least one of the concentrations studied, as the influence of a carrier should be apparent up to 10 times above its  $P_{50}$  concentration and below it to a lesser extent (12).

The presence of a carrier for CO and  $O_2$  in the placenta has been suggested (1, 7); in this situation, where diffusion distances are significant, there is at least a physiological role for a carrier to enable the fetus to obtain an adequate supply of  $O_2$ . In contrast, the diffusion distances

across the alveolar membrane in the lung are extremely small, approximately  $1.7 \mu\text{M}$  (15). In certain diseases the membrane does become thickened, but these conditions are relatively rare and the thickening usually occurs at a late stage in the disease process. Thus the evolutionary pressure required to develop a facilitated transport system in the lung is not strong.

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# Quantitative measurement of intrapulmonary and extrapulmonary right-to-left shunt

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JONES, HAZEL A., J. R. STRADLING, J. C. CLARK, E. E. DAVIES, AND A. ROZKOVEC. *Quantitative measurement of intrapulmonary and extrapulmonary right-to-left shunt.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 54(5): 1434-1438, 1983.—We have developed a new technique that enables the shunting of blood from the right to the left side of the circulation to be partitioned into a cardiac and a lung component. The effects of recirculation are minimal, and the method does not require on-line data analysis. Quantitative estimates of these components have been made in two normal dogs and in five patients with raised pulmonary arterial pressures, some of whom were known to have a patent foramen ovale. The results were compared with oxygen shunt measured during air breathing. A poorly soluble gas, nitrogen, radiolabelled with  $^{13}\text{N}$  in solution is injected first into a central vein while matched samples of blood are drawn from the pulmonary artery and the aorta. A second solution containing  $^{13}\text{N}$  is injected into the right ventricle and sampled from the aorta only. Standardized gamma-counting techniques were used to analyze both the injected radioactivity and the radioactivity in the samples. These two measurements enable us to calculate the total right-to-left shunt, the pulmonary shunt, and by subtraction the extrapulmonary cardiac shunt.

pulmonary shunt; cardiac shunt; nitrogen-13; oxygen shunt

THE QUANTITATION AND ANALYSIS of shunting through the lungs and through the heart, as well as ventilation-perfusion ( $\dot{V}/\dot{Q}$ ) mismatching, are important if one is to study the effects of potentially beneficial vasodilator drugs in patients with raised pulmonary arterial pressures, particularly if they have associated foramen ovale. The importance of this differentiation has been recognized for many years (12, 16), and several techniques based on the Fick principle have been tried. These include the oxygen method (1) and the use of poorly soluble gases that are virtually eliminated from the blood on passage through the lungs (4, 8, 18). However, in patients who are shunting blood from right to left, none of these methods can differentiate cardiac from lung shunts, which may result from atelectasis or new vessels formed in response to high pulmonary arterial pressures that bypass gas-exchanging areas (2). Low  $\dot{V}/\dot{Q}$  areas in the lung will interfere with all methods to a varying extent.

We have developed and tested a new technique that not only differentiates between cardiac and intrapulmonary shunting of blood but allows quantitative measure-

ments of each to be made. This enables the effective site of action of various drugs that affect total shunting in patients with raised pulmonary arterial pressures to be determined.

## THEORY

A diagrammatic representation of the system to be studied is shown in Fig. 1. This system consists of an input (I) and an output (O). Blood flow is in the direction shown by the arrows.

Blood entering at I can reach O by three possible routes. Let  $x$  be the fraction of blood shunted through the heart from right to left and  $y$  be the fraction of blood shunted through the lungs. This includes any areas of atelectasis and any new vessel formation. Thus  $1 - x - y$  will be the fraction of blood that passes through the gas-exchanging area of the lung and will completely lose a totally insoluble gas tracer.

Consider an injection of  $N$  molecules of insoluble tracer at A. Let  $F$  be the fraction of blood leaving O and returning at I, i.e., the fractional recirculation. Therefore

$$I_A = F \cdot O_A$$

Let  $C$  be the number of tracer molecules detected. At sampling site 1

$$C_{1A} = K(N + I_A) \quad (1)$$

where  $K$  is the proportionality constant of tracer detection. And at sampling site 2

$$C_{2A} = K(N + I_A)(x + y)$$

Therefore

$$C_{2A}/C_{1A} = x + y$$

where  $x + y$  is the fractional heart and lung shunt independent of recirculation.

Now consider an injection of  $N$  molecules of insoluble tracer at B. Input counts are not measurable in this case, and therefore  $C_{1B}$  is not relevant.

$$I_B = F \cdot O_B$$

$$C_{2B} = K \cdot O_B = K(N \cdot y + I_B(x + y)) \quad (2)$$

$$O_B = I_B/F$$

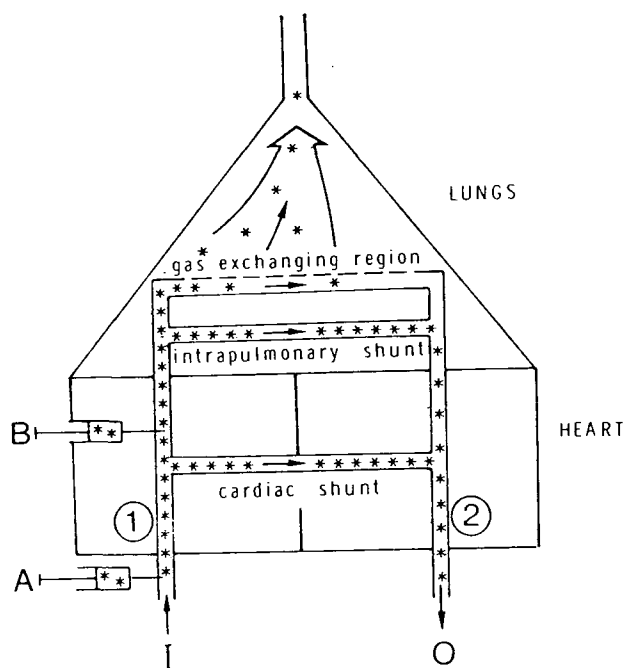


FIG. 1. Heart and lung system; 3 theoretical pathways by which blood can pass from the right to the left side of the circulation are shown. Sites of injection (A and B) and sampling (1 and 2) for the measurement of shunts are also shown.

Therefore

$$I_B/F = N \cdot y + I_B(x + y)$$

$$I_B(x + y - 1/F) = -N \cdot y$$

$$I_B = \frac{N \cdot y}{1/F - x - y}$$

substituting for  $I_B$  in Eq. 2

$$C_{2B} = K \left( N \cdot y + \frac{N \cdot y(x + y)}{1/F - x - y} \right) \quad (3)$$

Similarly for injection at A

$$I_A = F \cdot O_A$$

$$C_{2A} = K \cdot O_A = K \cdot (N + I_A)(x + y);$$

$$O_A = I_A/F$$

$$I_A/F = (N + I_A)(x + y)$$

$$I_A(x + y - 1/F) = -N(x + y)$$

$$I_A = \frac{N(x + y)}{1/F - x - y}$$

substituting for  $I_A$  in Eq. 1

$$C_{1A} = K \left( N + \frac{N(x + y)}{1/F - x - y} \right) \quad (4)$$

dividing Eq. 3 by Eq. 4

$$C_{2B}/C_{1A} = \frac{K \left( N \cdot y + \frac{N \cdot y(x + y)}{1/F - x - y} \right)}{K \left( N + \frac{N(x + y)}{1/F - x - y} \right)} = y$$

where  $y$  is the fractional lung shunt independent of recirculation. (Note that the fractional recirculation ( $F$ ) must be the same for injection at A and at B.)

The use of a gas with very low but finite solubility such as nitrogen (blood-gas partition coefficient = 0.015) means that the intrapulmonary retention is dependent on  $\dot{V}/\dot{Q}$  distribution as well as true shunt, retention being  $\lambda/\lambda + \dot{V}/\dot{Q}$  (6). Thus a "shunt" of 1.48% will be measured in a lung with a  $\dot{V}/\dot{Q}$  of 1.0 due to the solubility. The lower the  $\dot{V}/\dot{Q}$  of the unit, the greater will be the retention of nitrogen, such that the retention will be 2.9% for a  $\dot{V}/\dot{Q}$  of 0.5, 4.8% for a  $\dot{V}/\dot{Q}$  of 0.3, 13.0% for a  $\dot{V}/\dot{Q}$  of 0.1, and so on.

The use of a single gas does not allow the differentiation of the measured intrapulmonary shunt into  $\dot{V}/\dot{Q}$  distribution and pure anatomic shunt, but differentiation of the intrapulmonary and extrapulmonary component is unaffected by  $\dot{V}/\dot{Q}$ .

## METHODS

**Dog studies.** Two greyhound dogs were anesthetized with iv thiopental sodium (5 mg/kg body wt iv) and pentobarbital sodium (3–6 mg · kg<sup>-1</sup> · h<sup>-1</sup>). Catheters were placed under fluoroscopy in the inferior vena cava and right ventricle for injection of solution and in the main pulmonary artery and the aorta for withdrawal of blood. Repeat measurements of shunt were made with our new technique.

**Patient studies.** Four patients with primary pulmonary hypertension, confirmed by previous cardiac catheterization and pulmonary angiography, underwent repeat catheterization to study the effects of two drugs, prostacyclin and nifedipine. All patients gave their informed consent, and the study was approved by the Hammett Hospital Ethics Committee. Two of the patients were thought to have patent foramen ovale. None of the patients were in right heart failure at the time of the study, and there was no clinical indication of tricuspid regurgitation. Pressures measured in the right atrium were independent of the presence or absence of the catheter across the tricuspid valve, making catheter-induced regurgitation unlikely. Catheters were placed under fluoroscopic control into the aorta, the right ventricle, and the pulmonary artery via the femoral approach. A long line was inserted via an antecubital vessel into a central vein.

Measurement of shunt by our method were made at the end of a control period and in three cases at the end of an infusion of either prostacyclin into the pulmonary artery or after nifedipine sublingually, in a dose sufficient to lower pulmonary or systemic arterial pressure by at least 10 Torr. Just before these shunt measurements, paired 5-ml samples of systemic and pulmonary arterial blood were drawn over a 5-min period, during which time all expired gas was collected. Total expired volume was measured through a gas meter, and oxygen and carbon dioxide concentrations were analyzed by a mass spectrometer (Centronic 200 MGA). The blood samples were immediately placed in ice and were always analyzed within 30 min of withdrawal, to minimize the effect of oxygen metabolism by the blood on partial pressure of oxygen. The data enabled us to calculate the cardiac

output and oxygen shunt using the alveolar air equation (15). Saturations and gas tensions in the blood sample were measured on an oximeter (IL CO-oximeter 282) and gas analyzer (IL 613).

**Methodology.** Nitrogen radiolabelled with  $^{13}\text{N}$  was prepared in the M.R.C. cyclotron at Hammersmith Hospital by deuteron irradiation of carbon dioxide (3). This isotope of nitrogen emits positrons and has a half-life of 10 min. Solutions of approximately 1 mCi  $^{13}\text{N}$  in 10 ml isotonic saline were prepared for each injection by dissolving 0.1–0.2 ml of the gas in isotonic saline by vigorous shaking. The solution was then sterilized by millipore filtration, which also removed any undissolved microbubbles of gas. All counting of radioactivity was carried out using a two-position jig over a NaI/Tl scintillation counter (Fig. 2), which was situated in a room remote from the patient to avoid interference from exhaled radioactivity. Room background was counted at intervals with and without the removable lead filter in position. All counts were corrected for room background, radioactive decay, and decay occurring during the counting time (3).

Each measurement consisted of two parts that were identical, apart from the site of injection and the number of samples withdrawn. Two 30-ml syringes were connected to the pulmonary arterial and aortic catheters via manometer tubing (0.2 cm ID, 60 cm long). The whole system was flushed with heparinized saline and was bubble free. The syringes were placed in a dual-channel matched withdrawal pump (Harvard Apparatus model 2206). Withdrawal of blood was started, and 1 mCi of  $^{13}\text{N}$  in 10 ml isotonic saline was injected into the central vein over 5–10 s, followed by immediate withdrawal of 10 ml fluid into the same syringe, so that the residual activity

in the injection catheter could be counted. Both the initial and residual  $^{13}\text{N}$  were counted with the syringe mounted in position 1 on the jig and with the lead filter in position to attenuate the radioactivity reaching the detector (Fig. 2). The same volume of fluid in each measurement ensured identical counting geometry. The blood withdrawal was continued at a steady rate of 0.5 ml/s until a 30-ml sample had been collected in each syringe. The second part of the measurement was made approximately 5 min later by injecting the  $^{13}\text{N}$  solution into the right ventricle and sampling from the aorta only. Again initial and residual activities were counted in the injection syringe. Aortic blood was sampled in a manner identical to that for the first part of the measurement. All blood samples were counted with the 30-ml syringes containing 30 ml of fluid mounted in position 2 on the jig and with the lead filter removed, because the radioactivity in the samples was lower than that injected due to dilution with blood and also, in the case of the aortic sample, loss of  $^{13}\text{N}$  to the lung gas. Counting times were adjusted to give a low statistical counting error. After counting the radioactivity of all samples the blood was returned to the patient.

The total shunt and the intrapulmonary shunt were calculated from the equations derived in THEORY. As described in METHODS, blood was withdrawn from the pulmonary artery, because we wanted to be absolutely sure of a mixed venous sample. This does not affect the application of THEORY, because an identical sample would be obtained after injection at A (Fig. 1) whether the blood was withdrawn from position 1 or in the pulmonary artery. Thus  $C_{1A}$  was measured as counts in the pulmonary arterial sample, and  $C_{2A}$  and  $C_{2B}$  were measured as counts in the two aortic samples. Thus fractional total shunt =  $C_{2A}/C_{1A}$ . Because the injected activity ( $N$ ) was not the same for injections at A and B, the ratio of the injected counts was calculated to give the appropriate  $C_{1A}$  for the injection at B

$$\text{injected counts} = \text{initial} - \text{residual counts in syringe}$$

$N$  counts injected at A gives  $C_{1A}$  counts. Thus  $N^*$  counts injected at A would give  $C_{1A} \cdot N^*/N$  counts. Therefore the  $C_{1A}$  term must be multiplied by  $N^*/N$  for calculation of intrapulmonary shunt, where  $N^*$  are the counts injected at B

$$\text{fractional intrapulmonary shunt} = C_{2B} \cdot N / C_{1A} \cdot N^*$$

## RESULTS

**Dog studies.** The measurements of  $^{13}\text{N}$  shunting are shown in Table 1 both as the total measured shunt and as intrapulmonary shunt in the two dogs. The shunt

TABLE 1.  $^{13}\text{N}$  shunt measurements in dogs

| Dog No. | A     |     |      | B     |     |      | C     |     |      |
|---------|-------|-----|------|-------|-----|------|-------|-----|------|
|         | Total | IP  | EP   | Total | IP  | EP   | Total | IP  | EP   |
| 1       | 2.0   | 2.2 | -0.2 | 3.2   | 2.7 | +0.5 |       |     |      |
| 2       | 2.1   | 1.9 | +0.2 | 4.0   | 3.5 | +0.5 | 4.4   | 4.1 | +0.3 |

A, B, and C are repeat measurements separated only by time; IP, intrapulmonary; EP, extrapulmonary. Total shunt (as percent of total blood flow) differentiated into intra- and extrapulmonary components.

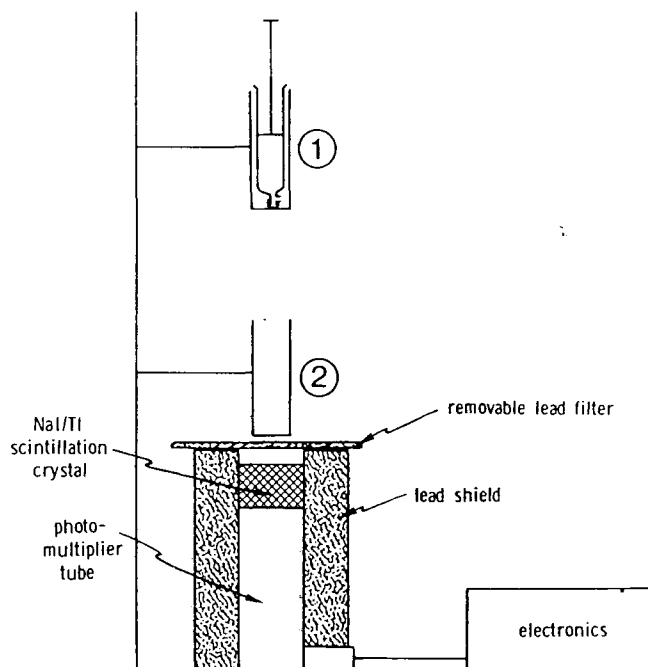


FIG. 2. Two-position jig used for all measurements of radioactivity. Position 1 is used for counting radioactivity in syringes containing injection and residue and position 2 for counting radioactivity in syringes containing pulmonary arterial and aortic samples.

TABLE 2. Oxygen and  $^{13}\text{N}$  shunt measurements before and after administration of vasodilator drug

| Patient | A                     |                       |     |      | B                     |                       |     |      |
|---------|-----------------------|-----------------------|-----|------|-----------------------|-----------------------|-----|------|
|         | $\text{O}_2$<br>shunt | $^{13}\text{N}$ shunt |     |      | $\text{O}_2$<br>shunt | $^{13}\text{N}$ shunt |     |      |
|         |                       | Total                 | IP  | EP   |                       | Total                 | IP  | EP   |
| JH      | 21.4                  | 13.9                  | 9.6 | 4.3  | 26.6                  | 22.0                  | 8.6 | 13.4 |
| MW      | 44.7                  | 66.0                  | 1.4 | 64.6 |                       |                       |     |      |
| SH      | 4.7                   | 2.4                   | 2.2 | 0.2  | 9.2                   | 3.7                   | 4.0 | -0.3 |
| DJ      | 6.2                   | 1.6                   | 1.7 | -0.1 | 10.2                  | 1.7                   | 1.7 | 0    |

Values expressed as percent of total blood flow. A, before vasodilator drug; B, after vasodilator drug; IP, intrapulmonary; EP, extrapulmonary.

measurements give increasing values with time. The intrapulmonary and total values of shunt for each paired measurement give an indication of the precision of the technique, because no cardiac shunting was suspected in these animals.

**Patient studies.** The measurements of  $^{13}\text{N}$  shunt are shown in Table 2 and compared with those for oxygen shunt before and after the administration of prostacyclin or nifedipine. When the  $^{13}\text{N}$  shunt is differentiated into intra- and extrapulmonary components, two of the patients (JH and MW) show that a significant amount of the shunt is through the heart, whereas the remaining patients exhibit purely intrapulmonary shunting. The measured intrapulmonary shunt comprises both anatomic shunt and areas of very low  $\dot{V}/\dot{Q}$ .

After the administration of the drug to three of the patients, no change in  $^{13}\text{N}$  shunt was demonstrated in patient DJ, shunting through the lungs was increased in SH, and shunting through the heart increased in JH.

## DISCUSSION

All presently available methods for measuring shunting of blood are subject to various limitations. The oxygen method, which compares oxygen content in mixed venous and arterial blood, if carried out during air breathing, tends to overestimate shunting due to areas of low  $\dot{V}/\dot{Q}$  (7). The use of a gas with very low solubility minimizes this effect. Oxygen breathing can minimize the influence of these areas (1) but may affect the shunt (5, 11), changing  $\dot{V}/\dot{Q}$  itself by reducing hypoxic vasoconstriction or by creating areas of atelectasis due to the washout of nitrogen from poorly ventilated areas, already prone to collapse. The oxygen measurement of shunt also includes any right-to-left shunting both through the heart and through lung vessels that bypass gas-exchanging units. The advantage of the oxygen method is that it is a steady-state method and therefore not subject to inaccuracies due to recirculation.

The double-indicator dilution techniques, which compare the passage of an insoluble with a soluble tracer through the system, are not steady-state methods and hence have additional limitations. The magnitude of the effect of low  $\dot{V}/\dot{Q}$  areas on the measurement of pulmonary shunt depends on the solubility of the "insoluble" tracer used.  $\text{SF}_6$ ,  $^{85}\text{Kr}$ , Xe, and tritium have all been used (8, 10, 13, 14, 17) and their retention in blood as a function of lung volume described (4). Probably a more important

limitation of this technique is the effect of recirculation on the data. Copley et al. (4) avoided the problem in their animal preparation by not allowing the recirculated blood to enter the system during the shunt measurement. Other authors have terminated measurement before the downslope of the soluble tracer curve deviated from a monoexponential (8, 10, 13) or used the Hamilton monoexponential extrapolation (9) to analyze the soluble tracer curve (14). The use of these methods assumes that the transit time is the same through all pathways. This cannot be true, especially when there is a right-to-left communication through the heart. Transit times through intrapulmonary shunts are also likely to affect this analysis, albeit to a lesser extent.

Our technique combines the use of a low-solubility gas,  $^{13}\text{N}$ , which has a blood gas partition coefficient of 0.015, with a method that is independent of recirculation. The solubility of  $^{13}\text{N}$  is such that only very low  $\dot{V}/\dot{Q}$  will contribute significantly to the measured shunt, the retention of gas being  $\lambda/\lambda + \dot{V}/\dot{Q}$  (6), where  $\lambda$  is the blood gas partition coefficient. Thus  $^{13}\text{N}$  will be equally partitioned between blood and gas at a  $\dot{V}/\dot{Q}$  of 0.015, and a homogeneous lung with a  $\dot{V}/\dot{Q}$  of 1 will give a  $^{13}\text{N}$  shunt of 1.48%. Farhi's equation (6) is, however, based on steady-state conditions. A true single-pass bolus technique would lead to problems of resorption of gas with high specific activity from areas with a low  $\dot{V}/\dot{Q}$  as pointed out by Copley et al. (4). Our technique is not truly steady state, nor is it a discrete bolus, because the injection takes place over 5–10 s and recirculation is included in the input signal. The fact that the time-activity profile in blood reaching the lung is not absolutely flat may lead to a small error; this is, however, minimized by the use of a gas of very low solubility, such as nitrogen.  $\dot{V}/\dot{Q}$  distribution within the lungs of our patients, which may well be affected by the drugs administered, will affect the intrapulmonary retention of  $^{13}\text{N}$  and hence our measurement of intrapulmonary shunt. However, in no way does  $\dot{V}/\dot{Q}$  distribution or resorption of gas affect our ability to distinguish between the intra- and extrapulmonary components of gas retention.

The measurements in both dogs show  $^{13}\text{N}$  shunt increasing with time. Because the dogs were anesthetized and supine, it is probable that areas of atelectasis and very low  $\dot{V}/\dot{Q}$  developed and caused increased  $^{13}\text{N}$  shunting through the lungs.

Measurements of oxygen shunt are influenced to a greater extent by low  $\dot{V}/\dot{Q}$  areas than are those of  $^{13}\text{N}$  shunt, and the values we obtained for oxygen shunt were higher in all but one of our patients (MW, Table 1). We are unable to explain this discrepancy, but because the measurements were made at different times and under different conditions the most probable reason is that the shunt was variable through the heart. Although the oxygen shunt measured closest in time to the  $^{13}\text{N}$  shunt was only 45%, a measurement made later in the study was 59%. The measurement of oxygen shunt is made with the patient breathing on a mouthpiece for 5 min, which may alter both breathing pattern and body position. Certainly the patient is aware that the measurement is being made, whereas he is not during the  $^{13}\text{N}$  measurement. Pulmonary arterial pressures are extremely labile in these patients, and this may contribute to the differ-

ences between the two methods.

As the measurement collects blood over a long sampling time, our sample will include recirculated blood. If a shunt is present, this blood will contain radioactivity. This does not affect the calculation of either the total shunt or the intrapulmonary shunt as shown in THEORY. The only possible error that the recirculation could introduce would be radioactivity returning to the pulmonary arterial but not to the aortic sample. This is unlikely to be a significant error, because the time-activity profile will be slurred on recirculation due to the vastly different transit times through the systemic circulation.

The presence of tricuspid regurgitation would affect the measurement of the intrapulmonary shunt by allowing some of the injected radioactivity to flow back into the right atrium and thence across a foramen ovale. The appropriate pressure measurements made in all patients before the start of the study (see METHODS) eliminates the possibility of this phenomenon.

*Patient JH* (Table 2) is a good example of the use of this technique to study the effects of a vasodilator drug. The total measured  $^{13}\text{N}$  shunt is 14% in the control situation; 9% of this is through the lungs and the rest through the heart. After the administration of the drug the shunting through the lungs was not altered, and it was purely the cardiac component of the shunt that was increased. This explains the other observations made at the time: although the drug increased cardiac output and decreased pulmonary arterial pressure, the patient's hy-

poxemia worsened. This is not surprising, because we were able to demonstrate that the fraction of blood supplied to the gas-exchanging units in the lungs did not increase.

This technique for the measurement and differentiation of shunts can of course be used with gases other than the cyclotron-produced  $^{13}\text{N}$  used here. Any gas with a sufficiently low solubility is suitable, provided that its concentration can be detected with accuracy by some method. Gas chromatography, for example, using  $\text{SF}_6$  would be ideal, because this gas is even less soluble than nitrogen.  $^{35}\text{SF}_6$ , which has a half-life of 86 days, could also be used in conjunction with the widely available liquid scintillation detectors for  $^{14}\text{C}$ . Radioactive tracers used in this way provide extremely accurate estimates of blood levels with a low radiation dose to the patient.

In summary, we believe that we have developed a potentially useful technique that can be used to study the effects of various drugs in patients with pulmonary hypertension. This technique involves the injection of a low-solubility tracer into a central vein and the right ventricle and the withdrawal of blood through catheters that have been introduced into the aorta, right ventricle, and pulmonary artery for other routine investigations, such as injections of drugs and measurements of pressure. The technique can be used with widely available detection systems.

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# Radioassay Problems Associated with the Clinical Use of a $^{82}\text{Rb}$ Radionuclide Generator

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The short-lived positron emitting radionuclide  $^{82}\text{Rb}$  ( $t_{1/2}$  1.27 min) is conveniently available from a  $^{82}\text{Sr}/^{82}\text{Rb}$  generator system. The parent nuclide ( $t_{1/2}$  25.5d) produced from the spallation of molybdenum, has associated with it varying quantities of other long-lived strontium radionuclides, namely  $^{84}\text{Sr}$ ,  $^{86}\text{Sr}$  and  $^{90}\text{Sr}$ . It is important therefore in the clinical use of such material that the levels of strontium radionuclides being administered to patients is carefully assayed and controlled. The problems associated with these measurements are discussed with special reference to the radiation dose received by the patient and the problems in resolving overlapping peaks with different FWHMs.

## Introduction

Strontium-82 ( $t_{1/2}$  25.5d)<sup>(1)</sup> can be used as a source of the short-lived positron emitter  $^{82}\text{Rb}$  ( $t_{1/2}$  76.4s)<sup>(1)</sup> in a generator system.<sup>(2-9)</sup> Such a generator is being extensively used at Hammersmith Hospital for myocardial studies in patients and experimental animals using the positron emission computerised axial tomograph (PECAT)<sup>(10)</sup>. These studies involve a continuous infusion of the generator eluate which in some cases amounts to a total of about 1500 ml of solution. As it is important to determine accurately the radiation dose to the patient from this infusion, great care has to be taken in assaying the generator. In particular the long-lived strontium radionuclides which accumulate in bone and have a long effective half-life<sup>(11)</sup> may be present due to breakthrough from the generator columns.

## Materials and Methods

The  $^{82}\text{Sr}$  used in these studies was produced by the irradiation of molybdenum with 800 MeV protons at the Los Alamos Meson Physics facility, where extensive processing was carried out concentrating the strontium radionuclides, and subsequent decay products.<sup>(12,13)</sup> However, by the time the processed  $^{82}\text{Sr}$  solution was delivered to the U.K., only the longer-lived strontium radionuclides remained, namely  $^{82}\text{Sr}$ ,

$^{84}\text{Sr}$  ( $t_{1/2}$  64.8d),  $^{86}\text{Sr}$  ( $t_{1/2}$  50.5d) and  $^{90}\text{Sr}$  ( $t_{1/2}$  28.5yr).

The clinical generator assembly which has been described in detail elsewhere,<sup>(14)</sup> consisted of two perspex columns in series. The primary column (5 × 1 cm diameter) was filled with Whatman chromatographic grade basic alumina supported on sintered polyethylene. The second smaller column (2 × 1 cm diameter) contained Bio-Rad Chelex-100 (200-400 mesh, preconditioned with 1.8% NaCl at pH 10) which performed a final chemical purification of the primary column eluate.

The solution of  $^{82}\text{Sr}$  obtained from Los Alamos in 0.5 M HCl was adjusted to pH 9-10, loaded onto the  $\text{Al}_2\text{O}_3$  column, and washed with >200 ml of 1.8% NaCl at pH 9-10 to remove the poorly absorbed radionuclides (e.g.  $^{58}\text{Co}$ ,  $^{84}\text{Rb}$ ,  $^{87}\text{Y}$ , etc). At this stage more than 99% of the  $^{82}\text{Sr}$  was retained by the column.

Clinically useful  $^{82}\text{Rb}$  was obtained from the column by elution with 1.8% NaCl solution, which was subsequently adjusted for pH and NaCl concentration by mixing the output flow with acidified water for injection. The final solution (pH 6.5-7.5) in 0.9% NaCl was then passed through a sterilising filter before being infused.

Because of the possible breakthrough from the column of strontium radionuclides it was extremely important that the exact quantity of each in the infused solution could be determined. From  $\gamma$ -ray



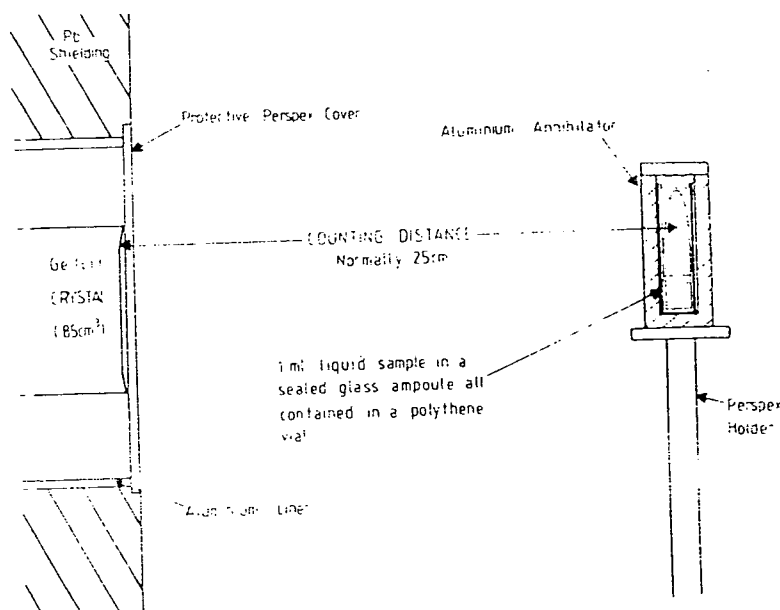


Fig. 1. Counting geometry for the MRC Ge(Li) detector

analysis.  $^{85}\text{Sr}$  was readily detectable by its 514 keV emission line, though as it will be shown this proved difficult to assay, as it was not completely resolved from the annihilation radiation. As the presence of the pure  $\beta$ -emitters  $^{89}\text{Sr}$  and  $^{90}\text{Sr}$  was a distinct possibility, it was decided to subject a portion of the  $^{82}\text{Sr}$  stock solution to mass-separation and at the same time to independently check the level of  $^{85}\text{Sr}$ .

#### Radioassay

On receipt of the  $^{82}\text{Sr}$  radioactivity in the U.K., sources were prepared for counting and examined using a Ge(Li) detector (See Fig. 1) and a 4096 multichannel analyser. The radioassay for  $^{82}\text{Sr}$  was performed by integrating the 777 keV photopeak. Using an absolute intensity of 13.6%<sup>(14,15)</sup> excellent

agreement ( $\pm 0.5\%$ ) with the Los Alamos assay was obtained for the total  $^{82}\text{Sr}$  activity delivered.

Though the 514 keV line of  $^{85}\text{Sr}$  was detected with the Medical Research Council (MRC) Ge(Li) detector it was not possible to resolve it completely from the annihilation radiation of  $^{82}\text{Rb}$  (see Fig. 2), and in early batches the  $^{85}\text{Sr}$  was assayed by a simple subtraction technique (using peak areas) and by a gross analysis when the  $^{82}\text{Sr}$  had completely decayed. After correcting these values for decay, there was an inexplicable difference with the Los Alamos data which had been obtained from computerised analyses of Ge(Li)  $\gamma$ -ray spectra using a derivative of the GAMANAL programme.<sup>(16)</sup>

Further studies at Hammersmith, resulted in an assay procedure that could resolve the 511 and

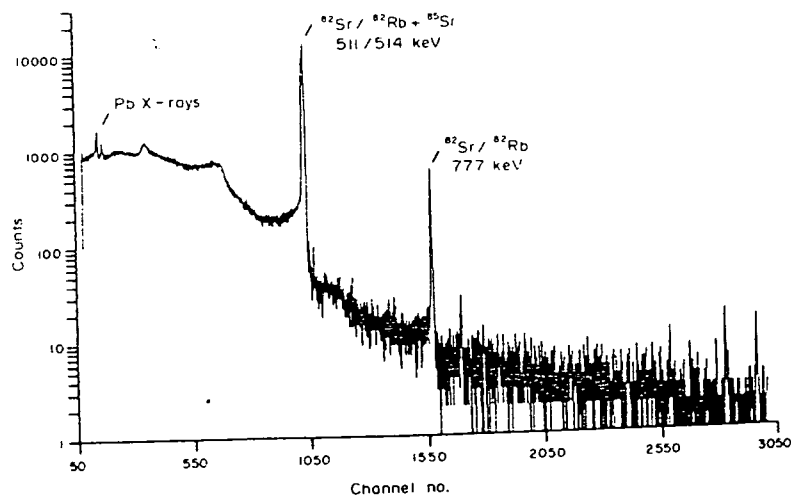


Fig. 2.  $\gamma$ -Ray spectrum of spallogenic produced  $^{82}\text{Sr}$  ( $0.493 \text{ keV ch}^{-1}$ ).

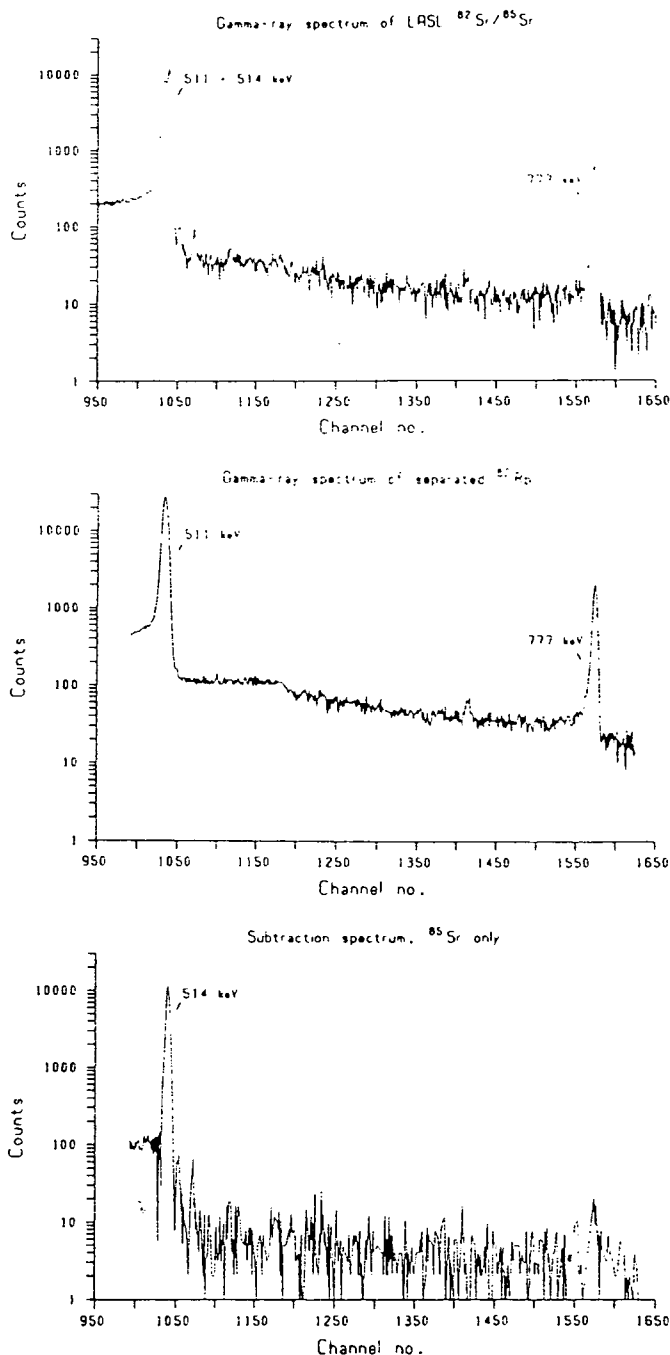


Fig. 3. Spectrum stripping technique for the resolution of the 511/514 keV lines.

514 keV lines by a  $^{82}\text{Rb}$   $\gamma$ -ray spectrum stripping technique, which was made possible by the use of a Nuclear Data (ND-66) analyser system. The method is illustrated in Fig. 3, and the results for a number of different batches of  $^{82}\text{Sr}$  are summarised in Table 1.

During the development of the spectrum strip procedure it became clear that even greater care than normal had to be taken in standardising all the parameters affecting the Ge(Li) detector efficiency. In

particular, as the endpoint energies of the emitted positrons from  $^{82}\text{Rb}$  are so high (up to 3.35 MeV) a relatively thick absorber (e.g.  $2.14 \text{ g cm}^{-2}$  of Al) was required in an attempt to annihilate the positrons within the fixed geometry of the source. However this thickness of annihilator material had a significant effect on the overall detection efficiency of the  $\gamma$ -rays, amounting to  $\approx 20\%$  reduction for the detection of the annihilation radiation.<sup>(17)</sup>

Table 1. Relative  $^{85}\text{Sr}$  content in various batches of spallogenic produced  $^{85}\text{Sr}$  (corrected to the date of calibration)

| LANL number and calibration date                 | GAMANAL value (Los Alamos) | $^{85}\text{Sr}$ spectrum strip analysis (Hammersmith) |
|--------------------------------------------------|----------------------------|--------------------------------------------------------|
| SR 74299C<br>31 Mar. '81                         | 165%                       | $105.6 \pm 3.4\%$ ,<br>where $n = 5$                   |
| SR 75873C<br>4 Aug. '81                          | 172%                       | $105.9 \pm 9.6\%$ ,<br>where $n = 4$                   |
| SR 78684C<br>20 Aug. '81                         | 180%                       | $105.6 \pm 1.5\%$ ,<br>where $n = 4$                   |
| $(n = \text{number of individual measurements})$ |                            |                                                        |

When this effect was taken into account the annihilation radiation could be used to assay for the  $^{85}\text{Sr}/^{82}\text{Rb}$  equilibrium using the published positron branching ratio of 96%<sup>(14,15)</sup>. However the value obtained was in disagreement with the assay value calculated using the 777 keV line. If the adoption of 13.6% for the 777 keV line is correct then the positron branching ratio measured during this series of experiments would appear to be closer to 75% ( $74.4 \pm 5.2\%$ ; where  $n = 5$ ) than to the literature value of 96%.

#### Mass-separation

Samples from two batches of Los Alamos material were subjected to mass-separation at the National Physical Laboratory (NPL) and details of the method have been published elsewhere.<sup>(13)</sup> The importance of using this procedure was that it was possible to radioassay separately for  $^{82}\text{Sr}$ ,  $^{85}\text{Sr}$  and the  $\beta$ -emitters  $^{89}\text{Sr}$  and  $^{90}\text{Sr}$ , using pure samples available only by this method.  $^{82}\text{Sr}$  and  $^{85}\text{Sr}$  were assayed using the MRC Ge(Li) detector, while  $^{89}\text{Sr}$  and  $^{90}\text{Sr}$  were assayed in a low background anti-coincidence shielded  $4\pi\beta$  proportional counter<sup>(18)</sup> at the NPL. The results of these experiments are given in Table 2.

#### Discussion

Before any determinations were made of the dose incurred by the patients in these clinical studies, it was important to have reliable measurements as to the exact quantities of the radionuclides involved.

The activity of the  $^{82}\text{Sr}/^{82}\text{Rb}$  equilibrium was measured using the Ge(Li) detector and integrating the 777 keV photopeak using an adopted abundance figure of 13.6%<sup>(14,15)</sup>. Assuming a constant flow infusion of  $^{82}\text{Rb}$  into the patient and basing calcu-

lations on the MIRD Tables for radiation dose at a steady state,<sup>(19)</sup> then the radiation dose to the patient was calculated to be  $1.9 \times 10^{-4}$  Gy (19 mrad)min<sup>-1</sup> to the myocardium,  $3.5 \times 10^{-4}$  Gy (35 mrad)min<sup>-1</sup> to the renal cortex and  $0.2 \times 10^{-4}$  Gy (2 mrad)min<sup>-1</sup> to the whole body, for a  $^{82}\text{Rb}$  generator giving 390 MBq (10.5 mCi)min<sup>-1</sup> at a steady state elution. This is not regarded as excessive for the clinical information which is obtained.<sup>(16)</sup> However, it appears from our measurements that the adopted value for the positron branching ratio for  $^{85}\text{Sr}$  may be in error by as much as 20%. If this is confirmed by further experiments,<sup>(17)</sup> it could mean an increase in the calculated radiation dose to the patient.

An additional source of patient radiation dose arose via the breakthrough of strontium radionuclides from the clinical generator. The total quantity of strontium radioactivity leached from a typical column into a litre of infused solution was found to be only 20 Bq (0.54 nCi)ml<sup>-1</sup>, thus making the separate assay for  $^{82}\text{Sr}$  and  $^{85}\text{Sr}$  a difficult task.

In addition, there appeared to be a discrepancy in the  $^{85}\text{Sr}/^{82}\text{Sr}$  activity ratio (see Tables 1, 2) as calculated using the "GAMANAL" assay for  $^{85}\text{Sr}$  with the spectrum-strip and mass-separator values. In order to find a reason for this difference, a study was made at the NPL of a similar computerised  $\gamma$ -ray analysis programme to GAMANAL which was capable of resolving overlapping  $\gamma$ -rays of similar energy. The GAMANAL programme does this by making the reasonable assumption that the peak widths of lines which are only 3 keV apart are comparable, i.e. the values for the full width at half the maximum (FWHM) are virtually equal.<sup>(16)</sup>

However, in the case of the annihilation radiation the FWHM of the  $\gamma$ -ray is much wider than the corresponding nuclear emitted photons because of Doppler broadening as the positron is brought to rest.<sup>(20)</sup> This increase in peak width is a function of both annihilator material and of positron energy.<sup>(20)</sup> In the case of the annihilation radiation from the very energetic  $^{82}\text{Rb}$  positrons, this increase in width measured with the MRC Ge(Li) detector amounted to a factor of 1.6 of the 514 keV width when aluminium annihilators were used (See Fig. 4).

When this information was incorporated into the computerised  $\gamma$ -ray analysis programme, it became clear that unless proper account was taken for the

Table 2. Examination of spallogenic produced  $^{82}\text{Sr}$  after mass-separation (activity relative to  $^{82}\text{Sr}$  at the date of calibration)

| LANL number and calibration date | GAMANAL value for $^{85}\text{Sr}$ (Los Alamos) | Mass-separated samples        |                                      |                                    | Notes                                       |
|----------------------------------|-------------------------------------------------|-------------------------------|--------------------------------------|------------------------------------|---------------------------------------------|
|                                  |                                                 | $^{85}\text{Sr}$ Ge(Li) assay | $^{89}\text{Sr}$ $4\pi\beta$ counter | $^{90}\text{Sr}$ , $^{90}\text{Y}$ |                                             |
| SR/63764<br>18 Dec. '79          | 180%                                            | $\approx 30\%$                | $\approx 0.5\%$                      | $< 0.01\%$                         | Results reflect preliminary nature of study |
| SR/74299C<br>31 Mar. '81         | 165%                                            | 103%                          | 1.85%                                | 0.018%                             |                                             |

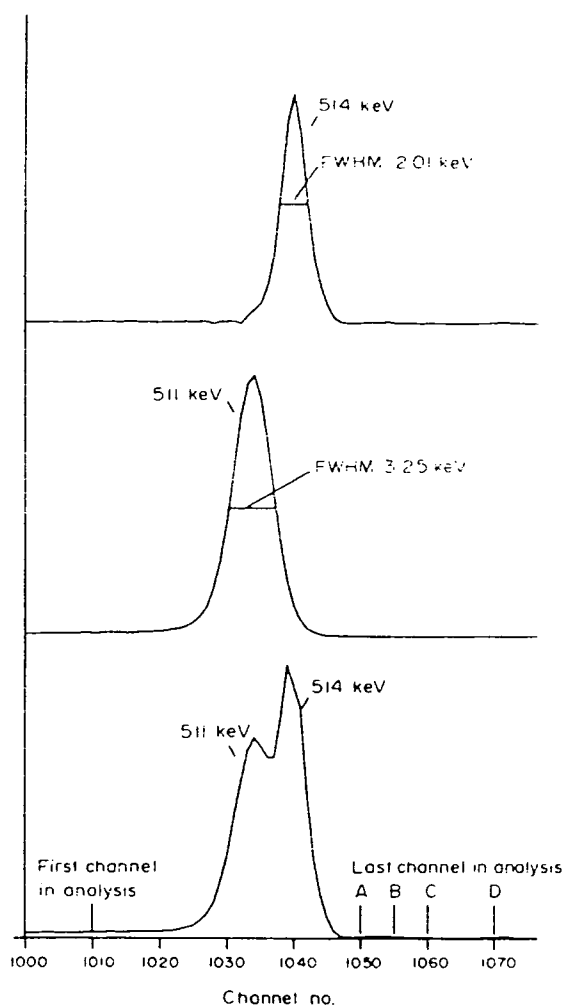


Fig. 4. Resolution comparison of the 511/514 keV lines showing increased peak width of the 511 keV annihilation radiation.

increased peak width of the 511 keV line, then its integration was underestimated and the 514 keV line overestimated by as much as 75% (See Table 3).

After these corrections were made to the GAMANAL figures for the  $^{85}\text{Sr}$  content in the  $^{82}\text{Sr}$  batches, it was found that all the analytical methods gave approximately the same ratio (i.e. 106% at the time of calibration).

Using this figure and those for  $^{85}\text{Sr}$  and  $^{86}\text{Sr}$  from Table 2, the total radiation dose to the bone tissue due to the breakthrough of strontium radionuclides from the generator have been calculated and appear in Table 4. These values were determined on the basis of the dosimetry study by Bigler *et al.* and clearly show that the majority of the radiation dose arises from the  $^{85}\text{Sr}$ / $^{82}\text{Rb}$  equilibrium. At the end of the generator "life" (approx. 100 days from the calibration date) the proportion of the longer-lived strontium radionuclides increases but  $^{82}\text{Sr}$  remains the predominant source of radiation dose.

### Conclusion

The  $^{82}\text{Sr}$  from Los Alamos is being extensively used in a  $^{82}\text{Rb}$  generator for myocardial studies using the PECAT and the results are very encouraging. The radiation dose to the patient has been calculated and is not regarded as excessive for the clinical information which is obtained.

Various analytical approaches have been made to estimate the proportion of other strontium radionuclides present, a mass separation technique being necessary for the assay of  $^{85}\text{Sr}$  and  $^{86}\text{Sr}$ . The various adopted methods employed for the measurement of the  $^{85}\text{Sr}$  content all give a value of around 106% at the time of calibration, and these are outlined below.

(a) Measuring the  $^{85}\text{Sr}$  when all of the  $^{82}\text{Sr}$  had decayed and correcting this data for decay to obtain a value at the Los Alamos date of calibration.

(b) Using a simple subtraction technique to correct the counts in the 511/514 keV lines for the  $^{82}\text{Sr}$  contribution using the published literature values for the 777 keV/511 keV ratio. A measurement with generator eluted  $^{82}\text{Rb}$  has shown this ratio to be 1:0.0914 (13.6% for 777 keV and 148.8% for 511 keV  $\pm$ ).

(c) Mass separating samples and individually measuring the collector foils of different mass number.

(d) Using a spectrum strip method with the aid of multichannel analyser that can store and retrieve the spectra of separated  $^{82}\text{Rb}$ .

(e) By examination of the  $^{82}\text{Sr}/^{85}\text{Sr}$  spectra using a computer programme which indicated that a discrepancy in the  $^{85}\text{Sr}/^{82}\text{Sr}$  ratio could be explained by the

Table 3. Quantitative analysis for the resolution of the 511/514 lines from the LANL  $^{82}\text{Sr}$  Batch SR-78684C

| Number of channels used for analysis (0.497 keV ch <sup>-1</sup> )* | Ratio of integrated counts for 511/514 |                                         |
|---------------------------------------------------------------------|----------------------------------------|-----------------------------------------|
|                                                                     | Similar peak widths                    | Adjusted peak widths                    |
|                                                                     | 511 514 2.5 keV (FWHM)                 | 511 514 3.25 keV (FWHM) 2.01 keV (FWHM) |
| 40 (A)                                                              | 1.22                                   | 2.50                                    |
| 45 (B)                                                              | 1.22                                   | —                                       |
| 50 (C)                                                              | 1.22                                   | —                                       |
| 60 (D)                                                              | 1.21                                   | 2.33                                    |

\* See Fig. 4.

Table 4. Radiation dosimetry for the breakthrough of strontium radionuclides from the  $^{89}\text{Sr}/^{90}\text{Rb}$  clinical generator  
Total radiation dose to bone tissue due to breakthrough†

| Radionuclide                    | Percentage on calibration date | Absorbed radiation dose to bone tissue for a 70 kg man*                          | Calibration date                             | ± 100 days                                   |
|---------------------------------|--------------------------------|----------------------------------------------------------------------------------|----------------------------------------------|----------------------------------------------|
| $^{89}\text{Sr}/^{90}\text{Rb}$ | 100%                           | $1.92 \times 10^{-4} \text{ Gy Bq}^{-1} (71.1 \text{ mrad } \mu\text{Ci}^{-1})$  | $1.9 \times 10^{-4} \text{ Gy (19 mrad)}$    | $1.9 \times 10^{-4} \text{ Gy (19 mrad)}$    |
| $^{90}\text{Sr}$                | ~106%                          | $2.05 \times 10^{-4} \text{ Gy Bq}^{-1} (76.6 \text{ mrad } \mu\text{Ci}^{-1})$  | $2.0 \times 10^{-4} \text{ Gy (20 mrad)}$    | $1.00 \times 10^{-4} \text{ Gy (10.9 mrad)}$ |
| $^{90}\text{Sr}/^{90}\text{Y}$  | ~0.02%                         | $4.00 \times 10^{-4} \text{ Gy Bq}^{-1} (14.8 \text{ mrad } \mu\text{Ci}^{-1})$  | $8.0 \times 10^{-4} \text{ Gy (0.08 mrad)}$  | $3.2 \times 10^{-4} \text{ Gy (0.32 mrad)}$  |
|                                 |                                | $1.59 \times 10^{-4} \text{ Gy Bq}^{-1} (59.00 \text{ mrad } \mu\text{Ci}^{-1})$ | $3.0 \times 10^{-4} \text{ Gy (0.3 mrad)}$   | $4.8 \times 10^{-4} \text{ Gy (4.8 mrad)}$   |
|                                 |                                | TOTAL = $2.14 \times 10^{-4} \text{ Gy (21.4 mrad)}$                             | $3.50 \times 10^{-4} \text{ Gy (35.0 mrad)}$ |                                              |

\* Data from Bigler *et al.* (22) and MIRD Pamphlets Nos 5 and 13 (19).  
† Assuming a litre infusion at  $\sim 30 \text{ ml min}^{-1}$  with a typical total breakthrough from the clinical generator of  $10 \text{ kBq of } ^{89}\text{Sr}$  ( $0.27 \mu\text{Ci}$ ).

fact that the width of the 511 keV line was not fully accounted for.

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# Measurement of Cerebral Blood Flow Using Bolus Inhalation of $C^{15}O_2$ and Positron Emission Tomography: Description of the Method and Its Comparison with the $C^{15}O_2$ Continuous Inhalation Method

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**Summary:** This article describes a rapid method for the regional measurement of cerebral blood flow using a single breath of  $C^{15}O_2$  and positron emission tomography. The technique is based on the bolus distribution principle and utilises a reference table for the calculation of flow. Seven subjects were studied using both this method and the  $C^{15}O_2$  continuous inhalation steady-state technique. The single-breath method gave flow values 20% higher than those obtained using the steady-state method. A simulation study was performed in an attempt to define the reasons for the difference between the two tech-

niques. Estimations were made of identified sources of error in the measurement of regional cerebral blood flow using the single-breath technique and compared with results from a similar study previously described for the steady-state technique. However, further comparative studies will be necessary to satisfactorily explain the difference between both techniques. **Key Words:** Continuous inhalation of  $C^{15}O_2$ —Positron emission tomography—Regional cerebral blood flow—Single-breath inhalation of  $C^{15}O_2$ .

The search continues for improved methods for measuring regional cerebral blood flow (rCBF) using techniques based on positron emission tomography (PET). Flow measurements are required to interpret the results of metabolic studies and in particular to define supply-demand relationships within cerebral tissue. In addition, a rapid means for measuring rCBF is needed. This would not only reduce the time scale of combined cerebral flow-metabolism studies but is essential for studying the transient cerebral vascular response to vasodynamic, chemical, and neurophysiological stimulation. The more common techniques developed to date for measuring rCBF with PET are the  $^{77}Kr$  clearance method described by Yamamoto et al. (1977), the  $C^{15}O_2$  continuous inhalation technique developed by Frackowiak et al. (1980), the  $H_2^{15}O$

clearance method reported by Huang et al. (1982), the ramp intravenous  $H_2^{15}O$  infusion technique of Ginsberg et al. (1982), and the intravenous  $H_2^{15}O$  bolus injection technique (Herscovitch et al., 1983; Raichle et al., 1983) initially developed by Raichle et al. (1981). The steady-state and clearance techniques require a condition of steady brain function during a scan period of several minutes. Hence these methods are unsuitable for monitoring rapid changes in cerebral vascular function.

This article describes a method for the rapid measurement of rCBF using PET and a systemic arterial bolus of  $H_2^{15}O$  produced by a single-breath inhalation of  $C^{15}O_2$ . The measurement used is based on the bolus distribution principle (Landau et al., 1955). The method developed in this study differs from that described in previous publications (Raichle et al., 1981; Ginsberg et al., 1982; Herscovitch et al., 1983; Raichle et al., 1983) in two respects. First, in our study a single breath of  $C^{15}O_2$  was used. Raichle and co-workers (Raichle et al., 1981, 1983; Herscovitch et al., 1983) employed an intravenous bolus injection of  $H_2^{15}O$ , while Gins-

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**Abbreviations used:** CBF, Cerebral blood flow; CV, coefficient of variation; CRT, cathode ray tube; PET, positron emission tomography; rCBF, regional cerebral blood flow.

berg et al. (1982) made use of a ramp infusion of H<sub>2</sub><sup>15</sup>O. Second, in contrast to the previous studies flow values were calculated using a reference table, a technique initially described by Kanno and Lassen (1979).

The uptake approach has been adopted since difficulties arise when attempting to use the tissue clearance of H<sub>2</sub><sup>15</sup>O to measure flow following systemic administration of tracers. These problems arise because the concentration of the recirculating H<sub>2</sub><sup>15</sup>O rapidly reaches that of the H<sub>2</sub><sup>15</sup>O in the tissue, at which time the recorded signal contains little or no flow information. In the study reported here, the bolus inhalation uptake technique was compared directly with the C<sup>15</sup>O<sub>2</sub> continuous inhalation steady-state method (Frackowiak et al., 1980).

### THEORY

The relationship between the time course of the arterial concentration of a diffusible radioactive tracer,  $a(t)$  (in  $\mu\text{Ci/ml}$ ), the cerebral blood flow (CBF) per unit volume,  $f$  (in  $\text{ml/ml/min}$ ), and the concentration of the tracer per unit volume of brain tissue,  $b(t)$  (in  $\mu\text{Ci/ml}$ ), is given by the convolution integral:

$$\begin{aligned} b(t) &= \int_0^t f a(s) e^{-(f/\lambda)(t-s)} ds \\ &= a(t) * f e^{-(f/\lambda)t} \\ &= \lambda a(t) * k e^{-kt} \end{aligned} \quad (1)$$

where  $k = f/\lambda$ ,  $\lambda$  is the equilibrium partition coefficient of the tracer between the tissue and the blood (ml/ml), the asterisk denotes the convolution integral, and  $s$  is the time variable of integration.

Integration of both sides of Eq. 1 between  $t_1$  and  $t_2$  gives

$$\int_{t_1}^{t_2} b(t) dt = \lambda \int_{t_1}^{t_2} a(t) * k e^{-kt} dt \quad (2)$$

When C<sup>15</sup>O<sub>2</sub> is administered as a single breath, the <sup>15</sup>O atoms exchange almost instantaneously with the oxygen of the water molecules in the lung under the influence of carbonic anhydrase (West and Dollery, 1962). With the use of H<sub>2</sub><sup>15</sup>O as the *in vivo* tracer, Eq. 1 can be solved by measuring  $a(t)$  and  $b(t)$  [corrected for the radioactive decay of <sup>15</sup>O ( $T_{1/2} \sim 2.1$  min)]. In the application of this model it is assumed that the partition coefficient for water ( $\lambda$ ) is equal to unity. Values of  $\int_{t_1}^{t_2} b(t) dt$  can be obtained by measuring the integral concentration of

H<sub>2</sub><sup>15</sup>O in the brain with a positron emission tomograph.

The component  $\int_{t_1}^{t_2} b(t) dt$  of Eq. 2 corresponds to the pixel value of the reconstructed tomogram obtained by the PET scanner during the period between  $t_1$  and  $t_2$ . This assumes that the time integral of the tomogram is given by the time integration of the projection data (Tsui and Budinger, 1978).

Continuous monitoring of the arterial blood concentration, using a detection system calibrated against the pixel response of the PET scanner, provides values of  $a(t)$ . Hence, values for the right-hand side of Eq. 2 can be calculated by inserting values for  $k$  between 0.01 and 2.00 in steps of 0.01. This provides a reference table with which the regionally measured pixel values (i.e., the left-hand side of Eq. 2) can be compared to determine the actual  $k$  values and hence the rCBF (Fig. 1). This approach is in essence the "early picture" method described by Kanno and Lassen (1979), which has been modified to scale the integral in absolute units.

### METHOD

The subject, while positioned within the PET scanner, exhaled to functional residual capacity and then inhaled 1 L of air containing between 70 and 100 mCi of C<sup>15</sup>O<sub>2</sub> through a mouthpiece. This was followed up to end expiration with room air. The 1-L anaesthetic bag containing the radioactive gas was surrounded by a 5-mm-

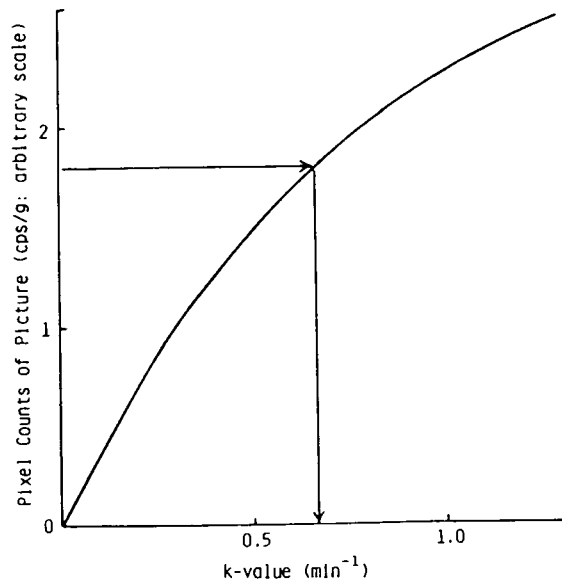


FIG. 1. Graphic representation of the reference table for the C<sup>15</sup>O<sub>2</sub> single-breath bolus distribution method. The curve represents the relation between pixel counts (ordinate) and CBF (abscissa). The pixel value was calculated by Eq. 2 using the arterial blood concentration curve measured by "beta counting" the continuously withdrawn arterial blood. The absolute arterial concentration was measured with a well counter calibrated against the positron emission tomographic scanner.

thick acrylic cylinder which absorbed the emitted positrons, thus protecting the skin and eyes.

Selection of the period of integration requires special consideration in order to optimise the signal (flow-related counts)-to-noise (tissue volume-related counts) ratio. Given the time course of the tracer in the tissue and the mechanical speed and sensitivity of the PET scanner used, this period was taken to be approximately 1.5 min starting 5 s after inhalation. Although ideally a shorter scanning time would probably be better, this could not be explored with the present relatively insensitive system. From statistical studies (Lammertsma et al., 1982), it was concluded that a reduction in count density would result in a large reduction in statistical precision.

A fine-gauge catheter was inserted into the radial artery at the beginning of the study and connected to manometer tubing (1.6 mm i.d.) which had been coiled on the surface of a plastic scintillator. This allowed the concentration of  $H_2^{15}O$  in the arterial blood to be measured by "beta counting" while being sampled using a constant-withdrawal pump. The tube length from the catheter to the coil was approximately 60 cm, and the speed of the pump (Harvard Apparatus Model 2206) was set at a constant-withdrawal rate of 10 ml/min. After recording the blood activity for 4–5 min, the tube was disconnected and an arterial blood sample withdrawn into a syringe. At this time the blood concentration was reasonably steady (Fig. 2). The concentration of this sample was measured with

a well counter that had been calibrated against the PET scanner as cps/ml in relation to cps/pixel. This sample, with an appropriate decay correction, in turn was used to scale the arterial curve in absolute units. The dispersion in the tube was measured experimentally using venous blood mixed with  $H_2^{15}O$ . The tube dispersion function  $d(r)$  obtained was used to correct the right-hand side of Eq. 2 (see the Appendix).

The plastic scintillator used for the  $\beta$ -ray detection was a Nuclear Enterprises type 102A, 3 mm thick and 20 mm in diameter. This detector has a high sensitivity for registering positrons but a low response to 511-keV annihilation photons. A lower energy discrimination threshold was used to reject the low energy continuum of scattered photons that emerged from the subject and penetrated the detector shielding. Only about 2 cm of lead was needed for the primary shielding around the detector. The arterial blood time activity data were recorded in 1-s intervals on a multichannel analyser (Hewlett-Packard model 4096).

The PET system used for the study was the whole-body ECAT II (EG&G Ortec). For these investigations, medium-resolution shadow shields and the medium-resolution scan mode were used (Phelps et al., 1978; Williams et al., 1979). This provides a sensitivity of  $12 \times 10^3$  cps  $\mu Ci/ml$  for a 20-cm-diameter uniform pool of activity. The scanner has a spatial resolution of 17 mm full width at half maximum in both the transverse and axial directions. A net scan time for data acquisition was set to 70 s. How-

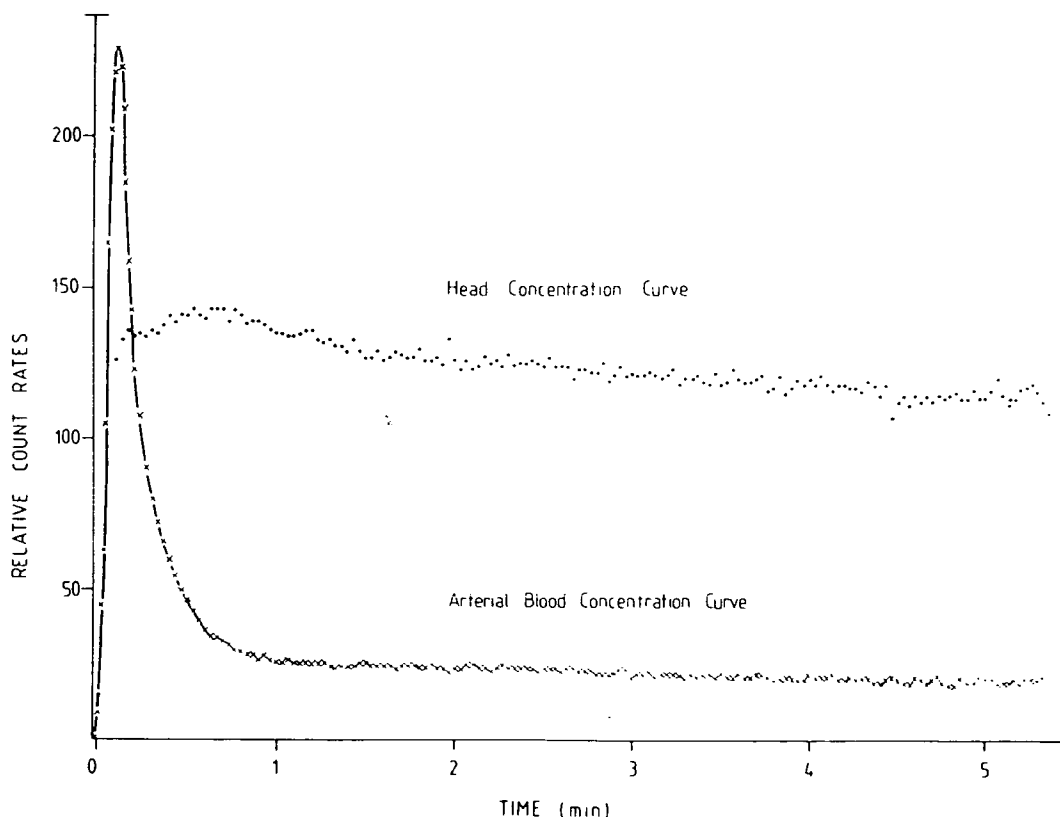


FIG. 2. Time course of the radioactivity concentration of the arterial blood and the head following a single breath of  $C^{15}O_2$ . Both curves were corrected for the decay of  $^{15}O$  ( $T_{1/2} = 2.1$  min). The arterial curve shows a steep rise and fall and then a steady level of recirculating activity. The head curve shows a very slow clearance, illustrating the difficulty in applying conventional clearance analysis techniques.



ever, this took a total of 90 s because of mechanical motion between different scan positions. The scan was started 5 s after the end of the single breath of C<sup>15</sup>O<sub>2</sub>. In addition to recording the arterial detector counts, the multichannel analyser also recorded the whole slice count rate from the PET scanner. An additional channel in this analyser was used to record the time at which the PET scan commenced. In this way the starting points of the head and blood data could be superimposed. Figure 2 shows a typical example of the arterial curve and PET true coincidence events (totals minus randoms). The total number of counts accumulated with the above procedure varied between 0.8 and 1.0 million per plane. In calculating rCBF, a pixel size of 2.5 × 2.5 mm<sup>2</sup> was used on the cathode ray tube (CRT) display and 7.5 × 7.5 mm<sup>2</sup> on the matrix printout. In addition to the C<sup>15</sup>O<sub>2</sub> scan a transmission scan was performed using an external <sup>68</sup>Ge ring source. The data from this scan were used to correct for attenuation in the C<sup>15</sup>O<sub>2</sub> scan.

### Subjects

Single-breath C<sup>15</sup>O<sub>2</sub> rCBF studies were carried out on three cerebral infarct patients, one brain tumour patient, and three normal volunteers. In all cases, they were followed immediately with the conventional steady-state C<sup>15</sup>O<sub>2</sub> rCBF study. The two measurements of rCBF were performed on the same anatomical plane. A total of nine planes in seven subjects were measured. Four planes were studied at a level of 4.5 cm above the orbitomeatal line (OM + 4.5 cm), two at OM + 6.0 cm, two at OM + 6.5 cm, and one at OM + 7.0 cm (Table 1).

### Analysis of errors

There are several possible factors which determine the accuracy of the C<sup>15</sup>O<sub>2</sub> single-breath technique: (1) the use of an incorrect value of the partition coefficient for water; (2) the limited extraction of water in a single pass, resulting in nonextracted intravascular (venous) H<sub>2</sub><sup>15</sup>O; (3) the contribution of activity contained within the arterial blood; (4) the inhomogeneity of tissue within the resolved

volume; and (5) the imposed statistical error. There are also possible technical errors that arise from (6) an inaccurate cross-calibration between the PET scanner and the well counter; (7) a nonlinearity of the count rates (deadtime) of the PET scanner; (8) an inaccurate correction for the dispersion of tracer within the tubing through which the arterial blood is withdrawn; and (9) misalignment, with respect to time, of the PET data and the recorded arterial curve. Of these factors, the first five intrinsic problems of the method were evaluated by simulation. The other factors will not be discussed here, since they can be avoided by careful selection of the instrumentation used and the design of the studies.

### Simulation

In the simulation, the right-hand side of Eq. 2 was expanded to include the terms involving the partition coefficient ( $\lambda$ ), the extraction fraction of water ( $E$ ), and the fractions of the total volume occupied by the arterial ( $V_a$ ) and venous ( $V_v$ ) blood and the tissue ( $V_t$ ):

$$A(f) = \int_0^f [(V_t + V_v\lambda)Efa(t)e^{-\lambda f} + V_v(1 - E)a(t) + V_a a(t)] dt \quad (3)$$

where  $V_v + V_a + V_t = 1$  and  $E = 1 - e^{-PS/f}$  (where  $PS$  is the permeability-surface area product). Each of the three itemised sources of error were evaluated by considering the difference in the values of rCBF obtained using both Eq. 2 and 3. These comparisons were carried out separately by keeping two of the three parameters,  $\lambda$ ,  $V_a$ , and  $E$ , constant and equal to the values used in Eq. 2.

The effect of the partition coefficient of the water ( $\lambda$ ) was examined under the condition that  $V_a = 0$ ,  $V_v = 0$ , and  $E = 1$ :

TABLE 1. Cerebral blood flow (CBF) studies measured by the C<sup>15</sup>O<sub>2</sub> single-breath (SB) method and the C<sup>15</sup>O<sub>2</sub> continuous inhalation (steady-state, SS) method

| Study no. | Subject | Age (years) | Level <sup>a</sup> | Diagnosis                  | Whole plane CBF |                 | Cortical tissue CBF |                 | Periventricular tissue CBF |                 |
|-----------|---------|-------------|--------------------|----------------------------|-----------------|-----------------|---------------------|-----------------|----------------------------|-----------------|
|           |         |             |                    |                            | SB <sup>b</sup> | SS <sup>c</sup> | SB <sup>b</sup>     | SS <sup>c</sup> | SB <sup>b</sup>            | SS <sup>c</sup> |
| 1         | G.K.    | 58          | 4.5                | Carotid stenosis           | 40.5            | 34.0            | 41.2                | 35.2            | 29.4                       | 22.8            |
| 2         | V.O.    | 33          | 4.5                | MCA stenosis               | 51.8            | 41.1            | 54.7                | 43.1            | 32.1                       | 28.3            |
| 3         | T.J.    | 41          | 4.5                | Normal                     | 49.6            | 35.3            | 53.1                | 36.6            | 24.3                       | 20.6            |
| 4         | T.J.    | 41          | 6.5                | Normal                     | 42.8            | 32.5            | 55.5                | 41.1            | 23.0                       | 17.7            |
| 5         | I.K.    | 35          | 4.5                | Normal                     | 40.4            | 41.7            | 42.9                | 46.3            | 32.0                       | 30.3            |
| 6         | I.K.    | 35          | 6.5                | Normal                     | 51.0            | 38.4            | 58.8                | 50.7            | 31.9                       | 23.4            |
| 7         | J.M.    | 54          | 7.0                | Microglioma                | 32.7            | 30.7            | 39.1                | 37.9            | 17.8                       | 16.2            |
| 8         | K.K.    | 33          | 6.0                | Normal                     | 42.9            | 40.9            | 46.8                | 44.9            | 32.1                       | 30.6            |
| 9         | A.G.    | 52          | 6.0                | Bilateral carotid stenosis | 48.8            | 34.4            | 50.4                | 36.5            | 32.0                       | 23.1            |

<sup>a</sup> Slice level shown by centimeters above the orbitomeatal line.  
<sup>b</sup> CBF in ml/100 ml/min by the C<sup>15</sup>O<sub>2</sub> single-breath method.  
<sup>c</sup> CBF in ml/100 ml/min by the C<sup>15</sup>O<sub>2</sub> continuous inhalation method.

$$A(f) = \int_{t_1}^{t_2} f a(t) * e^{-\lambda k t} dt \quad (4)$$

$A(f)$  was used as a value in the "look-up" reference table based on Eq. 2 to obtain a calculated  $k$  value ( $k_c$ ). This value was then converted to flow using the relationship  $f_c = \lambda k_c$ . The partition coefficient  $\lambda$  was varied from 0.8 to 1.1 in steps of 0.05.

The effect of limited extraction of the water in the capillary was examined under the condition that  $\lambda = 1$  and  $V_a = 0$ :

$$A(f) = \int_{t_1}^{t_2} [E a(t) * f e^{-f t} + V_v (1 - E) a(t)] dt \\ = \int_{t_1}^{t_2} [(1 - e^{-PS f}) a(t) * f e^{-f t} + V_v e^{-PS f} a(t)] dt \quad (5)$$

Similarly,  $A(f)$  in Eq. 5 was used as a value in the look-up reference table based on Eq. 2. However, instead of the extraction fraction  $E$ , the  $PS$  product was varied from  $PS = 100$  ml/100 g/min to infinity. The calculation was carried out for two values of the venous fraction ( $V_v$ ), 0.05 and 0.10, respectively.

The contribution of the arterial blood volume was evaluated assuming  $\lambda = 1$  and  $E = 1$ . The fraction of the tissue volume occupied by arterial blood was varied from 0 to 5% in 1% steps.

$$A(f) = \int_{t_1}^{t_2} [(1 - V_a) a(t) * f e^{-f t} + V_a a(t)] dt \quad (6)$$

$A(f)$  in Eq. 6 was used as a value in the look-up reference table for Eq. 2.

The effect of inhomogeneity of the tissues was evaluated using a compartment model which consisted of various mixtures of pure grey and pure white matter within a single image element:

$$A = W_1 A(f_1) + W_2 A(f_2) \quad (7)$$

where  $f_1$  and  $f_2$  are the flows of each component and  $W_1$  and  $W_2$  are the weighting factors such that  $W_1 + W_2 = 1$ . The flows determined for the summed value given by Eq. 7 were compared with the mean flow ( $f$ ), calculated as  $f = W_1 f_1 + W_2 f_2$ . The ratio of  $W_1$  to  $W_2$  was varied from 0 to 1 in steps of 0.05. The flows of each component were taken to be 70 and 20 ml/100 g/min.

The statistical error in the computation of flow can be determined from the statistical error in the original pixel counts. This approach was previously examined for the steady-state  $C^{15}O_2$  study (Lammertsma et al., 1982) and is also applicable for the single-bolus technique. This procedure assumes that changes in the distribution of radioactivity during the scan do not influence the statistical error of the tomogram. From the statistical error in the accumulated pixel value, the corresponding error in flow can be determined using Eq. 2 and appropriate values for  $k$ .

## RESULTS

From the total of nine planes studied it was not possible to obtain mean flow values for both pure

grey and white matter because, for the grey, the OM + 4.5 cm needed to be recorded and, for the white, OM + 6 – OM + 7 cm. Hence, mean hemispheric cerebral blood flow (CBF) values in each plane were considered representative of flow for the present study comparing the two methods. Where possible, mean CBF values for the cortical tissue, the periventricular tissue, and the whole plane were extracted. Mean CBF values were obtained by first calculating rCBF per pixel and then taking the average. Comparisons between the single-breath and the steady-state techniques are shown individually in Table 1 and summarised in Table 2. In calculating the mean CBF for the cortical tissue, the data within the frontal and occipital poles were excluded to avoid unknown contributions from the arterial and venous blood. For the cortical and the periventricular tissue, the single-breath technique gave values for CBF 18.9 and 19.5% higher than those obtained using the steady-state technique. The mean values for the entire planes showed the following relation between the two methods:

$$CBF_{SB} = 1.19 CBF_{SS} + 3.85 \text{ ml/100 ml min} \\ r = 0.90 (p < 0.001) \quad (8)$$

where  $CBF_{SB}$  is the CBF with the single-breath technique and  $CBF_{SS}$  is the CBF with the steady-state technique (Fig. 3). A statistical test on the slope of this relation in comparison to 1 (the line of identity) showed that the two methods were different, with a significance level of 0.005.

The rCBF data were further analysed by plotting profiles from the printout matrices for rCBF. For each plane, CBF profiles were plotted as cortical strips for each plane for both the left and right hemispheres (Fig. 4, top left). Each point of the profile is the mean of pairs of adjacent matrix elements, each element corresponding to an area  $7.5 \times 7.5$  mm<sup>2</sup>. The left and right profiles obtained from the single-breath and the steady-state techniques were superimposed on the same drawing. Similarly rCBF profiles along the coronal lines and the sagittal lines were obtained for the two methods (Fig. 4, left and top right). By comparing corresponding pixel values along the profile, correlation coefficients between the single-breath and the steady-state methods were calculated for each profile (Table 3). For the cortical profile data, 8 of the 18 profiles gave a correlation of  $>0.8$  for both hemispheres. In the coronal and sagittal profiles, of 36 data sets only 2 had a correlation of  $<0.8$ . One reason for such differences between the cortical contour and the coronal-sagittal line was that the former was very sensitive to the misalignment in repositioning the head in the

TABLE 2. Comparison of mean cerebral blood flow by both C<sup>15</sup>O<sub>2</sub> methods

| Region                 | Single-breath | Steady-state | Ratio |
|------------------------|---------------|--------------|-------|
| Whole plane            | 44.52         | 36.56        | 1.220 |
| Cortical tissue        | 49.17         | 41.36        | 1.189 |
| Periventricular tissue | 28.29         | 23.67        | 1.195 |

two studies. However, these CBF profiles were of use in identifying systematic differences between the two techniques rather than in the direct visual comparison of rCBF images (Fig. 5).

The results from the simulation of the systematic errors are shown in Figs. 6–9. These figures indicate that the errors move in directions similar to those in the steady-state method (Lammertsma et al., 1981a), but in general the range of the error is seen to be smaller. Using a 5% increase in the partition coefficient gave flow values 1 and 3.5% higher at flow rates of 20 and 70 ml/100 ml/min, respectively (Fig. 6). By varying the value of the *PS* product, the effect of limited water extraction was seen for low *PS* products (Fig. 7). With increasing venous blood volume, the effect was reduced. The contribution of the arterial blood volume to the flow signal was almost constant over the wide range of flow values considered and was almost proportional to the blood volume fraction itself. An arterial frac-

tion of 5% resulted in an approximate 5% overestimation of flow (Fig. 8). An underestimation of mean tissue flow results when mixtures of grey and white matter reside within a resolution element (Fig. 9). This effect was found to be smaller than with the steady-state technique. When considering a grey matter flow of 70 ml/100 ml/min and a white matter flow of 20 ml/100 ml/min, a maximum underestimation of 9% occurred for a white/grey ratio of 0.4.

Statistical errors were evaluated using the pixel value of the plane and referring to the experimental statistical response data of Lammertsma et al. (1982). In the present single-breath C<sup>15</sup>O<sub>2</sub> study, typical count densities were 3,000 and 1,500 counts per pixel for the cortical tissues and periventricular tissues, respectively. Relating these count densities to those in Fig. 2 of the Lammertsma article showed that the corresponding coefficients of variation (CVs) were 4.4–4.7%. From these pixel CVs, the corresponding flow CVs were obtained from the count–flow relationship shown in Fig. 1.

## DISCUSSION

The single-breath C<sup>15</sup>O<sub>2</sub> procedure is an attractive means for pulse-labelling the arterial blood with H<sub>2</sub><sup>15</sup>O. The instantaneous shift of the <sup>15</sup>O from the gas phase to H<sub>2</sub>O in the lung (West and Dollery, 1962) means that the protraction of an intravenous bolus of H<sub>2</sub><sup>15</sup>O that results from transit through the right side of the heart is avoided. In addition, radiochemical sterility problems associated with producing H<sub>2</sub><sup>15</sup>O for i.v. injection are avoided.

The general systematic administration of H<sub>2</sub><sup>15</sup>O results in a high level of recirculation such that traditional "washout" or "clearance" measurements are difficult. The tracer rapidly redistributes between the high and low flow tissue with the flow

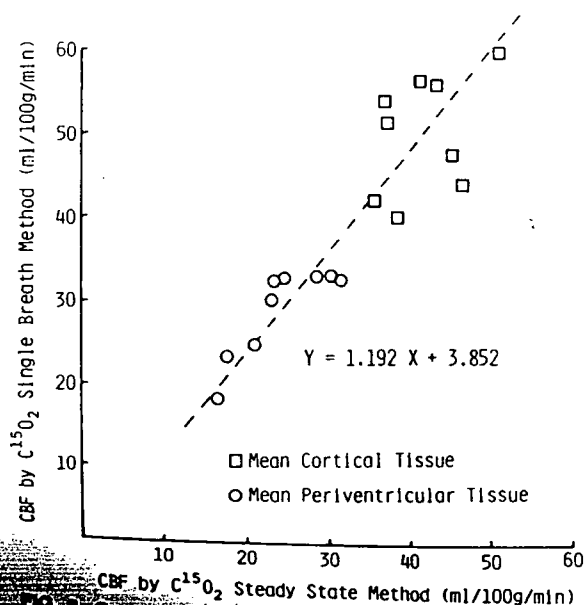


FIG. 1. Comparison of CBF values measured by the C<sup>15</sup>O<sub>2</sub> single-breath (ordinate) and steady-state (abscissa) methods. The dashed line shows the average value for the global regions. The squares and circles represent the average values for the cortical tissues and the periventricular tissues studied in the present work.

TABLE 3. Correlation coefficients between the two C<sup>15</sup>O<sub>2</sub> methods in the CBF profiles

| Study no. | Cortical contour |       | Coronal line |           | Sagittal line |       |
|-----------|------------------|-------|--------------|-----------|---------------|-------|
|           | Left             | Right | Anterior     | Posterior | Left          | Right |
| 1         | 0.47             | 0.83  | 0.95         | 0.96      | 0.72          | 0.94  |
| 2         | 0.67             | 0.89  | 0.96         | 0.84      | 0.82          | 0.94  |
| 3         | 0.65             | 0.67  | 0.91         | 0.87      | 0.85          | 0.81  |
| 4         | 0.84             | 0.48  | 0.89         | 0.96      | 0.84          | 0.92  |
| 5         | 0.55             | 0.52  | 0.86         | 0.87      | 0.93          | 0.77  |
| 6         | 0.73             | 0.85  | 0.85         | 0.98      | 0.87          | 0.81  |
| 7         | 0.87             | 0.89  | 0.95         | 0.88      | 0.92          | 0.96  |
| 8         | 0.74             | 0.77  | 0.85         | 0.97      | 0.87          | 0.94  |
| 9         | 0.93             | 0.96  | 0.95         | 0.89      | 0.94          | 0.81  |

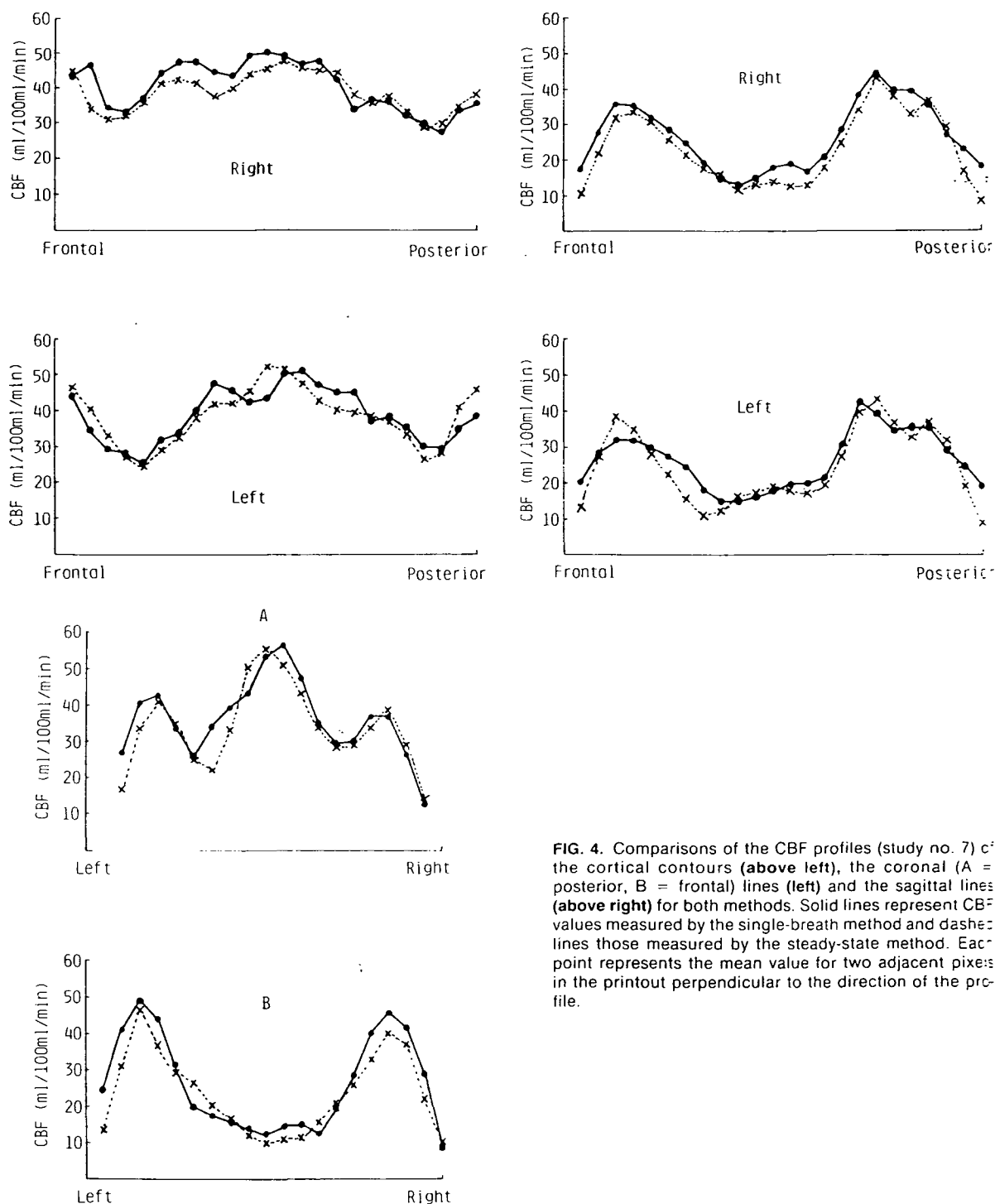


FIG. 4. Comparisons of the CBF profiles (study no. 7) of the cortical contours (above left), the coronal (A = posterior, B = frontal) lines (left) and the sagittal lines (above right) for both methods. Solid lines represent CBF values measured by the single-breath method and dashed lines those measured by the steady-state method. Each point represents the mean value for two adjacent pixels in the printout perpendicular to the direction of the profile.

information in the tissue signal rapidly becoming attenuated. We therefore adapted the bolus distribution principle using only the early uptake phase (Landau et al., 1955) rather than the clearance anal-

ysis using the whole kinetics (Huang et al., 1982). The tracer model used for the analysis was based on the early picture method described by Kanno and Lassen (1979). This technique has been modi-

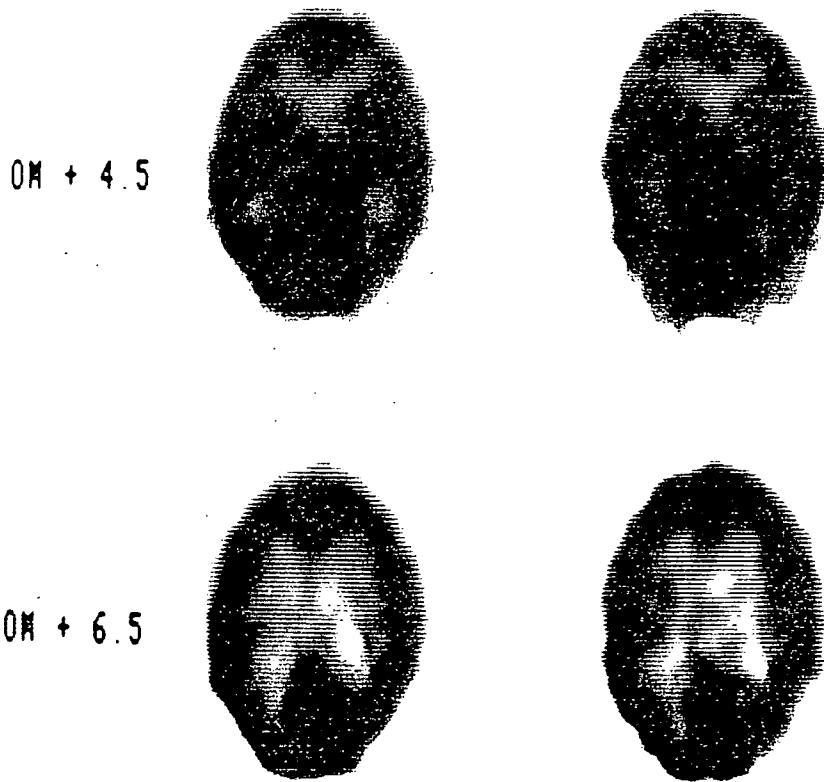


FIG. 5. Cerebral blood flow images measured at the level of the orbitomeatal line (OM) + 4.5 cm (top) and OM + 6.5 cm (bottom) by the C<sup>15</sup>O<sub>2</sub> single-breath method (right) and the C<sup>15</sup>O<sub>2</sub> steady-state method (left) in the same patient.

fied to deal with absolute tissue uptakes. It is different from procedures in previous reports (Raichle et al., 1981, 1983; Ginsberg et al., 1982; Herscovitch et al., 1983) in the way the tracer is administered

and in the computational algorithm based on a reference table.

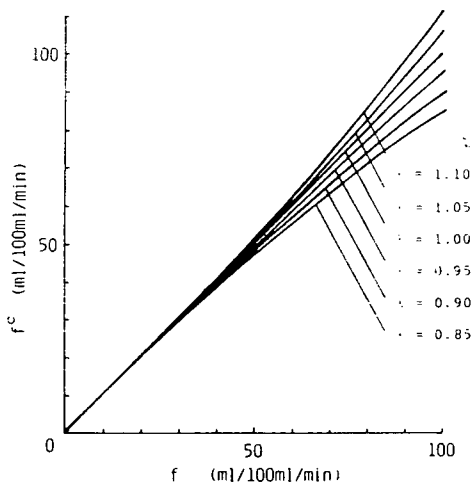


FIG. 6. Errors due to an incorrect partition coefficient evaluated by simulation studies. The single-breath CBF value ( $f^c$ ) (ordinate) and the given (true) CBF value ( $f$ ) (abscissa) are plotted for varying partition coefficients from 0.85 to 1.10 in increments of 0.05.

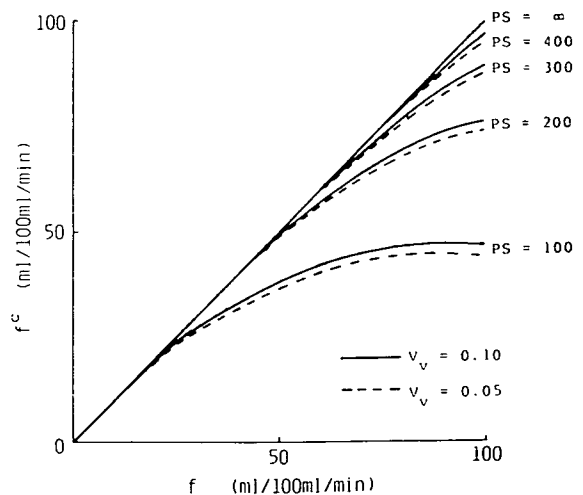


FIG. 7. Errors due to the limited extraction of water, assuming the partition coefficient is 1 and the arterial blood volume is 0. The true CBF ( $f$ ) is given on the abscissa and the CBF calculated from the reference table ( $f^c$ ) on the ordinate. The extraction of water ( $E$ ) is related to the permeability surface area product ( $PS$ ) according to  $E = 1 - e^{-PS/f}$ . The value of  $PS$  was varied from 100 ml/100 g/min to infinity. Two values of venous blood volume ( $V_v$ ) were used: 5% (dashed lines) and 10% (solid lines).

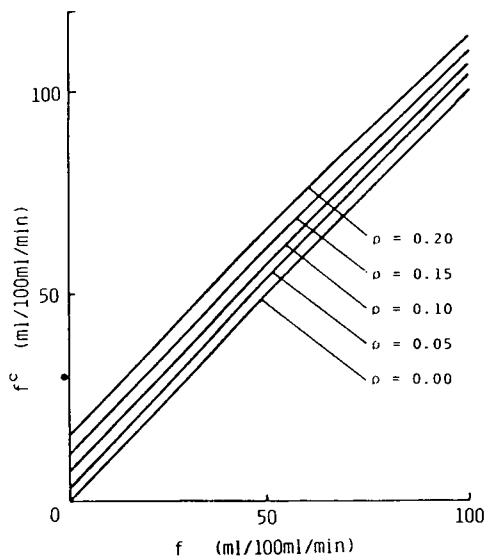


FIG. 8. Errors due to the contribution of the arterial blood volume, fixing other parameters, i.e., the partition coefficient is 1 and the extraction of water is 1. The true CBF ( $f$ ) is given on the abscissa and the CBF calculated from the reference table ( $f^c$ ) on the ordinate. The fraction of the arterial blood volume ( $p$ ) was varied from 0 to 0.2 in increments of 0.05.

#### Comparison with the steady-state method

In the present study we observed a significantly ( $p < 0.005$ ) higher value of rCBF using the single-breath method than with the steady-state method. One reason for the difference might be that the single-breath procedure requires more cooperation, and hence the subjects are more aroused than in the steady-state study. In addition, a breath hold, even as short as 10 s, immediately after  $C^{15}O_2$  inhalation might increase  $P_aCO_2$ . These states of arousal and the possible elevation of  $P_aCO_2$  would tend to increase the CBF. In practice the breath hold is not necessary, since most of the inspired  $C^{15}O_2$  exchanges instantaneously with the water pool.

It is doubtful, however, if these reasons could fully explain why the rCBF values were 20% higher than those obtained by the single-breath method. This was the main motivation in carrying out a simulation study for evaluating systematic errors. This analysis showed that most systematic errors were in the same direction as, but smaller than, those of the steady-state method. Only the overestimation due to the presence of arterial activity is higher in the single-breath method, the overestimation in the steady-state technique normally being negligible (Lammertsma and Jones, 1983).

In particular, the underestimation of mean flow due to the inhomogeneity of components in a resolution element was much lower than that in the

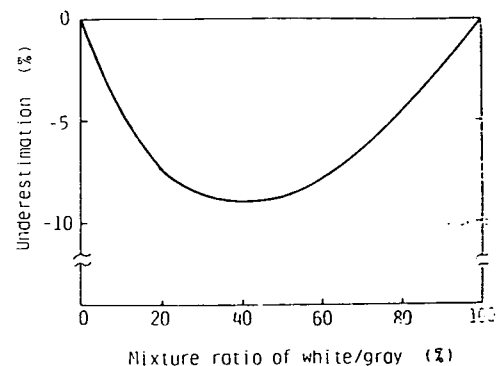


FIG. 9. Underestimation of CBF due to the mixture of inhomogeneous tissues in the same pixel. The simulation curve is given by Eq. 7 in the text, where  $f_1 = 70$  ml/100 ml/min as grey matter flow and  $f_2 = 20$  ml/100 ml/min as white matter flow. The abscissa gives the white/grey ratio. The ordinate represents the difference between the weighted mean and that calculated from the reference table.

steady-state method (Lammertsma et al., 1981b). A 9% underestimation at maximum was half that of the steady-state method. This possibly explains part of the overestimation of rCBF by the single-breath method in comparison with the steady-state technique.

An incorrect partition coefficient was shown to induce the same effect as in the steady-state method. Limited extraction of water resulted in less underestimation than in the steady-state method. The results of the simulation indicate that the observed higher values for flow in the single-breath method imply an underestimation in the steady-state method. More experimental clinical studies are necessary to establish this point.

#### Limitations of the PET device

It is clear from the reported experience with the ECAT II body PET scanner that the sensitivity of this generation of machines (12 kcps/ $\mu$ Ci/ml) is not optimal for transit studies of this nature. The sensitivity and mechanical movement should be such that data can be collected over a shorter time window of about 40 s following inhalation, during which the tissue activity reaches maximum (see Fig. 2). This would significantly improve the flow signal contained within the emitted data.

#### Arterial sampling

The dispersion of blood within the sampling tube was found to be insignificant in the present method. In the simulation, the distortion of Eq. A2 in the Appendix caused less than a 2.5% overestimation of flow at flow values greater than 20 ml/100 ml/min. An alternative approach would be to monitor

the lung or left ventricular activity externally, assuming that this signal would be proportional to the arterial concentration. Such a possibility awaits "time of flight" techniques for measuring intrathoracic activity to avoid the chest wall contribution.

## APPENDIX

### Measurement and correction for dispersion in the arterial blood sampling tube

The dispersion effect of the sampling tube on the arterial blood time-activity curve was experimentally measured under the same conditions as those used for the rCBF studies. A mixture of 20 ml of heparinised blood containing 4 mCi H<sub>2</sub><sup>15</sup>O was prepared. The catheter, sampling tube, and beta detector were set up as for the human studies. The lines were initially filled with saline. The tip of the catheter was submerged in inactive blood, and the withdrawal pump was switched on. The catheter tip was moved into the active blood after ~30 s. The count rate of the beta detector was recorded using the multichannel analyser. From the build-up phase of the recorded curve, the response function to the step input was determined. We defined the dispersion function  $d(t)$  as the time derivative of this function. Four experimental determinations of  $d(t)$  gave

$$d(t) = 24.7e^{-34t} + 0.82e^{-3t} \quad (A1)$$

where  $d(t)$  was normalised such that  $\int_0^\infty d(t)/dt = 1$ .

Correction for  $d(t)$  in this calculation of Eq. 2 was possible mathematically using the Laplace transform. The following terms were employed:

Dispersion function:  $d(t) = W_1e^{-k_1t} - W_2e^{-k_2t}$

Measured arterial curve:  $g(t) = a(t)*d(t)$

Tissue impulse response:  $h(t) = ke^{-kt}$

$A(S)$ ,  $D(S)$ ,  $G(S)$ , and  $H(S)$ : Laplace transforms of  $a(t)$ ,  $d(t)$ ,  $g(t)$ , and  $h(t)$ , respectively

Using these expressions,  $a(t)*ke^{-kt}$  in the integral of the right-hand side of Eq. 2 can be rewritten and solved as follows:

$$\begin{aligned} a(t)*h(t) &= \mathcal{L}^{-1}[A(S)H(S)] \\ &= \mathcal{L}^{-1}\left[\frac{G(S)H(S)}{D(S)}\right] \\ &= \beta_1g(t) + \beta_2g(t)*h(t) + \beta_3g(t)*r(t) \\ &\quad + \beta_4g(t)*h(t)*r(t) \end{aligned} \quad (A2)$$

where  $r(t) = \alpha e^{-t}$ ;  $\alpha = k_1k_2/(W_1 + W_2)$ ;  $\beta_1 = k/(W_1 + W_2)$ ;  $\beta_2 = (k_1/k_2)/(W_1 + W_2) - \beta_1$ ;  $\beta_3 = -\beta_1$ ;  $\beta_4 = 1 - (k - k_1 - k_2)/(W_1 + W_2)$ ; and  $\mathcal{L}^{-1}$  represents the inverse Laplace transform. Equation

A2 was used with Eq. 2 in calculating the reference table. This calculation does not enhance the statistical noise, unlike the usual deconvolution of  $d(t)$  from  $g(t)$ .

Finally a simulation study was carried out to evaluate the effect of such a dispersion on the CBF. If we ignore the dispersion in Eq. 2, that is, treat the measured  $g(t)$  as the true arterial curve  $a(t)$ , the integral on the right-hand side of Eq. 2 will decrease more for low values of  $k$ . However, it was found that the overestimations of CBF caused by this decrease in the reference table value were quite small, 2.5 and 1.5% at flows of 20 and 70 ml/100 ml/min, respectively. Thus, the error caused by the dispersion of the sampling tube, even if neglected, was revealed to be a minor factor.

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Scientific Note

## Blood flow in the feet of diabetic patients measured with a MWPC positron camera and inhalation of $C^{15}O_2$

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### 1. Introduction

Two basic detector configurations have been employed in *in vivo* positron emission tomography. One consists of a ring or polygon of detectors for the imaging of pre-selected slices of the body. Examples of this are the EG&G Ortec ECAT II (Phelps *et al* 1978), a single plane device employing a hexagon of 66 NaI detectors, and PETT VI, the design of Ter-Pogossian *et al* (1982) which consists of four rings each of 72 CsF detectors simultaneously yielding seven tomographic sections. The second type consists of opposed planar detectors which provide images of a larger section of the body from which desired planes may be subsequently selected at will. The Anger camera system described by Muehllehner *et al* (1976) and the device using two arrays of 127 NaI detectors devised by Burnham and Brownell (1972) are examples in this category.

The ability of planar detector systems to image a larger section of the body potentially enables more efficient use to be made of scanning time and administered dose. However, the limited field of view even of current Anger cameras and the high cost of significantly increasing their size has prompted the development of cheaper large-area devices such as those employing multiwire proportional chambers (MWPC). Detectors of this design for positron imaging have been described by Jeavons *et al* (1980) and Bateman *et al* (1980). The latter has been employed to investigate blood flow using the continuous inhalation of  $C^{15}O_2$ , a technique in routine use at the Medical Research Council Cyclotron Unit employing an EG&G Ortec ECAT II (Frackowiak *et al* 1980, Hopkins *et al* 1983). The radioactive gases are produced on line in the cyclotron. The positron camera used has an intrinsic spatial resolution of 6 mm (FWHM) (Bateman *et al* 1980) but, as will be discussed below, the effective resolution for the images presented was lower than this due to relatively poor contrast and statistics.

Tomographic devices designed to image transaxial slices through the body minimise scattered radiation by collimation. For area devices a much greater proportion of scattered radiation contributes to the image but the positron camera used in this study has been designed to be relatively insensitive to photons of energy less than 511 keV (Ott *et al* 1983), which is the opposite of the case for the Anger camera. Scatter

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contributes a broadcast (very low spatial frequency) background in the image and although this reduces contrast, imaging ability is retained in the form of unscattered events. For preliminary studies it was considered that imaging of the limbs, where scattering is as low as possible and sensitivity is important, would be more appropriate. This paper describes a study of blood flow in the feet of patients suffering from diabetes to investigate the extent of necrotic areas. The camera, with its associated computing, display facilities and gas rig, was built into a mobile container for convenient transfer to the Hammersmith Hospital site, with its source of isotopes from the MRC Cyclotron. This system was found to operate very satisfactorily.

## 2. Materials and methods

### 2.1. Positron camera

The positron camera consisted of two opposed detectors counting in coincidence with a resolving time of 52 ns. Each detector was made up of a stack of 20 multiwire proportional chambers (Bateman *et al* 1980). The  $x$ - $y$  coordinates of each event were determined by signals from mutually orthogonal lead (cathode) strips and the particular (anode) wire plane triggered specified the  $z$  coordinate (essential to avoid parallax errors in image reconstruction due to the finite detector depth).

The active area of each detector was  $30 \times 30 \text{ cm}^2$  although a limit was placed on the angle that recorded rays could make with the  $z$ -axis to ensure uniform detection efficiency over a volume of  $15 \times 15 \times 15 \text{ cm}^3$  in the centre of the field of view. This procedure was essential for the implementation of the deconvolution procedure (Townsend *et al* 1978).

The intrinsic spatial resolution was measured by the use of line sources of  $^{22}\text{Na}$ , contained in steel tubes of diameter 1 mm and length 50 mm, placed in the centre of the field of view. The reconstruction showed a FWHM of 6 mm (Bateman *et al* 1980). For an acceptable signal-to-noise ratio in imaging the activity distribution in patients, the rate limit is about 2000 cps in the fast coincidence rate, half of which are accepted in the image. This matched well with the direct data acquisition rate on to magnetic tape.

The part of the body to be imaged was placed centrally between the detectors, whose centres were separated by 43 cm (figure 1). Since the uptake of activity in these

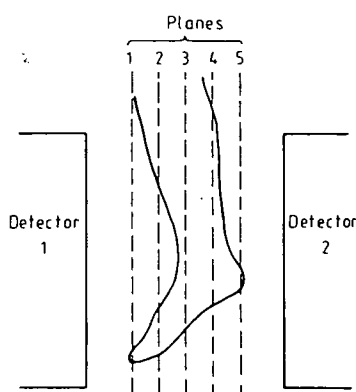


**Figure 1.** Approximate relationship between patient's foot, imaged planes and detectors (plan view). Spacing between planes = 4 cm.

studies was not confined to the region to be imaged, the detectors had to be shielded. If  $N_s$  is the 'singles' counting rate, assumed the same for each detector, and  $\tau$  the coincidence resolving time, then the random coincidence rate is given by  $N_s^2\tau$ . This squared dependence emphasises the importance of reducing the gamma-ray 'background' outside the field of view of the detectors. Each was therefore shielded around the sides and back with 5 cm thick lead bricks and additional shielding was placed around the pipes carrying radioactive gases from the cyclotron.

## 2.2. Data collection and processing

Steady-state images were obtained from continuous inhalation of  $C^{15}O_2$ . The label is transferred to  $H_2^{15}O$  in the lungs enabling regional tissue blood flow to be determined. For steady state inhalation of  $C^{15}O_2$  we can apply the equation (Frackowiak *et al* 1980):

$$FC = \left[ \frac{F}{V} + \lambda \right] Q \quad (1)$$

(since input of activity equals output), where  $F$  is the blood flow ( $ml\ min^{-1}$ ),  $C$  the arterial concentration of labelled water ( $MBq\ ml^{-1}$ ),  $Q$  the activity in tissue volume  $V$  ( $MBq$ ), and  $\lambda$  the decay constant of  $^{15}O$  ( $0.336\ min^{-1}$ ).

Arterial sampling was not carried out on the patients in this study so that absolute measurement of blood flow was not possible. However, from work using the Ortec ECAT II (Hopkins *et al* 1983) average blood flow per unit volume ( $F/V$ ) in diseased and normal resting legs of 16 patients with ulceration or liposclerosis was found to be  $0.073$  and  $0.011\ ml\ ml^{-1}\ min^{-1}$  respectively. In both cases,  $F/V$  is small compared with  $\lambda$  and equation (1) can be approximated to

$$Q = FC/\lambda \quad (2)$$

that is, activity is proportional to flow.

Patients wore a light, disposable plastic mask through which  $C^{15}O_2$  diluted with air, was breathed; gas was delivered at a rate of  $500\ ml\ min^{-1}$  with a specific activity of  $0.5\ MBq\ ml^{-1}$ . Data accumulation was begun after an equilibrium period of  $6 \pm 1\ min$  (from the start of inhalation) when a steady count rate had been achieved. The mean counting time was  $474 \pm 43\ s$  and was limited both by the dose received by the patients and their ability to remain still. The dose equivalent received by the lungs (the critical organ) was  $16\ mSv$  and the data rate corresponded to a steady state activity of  $3 \pm 0.5\ MBq$  in the field of view.

Prior to data accumulation, the following quantities were measured: (i) total 'singles' counting rate in each detector, (ii) total fast coincidence events, (iii) 'good' coincidence events actually contributing to the image, and (iv) accidental events associated with categories (ii) and (iii). Accidental coincidences were measured by delaying the signals from one detector relative to those from the other. For patients in this study  $3.0 \pm 0.1 \times 10^5$  coincidence events contributed to the image of which 25% were accidental coincidences.

In the manner described above (section 2.1)  $x$ ,  $y$  and  $z$  coordinates were attributed to each coincidence event and images were obtained by back projection. Raw images were obtained by forming histograms on the intersections of the positron decay vectors with ( $x$ - $y$ ) planes (parallel to the detector planes) whose position and spacing were chosen by the operator. Images were firstly smoothed and deconvoluted (filtered) with the point response function to enhance the contrast. Each of these planes contained

a 'blurred' contribution from activity outside the plane and a mathematical technique was applied to subtract this 'out-of-focus' contribution (Townsend *et al* 1978). In these preliminary studies no correction for tissue attenuation was applied. Five *x-y* planes were reconstructed for each scan, one mid-way between the detectors and two equally spaced 4 cm either side. The position and spacing of the planes could be selected retrospectively. The computer used was an LSI 11-23 with 256 kbytes of main memory and associated rigid disc, floppy disc and magnetic tape drives. The positron camera operated under computer control at all times, data being taken into memory on an event-by-event basis into a buffer store which, when full, was normally written directly to magnetic tape. For fast image processing an Analogic AP-400 array processor was utilised to carry out the Fourier transform calculations and operated at a rate of about two seconds per plane.

### 2.3. Description of patients

Two male patients suffering from diabetes who had developed gangrene of the feet were scanned prior to surgery. The procedure was approved by the research ethical committee of the Royal Postgraduate Medical School and the United Kingdom Administration of Radioactive Substances Advisory Committee. Both patients gave informed consent. Patient No 1 had gangrene in the fourth toe of the right foot which extended some way back into the foot (figure 2(a)). Patient No 2 had gangrene in the big toe of the left foot (figure 3(a)).

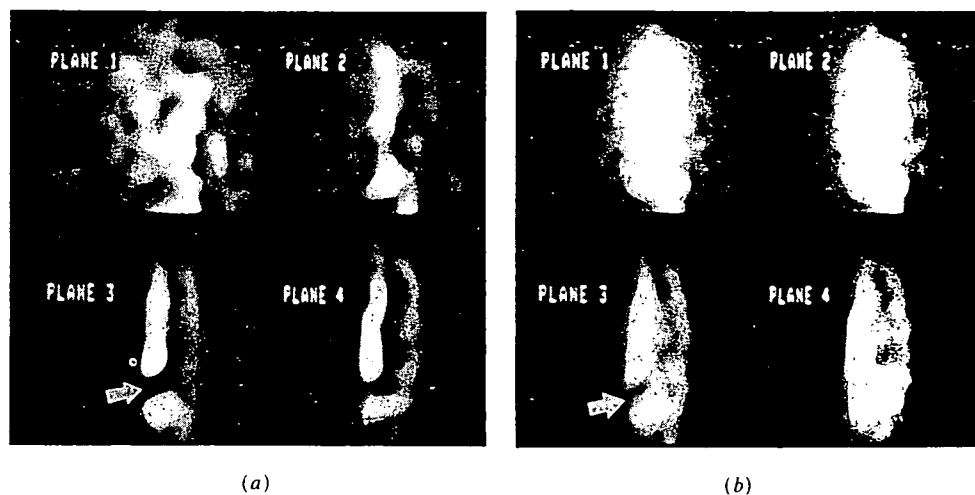


**Figure 2.** (a) Planes 1 to 4 for patient No 1 filtered and processed by the method of Townsend *et al* (1978) to remove off-plane 'blurring' (necrotic region shown arrowed). (b) Planes 1 to 4 for patient No 1 filtered but unprocessed.

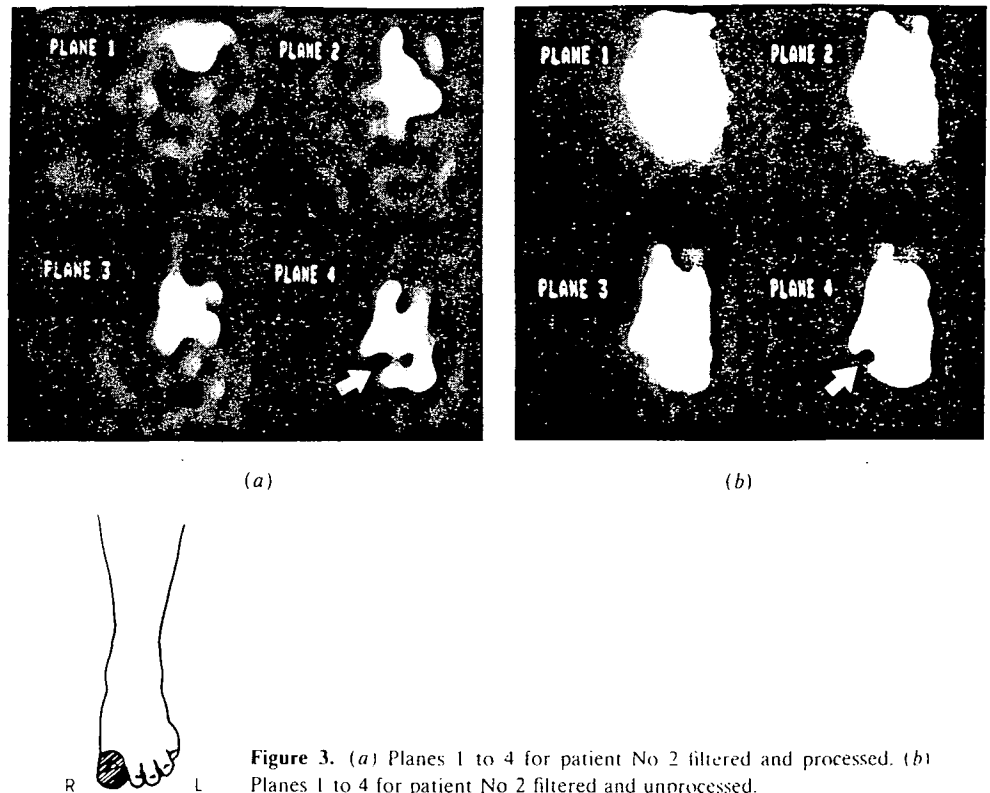


Figure 3. (a) Planes 1 to 4 for patient No 2 filtered and processed. (b) Planes 1 to 4 for patient No 2 filtered and unprocessed.

### 3. Results

The approximate relation between the imaged planes and the patient's foot is given in figure 1. Figure 2(a) shows  $C^{15}O_2$  (steady-state) images for patient No 1 which were filtered and processed using the algorithm of Townsend *et al* (1978). The region displayed extends from toes to ankle (for both patients). A necrotic region is clearly visible in plane 3 (arrowed) and the progressive focusing of this structure is apparent for successive planes (plane 5 is omitted because this region is not clearly displayed—similar to plane 2). Flow in the right side of the foot and in the unaffected toes is approximately twice that in the left side. The maximum flow (3.5 times the average for the whole foot in plane 3) occurs immediately above the gangrenous region. Figure 2(b) shows, for comparison, the images before subtraction of off-plane 'blurring'.

Planes 1 to 4 for patient 2 are shown in figure 3(a). In this case plane 4 gives the clearest demonstration of the gangrenous big toe (again arrowed). As in the case of patient No 1, there is a region of relatively high flow in the adjacent toes and even higher flow in the region just above the necrotic area (2.4 times the average). During subsequent surgery the latter was found to be the site of an abscess. Figure 3(b) similarly shows the unprocessed images.

### 4. Discussion

The MWPC camera used in this study has successfully delineated regions of impaired blood flow using the steady state  $C^{15}O_2$  inhalation technique with an acceptable

radiation dose and scanning period. The scans on both diabetic patients studied, shown in figures 2(a) and 3(a), demonstrate the striking way in which different structures are focused by moving from plane to plane. Areas of impaired blood flow (arrowed) are obvious from figures 2(b) and 3(b) before subtraction of off-plane 'blurring' but are made clearer after subtraction (figures 2(a) and 3(a)). Mean counts per pixel in these areas were reduced from 30% of the image maximum values to less than 5% by this process. This corresponded well with known areas from which the blood supply had been cut off, although the filtering process introduced a more mottled appearance in the background where maximum pixel counts on average rose from 12% to 15% of the image maximum. However, the qualitative rather than quantitative nature of the images should be emphasised at this stage. The cut-off frequency ( $F_C$ , expressed as harmonics of the 30 cm field) on the low pass filter must often be set to give lower spatial resolution than the 'physical' resolution of the system (corresponding approximately to  $F_C = 15$ ) due to poor contrast and statistics. For the images presented, a cut-off value of 10 was used, which corresponds to a spatial resolution of 12–14 mm (FWHM). If a value of  $F_C = 15$  were chosen, potentially more detail could be observed but noise oscillations would increase and there would be a danger of introducing confusing artefacts (Bateman *et al* 1982).

Designs of single and multiple-slice transaxial positron tomographs recently described (Burnham *et al* 1983, Hoffman *et al* 1983) have intrinsic spatial resolutions comparable with that of the positron camera employed in this study. However, the collimation inherent in these devices leads to relatively inefficient use being made of emitted rays because of the small solid angle of collection. The positron camera has a much wider angle of acceptance and although this is inevitably associated with a greater number of Compton scatter coincidence counts, its sensitivity to scattered radiation is reduced relative to the primary 511 keV gamma rays. Scatter (and accidental) fast coincidence events generate long range tails in the point response function but these are approximately constant in shape and can be modelled with reasonable accuracy. For instance, in a study of thyroid uptake this was carried out by placing a point source next to the section of the patient's body to be scanned (Bateman *et al* 1982). In the present study, the contrast in the object was lower than for the thyroid uptake studies and the point spread function was not modelled as accurately but the clear demarcation of relatively small necrotic regions is apparent.

Additional advantages of the camera are (i) the possibility of retrospectively selecting multiple slices through a relatively large section of the body and (ii) the potential of construction at a relatively much smaller cost than that of an Anger camera of comparable size.

In addition to the assessment of the appropriate method of image processing, an accurately quantitative analysis will only be feasible when arterial sampling is carried out and correction for gamma ray attenuation applied. In this particular study, the problem of attenuation is less important because of the relatively small regional variation in the thickness of the foot (a correction of 35% would correspond to 3 cm of tissue, taking  $\mu$  to be  $0.1 \text{ cm}^{-1}$ ). The accurate and simply-applied attenuation correction procedure, employing a ring source, in transaxial positron tomography (Huang *et al* 1979) is essential for accurate quantitation of radionuclide concentrations. A comparable procedure is not yet available for the positron camera and some work is needed to implement one. The future requirements of the system, apart from attenuation correction are: (i) larger area detectors, (ii) a greater detector efficiency (a factor of 2 increase appears to be technically possible which would give a factor of

4 in sensitivity) and (iii) at least two orthogonal views to improve the stereo information at low spatial frequencies.

### Acknowledgments

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# The Remotely-controlled Preparation of a $^{11}\text{C}$ -Labelled Radiopharmaceutical—[1- $^{11}\text{C}$ ]Acetate\*

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Remotely-controlled apparatus is described for the preparation of the radiopharmaceutical, [1- $^{11}\text{C}$ ]acetate, from cyclotron-produced [ $^{11}\text{C}$ ]carbon dioxide according to established radiochemistry. This apparatus features a multi-ported reaction vessel (fitted with an electrical stirrer), twelve solenoid valves (to direct fluid flows and hydraulically-powered syringes), one hydraulic oil pump and one heated water-bath (all operated at 24 V d.c.). These components are controlled either with a rotary-switch or with an "Apple II" microcomputer acting through a digital output card. An important advantage of the use of this apparatus over the use of manually-controlled apparatus is that it results in a much reduced radiation dose to the operator. Moreover it has been shown that [1- $^{11}\text{C}$ ]acetate can be prepared much more efficiently with the remotely-controlled apparatus than with corresponding manually-controlled apparatus. Thus the overall efficiencies (radiochemical yields uncorrected for decay) for the conversion of [ $^{11}\text{C}$ ]carbon dioxide into [1- $^{11}\text{C}$ ]acetate for injection are  $24 \pm 8$ ,  $39 \pm 7$  and  $47 \pm 8\%$  for manual, remote rotary-switch and remote microcomputer control, respectively. The high efficiency and consistent performance of the remotely-controlled apparatus have been found to permit useful flexibility in the design of clinical experiments with [1- $^{11}\text{C}$ ]acetate and positron emission tomography.

## Introduction

With the development of the quantitative technique of positron emission tomography,<sup>(1)</sup> radiopharmaceuticals labelled with short-lived positron-emitting radionuclides, such as  $^{13}\text{N}$  ( $t_{1/2} = 10$  min),  $^{11}\text{C}$  ( $t_{1/2} = 20.4$  min) and  $^{18}\text{F}$  ( $t_{1/2} = 110$  min), have become increasingly important for the non-invasive investigation of physiological function and disease. In order to supply such radiopharmaceuticals regularly for clinical studies, efficient, reliable and safe preparative procedures are required.

Within our Unit [1- $^{11}\text{C}$ ]acetate has proved a valuable radiopharmaceutical for the study of human myocardial metabolism.<sup>(2,3)</sup> Originally, the required doses of [1- $^{11}\text{C}$ ]acetate were prepared via the carbonylation of methylmagnesium bromide with cyclotron-produced [ $^{11}\text{C}$ ]carbon dioxide in a well-established procedure that uses manually-controlled apparatus.<sup>(2)</sup> Though this apparatus performs reliably, regular use of the apparatus exposes its operator to significant radiation; the average finger dose per preparation is 7 mSv (0.7 rem). In order to be able to prepare

[1- $^{11}\text{C}$ ]acetate more regularly and in greater safety we set out to achieve remote control of all the operations involved in the established preparation.<sup>(2)</sup> Here we report on the apparatus we have developed and compare its performance to that of the corresponding manually-controlled apparatus.

## Experimental and Results

### Preparation of methylmagnesium bromide

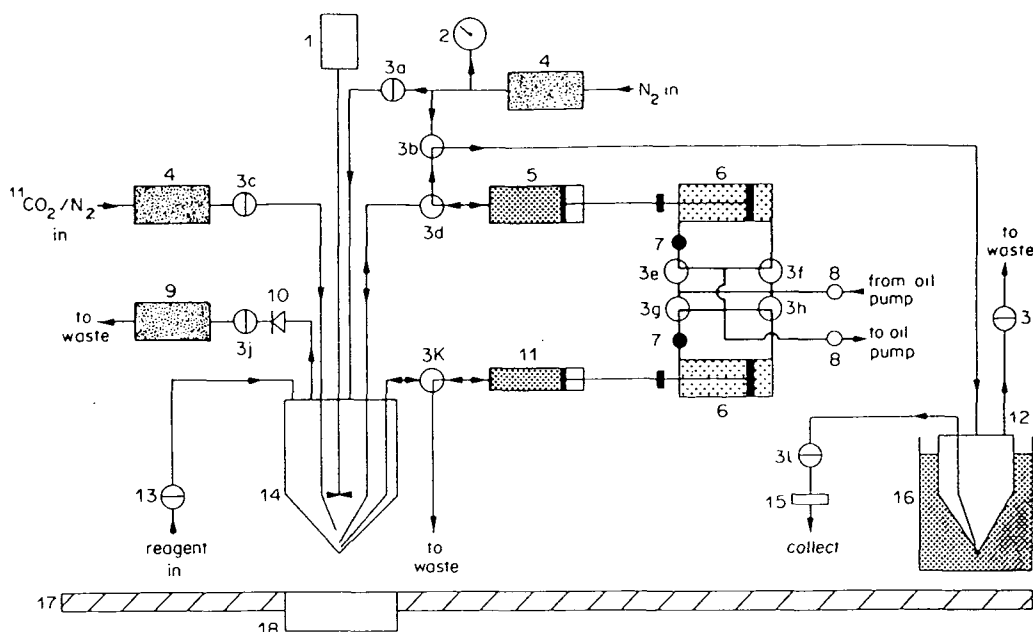
Solutions of methylmagnesium bromide (ca. 1.0 M) in diethyl ether were prepared and stored as described previously.<sup>(2)</sup>

### Production of [ $^{11}\text{C}$ ]carbon dioxide

[ $^{11}\text{C}$ ]Carbon dioxide was produced on the Medical Research Council cyclotron at Hammersmith Hospital by the  $^{14}\text{N}(\text{p}, \alpha)^{11}\text{C}$  nuclear reaction, using essentially the method described by Clark and Buckingham.<sup>(4)</sup> Nitrogen at  $100 \text{ kN m}^{-2}$  acted both as the bombarded substance and as the sweep gas for the target. The [ $^{11}\text{C}$ ]carbon dioxide was continuously trapped from the sweep gas (flow rate =  $80\text{--}100 \text{ mL min}^{-1}$ ) in a stainless steel tube ( $5 \text{ cm} \times 7 \text{ mm i.d.}$ ) that was filled with molecular sieve (4A, 60-80 mesh; B.D.H. Chemicals Ltd) and contained within an ionisation chamber. Bombard-

\* For a preliminary report of this work see, Pike V. W., Horlock P. L., Brown C. and Clark J. C. *Nucl. Med. Commun.* (abstr.) 4, 149 (1983).





Scheme 1. Apparatus for the remotely-controlled preparation of  $[1-^{11}\text{C}]$ acetate. 1—electrical stirrer; 2—pressure gauge (Budenberg); 3—solenoid valves (a–d and j–l, sub-miniature: Production Techniques Ltd. e–f, Joucomatic, i, Skinner); 4—magnesium perchlorate trap; 5—syringe with glass barrel and Teflon plunger (10 mL; Hamilton); 6—cylinder (double acting, stroke 11 cm, Scovill, Schrader Fluid Division); 7—oil flow restrictor; 8—self-sealing couplings (Swagelok Ltd); 9—soda lime trap; 10—poppet valve (opening pressure  $1.3 \text{ cN m}^{-2}$ ; Nupro); 11—syringe with glass barrel and Teflon plunger (1.0 mL; Hamilton); 12—glass vessel (capacity 15 mL) with multi-ported Teflon top, (Sovirel SVL 30 joint); 13—tap (Pharmaseal K75, KHS International); 14—glass reaction vessel (capacity, 15 mL) fitted with Teflon multi-ported top (Sovirel SVL 42 joint); 15—filter ( $0.22 \mu\text{m}$ , Millex FS, Millipore Corporation); 16—electrically-heated water bath; 17—lead wall (5 cm thick); 18—lead glass window. Connection tubes are Teflon (3 or 0.15 mm o.d.) with screw fittings (Altex Beckman Ltd) and flanged ends.

ment of nitrogen with protons (7.6 MeV, 40–50  $\mu\text{A}$ ) for 40–60 min usually gave 9–13 GBq (250–350 mCi) of trapped  $[^{11}\text{C}]$ carbon dioxide at the end of bombardment. The trapped  $[^{11}\text{C}]$ carbon dioxide was dispensed when required by heating the column of molecular sieve to  $220^\circ\text{C}$  and then passing nitrogen through the column at about  $20 \text{ mL min}^{-1}$ . Generally 80–90% of the trapped radioactivity was released into the nitrogen stream within 1–2 min.

#### Remotely-controlled preparation of $[1-^{11}\text{C}]$ acetate

Apparatus (Scheme 1) was built to enable  $[1-^{11}\text{C}]$ acetate solutions to be prepared remotely for clinical use, according to the sequence of operations listed in Table 1. These operations achieve the radiochemistry shown in Scheme 2, which is exactly that reported<sup>(2)</sup> previously. Many features of the apparatus are not readily apparent from Scheme 1 or Table 1 and are therefore described below.

(a) *Hydraulics.* Hydraulics are used to drive two double-acting cylinders, each linked to the piston of an adapted gas-tight syringe, so enabling solutions to be added to or withdrawn from the reaction vessel (Scheme 1).

The hydraulic system is powered by a “Minipack” oil pump (Smiths Hydraulics Industries Ltd). A

pressure-relief valve limits the pressure in the hydraulic circuit to *ca.*  $1 \text{ MN m}^{-2}$ . Oscillations in oil pressure and in flow are damped by means of an air reservoir (volume, 100 mL). These components are housed in a box that is fitted with a cooling fan and situated remote from the main apparatus (Scheme 1). Oil lines (6.35 mm, o.d.) leading to and from the box are connected to the main apparatus via two self-sealing couplings. These enable the hydraulic pumping system to be easily disconnected and, in principle, to be used to power syringes in other apparatus. No re-bleeding of the hydraulic system is necessary after breaking the self-sealing couplings.

The direction of syringe movement is controlled by solenoid valves (Table 1, Scheme 1). The rate of withdrawal of liquid from the reaction vessel into each syringe was set by pre-adjustment of a flow restrictor at one port of each hydraulic cylinder (Scheme 1) to give a linear motion of  $0.5 \text{ cm s}^{-1}$ . This slow linear motion enables an aqueous phase to be withdrawn slowly and precisely from the bottom of any two-phase system within the reaction vessel, via an appropriate dip-tube (Scheme 1). (Preliminary investigations indicated that such precise control would not be easy to achieve with pneumatically-powered syringes).

(b) *Electrics.* A 24 V d.c. supply is used to power

Table 1. Details of operations in the microcomputer-controlled preparation of [1-<sup>11</sup>C]acetate and their relationships to rotary-switch positions in the rotary-switch-controlled preparation

| Operation                                                                                                                     | Solenoid valves energised <sup>a,b,c</sup> | Duration (s)      | Corresponding rotary-switch position |
|-------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|-------------------|--------------------------------------|
| Flush with dry N <sub>2</sub>                                                                                                 | a, j                                       | ~600 <sup>d</sup> |                                      |
| Load reagent <sup>e</sup> (0.05 M MeMgBr in Et <sub>2</sub> O; 4.0 mL)                                                        | j                                          | ~60 <sup>d</sup>  |                                      |
| Standby                                                                                                                       | —                                          | ~300 <sup>d</sup> | Off                                  |
| [ <sup>11</sup> C]Carbonation <sup>f</sup> (with <sup>11</sup> CO <sub>2</sub> /N <sub>2</sub> ; at 20 mL min <sup>-1</sup> ) | c, j                                       | 120               | 1                                    |
| Add acid <sup>g</sup> (6 M HCl 0.8 mL)                                                                                        | j, k, h                                    | 20                | 2                                    |
| Phase separation                                                                                                              | j, k, h                                    | 30                | 2                                    |
| Withdraw acid <sup>h</sup>                                                                                                    | a, k, g                                    | ~30 <sup>d</sup>  | 3                                    |
| Add NaHCO <sub>3</sub> soln <sup>i,j</sup> (0.9% w/v; 10.0 mL)                                                                | d, f, j, h                                 | 60                | 4                                    |
| Phase separation                                                                                                              | d, f, j, h                                 | 30                | 4                                    |
| Withdraw aq. [1- <sup>11</sup> C]AcO <sup>-</sup> soln <sup>k</sup>                                                           | a, d, e                                    | ~30 <sup>d</sup>  | 5                                    |
| Transfer aq. [1- <sup>11</sup> C]AcO <sup>-</sup> soln <sup>k</sup>                                                           | f, i                                       | 20                | 6                                    |
| Remove ether at 60°C <sup>h</sup>                                                                                             | b, i                                       | 300               | 7                                    |
| Dispense aq. [1- <sup>11</sup> C]AcO <sup>-</sup> soln <sup>k</sup>                                                           | b, l                                       | 60                | 8                                    |

<sup>a</sup>Valve nomenclature corresponds to that in Scheme 1.<sup>b</sup>Two-port solenoid valves are open when energised.<sup>c</sup>The de-energised configurations of three-port solenoid valves are shown in Scheme 1.<sup>d</sup>Duration controlled by operator from microcomputer keyboard.<sup>e</sup>Reagent loaded through a hydrophobic filter (Millex FG, Millipore Corporation) connected to the manual valve (no. 13 in Scheme 1), which is thereafter kept closed.<sup>f</sup>Electrical stirrer operates automatically in microcomputer-controlled preparation.<sup>g</sup>Hydraulic oil pump operates automatically in microcomputer-controlled preparation.<sup>h</sup>Water-bath is maintained at 60°C.<sup>i</sup>N<sub>2</sub> supply is pre-set to 50 kN m<sup>-2</sup>.

the electrical stirrer, 12 solenoid valves, electrical heater and hydraulic oil pump.

(c) *Remote-control of apparatus.* (i) By rotary-switch. Originally a rotary-switch (comprised of 14 banks of 1-pole-12-way switches and with 8 position control) was used to control the radiosynthesis. Each position of the rotary-switch energises a particular set of solenoid valves and corresponds to either one or two operations (Table 1). Separate toggle switches were used to control the electrical stirrer and to allow syringe movement to be started and stopped during phase separations (rotary-switch positions 3 and 5; Table 1). The timing of all operations was controlled by the operator to match as nearly as possible the durations shown in Table 1.

(ii) By microcomputer control. Subsequently a microcomputer ("Apple II europlus"; Apple Computer Inc.) was programmed\* in "Applesoft BASIC" to control and time operations (Table 1) in the radiosynthesis via an interface (digital output card; MC Computers) with the electrical components of the apparatus. The digital output card fits into any one of option slots 1-6 within the microcomputer,

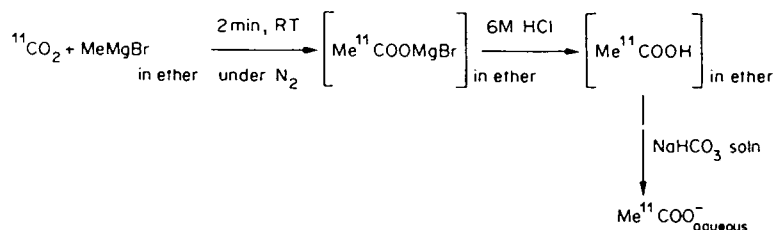
is powered from a +5 V power rail and gives output via a 40-way ribbon cable supplied with the card.

With microcomputer control of the apparatus the preparation of [1-<sup>11</sup>C]acetate requires only two operator interventions, each to control syringe movement during the withdrawal of an aqueous phase from the reaction vessel. Each removal of an aqueous phase is monitored visually through the lead glass window (Scheme 1) and syringe movement (stop, forward and reverse) controlled from the microcomputer keyboard. Aqueous phase withdrawals are the only operations not having programmed durations (Table 1).

#### Performance of the remotely-controlled apparatus

Measurements (from the National Radiological Protection Board) show that the average finger dose received by an operator of the manual apparatus<sup>21</sup> during one preparation of [1-<sup>11</sup>C]acetate is ca. 7 mSv (0.7 rem), whereas during one rotary-switch or microcomputer-controlled preparation an operator receives a finger dose of <2 mSv (0.2 rem).

The efficiency of the apparatus under rotary-switch control and under microcomputer control was compared to that of the manually-controlled apparatus previously described.<sup>(2)</sup> Parameters measured were

Scheme 2. The radiosynthesis of [1-<sup>11</sup>C]acetate from cyclotron-produced [<sup>11</sup>C]carbon dioxide.

\* The computer programme is available from the authors on request.

Table 2. Radiochemical yields, preparation times, and efficiencies for the preparation of [ $^{11}\text{C}$ ]acetate by manual, rotary-switch and microcomputer-control

| Control of preparation | No. of studies | Radiochemical yield <sup>a</sup> of [ $^{11}\text{C}$ ]AcO <sup>-</sup> (mean $\pm$ SD) <sup>a</sup> , % | Preparation time (min) | Efficiency <sup>b</sup> of preparation (mean $\pm$ SD) <sup>c</sup> , % |
|------------------------|----------------|----------------------------------------------------------------------------------------------------------|------------------------|-------------------------------------------------------------------------|
| Manual <sup>c</sup>    | 35             | 48 $\pm$ 16                                                                                              | 20                     | 24 $\pm$ 8                                                              |
| Rotary-switch          | 10             | 60 $\pm$ 10                                                                                              | 12.5                   | 39 $\pm$ 7                                                              |
| Microcomputer          | 8              | 70 $\pm$ 11                                                                                              | 11.5                   | 47 $\pm$ 8                                                              |

<sup>a</sup>Decay-corrected and based on the activity of  $^{11}\text{CO}_2$  dispensed into MeMgBr/Et<sub>2</sub>O.

<sup>b</sup>Non-decay-corrected radiochemical yield of [ $^{11}\text{C}$ ]AcO<sup>-</sup> from  $^{11}\text{CO}_2$ .

<sup>c</sup>The data for manual control were obtained using the apparatus described in Ref. 2.

the decay-corrected radiochemical yield of [ $^{11}\text{C}$ ]acetate from the [ $^{11}\text{C}$ ]carbon dioxide dispensed and the preparation time. Several preparations were examined for each form of control (Table 1). From the measured parameters the average overall efficiency (the non-decay corrected yield) and its standard deviation were calculated for each method of preparation (Table 2).

Solutions of [ $^{11}\text{C}$ ]acetate, prepared in the remotely-controlled apparatus, were all found to be >96% radiochemically pure by the chromatographic methods described previously,<sup>(12)</sup> and to have physiologically acceptable pH (pH 7–8) and tonicity. All randomly selected preparations passed independent (SafePharm Ltd) tests for apyrogenicity (the rabbit pyrogen test) and sterility.

The apparatus is very reliable; no failures have been recorded in 40 production runs.

### Discussion

The development of the remotely-controlled apparatus described in this paper achieved its main objective, a drastic reduction in the finger dose received by the chemist during a single preparation of [ $^{11}\text{C}$ ]acetate. This achievement permits any one operator to prepare [ $^{11}\text{C}$ ]acetate more regularly than previously.

A bonus arising from the adaptation of the preparation of [ $^{11}\text{C}$ ]acetate from manual to remote control is the much increased efficiency of the preparation (Table 2); indeed the microcomputer-controlled preparation is nearly twice as efficient as the manually-controlled preparation previously reported<sup>(2)</sup> (Table 2). Two factors contribute almost equally to the increased efficiencies of each of the remotely-controlled preparations, their greater radiochemical yields and their reduced preparation times (Table 2). The increased radiochemical yields are not the result of any formal change in radiochemistry or protocol, since great care was taken to reproduce the already optimised radiochemistry and protocol of the manual preparation;<sup>(2)</sup> they are the result of the more reproducible precision with which each stage of the preparation is performed under remote control. Remote control reduces the preparation time considerably because many operations (e.g. valve-switching), which must be performed sequentially

under manual control, are performed simultaneously and instantly under remote control. The timing of operations under microcomputer control is somewhat more precise than under rotary-switch control and this is reflected in the slightly shorter preparation time and in its very small standard deviation (12 s,  $n = 8$ ).

Overall, microcomputer-control was found to be more efficient than rotary-switch control (Table 2). This benefit need not be at great expense: in principle, a dedicated microprocessor could act as a cheap substitute for the microcomputer. Nevertheless, we found the microcomputer to be very convenient for the initial development of programmed control and to have the further advantage of enabling graphics to be used to monitor the radiosynthesis.

In practice, the average yield of [ $^{11}\text{C}$ ]acetate obtained for injection from the remotely-controlled apparatus was 5.2 GBq (141 mCi) under microcomputer control and 4.3 GBq (117 mCi) under rotary-switch control. It is known that a dose of about 370 MBq (10 mCi) of [ $^{11}\text{C}$ ]acetate must be administered to a patient in order to obtain good images of myocardium and meaningful quantitative data by means of positron emission tomography.<sup>(13)</sup> The production of greater than 3.3 GBq (90 mCi) of [ $^{11}\text{C}$ ]acetate makes it possible to administer two doses of [ $^{11}\text{C}$ ]acetate, each of about 370 MBq (10 mCi), up to at least 60 min (i.e. 2–3 half-lives of  $^{11}\text{C}$ ) apart. This possibility has been exploited in the study of transient myocardial ischemia.<sup>(12,3)</sup> Thus, for example, information on the myocardium may be gained with the patient at rest from the first dose of [ $^{11}\text{C}$ ]acetate and with the patient at exercise from the second dose. That remote control enables [ $^{11}\text{C}$ ]acetate to be produced *routinely* in activities exceeding 3.3 GBq (90 mCi) therefore permits greater flexibility in the design of clinical experiments and enables more information to be gained from each study.

We are aware that the activity of [ $^{11}\text{C}$ ]acetate that could be prepared in a modern cyclotron facility, where high energy (*ca.* 20 MeV) protons are available, is many times higher than that reported here, because much higher activities (74–110 GBq; 2–3 Ci) of [ $^{11}\text{C}$ ]carbon dioxide could be produced. (Only 7.6 MeV protons are presently available within our Unit). Even so the use of high initial radioactivity

would also make the use of remotely-controlled apparatus, similar to that reported here, very desirable if not essential in order to limit radiation exposure to personnel.

Remotely-controlled procedures, of diverse sophistication, have been reported for the preparation of several radiopharmaceuticals that are widely applied in positron emission tomography, including [1-<sup>11</sup>C]palmitate,<sup>(5)</sup> 2-deoxy-2-[<sup>18</sup>F]fluoro-D-glucose,<sup>(6)</sup> [1-<sup>11</sup>C]2-deoxy-D-glucose,<sup>(7)</sup> L-[*S*-methyl-<sup>11</sup>C]methionine,<sup>(8)</sup> and 3-[<sup>11</sup>C]methyl-D-glucose.<sup>(9)</sup> Though these procedures are generally claimed to be reliable and to reduce radiation doses to personnel, a detailed comparison between a manually-controlled procedure and a corresponding remotely-controlled procedure has not been made hitherto. This study clearly demonstrates that the adaptation of procedures for the labelling of radiopharmaceuticals with short-lived positron-emitting radionuclides (<sup>13</sup>N, <sup>11</sup>C and <sup>18</sup>F) from manual to remote control can provide several benefits besides the desired reduction in radiation exposure to personnel. These benefits include reduced preparation time, increased yield, greater reproducibility and consequently more flexibility in the design of clinical experiments. The approaches we have taken towards the design and construction of the remotely-controlled apparatus reported here could equally well be applied to the remote control of the preparations of other radiopharmaceuticals that require similar operations. Important examples are the preparations of [1-<sup>11</sup>C]palmitate<sup>(5,10)</sup> and D,L-3-methyl-[1-<sup>11</sup>C]heptadecanoate.<sup>(11)</sup>

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# IX-7. Dopamine Receptor Binding in Schizophrenic Patients as Measured with <sup>11</sup>C-Methylspiperone and PET

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An increase in dopamine receptor density assessed by spiperone binding has been found in post-mortem brains of schizophrenic patients (1), thus supporting the hypothesis that schizophrenia might be related to a disturbance in the postsynaptic dopaminergic receptor (2). To test this hypothesis in vivo, we used the butyrophenone derivative 3-[<sup>11</sup>C]-methylspiperone and positron emission tomography to measure dopamine receptor binding in schizophrenic patients and normal controls. In the striatum this tracer is mainly bound to D2 dopamine receptors whereas activity in the cortex is thought to reflect binding to 52 serotonin receptors (3,4).

## Patients and Methods

Twelve normal volunteers, 7 females and 5 males, ranging in age from 20-64 years (mean 45 ± 17) and five schizophrenic patients, 2 females and 3 males, in the age range from 21-63 years (mean 37 ± 16) were studied. All patients fulfilled the present-state examination criteria for the diagnosis of schizophrenia. Four of the five schizophrenic patients were freshly diagnosed, had never received neuroleptic medication and displayed positive psychotic symptoms at the time of the scan. One male patient aged 21 had had a psychotic episode one year prior to the study and had then received neuroleptics for several months. At the time of the scan, he had been drug-free for 6 months and was symptom-free.

As four of the schizophrenic patients were under the age of 40 (21-37, mean 30 ± 7) the main points of comparison were made between them and the six normals in this age group (20-38, mean 29 ± 7).

Scans were performed on a single plane ECAT II scanner. 2.5-19 mCi of 3-[<sup>11</sup>C]methylspiperone were injected intravenously. The total amount of methylspiperone injected ranged from 0.03-0.14 mg in the patient group and 0.02-0.13 mg in the control group. In most studies, immediately after injection a series of consecutive scans was initiated alternating between 2 planes 2.5 and 4.5-5cm above and parallel to the OM-line. Depending on the amount of activity, subjects were scanned for 65-85 minutes after injection. In two schizophrenic patients and one normal volunteer only one scanning cycle of 2 planes was performed at 60 minutes.

Regions of interest were taken from the cerebellum in the +2.5cm plane and the striatum and cerebral cortex in the +4.5/+5.0cm plane. Integrated cerebellar values were taken at times corresponding to the midscantimes of the striatal plane. Striatum/cerebellum and cortex/cerebellum ratios could then be calculated and plotted against time. The ratio at 60 minutes was used to compare patient and control groups.

### Results

The striatum/cerebellum ratios and cortex/cerebellum ratios increased linearly with time; ratios at 60 minutes after injection showed an age-dependent decline as has been observed by other investigators (5). Ratios of the schizophrenic patients were not significantly different from normal controls, whether patient and control groups were considered as a whole or whether only the under 40 age group was looked at.

| Ratio                   | <u>Controls</u> |                  | <u>Schizophrenics</u> |                  |
|-------------------------|-----------------|------------------|-----------------------|------------------|
|                         | all<br>n = 12   | <age 40<br>n = 6 | all<br>n = 5          | <age 40<br>n = 4 |
| striatum/<br>cerebellum | 3.14 ± 0.61     | 3.57 ± 0.45      | 3.29 ± 0.30           | 3.24 ± 0.33      |
| cortex/<br>cerebellum   | 1.60 ± 0.21     | 1.73 ± 0.15      | 1.64 ± 0.12           | 1.60 ± 0.10      |

As a correlation was found between ratios and the total amount of methylspiperone injected (6) ratios were also corrected to a common injected dose of 0.01mg. There were again no differences between patient and control groups.

### Conclusions

The data available do not support the hypothesis of dopamine receptor supersensitivity in schizophrenia. However, the linear increase in ratios means that specific binding has not reached equilibrium in the scanning period whose length is dictated by the half-life of the  $^{11}\text{C}$ -label ( $t_{1/2} = 20\text{min}$ ). It is therefore possible that the two groups differ in striatal receptor density but, especially if differences are only small, a longer lived label will be necessary to test this hypothesis (e.g.  $^{18}\text{F}$  with a  $t_{1/2}$  of 110 min).

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# Brain dopamine metabolism in patients with Parkinson's disease measured with positron emission tomography

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**SUMMARY** L-[<sup>18</sup>F] fluorodopa was administered in trace amounts intravenously to healthy control subjects and to patients with Parkinson's disease. Striatal uptake of radioactivity was measured using positron emission tomography. The capacity of the striatum to retain tracer was severely impaired in patients compared to controls. This may reflect a reduction of striatal dopamine storage in Parkinson's disease. Patients showing the "on/off" phenomenon had an even greater decrease of striatal storage capacity.

Positron emission tomography (PET) is a scanning technique which allows measurement in absolute units of the regional concentration of positron emitting isotopes in the brain or other organs.<sup>1</sup> When these isotopes are attached to suitable tracers and inhaled or injected intravenously it is possible to explore their fate by measuring the uptake of radioactivity over time in an organ such as the brain. Recently a method has been developed to label an analogue of levodopa (6-L-fluorodopa) with the positron emitting isotope fluorine-18.<sup>2</sup> This tracer can be used to study regional dopamine metabolism in brain *in vivo* by measuring tomographically the accumulated radioactivity with PET.<sup>3-5</sup>

We have applied this method to a group of healthy control subjects and to patients with Parkinson's disease. Because of severe loss of nigrostriatal dopaminergic neurons that characterises Parkinson's disease, the dopamine content in striatum of these patients is markedly diminished.<sup>6</sup> The aim of the present study was to investigate storage capacity for dopamine in striatum of healthy individuals and subjects with Parkinson's disease.

## Methods

### Tracer

The isotope fluorine-18 (half life 110 min) was produced in

the MRC cyclotron at the Hammersmith Hospital, London. Labelled L-[<sup>18</sup>F] fluorodopa was prepared according to the technique of Firnau *et al*<sup>2</sup> (but without the final stage of separation of isomers). Therefore a mixture of 2-, 5- and 6-L-[<sup>18</sup>F] fluorodopa was used. The relative isomeric proportions were 35, 5 and 60% respectively. The radioactivity, 2-6 mCi, was associated with 8-10 mg L-fluorodopa. The estimated mean specific activity was  $103.0 \pm 22.9$  mCi/mmol. This mixture was injected intravenously in a volume of 10 ml over two minutes using a constant infusion Harvard pump.

### Construction of arterial curve

A Teflon (gauge 21) cannula was inserted into one radial artery, and 3 ml blood samples were taken at 20 second intervals during the first three minutes following tracer injection, and then every 30 to 60 seconds for a further seven minutes. Arterial sampling times were then gradually spaced out via 5 and 10, to 20 minute intervals. Usually a total of 25 samples were taken. The samples were spun and the concentration of isotopes in plasma was measured in a well-counter cross calibrated with the tomograph.

### Scanning technique

The positron emission tomograph used was the ECAT-II (EG & G Ortec). This is a whole body single slice machine with a spatial resolution of 17 mm × 17 mm FWHM (full width half maximum) and a slice thickness of 16 mm (FWHM).<sup>7</sup> Serial scans were started in most subjects about one hour after the tracer had been given. Some subjects were scanned from the moment at which tracer was injected. Owing to the slow uptake of the tracer by brain tissue, the relatively small volume of the striatum and the relatively low sensitivity of our scanner, 10 minute scans were needed to obtain sufficient counts to reconstruct one tomographic image. Only one cross-section was scanned (5 cm above and

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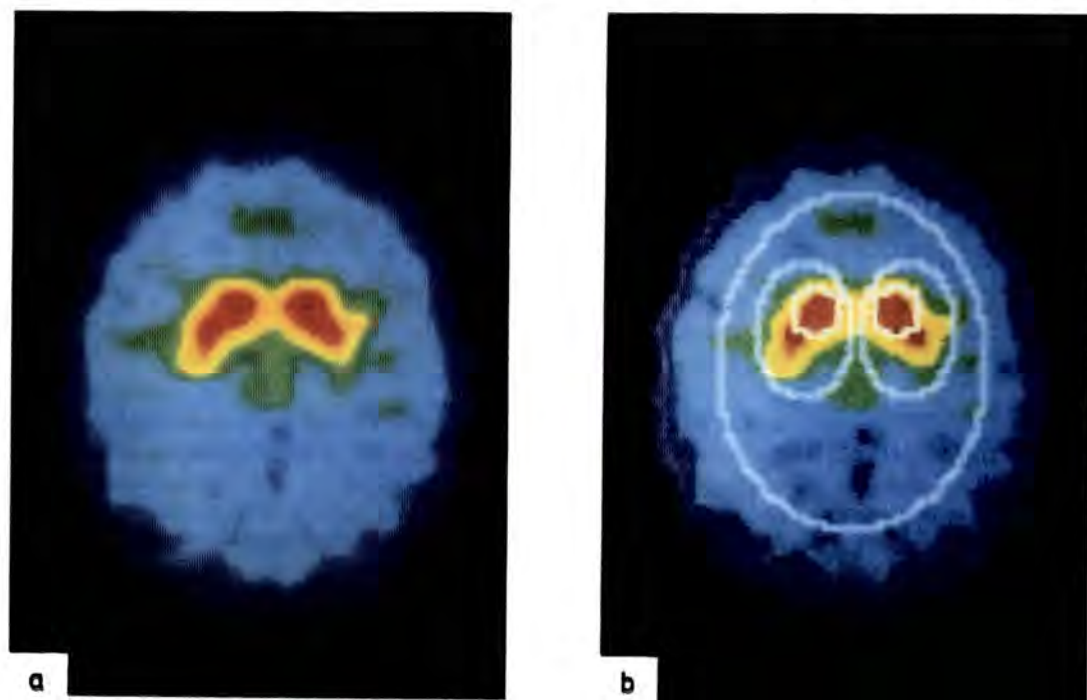


Fig 1  $L$ - $[^{18}F]$ fluorodopa uptake in a cross-section through both striata of the brain of a normal volunteer (a). The image is a summation of all the scans (after correction for attenuation) between 100 and 220 min after administration of the tracer. In this and following illustrations the top of the image indicates the front of the brain, viewed from above. (b) illustrates how the regions of interest were positioned on each image (see text for explanation).

parallel to the orbito-meatal line) at the level of the body of the striatum. The same transaxial tomographic plane was measured in a sequence of consecutive 10 minute scans for approximately two hours. A transmission scan, using an external ring source (Germanium 68/Gallium 68), was used to correct the measured emission data for tissue attenuation. After data collection the images were reconstructed using standard computer processing for all of the 10 minute scans (fig 1a). The picture element (pixel) response within each reconstructed tomographic image relates directly to the regional concentration of fluorine-18 in the tissue examined, and was corrected for physical decay from the time of injection. This procedure allowed us to follow the changes in concentration of radioactivity over time in the striatal region and in regions of the surrounding brain.

#### Data analysis

The tissue concentration of fluorine-18 in both striata and surrounding brain were obtained from regions of interest (ROIs) defined on the images of the emission scans. An example is given in fig 1b. The left and right striatal ROIs were obtained by summing all sequential images and determining a circular area (49 pixels; each pixel is  $2.5 \text{ mm} \times 2.5 \text{ mm}$ ) containing the maximum concentration of isotope. Striatal ROIs thus determined were placed on each 10 minute scan separately to obtain the time course of radioactivity. The average value from left and right striatum in

any one slice was taken as the "striatal value". The "surrounding brain value" was obtained as follows. A large ROI was placed on the image of the transmission scan just inside the rim representing the junction of skull and brain. This large ROI was then used to determine the outer border of the brain in each 10 minute emission scan. Two oval ROIs (radii 8 and 9, totalling 223 pixels) which completely encircled the two centrally located striatal regions with high activity were then subtracted from this large ROI to obtain the "surrounding brain value" (fig 1b). From the "striatal" and "surrounding brain" values a ratio was obtained for each 10 minute scan.

#### Patients and normal controls

Six healthy volunteers and 12 patients with Parkinson's disease were studied (table 1). The patients were divided into two groups. The first group comprised seven patients who had had the disease for only a relatively short time ("early" patients). Three of these patients were on regular treatment with a stable and sustained therapeutic response: their medication was stopped one day before the PET scan. The other four patients in this group had not been treated. The second group comprised five patients who had had the disease for longer, were more severely disabled, and whose response to levodopa treatment fluctuated ("on/off" patients). All of the second group were taking levodopa, but their response to treatment was variable. Throughout each day they had



Table 1 Clinical and scan data of normal controls and patients with Parkinson's disease

| Sex              | Age (yr) | $^{18}\text{F}$ Dopa<br>$\text{mCi/kg } 10^{-2}$ | Disease duration<br>(yr) | "On/Off" | Total disability<br>score* | Usual drugs<br>(daily dose)†                 | Time off drugs<br>before scan |
|------------------|----------|--------------------------------------------------|--------------------------|----------|----------------------------|----------------------------------------------|-------------------------------|
| <b>Controls</b>  |          |                                                  |                          |          |                            |                                              |                               |
| M                | 42       | 2.15                                             |                          |          |                            |                                              |                               |
| M                | 32       | 3.48                                             |                          |          |                            |                                              |                               |
| M                | 65       | 3.08                                             |                          |          |                            |                                              |                               |
| F                | 30       | 6.10                                             |                          |          |                            |                                              |                               |
| F                | 64       | 11.05                                            |                          |          |                            |                                              |                               |
| M                | 23       | 7.79                                             |                          |          |                            |                                              |                               |
| Mean             | 42.7     | 5.61                                             |                          |          |                            |                                              |                               |
| ± SD             | ± 18.0   | ± 3.39                                           |                          |          |                            |                                              |                               |
| <b>Group I‡</b>  |          |                                                  |                          |          |                            |                                              |                               |
| M                | 71       | 3.44                                             | 1                        |          | 400                        | Levodopa (500 mg)                            | 24 hrs                        |
| M                | 62       | 5.50                                             | 5                        |          | 530                        | Levodopa (600 mg)<br>orphenadrine (50 mg)    | 24 hrs                        |
| M                | 64       | 5.66                                             | 6                        |          | 450                        | Levodopa (600 mg)                            | 24 hrs                        |
| M                | 32       | 4.83                                             | 2.5                      |          | 255                        | Nil                                          |                               |
| M                | 46       | 5.59                                             | 3                        |          | 255                        | Nil                                          |                               |
| M                | 76       | 3.82                                             | 3                        |          | 710                        | Nil                                          |                               |
| F                | 74       | 9.62                                             | 2                        |          | 850                        | Nil                                          |                               |
| Mean             | 60.7     | 5.49                                             | 3.2                      |          | 493                        |                                              |                               |
| ± SD             | ± 16.2   | ± 2.02                                           | ± 1.7                    |          | ± 224                      |                                              |                               |
| <b>Group II§</b> |          |                                                  |                          |          |                            |                                              |                               |
| M                | 68       |                                                  | 10                       | +        | 1245                       | Levodopa (500 mg)<br>bromocriptine (22.5 mg) |                               |
|                  |          |                                                  |                          |          |                            | selegiline (5 mg)                            | 12 hrs                        |
| M                | 61       | 9.00                                             | 10                       | +        | 735                        | Levodopa (400 mg), pergolide<br>(20 mg)      | 24 hrs                        |
| M                | 47       | 7.06                                             | 8                        | +        | 750                        | Levodopa (600 mg)                            | 12 hrs                        |
| M                | 41       | 5.82                                             | 12                       | +        | 450                        | Levodopa (400 mg)                            |                               |
|                  |          |                                                  |                          |          |                            | orphenadrine (400 mg)                        | 24 hrs                        |
| M                | 42       | 5.35                                             | 7                        | +        | 1845                       | Levodopa (900 mg)                            | 24 hrs                        |
| Mean             | 51.8     | 6.81                                             | 9.4                      |          | 1005                       |                                              |                               |
| ± SD             | ± 12.1   | ± 63                                             | ± 2.0                    |          | ± 550                      |                                              |                               |

\*Maximum disability score on NYU scale is 2000.

†Levodopa dosage taken in combination with peripheral decarboxylase inhibitor.

‡Group I patients comprise those with early disease or showing sustained response to levodopa therapy.

§Group II comprises "on/off" patients.

periods of mobility (often with dyskinesias) alternating with periods of immobility. Such swings from mobility to immobility generally were related to the time at which levodopa was taken. Drugs were stopped the night before the PET scan in three "on/off" patients, but two could only tolerate a few hours without treatment. Each control subject and patient received 75 mg carbidopa orally about 30 minutes before the tracer was injected. Just before the patients were scanned they were examined and rated according to the New York University disability scale.<sup>8</sup> The two groups of patients did not differ in respect of age or administered dose of tracer (table 1). However, patients in group II had suffered from the disease three times as long as those in group I ( $p < 0.001$ ) and were more disabled ( $p < 0.05$ ).

Written informed consent was obtained from each patient and healthy control. The project was approved by the Research Ethics Committee of the Hammersmith Hospital and the Maudsley Hospital, and permission for use of the isotope was obtained from the UK Administration of Radioactive Substances Advisory Committee.

## Results

Figure 2 illustrates the time course of activity in striatum and surrounding brain in a control subject and an "on/off" patient. From 70 minutes onwards stri-

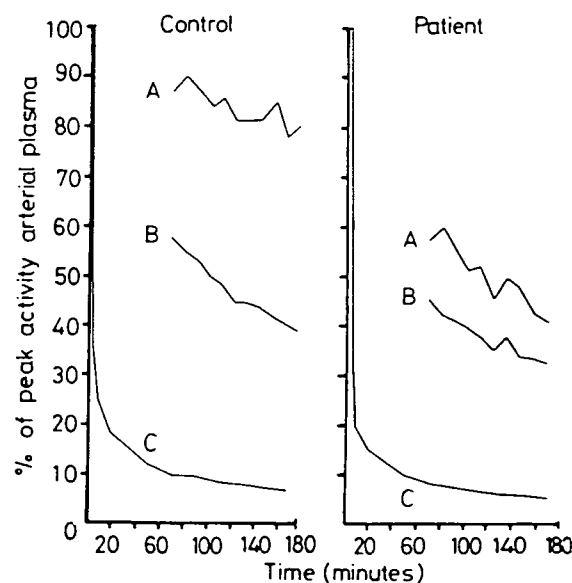


Fig 2 Striatal (A), surrounding brain (B) and arterial plasma (C) activity at 10 min intervals after L-[ $^{18}\text{F}$ ] fluorodopa administration in a control subject and a patient with Parkinson's disease, expressed as a percentage of the arterial plasma peak activity. For clarity the striatal and surrounding brain values have been multiplied by a factor of 10.

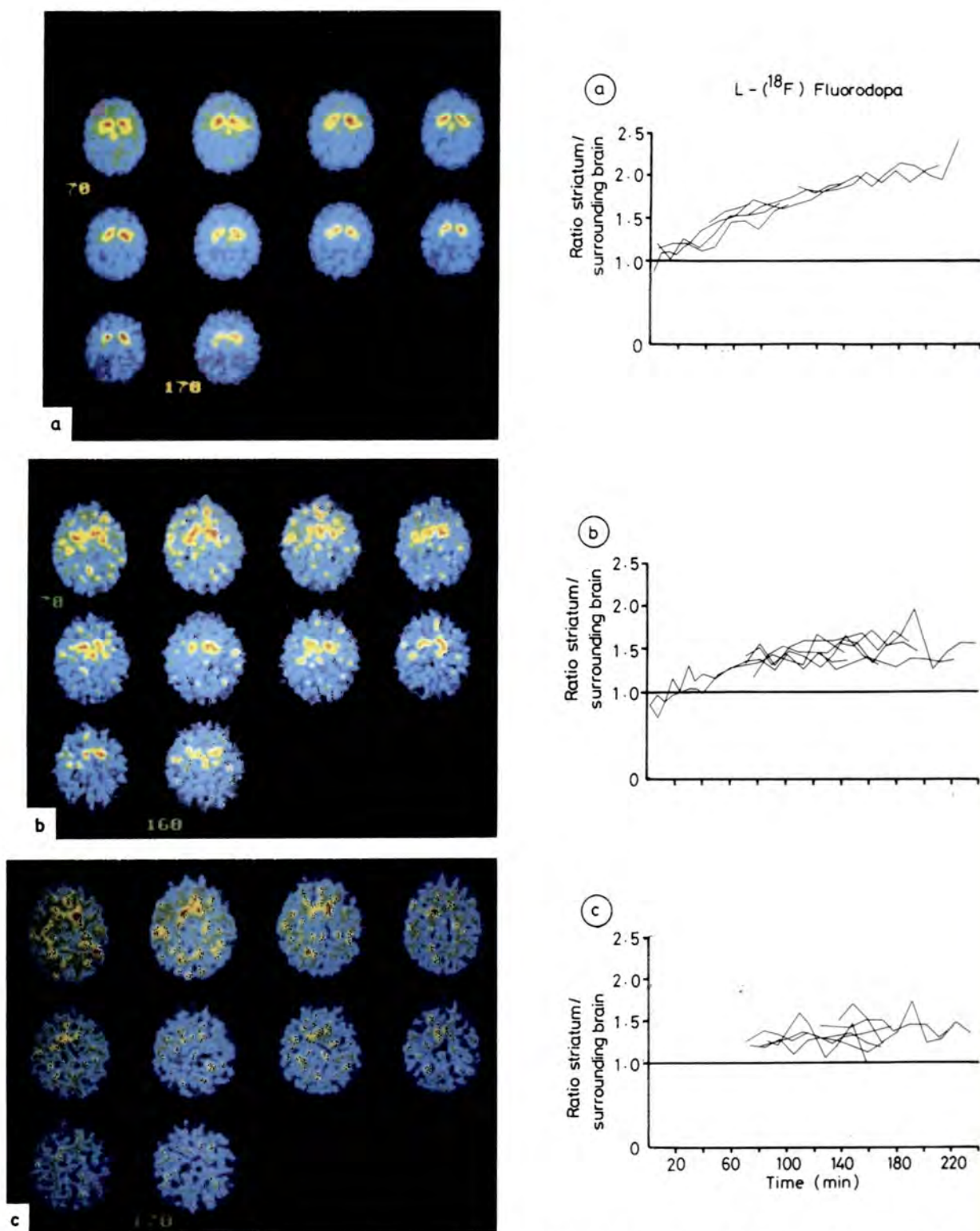


Fig 3 Left hand panels (a, b, c) show sequences of 10 min scans from 70 to 170 min. (a) a normal control; the contrast between striatal uptake of L-<sup>18</sup>F fluorodopa and that of surrounding brain increases constantly with time. (b) a patient with "early" Parkinson's disease; the contrast between uptake of the tracer in striatum and surrounding brain is also obvious, although the images are noisier owing to lower count rates, and there is little increase in contrast over time. (c) a case of Parkinson's disease showing the "on/off" phenomenon; the contrast between striatum and surrounding brain is hardly visible and the image reconstruction is very noisy owing to the low count rates. Right hand panel shows the ratios of activity in striatum to that in surrounding brain, for (A) normal subjects ( $n = 6$ ) (top), (B) patients with "early" Parkinson's disease ( $n = 7$ ) (middle), and (C) Parkinsonian patients with the "on/off" phenomenon ( $n = 5$ ) (bottom).

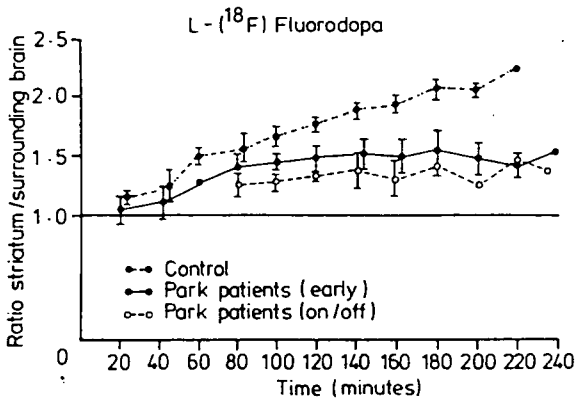


Fig 4 The average ratios of radioactivity (striatum versus surrounding brain), plotted at intervals of 20 min, are shown for (A) normal subjects ( $n = 6$ ), (B) patients with "early" Parkinson's disease ( $n = 7$ ), and (C) Parkinsonian patients with the "on/off" phenomenon ( $n = 5$ ). Means  $\pm$  SD are shown.

atal activity in the control subjects decreased only slightly (from 8.7% of arterial peak plasma value at 70 min to 8.0% at 180 min); activity in surrounding brain decreased more rapidly (from 5.8% at 70 min to 3.9% at 180 min). In the patients striatal activity followed the pattern of surrounding brain activity (striatal activity fell from 5.7% to 4.1%, and surrounding brain activity from 4.6% to 3.3%, between 70 and 170 min). The time course of the decline in surrounding brain activity was similar in controls and patients. The peak of this activity in those subjects whose scans were started immediately after administration of the tracer occurred in controls at  $28.8 \pm 5.3$  min ( $n = 3$ ) and in patients at  $29.3 \pm 0$  min ( $n = 2$ ) after injection. The percentage decline of activity in surrounding brain from about 2 to 3 hours after administration in those subjects who were scanned in that time period was  $15.9 \pm 2.0$  ( $n = 3$ ) and  $15.7 \pm 2.9$  ( $n = 7$ ) in controls and patients respectively.

Table 2 Average ratios of  $[L-^{18}F]$  Fluorodopa uptake in striatum versus surrounding brain from 100 to 200 min after administration in patients with Parkinson's disease

|          | Stable response patients | "On/Off" patients |
|----------|--------------------------|-------------------|
|          | 1.58                     | 1.35              |
|          | 1.49                     | 1.29              |
|          | 1.43                     | 1.22              |
|          | 1.47                     | 1.42              |
|          | 1.38                     | 1.33              |
|          | 1.61                     |                   |
|          | 1.34                     |                   |
| Mean     | 1.47                     | 1.32              |
| $\pm$ SD | $\pm 0.10$               | $\pm 0.07$        |
|          | $p < 0.02^*$             |                   |

\*Student's  $t$  test.

The ratio of activity in striatum compared to surrounding brain increased steadily over time in the normal subjects (fig 3a) for at least 120 min after injection of the tracer. Thereafter the ratio continued to increase, but the overall absolute count rates were extremely low by then, because of physical decay of the isotope, and only two subjects were studied at this time.

In contrast all Parkinsonian patients showed a lower ratio of activity for each time point after administration of the tracer (fig 3b, c). The variability of

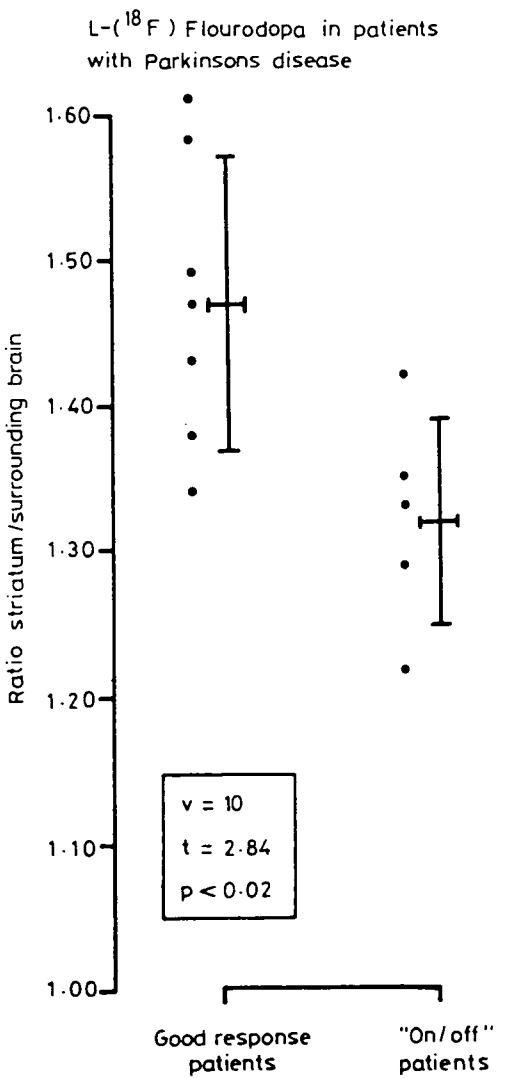


Fig 5 The mean ratio of radioactivity (striatum versus surrounding brain) for each patient between 100 and 200 min after administration of  $L-^{18}F$  fluorodopa. The bars indicate means and standard deviations. The "on/off" group of patients differed significantly from the group of patients with a stable response to treatment. Two sample Student's  $t$  test ( $v = 10$ ;  $t = 2.84$ ;  $p < 0.02$ ).

this ratio in the patient group was considerably larger than in the control subjects. This may have reflected the lower overall absolute activity in the striatum of patients, particularly towards the later part of the study, when the absolute count rates of isotope became very low.

Averaged ratios at 20 minute intervals for each group of subjects are shown in fig 4. The average ratio in the control subjects progressively increased throughout the period of study. In contrast, in the patient groups the average ratio plateaued about 100 min after isotope administration. In the group of patients with "early" disease, the ratios were not different whether the patients were treated or not. The ratios for patients with the "on/off" phenomenon were consistently lower than those for patients with "early" disease.

For each patient the average ratio (of all available ratios between 100 and 200 minutes after administration of the tracer) was calculated (table 2). "On-off" patients had a lower mean ratio compared with "early" patients (fig 5).

## Discussion

This study confirms previous reports<sup>3-5</sup> that accumulation of radioactivity after administration of fluorine-18 labelled levodopa takes place preferentially in the striatum of healthy controls and in patients with Parkinson's disease. By observing the time course of this uptake, we found a striking difference between controls and patients.

PET can only determine the concentration of positron emitting isotopes in a certain volume of tissue. The detected radioactivity (in this study fluorine-18) may arise from L-[<sup>18</sup>F] fluorodopa itself or one of its metabolic products, notably fluorinated dopamine (DA), homovanillic acid (HVA), 3,4-dihydroxy phenylacetic acid (DOPAC) or 3-methoxy, 4-hydroxy-phenylalanine (3-OM-dopa). The accumulation of the isotope is greatest in striatum, which has the highest concentration of native dopamine and related anabolic and catabolic enzymes. Fluorodopa has been shown to behave biochemically like levodopa and fluorodopamine is stored in the striatum.<sup>9-11</sup> Further, in primates much of the striatal radioactivity measured an hour after injection of L-[<sup>18</sup>F] fluorodopa is due to [<sup>18</sup>F]-fluorodopamine.

Horne *et al*<sup>12</sup> have shown in rats pretreated with carbidopa that L-[<sup>14</sup>C] dopa accumulated in striatal tissue mainly in the form of dopamine; dopa, HVA and DOPAC together constituted a small fraction of tissue radioactivity from 1 to 4 hours after intravenous administration. 3-OM-dopa rose slowly but steadily in arterial plasma, was capable of passing the blood brain barrier, and accounted for a considerable

fraction of brain tissue activity. However, 3-OM-dopa was distributed uniformly throughout the brain. Furthermore, lesions of substantia nigra markedly reduced striatal activity following administration of L-[<sup>14</sup>C] dopa. Hefli *et al*<sup>13</sup> reported that lesioning of substantia nigra and medial forebrain bundle in rats reduced dopamine concentration by 95% compared to the unlesioned contralateral side, and also demonstrated that dopamine formation from exogenous levodopa in striatum occurs mainly, but not exclusively, within dopaminergic nerve terminals. The clinical effects of levodopa administered in pharmacological doses are exerted only after it is decarboxylated to dopamine within the striatum.<sup>14</sup> Reserpine depletes intraneuronal vesicular storage sites of dopamine and other monoamines, and pretreatment of rats with reserpine decreased striatal activity after L-[<sup>14</sup>C] dopa administration by 48% at two hours.<sup>15</sup> This reserpine effect has also been demonstrated in monkey brain after L-[<sup>18</sup>F] fluorodopa.<sup>3</sup>

On the basis of these animal experiments we believe that the initial activity seen in the human striatum is an indication of its capacity to convert L-[<sup>18</sup>F] fluorodopa to L-[<sup>18</sup>F] fluorodopamine. The absolute concentration of tracer in striatum of normal human controls reached a plateau between 30 and 45 min, and thereafter decreased slightly.<sup>16</sup> The ratio of activity between striatum and surrounding brain continued to rise steadily from 0-4 hours after injection of the tracer in normal controls. This was due to a greater decrease of activity in surrounding brain rather than to an increase of activity in striatum. Our study therefore indicates that activity derived from L-[<sup>18</sup>F] fluorodopa is retained in human striatum for up to four hours after injection. This suggests that the activity seen in striatum throughout the major part of the period after injection of L-[<sup>18</sup>F] fluorodopa represents stored L-[<sup>18</sup>F] fluorodopamine.

In contrast to normal subjects, patients with Parkinson's disease showed a different time course of striatal activity after injection of labelled L-fluorodopa. The ratio of striatal to surrounding brain activity failed to rise after about 100 min from the injection, indicating that the net accumulation of activity within the striatal and surrounding brain tissue occurred at the same rate. The relative failure of Parkinsonian patients to selectively retain the tracer in the striatum suggests inability to store L-fluorodopamine, due to loss of nigrostriatal dopamine terminals.

Patients with longstanding disease and fluctuating "on/off" clinical response to levodopa treatment had a significantly lower "storage capacity" compared with "early" patients, either untreated or showing sustained clinical response. The ratio of activity between striatum and surrounding brain from about 100 min onwards was consistently lower in the young-

ger group of "on/off" patients than in the older group of "early" patients. This finding, together with the tight pattern of striatum to surrounding brain ratios within the control group despite wide age variations, would imply that these ratios are not age dependant.

Reches and Fahn<sup>17</sup> and Gervas *et al*<sup>18</sup> found in rats that 3-OM-dopa inhibits striatal uptake of levodopa in a dose-dependant manner. The difference in striatal uptake of the tracer between our three groups of human subjects could conceivably be due to circulating 3-OM-dopa. This would be expected to be present in higher concentrations in the "on-off" than "early" patients, and in patients than controls, as they had used higher pharmacological doses of levodopa and peripheral decarboxylase inhibitor before the PET scans were performed. However, if 3-OM-dopa is indeed capable of impairing transfer of levodopa across the blood-brain barrier in man, this should only affect overall levodopa uptake into brain, and not the ratio of levodopa concentration between striatum and surrounding brain. We have recently demonstrated (Leenders *et al*, unpublished observation) that the intravenous administration of a mixture of amino acids, although inhibiting uptake of injected L-[<sup>18</sup>F] fluorodopa into brain, has no effect on the ratio of striatal to surrounding brain radioactivity. In keeping with this observation, the four previously untreated Parkinsonian patients in this study showed the same ratio of activity between striatum and surrounding brain as the three who showed a sustained response to levodopa therapy.

Elsewhere<sup>19</sup> we have suggested that the development of "on/off" fluctuations in patients with Parkinson's disease on chronic levodopa therapy is due to progressive loss of the capacity to store dopamine in the striatum. Our results provide further evidence that as Parkinson's disease progresses, there is indeed a continuing decline of striatal storage capacity for dopamine. Patients in an advanced stage of the disease are therefore less capable of maintaining a constant dopamine pool in striatal tissue, and levels of striatal dopamine thus became more closely dependent upon the rate at which levodopa is delivered to striatum at any given time. A constant and sufficiently high level of plasma levodopa as provided by continuous intravenous infusion has been shown to abolish clinical fluctuations in patients with severe swings on oral therapy.<sup>20-22</sup> It has been proposed that such infusions may owe their success to their ability to compensate for a lack of storage capacity for striatal dopamine.

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# *Progress in radiopharmacy*

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## POSITRONS FROM GENERATORS

J.C. CLARK

In the late 1950s when what we now know as nuclear medicine was in its infancy the detection of human brain tumours by radioisotope scanning was being pioneered by a team at Massachusetts General Hospital lead by Gordon Brownell (1). They had chosen, for reasons that are now probably much more widely appreciated, to make use of the high degree of spatial resolution achievable when the two time coincident  $180^\circ$  correlated gamma rays (due to positron annihilations) were detected external to the subject with a pair of scintillation counters electronically set in time coincidence. The radionuclides  $\text{As}^{74}$  (17.7d) and  $\text{Cu}^{64}$  (12.7h) were the only positron emitters commercially available at the time and their supply was not without problems. Several nuclear research groups, particularly those at the Oak Ridge and Brookhaven National Laboratories, began to look for alternative positron emitting radionuclides. Attention was drawn to several nuclear relationships involving long or medium half-life parents and short-lived positron emitting daughters. Such relationships immediately offered the possibility of a positron generator system with a shelf life being that of the parent. The first generator of this kind, described by Gleason as a "Positron Cow" in 1960 (2), was based on the  $\text{Ge}^{68}$  (271d)/ $\text{Ga}^{68}$  (68 min) parent daughter relationship.

Over the years other positron generator systems have been studied, some of which are shown in table 1.

Without doubt the two most exhaustively studied and clinically used positron generators are  $\text{Sr}^{82}/\text{Rb}^{82}$  and  $\text{Ge}^{68}/\text{Ga}^{68}$  and this review will concentrate most of its attention on them.

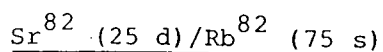


TABLE I.  
GENERATORS FOR POSITRON EMITTERS

| Parent            | Half-Life | Daughter           | Half-life | Decay Mode %<br>of Daughter        | E $\beta^+$ Mev | Gamma<br>MeV (%)       |
|-------------------|-----------|--------------------|-----------|------------------------------------|-----------------|------------------------|
| $^{44}\text{Ti}$  | 47 y      | $^{44}\text{Scg}$  | 3.9 h     | $\beta^+(95)\text{EC}(5)$          | 1.5             | 11.6 (100)             |
| $^{52}\text{Fe}$  | 8.3 h     | $^{52}\text{Mn}^m$ | 21.1 m    | $\beta^+\text{EC}(98)\text{IT}(2)$ | 2.6             | 1.43 (98)              |
| $^{62}\text{Zn}$  | 9.2 h     | $^{62}\text{Cu}$   | 9.7 m     | $\beta^+(98)\text{EC}(2)$          | 2.9             | Nothing<br>significant |
| $^{68}\text{Ge}$  | 271 d     | $^{68}\text{Ga}$   | 68 m      | $\beta^+(90)\text{EC}(10)$         | 1.9             | 1.08 (3)               |
| $^{72}\text{Se}$  | 8.4 d     | $^{72}\text{As}$   | 26 h      | $\beta^+(77)\text{EC}(23)$         | 3.3             | 0.83 (80)              |
| $^{82}\text{Sr}$  | 25 d      | $^{82}\text{Rb}$   | 75 s      | $\beta^+(96)\text{EC}(4)$          | 3.4             | 0.78 (15)              |
| $^{118}\text{Te}$ | 6.0 d     | $^{118}\text{Sb}$  | 3.5 m     | $\beta^+(75)\text{EC}(22)$         | 2.7             | 1.23 (3)               |
| $^{122}\text{Xe}$ | 20.1 h    | $^{122}\text{I}$   | 3.6 m     | $\beta^+(77)\text{EC}(23)$         | 3.1             | 0.56 (18)              |
| $^{128}\text{Ba}$ | 2.43 d    | $^{128}\text{Cs}$  | 3.6 m     | $\beta^+(61)\text{EC}(39)$         | 2.9             | 0.44 (26)              |

TABLE II  
RUBIDIUM 82 GENERATORS

| Column                                         | ELUANT             | <sup>82</sup> Rb YIELD % | Sr-BREAKTHROUGH/ml | REF                         |
|------------------------------------------------|--------------------|--------------------------|--------------------|-----------------------------|
| Bio-Rex-70                                     | NH <sub>4</sub> Ac | 72                       | 10 <sup>-5</sup>   | Yano 1968 [3]               |
| Chelex-100                                     | NH <sub>4</sub> Cl | 90                       | 10 <sup>-7</sup>   | Grant 1975 [7]              |
| Bio-Rex-70                                     | NaCl               | 72                       | 10 <sup>-7</sup>   | Yano 1977 [8]               |
| Al <sub>2</sub> O <sub>3</sub>                 | NaCl               | 76                       | 10 <sup>-7</sup>   | Yano 1979 [9]               |
| ZrO <sub>2</sub>                               | NaCl               | 70                       | 10 <sup>-8</sup>   | Kulpathipanja<br>1979 [12]  |
| Al <sub>2</sub> O <sub>3</sub> +<br>Chelex-100 | NaCl               | 66                       | 10 <sup>-9</sup>   | Horlock 1981 [5]            |
| Al <sub>2</sub> O <sub>3</sub>                 | NaCl               | 90                       | 10 <sup>-8</sup>   | Yano 1981 [4]               |
| SnO <sub>2</sub>                               | NaCl               | 70                       | 10 <sup>-9</sup>   | Neirineckx 1981 [10]        |
| Al <sub>2</sub> O <sub>3</sub> +<br>Chelex-100 | NaCl               | 40                       | 10 <sup>-9</sup>   | Vallabhajousla<br>1981 [11] |
| SnO <sub>2</sub>                               | NaCl               | 80                       | 10 <sup>-9</sup>   | Gennaro 1984 [6]            |



The first  $\text{Rb}^{82}$  generator was described by Yano and Anger in 1968 (3). It was based on the weakly acidic cation exchange resin BIOREX 70 and ammonium acetate as the eluant.

Over the intervening years other ion exchange materials and eluant combinations have been studied (4-12). Some of these are listed for quick reference in table 2.

The early organic resin generators suffered from the following major drawbacks. Although their performance at low levels of  $\text{Sr}^{82}$  seemed quite good, radiation damage at high parent loadings caused breakdown of the resin which led to a serious degradation in their performance. In particular the Sr breakthrough increased. In addition the eluants selected for these early systems were generally the preferred solution to the chemical problem and rarely acceptable as an infusate in man, e.g.  $\text{NH}_4\text{Cl}$  (13). The use of inorganic ion exchange materials to successfully overcome the radiation damage problems associated with organic ion exchangers has been known for many years. The most commonly encountered example in radiopharmacy is the  $\text{Mo}^{99}/\text{Tc}^{99\text{m}}$  chromatographic alumina radionuclide generator which is conveniently eluted with physiological saline (0.9% NaCl).

The search for an inorganic ion exchange material for use in the  $\text{Sr}^{82}/\text{Rb}^{82}$  generator was stimulated by two other factors, namely the upsurge in positron emission tomographic capability using the ring detector approach (14,15) and the commissioning of the beam dump target stations at the large linear accelerators at Los Alamos, LAMPF (16), and Brookhaven, BLIP (17), which were demonstrated to be capable of making hundreds of millicuries of the parent  $\text{Sr}^{82}$  (18).

Two reports on the study of potential candidates in inorganic ion exchangers by Brihaye et al (19) and Neirinx et al (20) concluded that tin dioxide should form the basis of an acceptable generator. They and other groups have moved on to test this hypothesis with the result that almost all clinical studies using  $\text{Rb}^{82}$  rely on the  $\text{SnO}_2/0.9\% \text{ NaCl}$  system. Only the Donner Laboratory group in Berkeley, California, appear to be persisting with the  $\text{Al}_2\text{O}_3$  generator (4,21). At Hammersmith we employed

TABLE III

DISTRIBUTION COEFFICIENTS (Kd) OF Sr(II) and Rb(I)

(Tris buffer/HCl solutions room temperature 12 h equilibration)

Data shown for pH = 7

| <u>Adsorbant</u>              | <u>Sr(II)</u> | <u>Rb(I)</u> | <u>Sr(II)/Rb(I)</u> |
|-------------------------------|---------------|--------------|---------------------|
| "Oxtain" *                    |               |              |                     |
| ( $\alpha$ -tin dioxide)      | 20000         | 1            | 20000               |
| Basic $\text{Al}_2\text{O}_3$ | 3980          | 25           | 160                 |
| Titanium Vanadate             | 31600         | 39800        | 0.8                 |
| "Polyan M" *                  |               |              |                     |
| (Polyantimonic acid)          | 2800          | 320          | 9                   |
| Antimony (V)                  |               |              |                     |
| hexacyanoferrate (II)         | 7550          | 110          | 72                  |

Ref: Brihaye C., Guillaume M. and Cognau M. Radiochem.Radioanal.Lett., 48, 157-164, (1981).

\* Applied Research Laboratories (see page 4).

the  $\text{Al}_2\text{O}_3$ /1.8% NaCl pH 9 - 10 system together with a Chelex-100 safety trap column (5) until we too transferred to the  $\text{SnO}_2$ /0.9% NaCl pH 6.5 - 7 system.

Table 3 and 4 summarize the Brihaye and Neirinx data respectively. Although not acquired in exactly the same way, the data are comparable. It is clear from this data why  $\text{SnO}_2$  is now most favoured. However a complication arises. Tin dioxide exists in at least two different forms,  $\alpha$  and  $\beta$ , depending upon its method of preparation (22) and these exhibit different adsorption characteristics for both anions and cations. Fortuitiously, Brihaye and Neirinx both carried out their distribution studies using the  $\alpha$  form from the same manufacturer (Oxtain Applied

TABLE IV  
DISTRIBUTION COEFFICIENTS (Kd) OF Sr(II) AND Rb(I)

(0.9% NaCl solutions at 60 min equilibration)

| <u>Adsorbant</u>                     | <u>pH</u> | <u>Sr(II)</u> | <u>Rb(I)</u> | <u>Sr(II)/Rb(I)</u> |
|--------------------------------------|-----------|---------------|--------------|---------------------|
| Basic Al <sub>2</sub> O <sub>3</sub> | 8         | 7000          | 9            | 800                 |
| Hydrous Zirconium oxide              | 7         | 800           | 5            | 200                 |
| Hydrous Titanium oxide               | 8         | 56000         | 56           | 1000                |
| Titanium peroxide                    | 7         | 43000         | 162          | 300                 |
| Polyantimonic acid                   | 3         | 75000         | 3            | 25000               |
| Hydrous tin oxide                    | 7.2       | 43000         | 2.5          | 21000               |

Ref: Neirinx R.D., Kronauge J.F. and Loberg M.D. Int.J.Appl.Radiat.Isot.,  
34, 721-725, (1983).

Research, Jodoigne, Belgium). The  $\beta$  form available from a different supplier (Farmilatia Carlo Erba, Milan, Italy), is quite unsuitable for the preparation of a  $\text{Sr}^{82}/\text{Rb}^{82}$  generator (23).

The performance of a typical  $\text{SnO}_2/\text{Rb}^{82}$  generator is described in detail by Gennaro et al (6).

In our early experiences with the  $\text{SnO}_2/0.9\% \text{ NaCl}$  system we retained the concept of a Chelex-100 safety column working on the assumption that if an added safety factor on the  $\text{Sr}^{82}$  breakthrough could be achieved with a replaceable Chelex-100 column operating in a low radiation dose situation, some advantages could be gained. It turned out however that the radiochemical purity improvements were totally out balanced by the inability to maintain the generator system sterile when a Chelex column was incorporated into it. It was found that  $\text{SnO}_2$  even when loaded with  $\text{Sr}^{82}$  could be sterilized using isotonic sodium hypochlorite (Milton) whereas all attempts either chemically or by  $\gamma$  irradiation to sterilize Chelex-100 resulted in a severe degradation of its ability to retain strontium.

The problems of maintaining a generator of this type in the clinical situation bear some examination in the context of achieving a good pharmaceutical practice and recording of quality control data. The main considerations are the following:

1. The long physical and biological half-life of the parent  $\text{Sr}^{82}$  which is always accompanied by  $\text{Sr}^{85}$  (65d) usually in at least equal proportions (18,5). This factor demands an extremely high level of scrutiny of test eluates for  $\text{Sr}^{82}$  and  $\text{Sr}^{85}$  breakthrough before and after each clinical session (24).
2. As the generator systems are almost always used in an infusion mode and the potential for large volume infusions exists, the eluant must be physiologically acceptable, especially in the case of cardiac studies and be maintained sterile and apyrogenic. Tests for pyrogenicity can be achieved quickly in house ("Pyrogent", Byk Mallinckrodt) but sterility and rabbit pyrogen tests cannot and the use of membrane filters for terminal sterilization is not without its problems. It is apparent that whenever the eluant reservoir is changed,

(in our case commercially produced bags of isotonic saline for injection are employed), there is a risk of infecting the generator as it is quite impractical to carry out this change in a sterile environment. As is usually the case, if something can go wrong, eventually it will, resulting in our generator occasionally failing to stand the tests for sterility and inevitably pyrogenicity. However since we introduced the isotonic hypochlorite treatment between clinical sessions followed by an exhaustive wash with sterile isotonic saline, we have not encountered sterility or pyrogen problems.

In conclusion, it is fair to say that clinically acceptable systems are now well established and those clinical researchers who have had the opportunity to carry out studies with  $\text{Rb}^{82}$  are without exception highly enthusiastic. The commercial potential of a  $\text{SnO}_2$  generator system is being assessed (The Squibb Inst. for Medical Research, New Brunswick, NJ, USA).

There remains however one vital point, this is the long-term supply of  $\text{Sr}^{82}$  and the only facility that has the potential to ensure this at the present time is the one at Los Alamos.

#### $\text{Ge}^{68}$ (271d)/ $\text{Ga}^{68}$ (68m)

The first description of a chemical system to separate  $\text{Ga}^{68}$  from its parent  $\text{Ge}^{68}$  appeared in 1960 (2). What was then termed a "Positron Cow" because it could be "milked" would now of course be referred to as a radionuclide generator. The separation employed a solvent extraction procedure.  $\text{Ga}^{68}$  was extracted into a 25% solution of acetylacetone in cyclohexane from a  $\text{Ge}^{68}$  solution at pH 4.5. Back extraction into 0.1  $\text{NHCl}$  resulted in a  $\text{Ga}^{68}\text{Cl}_3$  solution essentially ready for conversion to the  $\text{Ga}^{68}$  (EDTA) complex.

This last feature raised some irony as radiopharmaceutical chemists have, for the last ten years, been attempting to perfect a chromatographic generator to produce ionic  $\text{Ga}^{68}$  (III), more about which below.

About a year after Gleason's report was published a chromatographic  $\text{Ga}^{68}$  "Cow" was described by Greene and Tucker (25). Their successful use of  $\text{Al}_2\text{O}_3$  for chromatographic  $\text{Te}^{122}/\text{I}^{122}$

and  $\text{Mo}^{99}/\text{Tc}^{99\text{m}}$  generators (26) led to attempts to make a chromatographic  $\text{Ge}^{68}$  generator. The resulting system was quite revolutionary. The  $\text{Al}_2\text{O}_3$  column loaded with  $\text{Ge}^{68}$  could be eluted with 0.005 M EDTA pH 7 to produce the  $\text{Ga}^{68}$  EDTA complex directly for use in "positron brain scanning". A generator (New England Nuclear, North Billerica, USA) based on this system is still available commercially. However, in order to utilize  $\text{Ga}^{68}$  in more subtle radiopharmaceutical preparations, ionic  $\text{Ga}^{68}$  (III) is an essential prerequisite. It is possible to rid the  $\text{Ga}^{68}$  of its EDTA "cloak" but it is a task best avoided due to significant decay losses and increased prelabelling chemical manipulators to ensure total removal of EDTA (27,28). Again the advent of the new generation of positron tomographs (14,15) spurred on the radiopharmaceutical chemist to perfect a generator for ionic  $\text{Ga}^{68}$  (III).

The literature has many reports of ionic  $\text{Ga}^{68}$  generators in development (29-32) but the one which now is generally accepted as the "state of the art" was first described by Loc'h et al in 1980 (33).

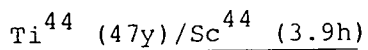
Several groups have duplicated the work and a commercial generator based on Loc'h's chemistry is now available (New England Nuclear, North Billerica, USA) The chromatographic separation of  $\text{Ga}^{68}$  from  $\text{Ge}^{68}$  is accomplished using a  $\text{SnO}_2$  column eluted with 3-5 ml 1N HCl. Elution efficiencies are high (75-80%) and  $\text{Ge}^{68}$  breakthrough acceptably low 0.0002% to 0.003% (33,34).

For those contemplating making a generator of this type, the choice of  $\text{SnO}_2$  of the acid resistant  $\beta$  form is vital and the  $\alpha$  form has been shown to be quite unsuitable (23).

In a recent comparison of  $\text{Ge}^{68}/\text{Ga}^{68}$  generator systems by McElvany et al (34), the only reservation about the performance of the  $\text{SnO}_2/\text{HCL}$  generator was that their studies indicated that even the small traces of metal impurities, principally tin at 3  $\mu\text{gm}$  in a 5 ml elution, could interfere with the efficient labelling of radiopharmaceuticals where limited numbers of chelating sites would be available, e.g. antibodies and proteins linked to bifunctional chelates. The less sophisticated radio-

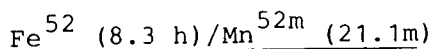


pharmaceuticals apparently do not suffer from this effect and satisfactory labelling has been reported for human serum albumin microspheres (35), transferrin (36) red cells (36), citrate and pyrophosphate (36) DTMP (37), Alizarin (37),  $\text{Fe}(\text{OH})_3$  (37), Alizarin-red-S (37). Unlike  $\text{Sr}^{82}$ ,  $\text{Ge}^{68}$  can be produced reasonably effectively by cyclotrons. However, its production by spallation at BLIP and LAMPF should be very attractive providing the costing of irradiation time is based only the parasitic beam dump concept!



A  $\text{Sc}^{44}$  generator was described by Greene and Hillman in 1967 (38). However, the production of the 46y parent  $\text{Ti}^{44}$  was not described. Its cyclotron production by the  $\text{Sc}^{45} (p,2n) \text{Ti}^{44}$  would appear possible but rather laborious. Production at one of the high energy proton accelerators (16,39,40) by spallation would be worthy of investigation should a demand arise.

The generator system is based on anion exchange (Dowex 1,  $\text{Cl}^-$  form) column of some 15 ml volume and 2 cm diameter.  $\text{Ti}^{44}$  is loaded, after treatment with hydrogen peroxide, onto the resin bed in 0.1 M oxalic acid. After washing the column with 40 ml of 0.1 M oxalic acid/0.2 M hydrochloric acid and allowing an appropriate ingrowth of  $\text{Sc}^{44}$  it was eluted with 30 - 50 ml of the same solution. Oxalate was decomposed by hydrogen peroxide treatment of the  $\text{Sc}^{44}$  containing oxalic acid residue resulting from boiling down the eluate to dryness. The elution yield reported was 60 to 70% but the breakthrough of this early system was 0.02 - 0.1%. With a 44y parent this could explain why little has been heard of this generator system since.



$\text{Fe}^{52}$  has been used for many years in haemopoetic studies. Consequently many potential human radionuclide generators have been made! However, despite repeated studies no evidence for the separation of  $\text{Mn}^{52\text{m}}$  from  $\text{Fe}^{52}$  has been observed in man. The cyclotron production of  $\text{Fe}^{52}$  for practicable  $\text{Mn}^{52\text{m}}$  generator preparation would be prohibitively slow and expensive. However, its production parasitically on a linear accelerator being

operated for other purposes, as BLIP (39) and LAMPF (40) are, seems particularly practicable. Using a manganese target the  $\text{Mn}^{55} (p,4n) \text{Fe}^{52}$  reaction at  $E_p = 70 \text{ MeV}$  a yield of  $98 \mu\text{Ci } \mu\text{Ah}^{-1}$  is achieved resulting in typical production batches of  $60 \text{ mCi}$  (39). With a nickel target the  $\text{Ni}^{58} (p,3p4n) \text{Fe}^{52}$  reaction at  $E_p = 193 \text{ MeV}$  a yield of  $50 \mu\text{Ci } \mu\text{Ah}^{-1}$  results in a typical production of  $33 \text{ mCi}$  (39). However reports from LAMPF at Los Alamos where irradiation of nickel targets can be carried out at  $E_p = 800 \text{ MeV}$  recovered yields of over  $1 \text{ Ci}$  of  $\text{Fe}^{52}$  have been reported (40).

A chemical generator system was described by Atcher et al in 1978 (41) and by Ku et al in 1979 (42). The generator column consists of a  $0.6 \text{ ml}$  bed of anion exchange resin (Bio Rad AG1 x 8, 200 - 400 mesh) contained in a  $1 \text{ ml}$  plastic tuberculin syringe barrel.  $\text{Fe}^{52}$  is loaded onto the column in  $8\text{N}$  hydrochloric acid and strongly absorbed as the  $\text{FeCl}_4^-$  complex. After a further  $5 \text{ ml}$  wash with  $8\text{N}$   $\text{HCl}$  the generator is ready for elution. Approximately  $90\%$  of the  $\text{Mn}^{52\text{m}}$  available is eluted in  $2 \text{ ml}$  of  $8\text{N}$   $\text{HCl}$ . The eluate is quickly evaporated to dryness and the  $\text{Mn}^{52\text{m}}$  residue taken up in  $3 \text{ ml}$  of sodium acetate buffer ( $\text{pH } 5.6$ ).

Terminal sterilization by a  $0.45 \mu\text{m}$  bacterial filter is reported. With an eluant of  $8\text{N}$   $\text{HCl}$  problems with bacterial endotoxins should be no problem. However care should be taken to eliminate any ion exchange resin decomposition products that could give rise to adverse pyrogenic reactions in the proposed clinical use of  $\text{Mn}^{52\text{m}}$  for myocardial imaging. No  $\text{Fe}^{52}$  breakthrough as detected at an upper limit of  $1 \times 10^{-7}$  based on the minimum detectable  $\text{Fe}^{52}$  activity of  $0.001 \mu\text{Ci}$ . However  $\text{Mn}^{52\text{g}}$  ( $5.67 \text{ d}$ ) will inevitably remain in the decayed  $\text{Mn}^{52\text{m}}$  eluate as  $2.2\%$  of its decays result in  $\text{Mn}^{52\text{g}}$  however if care is exercised in the selection of the post elution regrowth period it should be possible to maintain a figure of  $3.2 \times 10^{-4}\%$  of  $\text{Mn}^{52\text{g}}$  relative to  $\text{Mn}^{52\text{m}}$  (39).

$\text{Zn}^{62}$  (9.2h) /  $\text{Cu}^{62}$  (9.7 m)

A radionuclide generator based on this nuclear relationship was described by Robinson in 1976 (43). The parent  $\text{Zn}^{62}$  is most effectively produced by the  $\text{Cu}^{63}$  (p,2n)  $\text{Zn}^{62}$  reaction. A thin copper target is bombarded with 22 MeV protons. The  $\text{Zn}^{62}$  is recovered from the target by a chloride complex anion exchange procedure. The generator column 1 cm diameter 2 cm long filled with Dowex 1 x 8 200 - 400 mesh is loaded with  $\text{Zn}^{62}$  in 2N HCl. Elutions are carried out using 0.1N HCl containing 100 mgm/ml NaCl and 1  $\mu\text{gm/ml}$  Cu(II) carrier. Over 85% of the available  $\text{Cu}^{62}$  was recovered in 3 ml of eluate and the  $\text{Zn}^{62}$  breakthrough was less than 1  $\mu\text{Ci/mCi}$  of  $\text{Cu}^{62}$ .

Robinson also reported the rapid preparation of some potential  $\text{Cu}^{62}$  radiopharmaceuticals (43,44). These included colloidal  $\text{CuS}^{62}$  and  $\text{CuO}^{62}$  both of which were shown in rat and dog studies to be highly extracted by the liver.  $\text{CuS}^{62}$  was incorporated into macro aggregated albumin (MAA) using the heat denaturing procedure. Tin (II) coated human serum albumin microspheres (HAM) were labelled by the adjustment of the generator eluate to pH3, adding the "instant" HAM and heating to 121° C for 10 min. Both  $\text{Cu}^{62}$  MAA and  $\text{Cu}^{62}$  HAM were shown to be over 85% extracted by the lung in rats at 5 min. Perfusion images of the dog lung were reported to be of good quality. Finally a series of  $\text{Cu}^{62}$  "chelates" were studied and although while not exhaustively assessed showed some interesting radiopharmaceutical properties.

$\text{Se}^{72}$  (8.4 d) /  $\text{As}^{72}$  (26. h)

The radionuclide generator separation of this pair was being studied at Brookhaven in 1958 (45). Several other groups have studied the problems involved. All came to the conclusion that due to the wide range of oxidation states that both the parent and the daughter can adopt in solution a practicable chromatographic generator was extremely difficult to perfect. Reports of this work have apparently not appeared in the literature.

Te<sup>118</sup> (6.0 d)/Sb<sup>118</sup> (3.5 m)

Only preliminary reports of the production of Te<sup>118</sup> and its potential as a parent for Sb<sup>118</sup> have appeared (39). No generator system has yet been described. It would be anticipated that all the problems of the Se<sup>72</sup>/As<sup>72</sup> system will have to be overcome. This together with the 3.5 m daughter half-life leads one to suspect that a practical system is a long way off.

Xe<sup>122</sup> (20.1 h)/I<sup>122</sup> (3.6 m)

Over the years radioiodines have continued to fill the gap between simple inorganic radionuclides and the classic labelled molecule where no potential perturbing atoms are introduced. For single photon emission tomography I<sup>123</sup> (13.2h) has very good photon emission characteristics and for positron emission tomography cyclotron produced I<sup>121</sup> (2.12h) could be useful. Away from a cyclotron a generator produced positron emitting iodine radionuclide could have some attraction. Xe<sup>122</sup> is produced as a by product of Xe<sup>123</sup> production for I<sup>123</sup> generation. A radionuclide generator for the recovery of I<sup>121</sup> has been described by Richards and Ku in 1979 (46). Unlike most other radionuclide generators which operate in the solid/liquid phases this generator operates in the gas/solid phase. The Xe<sup>122</sup> is transferred back and forth between a metal reservoir and a glass growth chamber cryogenically. The Xe<sup>122</sup> is held in the growth chamber at -196° C (LN<sub>2</sub>) for a 5 to 10 min. ingrowth period. It is then transferred to the metal reservoir by cooling it to -196° C whilst warming the glass growth vessel. After operating the appropriate valves 1.4% sodium bicarbonate is introduced to the growth vessel via a septum and the I<sup>121</sup> solution recovered by vacuum transfer. Elution efficiency is quoted to be about 40% but it is not clear how this figure is arrived at. Radioxenon breakthrough is reported to be less than 0.1%. Rapid iodinations, some using lactoperoxidase and chloramine-T, have been reported (39,47,48) to achieve 85 - 90% yields in 1 to 3 min. Further exploitation of this rather unusual generator system will probably pose many quality control headaches!

$\text{Ba}^{128}$  (2.43 d)/ $\text{Cs}^{128}$  (3.6 m)

This radionuclide generator is analogous to the  $\text{Sr}^{82}/\text{Rb}^{82}$  system. The production of  $\text{Ba}^{128}$  has been described by Lagunas-Solar et al in 1982 (49,50) by the proton irradiation of  $\text{CsCl}$  with 67 MeV protons. Although the positron emission % of  $\text{Cs}^{128}$  is not as good as  $\text{Rb}^{82}$  (see table 1) and the myocardial extraction of  $\text{Cs}$  is about half that of  $\text{Rb}$ . The 3.6 min. half-life may offer some advantages.

#### CONCLUDING DISCUSSIONS

Without doubt the major factor that stimulated a revival of interest in "Positron Generators" was the advent of Positron Emission Transaxial Computed Tomography PETT (14,15).

We now have the two highly developed generator systems for  $\text{Rb}^{82}$  and  $\text{Ge}^{68}$  which fulfil complementary roles in the clinical application of PETT. The radiopharmaceutical labelling potential of  $\text{Ga}^{68}$  has been demonstrated and more developments should be anticipated.

One problem area with these generators with a long parent half-life which has received little attention is that of long-term maintenance of sterility (28,37,51). The collaboration of the radiopharmacist with the generator radiochemist to help resolve this problem would seem most appropriate.

What of the rest of the positron generator family listed in table 1? It is fair to say that we do not anticipate hearing much more about some of them for a variety of reasons.

However the  $\text{Zn}^{62}/\text{Cu}^{62}$  generator would appear to have more potential for exploitation. The parent half-life is not impractically short at 9.2 h. In fact it is just twice that of  $\text{Rb}^{81}$  which we and others are distributing to diagnostic clinics as the  $\text{Rb}^{81}/\text{Kr}^{81m}$  generator (52). There would seem to be scope for investigation the potential for radiopharmaceutical labelling with  $\text{Cu}^{62}$ , at least as developmental radiopharmaceutical projects, at centres remote from the producing cyclotron.

Unfortunately  $\text{Fe}^{52}$  and  $\text{Xe}^{122}$  the parents of  $\text{Mn}^{52m}$  and  $\text{I}^{122}$  respectively, can only be made in sufficient quantities at the present time on the giant accelerators in the USA. The logistics

of transportation to Europe with the 8.3 h. and 20.1 h. half-lives would preclude them for all practical purposes. However some thought should be put into the production potential of the very large accelerators in Europe, for example Swiss Institute of Nuclear Research (SIN), Villigen, Switzerland and Spallation Neutron Source (SNS), Science and Engineering Research Council, Rutherford and Appleton Laboratories, Chilton, Didcot, Oxon, before we totally dismiss these two generator systems.

In conclusion it is clear that positron generators have been demonstrated to complement the in house cyclotron in PETT studies and it is not totally unrealistic to expect to see some clinical PETT diagnostic tests based on generator produced positron radio-pharmaceuticals.

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## Current Methodology for Oxygen-15 Production for Clinical Use\*

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The radionuclide oxygen-15, half-life 2.05 min, is used in simple chemical forms to study oxygen metabolism, blood flow and blood volume in man,<sup>(1)</sup> using the technique of positron emission tomography (PET).<sup>(2)</sup>

The production of <sup>15</sup>O and the preparation of [<sup>15</sup>O]O<sub>2</sub>, [<sup>15</sup>O]CO<sub>2</sub>, [<sup>15</sup>O]CO and [<sup>15</sup>O]H<sub>2</sub>O is now well established in several PET centres in Europe, North America and Japan. In order to provide a practical design and operational data base for others intending to make use of these techniques an EEC task group representing four European laboratories routinely using <sup>15</sup>O in PET studies was set up to review the current practice of <sup>15</sup>O production purification and quality control of the clinically useful products.

### Radionuclide Production

#### Choice of nuclear reaction

Oxygen-15 may be produced by the <sup>14</sup>N(d, n)<sup>15</sup>O,<sup>(3)</sup> ( $Q > 0$ ), <sup>16</sup>O(p, d)<sup>15</sup>O<sup>(4-6)</sup> (threshold energy = 14 MeV) and <sup>15</sup>N(p, n)<sup>15</sup>O<sup>(7,8)</sup> (threshold energy = 3.7 MeV) nuclear reactions.

The <sup>14</sup>N(d, n)<sup>15</sup>O reaction is most widely used. The <sup>16</sup>O(p, d)<sup>15</sup>O reaction is used when only high energy protons are available ( $E_p > 20$  MeV). At least three centres in North America are now using this reaction to produce <sup>15</sup>O<sub>2</sub> and H<sub>2</sub><sup>15</sup>O but due to the high oxygen content of the target the production of C<sup>15</sup>O<sub>2</sub> by combustion of carbon is difficult to control. The production of C<sup>15</sup>O by combustion is avoided due to the hazardous amounts of carbon monoxide that would be produced.

The <sup>15</sup>N(p, n)<sup>15</sup>O reaction must be used when only low energy protons are available ( $E_p < 15$  MeV). The use of enriched <sup>15</sup>N<sub>2</sub> to produce <sup>15</sup>O<sub>2</sub> for both bolus and steady state metabolic studies has been demonstrated<sup>(8)</sup> using 4 mL volume gas target irradiated with a very well regulated 8-11 MeV proton beam. The running cost, at a flow rate of 4 mL/min, of U.S.\$ 1.00 per minute may not represent an unreasonable fraction of the total cost of a PET clinical study.

The <sup>14</sup>N(d, n)<sup>15</sup>O reaction will be of principal concern in this report, as the experience with the other

reactions is currently limited to only a few centres, none of which are in Europe.

#### Selection of charged particle energy

The energy of the deuterons incident on the nitrogen target gas should be adjusted to about 7 MeV either by adjustment of the accelerator output, degrader foil thickness of both. This energy should minimise the production of the longer lived radionuclidic impurities <sup>13</sup>N (half-life = 10.0 min) and <sup>11</sup>C (half-life = 20.4 min) by the <sup>14</sup>N(d, t)<sup>13</sup>N (threshold energy 4.9 MeV) and <sup>14</sup>N(d,  $\alpha$ n)<sup>11</sup>C (threshold energy 5.8 MeV) reactions.

#### Target design and gas delivery tubes

The target vessel used to contain the nitrogen during irradiation is usually made of aluminium alloy. The cross sectional dimensions of the target will depend on the size and shape of the deuteron beam and the length dependant on the chosen operating pressure.

The pressure is usually determined by the dimensions (diameter and length) of the radioactive gas delivery pipe. The choice of these parameters may be aided using the computer programme GASLIN.<sup>(9)</sup> Typically tubes with internal diameters from 1.5 to 4 mm and up to a few hundred metres length have been found to be practicable. Stainless steel, PTFE and polypropylene tubes have been used satisfactorily. Nylon should be avoided due to its lack of chemical and radiation resistance. A titanium beam

\* A report from an EEC task group.

entry window 0.025–0.050 mm thick and 25–30 mm diameter is fitted with a nitrile, viton or metal sealing ring to a 30–50 mm diameter cylindrical vessel some 200–250 mm long, filled with nitrogen gas between 3 and 1.5 bar (abs) respectively. As with all gas target and accelerator beam windows, provision must be made for their cooling. This is achieved with air or preferably helium jets with flow rates of 10–20 L min<sup>-1</sup> at 1–1.5 bar (abs) with recirculation in the case of helium.

### Target gases

#### <sup>15</sup>O<sub>2</sub> production

In-target production of <sup>15</sup>O<sub>2</sub> is achieved by irradiating nitrogen containing 0.2–4% (vol) oxygen. The lower level is quite critical as the recovered yield of <sup>15</sup>O<sub>2</sub> falls dramatically when lower concentrations are used but may be necessary when higher specific activities are required as e.g. in some C<sup>15</sup>O labelling procedures. H<sub>2</sub><sup>15</sup>O may also be prepared from <sup>15</sup>O<sub>2</sub>. <sup>15</sup>O<sub>2</sub> production yields of 0.2–0.3 GBq min<sup>-1</sup> μA<sup>-1</sup> (5–8 mCi min<sup>-1</sup> μA<sup>-1</sup>) using a nitrogen/2% oxygen target gas can be achieved at flow rates of 500 mL min<sup>-1</sup>.

#### C<sup>15</sup>O<sub>2</sub> production

In-target production of C<sup>15</sup>O<sub>2</sub> is generally preferred to the less efficient chemical conversion of <sup>15</sup>O<sub>2</sub> using hot activated charcoal. The target gas is nitrogen containing 2–2.5% carbon dioxide. C<sup>15</sup>O<sub>2</sub> production yields of 0.2–0.3 GBq min<sup>-1</sup> μA<sup>-1</sup> (5–8 mCi min<sup>-1</sup> μA<sup>-1</sup>) using nitrogen/2.5% carbon dioxide target gas can be achieved at flow rates of 500 mL min<sup>-1</sup>.

#### Target gas supply

Target gas mixtures are generally purchased from commercial gas suppliers and are either certified mixtures made from medical grade gases of the mixtures of N45 (99.995%) gases depending on the requirements of the local regulating authorities. Whichever type of mixture is used it must be adequately labelled to avoid accidental misuse.

### Target Operating Criteria

Deuteron beam currents of up to 50 μA have been found to be practicable. The target windows being the principal limitation. It is possible to use the same target vessel for <sup>15</sup>O<sub>2</sub> and C<sup>15</sup>O<sub>2</sub> production provided that adequate evacuation or flushing of the target and its supply pipes is achieved between irradiations. For C<sup>15</sup>O production the risk of accidental inclusion of oxygen or carbon dioxide with the inherent risk of producing high levels of carbon monoxide precludes the use of a common target in this case.

### Processing and Purification

#### <sup>15</sup>O<sub>2</sub>, C<sup>15</sup>O, and H<sub>2</sub><sup>15</sup>O

The main chemical and radiochemical impurities

ozone, nitrogen oxides and C<sup>15</sup>O<sub>2</sub>, N<sub>2</sub><sup>15</sup>O are removed using two absorber columns in series. The first 25 mm dia × 120 mm long is filled with soda lime and a second similarly dimensioned column is filled with 1.5 mm granules of activated charcoal.

When C<sup>15</sup>O is produced by the oxidation of a 250 × 15 mm column of 60 mesh dried activated charcoal at 1000°C with previously purified <sup>15</sup>O<sub>2</sub>, the presence of <sup>16</sup>O<sub>2</sub> carrier inevitably leads to the production of potentially hazardous quantities of carbon monoxide. For example if a nitrogen target containing 0.25% oxygen is used, stable carbon monoxide at levels of at least 0.5% are produced. Under these conditions a yield of C<sup>15</sup>O of 37 MBq min<sup>-1</sup> μA<sup>-1</sup> (1 mCi min<sup>-1</sup> μA<sup>-1</sup>) can be achieved and by using a 20:1 dilution a carbon monoxide concentration of 0.025% can be administered safely for periods of up to 15 min. Carboxyhaemoglobin measurements in the blood samples taken from subjects exposed in this way at SHFJ, Orsay, have been found to exhibit acceptable levels of carbon monoxide.

Alternative methods using no added oxygen have been proposed<sup>(10,11)</sup> but no data is available for the stable carbon monoxide produced. At present there is no experience with these systems in Europe. The in target production of C<sup>15</sup>O at high specific activity<sup>(11)</sup> should be studied in more detail.

H<sub>2</sub><sup>15</sup>O is readily prepared by the palladium catalysed reaction of <sup>15</sup>O<sub>2</sub> with hydrogen.<sup>(3,12)</sup> A flow of purified <sup>15</sup>O<sub>2</sub> in nitrogen is mixed with hydrogen and passed over a few pellets of palladium/alumina catalyst (Engelhard Deoxo Model D) the resulting water vapour is trapped by bubbling the nitrogen carrier through sterile isotonic saline.

The catalyst also generates a small amount of ammonia which causes the pH of the saline to rise to 9. Consequently the use of a physiological buffer may be desirable for some applications. Batches of over 3.7 GBq (100 mCi) can be readily be prepared with deuteron beam currents of 20 μA. Two alternative methods for the preparation of H<sub>2</sub><sup>15</sup>O have been described on involving the exchange reaction between C<sup>15</sup>O<sub>2</sub> and water<sup>(10)</sup> and the other employing in-target reaction of <sup>15</sup>O atoms with hydrogen using a nitrogen/5% hydrogen target gas.<sup>(13)</sup> However no experience of these techniques exist in Europe.

#### C<sup>15</sup>O<sub>2</sub>

During the irradiation of nitrogen/2–2.5% carbon dioxide the chemical and radiochemical impurities carbon monoxide and <sup>15</sup>O<sub>2</sub> are observed. Earlier attempts to reduce these have now been shown to be unreliable. The use of an oxidising column composed of a mixture of copper and iron oxides supported on kaolin reduces the <sup>15</sup>O<sub>2</sub> and carbon monoxide to <0.02% and <10 μL/L respectively.<sup>(14)</sup> Nitrogen-13 will inevitable be present in all systems employing carbon dioxide in the target gas because of the <sup>12</sup>C(d,n)<sup>13</sup>N reaction (threshold energy 0.4 MeV). For applications where >0.5% <sup>13</sup>N<sub>2</sub> cannot be toler-

ated the less efficient low temperature combustion of activated charcoal with  $^{15}\text{O}_2$  must be employed. The use of the copper/iron oxide column after the carbon furnace being recommended.

### Quality Control

#### Radiogas chromatography

A radiogas chromatograph is employed to analyse for the chemical and radiochemical atmospheric gas impurities.<sup>(3,15)</sup> typically a dual column chromatograph equipped with a thermal conductivity detector for the stable gas analysis and a small sensitive volume flow-cell beta particle detector, a well shielded gamma ray scintillation or Geiger-Mueller counter for the radioactive gas analysis. The two columns most commonly used are

(a) 1500 × 4 mm Porapak-Q 80–100 mesh (preconditioned at 250°C);

(b) 3000 × 4 mm Molecular Sieve 5A 80–100 mesh (preconditioned at 350°C).

These columns when operated at between 25 and 80°C with helium carrier gas flow rate of approx 10 mL min<sup>-1</sup> provide adequate separation of the gases  $^{15}\text{O}_2$ ,  $^{13}\text{N}_2$ ,  $\text{C}^{15}\text{O}_2$ ,  $\text{N}_2^{15}\text{O}$  and  $^{13}\text{N}_2\text{O}$  when used in combination. Recently the use of a concentric dual column, containing the above packings (CTR1 Alltech Assoc.) has been proposed and some simplification of the analytical procedures may be possible.<sup>(10)</sup>

#### Decay curve analysis

Radionuclidic purity can be controlled by decay curve analysis of a gas sample. A gas sample can be measured repeatedly, e.g. with a coincidence set-up, consisting of two NaI(Tl) detectors in a 180° geometry and the decay curve obtained can be analysed according to Cumming.<sup>(16)</sup> Using 7.5 MeV deuterons and  $^{13}\text{N}$  activity is 0.5% and the  $^{11}\text{C}$  is 0.004% of the  $^{15}\text{O}$  activity.

Radiochemical purity of  $^{15}\text{O}_2$  and  $\text{C}^{15}\text{O}_2$  products

|                           | $^{15}\text{O}_2$ | $\text{C}^{15}\text{O}_2$            |
|---------------------------|-------------------|--------------------------------------|
| $^{15}\text{O}_2$         | 100               | <0.03 <sup>a</sup> –0.2 <sup>b</sup> |
| $^{13}\text{N}_2$         | 0.5               | 0.5                                  |
| $\text{C}^{15}\text{O}$   | <0.3              | <0.09                                |
| $\text{C}^{15}\text{O}_2$ | <0.3              | 100                                  |
| $\text{H}_2^{15}\text{O}$ | 0.5               | <0.6                                 |
| $^{13}\text{N}_2\text{O}$ | <0.3              | —                                    |

<sup>a</sup>For an oxidising column used for several hours.

<sup>b</sup>For a freshly oxidised oxidising column.

Levels of chemical impurities found in  $^{15}\text{O}_2$  and  $\text{C}^{15}\text{O}_2$  purified products  $\mu\text{L/L}$  and threshold limit values (TLV) in  $\mu\text{L/L}$

|               | $^{15}\text{O}_2$ | $\text{C}^{15}\text{O}_2$ | $\text{C}^{15}\text{O}$ | TLV |
|---------------|-------------------|---------------------------|-------------------------|-----|
| $\text{O}_3$  | 0.01              | 0.01                      | —                       | 0.1 |
| $\text{NO}_2$ | 0.01              | 0.02                      | —                       | 5   |
| $\text{CO}$   | <1                | 2–9                       | 5000                    | 50  |

#### Chemical analysis

The chemical analysis of the noxious gases, ozone, nitrogen dioxide, and carbon monoxide down to the levels necessary for safety prior to the administration of a radioactive gas to man cannot be carried out satisfactorily by gas chromatography.

Instrumental methods, employing chemiluminescence, of high sensitivity are available for the estimation of ozone down to  $\mu\text{L/L}$  levels. Unfortunately the detectors are also radiation sensitive especially to the high energy positrons emitted by the radionuclides under review. Post radioactive decay measurement of ozone is not recommended due to the difficulty in storing ozone samples reliably. The classical iodometric analysis for ozone is recommended.<sup>(17)</sup> The only likely interference in the systems under present discussions being due to nitrogen dioxide.

Ozone will be potentially present in all irradiated mixtures containing oxygen. Particularly large amounts will be present in the irradiations of 100% oxygen as used with the  $^{16}\text{O}(\text{p}, \text{d})^{15}\text{O}$  reaction.<sup>(6)</sup> Instrumental methods of analysis for nitrogen dioxide are available but are not appropriate for the same reasons as above for ozone. A colorimetric estimation is available with adequate sensitivity for the present purposes based on an azo dye forming reagent.<sup>(17)</sup> Ozone interferes to a small extent. Nitrogen dioxide will always be present in irradiated nitrogen/oxygen mixtures used for  $^{15}\text{O}_2$  production. Therefore the proof of absence of this noxious gas following the purification processes is essential.

The sensitivity of the thermal conductivity detectors in the gas chromatograph can be considered marginally adequate for routine carbon monoxide analysis. More sensitive methods such as non dispersive i.r. photometry using a large folded path gas cell<sup>(14)</sup> or the use of an electrochemical detector specific for CO e.g. "Ecolyser" (Energetic Science Inc., New York), should be considered especially for checking the status of the copper/iron oxide column.

### Recommended Good Manufacturing Procedures in Oxygen-15 Production for Clinical Use

(1) Incoming gas supplies should be checked for correct contents and labelling especially if uncertified mixtures are used. The cylinders should be stored in an area where they cannot be tampered with.

(2) The energy of the accelerator beam if variable should be checked with the normal operating parameters of the cyclotron.

(3) Where the target window is used to significantly degrade the deuteron energy, regular checks on its condition, and a preventative maintenance schedule designed, based on these checks instituted.

(4) The reagents used in the purification systems should be checked and changed (or reoxidised for the

copper/iron oxide column) according to a regular schedule. The frequency of these checks will depend on the number of hours the systems are in use.

However, a good starting point would be to carry out weekly inspections of furnace tubes and changing of the room temperature reagents. Whilst checking the furnaces an inspection of the thermal regulation system should be carried out.

(5) The heart of the routine quality control procedure is the radiogaschromatograph. This should be maintained in accordance with the manufacturers recommendations and the performance of the columns checked using a calibration gas mixture of all the expected inactive gases.

(6) Where  $C^{15}O$  must be used the usual quality control checks should be followed by strictly regulated dispensing procedure to enable the chemical dose of carbon monoxide to be prescribed.

(7) Where  $H_2^{15}O$  samples are prepared for injection the system used should be shown to be capable of providing samples which are sterile and pyrogen free. One of the best ways of achieving this is to use disposable sterile components for those parts of the system that come into contact with the infusate and sterilise the incoming gases by filtration. As only retrospective testing of these samples is possible, random batches should be taken for biological testing.

(8) It is recommended that an operating manual should be provided for the production systems. This should be accompanied by an operations log book so that records of patient administration can be kept together with QC data and any information on the system's malfunctions and the operator's rectifying actions.

(9) Finally it should be stressed that as with all short half-life preparations ultimately destined for human use, the staff responsible for the preparations

should be adequately trained in the procedures both technically, and emotionally, thus being able to deliver high quality samples often under the pressures of both rapid radioactive decay and urgent clinical need!

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## Rapid analysis for metabolites of $^{11}\text{C}$ -labelled drugs: fate of [ $^{11}\text{C}$ ]-S-4-(*tert.*-butylamino-2-hydroxypropoxy)-benzimidazol-2-one in the dog

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### ABSTRACT

Positron emission tomography (PET) requires the use of compounds labelled with short-lived, positron-emitting isotopes (e.g.,  $t_{1/2}$  of  $^{11}\text{C}$  ~ 120 min). As the concentration of unbound, non-metabolised drug is required as the input function for modeling, this presents particular problems for the study of the kinetics and metabolism of such compounds. We have now developed a rapid extraction procedure, followed by high-performance liquid chromatography using a short analytical column coupled to an on-line  $\gamma$ -detector to determine the metabolism and kinetics of a non-selective  $\beta$ -adrenergic antagonist of high affinity, S-4-(*tert.*-butylamino-2-hydroxypropoxy)benzimidazol-2-one. This antagonist is potentially well suited to the non-invasive localisation of  $\beta$ -receptors *in vivo*. The ligand was rapidly taken up into the  $\beta$ -receptor pool or excreted in urine, with less than 5% of the drug converted to metabolites. Plasma protein binding was only 16%. No significant metabolism of the ligand was observed in the anaesthetised dog, and, therefore, no correction for blood metabolite concentration is required for kinetic analysis of the  $^{11}\text{C}$ -labelled ligand during PET studies in this species. The analytical method reported here should be widely applicable: quantification of metabolites enables accurate estimation of the input function and is critical to the interpretation of PET data.

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## INTRODUCTION

Positron emission tomography (PET) is a powerful method for determining the distribution and kinetics of radiolabelled ligands *in vivo* [1,2]. Positron-emitting isotopes (such as  $^{11}\text{C}$  and  $^{18}\text{F}$ ) are chemically incorporated into a suitable ligand. Following administration, the ligand will distribute between tissue compartments including the plasma and tissue receptor pool. The regional concentrations of radioligand are determined from the PET signal, which arises from radioactive decay of the positron-emitting isotope. The PET signal in plasma or tissue will represent both the parent radioligand and any labelled metabolites formed *in vivo*, either free or bound. However, it is the concentration of unbound ligand in plasma which is required as the input function for modeling receptor ligand kinetics [3,4]. Thus, any metabolites (or impurities from the original synthesis) must be measured and a suitable correction made to the input function.

*S*-4-(*tert*-Butylamino-2-hydroxypropoxy)benzimidazol-2-one (*S*-I) (Fig. 1) is a non-selective  $\beta$ -adrenergic antagonist of high affinity [5]. Because of its low lipophilicity, it exhibits low non-specific binding and does not accumulate in cells. The racemic form of the ligand (*RS*-I) has been proposed as a specific antagonist of surface  $\beta$ -receptors [6]. It has been used in competition studies to determine the binding constants of unlabelled  $\beta$ -adrenergic antagonists [5]. It has been labelled with the positron-emitting isotope  $^{11}\text{C}$  [7] in order to visualise  $\beta$ -receptor distribution and density in the lung and heart.

In order to determine the input function in PET studies, a knowledge of the clearance and metabolism of  $^{11}\text{C}$ -labelled drugs is critical [8,9]. We have developed extraction and analytical techniques, based on high-performance liquid chromatography (HPLC), to investigate the possible metabolism of this ligand in the rat and dog *in vivo*. Initial assessment of the metabolism of the ligand was carried out using both [ $^3\text{H}$ ]-*S*-I and [ $^{14}\text{C}$ ]-*RS*-I to enable detailed analysis of any metabolites and to establish the rapid techniques required when using  $^{11}\text{C}$ -labelled ligands ( $t_{1/2}$  of  $^{11}\text{C} \sim 20$  min). Subsequently, studies were performed with [ $^{11}\text{C}$ ]-*S*-I using a short analytical HPLC column coupled to an on-line  $\gamma$ -detector.

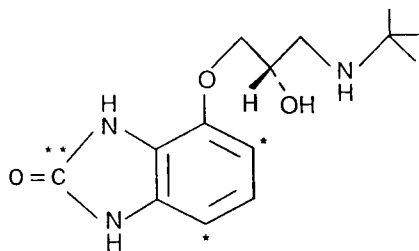


Fig. 1. Structure of *S*-I. The positions of the radiolabels are marked: (\*)  $^3\text{H}$ ; (\*\*)  $^{11}\text{C}$  or  $^{14}\text{C}$ .

## EXPERIMENTAL

*Radioligands*

Isotopically labelled ligand [ $^{14}\text{C}$ ]-4-(*tert.*-butylamino-2-hydroxypropoxy)-benzimidazol-2-one was synthesised in the MRC Cyclotron Unit at Hammer-smith Hospital by reacting 1-(3-*tert.*-butylamino-2-*S*-hydroxypropoxy)-2,3-diaminobenzene with [ $^{14}\text{C}$ ]phosgene as previously described [7]. The radioligand was purified by HPLC. The specific activity was  $\leq 10$  TBq/mmol and the radiopurity greater than 99.98% on all occasions. The  $^3\text{H}$ -labelled ligand ([ $^3\text{H}$ ]-*S*-I) was obtained from Amersham International (Amersham, UK) (specific activity 1.11–2.22 TBq/mmol, radiochemical purity 99.1%). A racemic form of the ligand labelled with  $^{14}\text{C}$  ([ $^{14}\text{C}$ ]-*RS*-I, 3.6 MBq/mmol, purity 96%) was a kind gift from Ciba-Geigy (Basle, Switzerland).

*Animal studies*

Five greyhound dogs were studied. Two females (weight 27.6 and 29.4 kg) and two males (weight 28.5 and 32.6 kg) were infused with [ $^{14}\text{C}$ ]-*S*-I. One female (weight 25.5 kg) was infused with [ $^3\text{H}$ ]-*S*-I. Anaesthesia was induced with thiopental sodium (5 mg/kg) and maintained with nitrous oxide–oxygen with 0.75% halothane (tidal volume 20 ml/kg, fifteen breaths per min). For injection of radioligand, a catheter (8F) was placed in the inferior vena cava via the femoral vein. Catheters were placed in the aorta (8F) via a femoral artery and in the pulmonary artery (6F) via an internal jugular vein for blood sampling and pressure monitoring. One animal received 37 MBq [ $^3\text{H}$ ]-*S*-I (223 ng/kg) in 10 ml over 20 s and the others 240–750 MBq of [ $^{14}\text{C}$ ]-*S*-I (173–215 ng/kg). Blood samples (5 ml) were withdrawn simultaneously from the aorta and pulmonary artery at 1-min intervals up to 30 min, at 5-min intervals up to 50 min and 10-min intervals to 125 min. The chest was opened 10 min before injection to obtain sequential lung tissue biopsies and the bladder was catheterised for collection of urine ( $^3\text{H}$  study only). Seven male Sprague–Dawley rats (220–280 g) were anaesthetised with oxygen–nitrous oxide–isoflurane (BOC, London, UK) and a catheter (0.96 mm) was inserted into a tail vein. The rats were allowed to recover consciousness in a restraining cage. The radioligand ( $^3\text{H}$ ,  $n = 3$ , 0.25–2500  $\mu\text{g/kg}$ ;  $^{14}\text{C}$ ,  $n = 4$ , 1.1–2.8  $\mu\text{g/kg}$ ) *S*-I or ( $^{14}\text{C}$ ,  $n = 1$ ) *RS*-I (5 mg/kg) was injected into the tail vein. At intervals (5, 10, 15 and 30 min) following injection animals were reanaesthetised and the maximum possible amount of blood (5–8 ml) was withdrawn by cardiac puncture at this time. Urine samples were collected through a needle inserted into the bladder.

*Extraction of  $^3\text{H}$ - and  $^{14}\text{C}$ -labelled compounds*

All solvents were of HPLC grade and obtained from Rathburn (Walkerburn, UK). Other chemicals were of AnalaR grade (BDH, Poole, UK). All blood samples containing [ $^3\text{H}$ ]-*S*-I or [ $^{14}\text{C}$ ]-*RS*-I were cooled in ice immediately on collec-



tion and centrifuged (3 min, 3000 g, 4°C) to obtain the plasma, which was then frozen at  $-80^{\circ}\text{C}$  for subsequent analysis. Following thawing, 20% trichloroacetic acid was added to each sample in a ratio of 1:2 to precipitate the protein, and the supernatant, after centrifuging, was passed through a solid-phase  $\text{C}_{18}$  adsorption cartridge (Sep-Pak, Waters Assoc., Chester, UK) and sequentially washed with 5 ml each of water, water-methanol (1:1, v/v) and methanol. Each wash was collected separately and the solvent was removed under vacuum. Lung samples (between 0.5 and 1.1 g) were homogenised in 2 ml of 0.2 M hydrochloric acid, centrifuged (10 min, 1000 g) and the supernatant removed and treated as above. Urine (10 ml) was extracted directly onto a  $\text{C}_{18}$  Sep-Pak cartridge as for plasma.

#### *High-performance liquid chromatography*

Samples were reconstituted in the mobile phase (1 ml) and HPLC was carried out on a Waters gradient elution system, eluting at 1 ml/min on a  $\mu$ Bondapak  $\text{C}_{18}$  column (30 cm  $\times$  0.8 cm, 10  $\mu\text{m}$  particle size, Waters Assoc.) with aqueous acetic acid (5%, v/v) isocratically at ambient temperature for 5 min followed by a 20-min linear gradient to 40% propan-2-ol in aqueous acetic acid. The radioactive HPLC fractions (from blood samples collected at 5, 10, 60 and 120 min) were further purified. HPLC solvent was removed under vacuum and then the samples were reconstituted in 500  $\mu\text{l}$  of aqueous trifluoroacetic acid (0.04%). These samples were then chromatographed on a Nova-Pak ODS column (15 cm  $\times$  0.4 cm, 4  $\mu\text{m}$  particle size, Waters Assoc.) eluting at ambient temperature at 1 ml/min with water-trifluoroacetic acid (100:0.04, v/v) isocratically for 5 min followed by a 20-min linear gradient to acetonitrile-water-trifluoroacetic acid (15:85:0.04, v/v). The HPLC eluent was monitored at 254 nm and by scintillation counting in 10-ml Instagel (Packard Instrument, Meriden, CT, USA) per 100  $\mu\text{l}$ . Extraction and HPLC yields were determined by adding [ $^3\text{H}$ ]-S-I to blood and lung tissue and extracting as above.

#### *Rapid analysis of $^{11}\text{C}$ -labelled compounds*

Untreated plasma (2–3 ml) was passed through a Sep-Pak solid-phase  $\text{C}_{18}$  cartridge, washing with 5 ml water and eluting with 5 ml of methanol. The methanol fraction was reduced to 0.3 ml by rotary evaporation and made up to 1.5 ml with water containing unlabelled S-I as a carrier. The sample was then centrifuged (1000 g, 2 min) before injection of 1.4 ml onto a Brownlee cartridge containing MCH10 packing (Varian Assoc.). Radioligand was eluted at 2 ml/min with a 5-min linear gradient from 20 to 50% methanol in 5 mM aqueous sodium octanesulphonate (pH 6). The eluent was passed through a 1.2-ml PTFE coil inserted into the well of a NaI (TI) well-type  $\gamma$ -ray detector (NE Technology, Edinburgh, UK) linked to a rate meter.

*Over-pressured thin-layer chromatography (OPTLC)*

Samples of rat urine and plasma which had been partially purified through a Sep-Pak were also analysed by OPTLC (Chrompress, Newman-Howell Assoc., Winchester, UK) by Dr. David Saynor at Glaxo (Ware, UK) using the method of the Orsay PET group [10]. Samples were dissolved in 100  $\mu\text{l}$  of OPTLC buffer and duplicate 20- $\mu\text{l}$  aliquots were chromatographed on a plastic backed silica TLC plate (Merck, Darmstadt, Germany), developing in acetonitrile-ethylamine-water (100:4:396) buffered to pH 4 with 125 mM sodium acetate-acetic acid. Radioactivity on the TLC plate was quantified on a multi-wire proportional counter (Autograph, Oxford Positron Systems, Oxford, UK).

*Plasma protein binding*

Plasma protein binding was measured by introducing paired aliquots (1 ml) of dog plasma containing 0.125–32  $\mu\text{g/l}$  [ $^3\text{H}$ ]-S-I into one side of the cells of a multiple equilibrium dialysis system (Dianorm, Berne, Switzerland). Samples were dialysed against phosphate-buffered saline through 0.025-mm Visking tubing for 24 h. Each side of a cell was sampled and the  $^3\text{H}$  activity measured by liquid scintillation counting.

## RESULTS

A simple extraction protocol was developed for S-I (using the  $^3\text{H}$ -labelled ligand) based on  $\text{C}_{18}$  solid-phase extraction and reversed-phase HPLC on a semi-preparative  $\mu\text{Bondapak}$  column. The ligand eluted from the Sep-Pak cartridge in methanol-water (1:1, v/v) and eluted on HPLC as a single peak with a retention time of 29 min ( $\mu\text{Bondapak}$  column) or 25 min (Nova-Pak column). The extraction yields for this ligand throughout the procedure were over 70% for both plasma and lung homogenates and over 80% for urine. Protein binding in plasma was approximately 16% at all concentrations of S-I studied. There was no evidence for *ex vivo* metabolism when S-I was incubated with plasma or lung homogenate: a single peak of radioactivity post HPLC was always obtained using both the  $\mu\text{Bondapak}$  and Nova-Pak HPLC system.

When [ $^3\text{H}$ ]-S-I was infused into an anaesthetised dog, the plasma radioactivity fell rapidly (Fig. 2). At all time points up to 2 h, >95% of the radioactivity recovered in the plasma was found in the methanol-water (1:1, v/v) Sep-Pak wash and chromatographed on HPLC as a single peak with a retention time of 29 min on the  $\mu\text{Bondapak}$  column (Fig. 3). When this purified substance was chromatographed further on a Nova-Pak column, a single peak of radioactivity was observed, eluting at 25 min. These data show that plasma radioactivity consisted almost entirely of unmetabolised S-I. From 60 min, there was evidence for the presence of a more polar species than S-I; a radioactive peak appeared in the Sep-Pak water wash and eluted on HPLC ( $\mu\text{Bondapak}$ ) at 19 min. This represented <2.5% of the unmetabolised S-I present in plasma at this time (*cf.* Fig. 2).

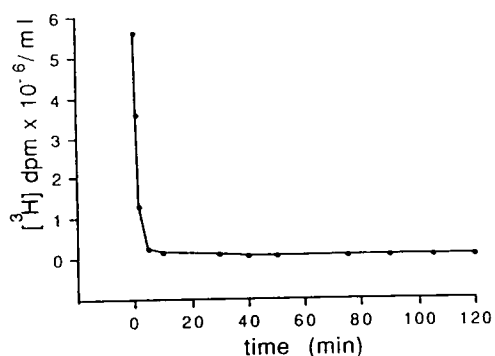


Fig. 2. Radioactivity-time curve for plasma, following infusion of [ $^3\text{H}$ ]-S-I into a single dog.

and could arise either from a metabolite or from trace impurity in the injected drug. The plasma radioactivity declined bi-exponentially. The initial phase represents distribution of the ligand mainly onto  $\beta$ -receptors (particularly in the lung). The terminal elimination phase was very prolonged. This was due largely to urinary excretion, as  $>70\%$  of the dose was recovered unchanged in the urine. Although only 6% of the dose was recovered in the urine over 2 h, this was due presumably to the very slow elimination caused by the long off-time from the  $\beta$ -receptors, as the renal clearance of S-I in the greyhound (calculated from the ratio of amount of unchanged S-I excreted in the urine to the area under the

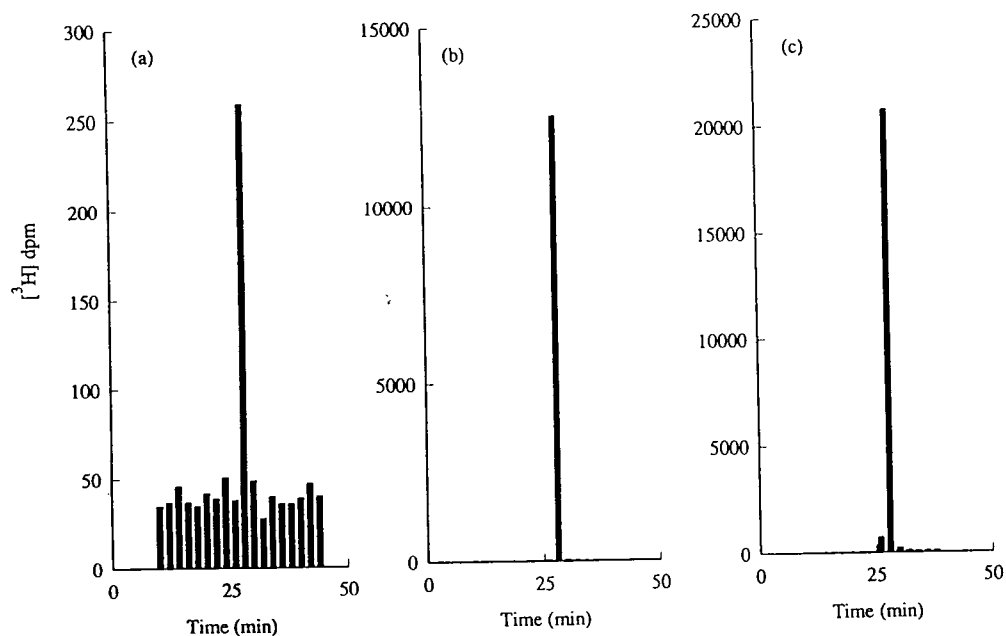


Fig. 3. HPLC radioprofile ( $\mu$ Bondapak column) for dog plasma (a) and homogenised lung tissue (b) taken at 60 min following infusion of [ $^3\text{H}$ ]-S-I. The radioprofile of 60–90 min urine from the same dog is also shown (c). The time for the dead volume to be voided was 11 min. In each case, the radioactivity was contained in only one fraction corresponding to authentic S-4-(*tert.*-butylamino-2-hydroxypropoxy)benzimidazol-2-one.

plasma concentration–time curve of radiolabel over the same interval) was similar to creatinine clearance. When uptake to the receptors was blocked, the elimination rate constant of *S*-I increased considerably (data not shown), showing that at the tracer dose used in this study, elimination was distribution limited.

Serial lung biopsies contained only unchanged *S*-I. Similarly, >95% of the urinary radioactivity chromatographed as unchanged *S*-I on HPLC. There was some evidence for small amounts of more polar radiolabelled compounds in the urine, but these accounted for <1% of unmetabolised *S*-I at any time. The overall recovery of radiolabel in each case was >70%, which was similar to recoveries obtained in control studies, making it unlikely that any metabolites had been selectively lost on extraction.

Similar data were obtained from four rats infused with [ $^3\text{H}$ ]-*S*-I. Unchanged ligand accounted for >95% of the radioactivity recovered in plasma and lung homogenates at all times. Because of the small plasma volumes (and the consequent low counts) available, it is possible that small amounts of metabolites (<2%) would not have been observed. To determine whether the position of radiolabelling or the use of a racemic mixture could affect the results, a single rat was infused with [ $^{14}\text{C}$ ]-*RS*-I and a sample of blood and urine obtained after 5 min. There was no difference in the recovery of radiolabel during the extraction, and, again, there was no evidence for metabolism at this time.

Samples of plasma (taken at 5 min) and urine (post Sep-Pak) from the rat study were also analysed by normal phase using the technique of OPTLC. A single radioactive spot was present for each sample eluting in the same position as authentic *S*-I ( $R_F = 0.7$ ). There was no evidence for the presence of metabolites.

The extraction protocol used for [ $^3\text{H}$ ]-*S*-I and [ $^{14}\text{C}$ ]-*RS*-I was modified for use with the short-lived  $^{11}\text{C}$ -labelled ligand. Plasma was extracted directly with a  $\text{C}_{18}$  Sep-Pak, and a short reversed-phase Brownlee HPLC cartridge (packed with MCH10) was used for analysis. *S*-I eluted with a retention time of 7 min. The elution system was buffered to pH 6 to overcome any possibility of acid-catalysed degradation. Unlabeled *S*-I was included as a carrier, and also to act as a UV-absorbing marker. An on-line  $\gamma$ -detector was set up to monitor  $^{11}\text{C}$  radioactivity. The extraction–HPLC is rapid, taking only 25 min ( $\sim$  one half-life) from collection of blood to HPLC quantification of the ligand. The system was evaluated with plasma samples obtained from the dog studied with [ $^3\text{H}$ ]-*S*-I; a single peak was observed on HPLC (7 min), with a recovery of >80%.

Samples from four dogs infused with [ $^{11}\text{C}$ ]-*S*-I were analysed with this technique. In each case, only unchanged *S*-I was detected in plasma (Fig. 4). Recoveries in excess of 90% were obtained, indicating that any putative metabolites had not been lost on extraction. In two experiments in the rat, using either  $^{11}\text{C}$ -labelled *RS*-I or *S*-I and analysing plasma by the rapid technique, no metabolites were observed at 5 min; however, small amounts (<5%) of a polar material were observed at 15 min post infusion.

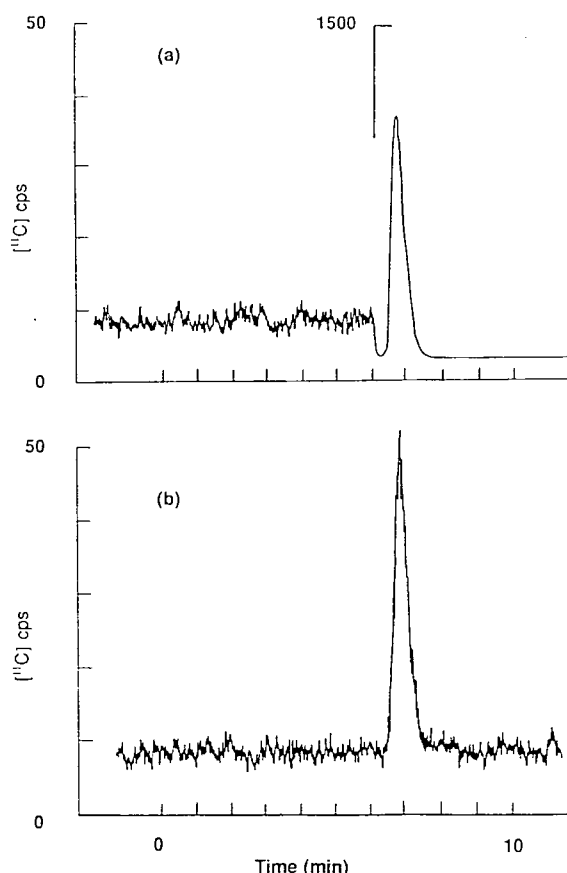


Fig. 4. HPLC radioprofile for dog plasma taken at 10 min (a) and 60 min (b) following infusion of [ $^{11}\text{C}$ ]-S-I. A short HPLC column was used and  $^{11}\text{C}$  radioactivity was monitored on-line with a NaI (Tl) well-type  $\gamma$ -ray detector linked to a rate meter. The void volume was 3 ml. A single radioactive peak was detected eluting at the same position as the authentic material. The sensitivity of the detector was decreased by 30-fold immediately before the elution position of S-I for the 10-min sample.

## DISCUSSION

The interpretation of data obtained *in vivo* from the PET scanner is based on mathematical models which allow the determination of receptor density and kinetics [3,11]. This modelling depends critically on the accurate estimation of the input function, which, in the case of the lung, is the free S-I present in the mixed venous blood, and for the heart, that present in the arterial blood. Although protein binding will reduce the proportion of free ligand as a fraction of the total radioactivity in the plasma, in the case of S-I such binding is minimal. Radioactivity which is present in plasma, either as an impurity co-injected with ligand or as metabolites produced *in vivo*, will, however, lead to inaccuracies in the measured input function. This is particularly important with a ligand such as S-I which is thought to be taken up into the  $\beta$ -receptor pool within minutes of administration. Metabolites (or impurities) which are not as avidly sequestered may

consequently be cleared from the circulation more slowly than the parent ligand. The input function for *S*-I would therefore need to be corrected for a progressively decreasing proportion of the parent ligand to other radiolabeled species in plasma.

It is well known that receptor binding, metabolism and clearance of enantiomers can differ [12,13]. Thus, infusion of a racemic mixture may lead to different plasma concentrations of each enantiomer. This is a particularly important consideration for PET studies, because of the dependence on mathematical modelling which requires an accurate input function for the ligand. In this study, we have used the biologically active *S*-I, which has an 80-fold greater affinity for  $\beta$ -receptors than its *R*-enantiomer [6].

No metabolism of *S*-I was observed in the dog during the 2 h of these studies. The high extraction yields excluded the possibility that metabolites had been lost on extraction. No differences were observed when three different isotopically labelled forms of the ligand ( $^3\text{H}$ ,  $^{11}\text{C}$  and  $^{14}\text{C}$ ) were used, showing that the position of the radiolabel does not affect the metabolism of the ligand (*e.g.* through an isotope effect on the  $\text{C}-^3\text{H}$  bonds). To determine whether our reversed-phase HPLC systems (three used overall) could have failed to resolve metabolites from the parent ligand, an additional study using normal-phase chromatography was carried out on samples of rat plasma and urine using OPTLC: only unchanged *S*-I was observed. In our preliminary extraction procedures, there was a possibility that the acid extractions (using trichloroacetic acid) and HPLC conditions could have resulted in the hydrolysis of a labile metabolite, such as an *N*-glucuronide, back to the parent compound. Our final rapid extraction procedure (used for the  $^{11}\text{C}$ -labelled ligand) was carried out without acid precipitation and using neutral solution. Using this method no metabolites were observed in the dog.

In contrast to our data on *S*-I, Delforge *et al.* [14] using OPTLC to analyse for metabolites, found that infusion of racemic [ $^{11}\text{C}$ ]-*RS*-I into the dog rapidly led to a high metabolite-to-parent drug ratio in blood; at 5 min, less than 12% of the total radioactivity in blood was unchanged *RS*-I. In a single experiment in the rat, also using [ $^{14}\text{C}$ ]-*RS*-I and with analysis by OPTLC, we still did not observe any significant metabolism at 5 min. However, using our rapid HPLC procedure (under neutral extraction conditions), small amounts (<5%) of  $^{11}\text{C}$ -labelled *S*-I polar substances were detected in rat plasma after 15 min. The presence of radio-labelled impurities in the  $^{11}\text{C}$  preparation would, if the plasma elimination rate constant of the impurity were greater than that of *RS*-I, give results consistent with the formation of metabolites. Quality control of the [ $^{11}\text{C}$ ]-*S*-I used in our study showed that the ligand was essentially pure.

In summary, we have developed a rapid extraction and purification protocol suitable for the analysis of [ $^{11}\text{C}$ ]-*S*-I. This ligand is not metabolised to any significant extent *in vivo* in the dog. Such information is essential in the use of [ $^{11}\text{C}$ ]-*S*-I to investigate the behaviour of the different  $\beta$ -adrenergic receptor subtypes and

the kinetics of unlabelled  $\beta$ -receptor antagonists in this species *in vivo*. The analytical procedures described here should be generally applicable for a number of drugs (and their possible metabolites) labelled with short-lived radioisotopes.

#### ACKNOWLEDGEMENTS

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## REGIONAL CEREBRAL BLOOD FLOW DURING VOLITIONAL BREATHING IN MAN

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### SUMMARY

1. Positron emission tomographic imaging of brain blood flow was used to identify areas of motor activation associated with volitional inspiration in six normal male subjects.

2. Scans were performed using intravenous infusion of  $\text{H}_2^{15}\text{O}$  during voluntary targeted breathing and positive pressure passive ventilation at the same level.

3. Regional increases in brain blood flow, due to active inspiration, were derived using a pixel by pixel comparison of images obtained during the voluntary and passive ventilation phases.

4. Pooling data from all subjects revealed statistically significant increases in blood flow bilaterally in the primary motor cortex (left, 5.4%; right, 4.3%), in the right pre-motor cortex (7.6%), in the supplementary motor area (SMA; 3.1%) and in the cerebellum (4.9%).

5. The site of increased neural activation in the motor cortex, associated with volitional inspiration, is consistent with an area which when stimulated, either directly during neurosurgery or transcranially with a magnetic stimulus, results in activation of the diaphragm.

6. The presence of additional sites of neural activation in the pre-motor cortex and SMA appears analogous to the results of studies on voluntary limb movement. The site of the increase in the SMA was posterior to that previously reported for arm movements. These areas are believed to have a role 'upstream' of the motor cortex in the planning and organization of movement.

7. This technique provides a means of studying the volitional motor control of respiratory related tasks in man.

### INTRODUCTION

The automatic generation of a respiratory rhythm within pontomedullary structures and its projection to spinal motoneurons via the bulbospinal tract has

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been extensively investigated and reviewed (von Euler, 1986; Feldman, 1986). It is also recognized that the same spinal motoneurons can be activated from higher centres in the central nervous system (Aminoff & Sears, 1971; Hugelin, 1986) allowing modulation of breathing for behavioural purposes. In man, such non-automatic control of breathing is crucial for the performance of respiratory motor acts such as breath-holding as well as the production of speech. Even during quiet breathing, there is evidence that the level of arousal forms an important component of the overall respiratory drive (Fink, Katz, Rheinhold & Schoolman, 1962; Shea, Walter, Pelley, Murphy & Guz, 1987) and it has been suggested that some ventilatory responses, hitherto thought to be reflex in nature, may depend in part on a neural drive originating from above the brain stem (Eldridge, Milhorn & Waldrop, 1981; Murphy, Mier, Adams & Guz, 1990).

Studies in anaesthetized animals allow efferent projections from midbrain or forebrain areas to respiratory spinal motoneurons to be identified (Colle & Massion, 1958; Bassal & Bianchi, 1982; Lipski, Bektas & Porter, 1986) but tell us little about their role in the conscious state. Studies in conscious animals to address this question have only recently been attempted (Orem & Nettick, 1986) and are difficult to design and interpret. Consequently, much of what is known about the neurophysiological basis for respiratory control related to consciousness comes from observations in patients with defined neurological abnormalities (Plum & Leigh, 1981).

Despite the fact that humans clearly possess highly developed motor skills for volitional control of respiratory muscles, relatively little is known about the presumed areas in the cerebral cortex associated with this function. Foerster (1936) found an area in the primary motor cortex which, when stimulated electrically in conscious man during neurosurgery, resulted in contraction of the diaphragm (hiccough). More recently, Gandevia & Rothwell (1987) identified a short-latency electromyogram (EMG) response in the diaphragm following transcranial electrical stimulation at the vertex. Maskill, Murphy, Mier, Owen & Guz (1991), who used focal transcranial magnetic stimulation, reported the best site for activating the contralateral diaphragm to lie approximately 3 cm lateral to the vertex. Such studies support the existence of an oligosynaptic (probably corticospinal) excitatory projection between the cortex and the main inspiratory muscle in humans, but as in animal studies they permit only a limited interpretation in terms of the voluntary control of breathing.

Positron emission tomography (PET) allows the non-invasive measurement of regional cerebral blood flow (rCBF) and has been used to define areas of increased neural activity associated with specific motor or cognitive tasks in conscious man (Raichle, 1987). The aim of the present study was to use PET to identify those areas of the brain showing an increased blood flow (and hence neural activity) associated with volitional inspiration in normal subjects. The objective was to perform scans during 'active' inspiration and matched 'passive' inspiration with intermittent positive pressure ventilation (IPPV) against relaxed respiratory muscles. The reason for this approach was to control for, as far as possible, afferent feedback from the lungs and chest wall occurring during inspiration, thus only identifying sites dominated by 'centrally generated' neural activity.

This study has been presented in a preliminary form (Colebatch, Adams, Murphy, Martin, Lammertsma, Tochon-Danguy, Clark, Friston & Guz, 1991*a*).

## METHODS

Six right-handed male subjects aged 24–60 years with no known respiratory or neurological abnormalities were studied. Local ethical committee approval was obtained and each subject gave informed consent. Three of the subjects were co-authors (L. A., K. M. and A. G.) and the remainder were respiratory physiologist colleagues not involved with this study.

*Training*

All the subjects were trained to perform the required tasks prior to scanning. Typically this required four to six sessions but no subject was unable to achieve satisfactory performance. For the 'active' respiratory task the subjects practised adopting a target respiratory volume and frequency, initially with auditory cues, and then maintaining this pattern without any external cues. For the 'passive' task, the subjects were taught to remain relaxed during IPPV. The adequacy of relaxation was judged from recordings of upper airway pressure (Datta, Shea, Horner & Guz, 1991) and initially this was displayed to the subjects on an oscilloscope.

*Experimental protocol*

**Ventilation.** The experimental arrangement is shown schematically in Fig. 1. The subjects inspired through a tightly-fitting nasal mask to a volume of 1.5–2 l with a frequency of 10–12 min<sup>-1</sup>; expiration was passive. Inspiratory and expiratory airflows and volumes were measured with an ultrasonic flowmeter (Branta, UK) and tidal  $P_{\text{CO}_2}$  was monitored using an 'in-line' infra-red analyser (Hewlett Packard 47210A, USA). Sufficient additional deadspace, in the form of 2.5 cm i.d. anaesthetic tubing (approx. 1.5 m), was added to maintain end-tidal  $P_{\text{CO}_2}$  ( $P_{\text{ET,CO}_2}$ ) at about 38 mmHg despite the voluntary over-ventilation. The pressure within the nasal mask was measured (Statham P23B, USA) as an index of upper airway pressure. Initially, subjects targeted their inspiratory and expiratory durations to the audible cycling of a mechanical ventilator and were given verbal feedback about the adequacy of their achieved tidal volume relative to the desired level. Subjects then maintained this pattern without any cues for the 4 min duration of each PET scan. For the 'passive' task, an intermittent positive pressure ventilator (Pneupac, UK) was connected to the terminal end of the dead space and set to deliver a similar inspiratory volume and frequency. While in the relaxed state, subjects were ventilated for the duration of the scan and were instructed not to sleep.

In order to assess the possible involvement of accessory muscles of respiration during voluntary breathing, three subjects performed the 'active' task, at a separate time (under exactly the same experimental conditions as described above), with EMG surface electrodes placed over the sternomastoid muscle; EMG signals were processed through an isolated differential amplifier of local design (bandwidth 10–1000 Hz).

**PET scanning.** Subjects were scanned six times consecutively in each session. Radiolabelled water ( $\text{H}_2^{15}\text{O}$ ) was used as a tracer of cerebral blood flow.  $\text{H}_2^{15}\text{O}$  was produced continuously by the catalytic reaction of  $^{15}\text{O}_2$  and hydrogen and was infused intravenously in a concentration of approximately 8 mCi ml<sup>-1</sup>. Preparation for scanning included insertion of cannulae into the left radial artery (under local anaesthesia; bupivacaine 1% s.c.) and also into the right antecubital vein. A polyurethane head mould was fitted to minimize head movement. Subjects lay supine in a quiet darkened room with eyes closed and ears unplugged and with the head placed in the scanner so that the lowermost plane was approximately parallel to, and 20 mm above, the orbitomeatal line.

Scanning was performed with an ECAT 931-08/12 (CTI Inc., Knoxville, USA) the physical characteristics of which have been described previously (Spinks, Jones, Gilardi & Heather, 1988). The scanner collects data in fifteen contiguous transverse planes, with a total axial field of view of 10.4 cm. Transmission data were collected first, over a period of 20 min, using an external  $^{68}\text{Ge}$  ring source generating positrons. These data are required to correct subsequent emission scans for the effects of radiation attenuation by the tissues of the head. This period also served to accustom subjects to the environment.

For each measurement of rCBF, scans were collected sequentially over 3.5 min, with a 0.5 min background scan followed by twelve scans of 5 s duration and twelve of 10 s. The  $\text{H}_2^{15}\text{O}$  infusion, at a rate of 10 ml min<sup>-1</sup>, began immediately after the background scan and continued for 1 min. The scans collected in the 110 s following the start of the tracer infusion were added for calculation of the rCBF images. Arterial blood was drawn continuously during scanning at a rate of 5 ml min<sup>-1</sup>.

and radioactivity was measured every second. Corrections to the arterial radioactivity (input) curve for delay and dispersion in the brachial vessels and tubing were made and an average partition coefficient of 0.95 for the relative volume of distribution of  $\text{H}_2^{15}\text{O}$  between brain 'tissue' and blood was assumed. The protocol and calculations were modified from those described by Lammertsma, Cunningham, Deiber, Heather, Bloomfield, Nutt, Frackowiak & Jones (1990) for  $\text{C}^{15}\text{O}_2$  inhalation.

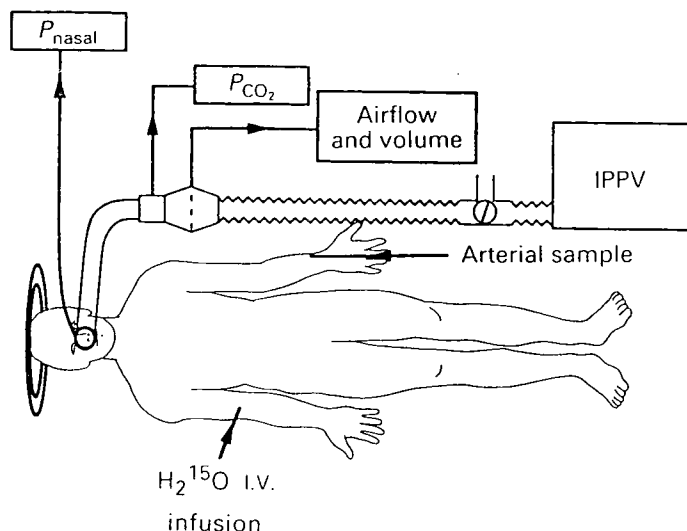


Fig. 1. Schematic representation of the experimental arrangement for measurement of changes in rCBF by PET scanning resulting from voluntary breathing. Subjects remained still, with the head positioned in the scanner and eyes and mouth closed, and breathed through a nasal mask via a deadspace (to maintain normocapnia), either volitionally from room air ('active' inspiration), or with relaxed respiratory muscles ('passive' inspiration) using 'matched' intermittent positive pressure ventilation (IPPV): expiration was always passive. Respiratory airflow (integrated to volume) and  $P_{\text{CO}_2}$  were measured continuously with appropriate 'in-line' transducers. Pressure within the nasal mask ( $P_{\text{nasal}}$ ) provided an index of relaxation during IPPV. Radiolabelled water ( $\text{H}_2^{15}\text{O}$ ) was infused over 1 min via the right antecubital vein and blood was sampled continuously from the left radial artery to allow quantification of rCBF.

Six scans were collected for each subject, with rest periods of 10–12 min between scans to allow for decay of radioactivity from the previous measurement ( $T_{1/2}$  for  $^{15}\text{O} = 2.1$  min). The scans were performed under 'active' and 'passive' conditions alternately and with the order reversed in half the subjects. A stable ventilatory pattern was established during the background scan of 30 s and maintained for a further 3.5 min until the last blood sample was collected. Scans were reconstructed using a Hanning filter with a cut-off frequency of 0.5 maximum resulting in an image resolution of  $8.5 \times 8.5$  mm (at full width half maximum) and a slice thickness of 6.75 mm. The reconstructed images contained  $128 \times 128$  picture elements (pixels) each  $2.05 \times 2.05$  mm in size.

The images of CBF were automatically reorientated and resized in height, length and width to match best a reference set of images. This set of images had been aligned previously with respect to the intercommissural plane using the method of Friston, Passingham, Nutt, Heather, Sawle & Frackowiak (1989) and rescaled to correspond to the standard brain dimensions used by Talairach & Tournoux (1988) in their stereotaxic atlas. The final image consisted of twenty-six slices at 4 mm intervals with plane 8 being the intercommissural plane. Each image was smoothed with a Gaussian filter 10 pixels wide, to accommodate variations in functional and gyral anatomy.

Subjects differ in the level of overall CBF, but activation results in an additional component which is independent of the global flow. Differences in global flow were therefore accounted for pixel by pixel in each scan by normalizing to a standard mean CBF of  $50 \text{ ml (100 ml brain volume)}^{-1} \text{ min}^{-1}$ , using a method based on analysis of covariance with global flow as covariant (Friston, Frith, Liddle, Dolan, Lammertsma, & Frackowiak, 1990). For every pixel, this analysis generated an adjusted

mean rCBF for each of the two conditions ('active' and 'passive') and the error variance was used to compare the two conditions in a manner equivalent to a paired *t* test. In each of three subjects, one pair of scans was technically unsatisfactory and hence only fifteen individual pairs of scans contributed to each condition mean. The method of Friston, Frith, Liddle & Frackowiak (1991) was used to produce 'statistical images' of rCBF change with significance criteria set at  $P < 0.05$  and  $P < 0.01$ . Only significant increases in rCBF in the 'active' compared to 'passive' condition were considered further. The most significant pixel within such regions of increased flow was used to determine anatomical location by reference to the corresponding locus in the atlas of Talairach & Tournoux (1988). Where foci occurred within the same anatomical structure (e.g. within the motor cortex) on multiple planes, 'weighted mean' co-ordinates were calculated (Colebatch, Deiber, Passingham, Friston & Frackowiak, 1991*b*). Average flow increases were quantified at the site of the most significant pixel using the images of normalized blood flow.

## RESULTS

*Subjects' comments*

The subjects confirmed that during the scans they had remained awake with eyes closed and had experienced little or no discomfort. All subjects were confident that they had been able to maintain a fairly uniform pattern of breathing during the 'active' task and that they had kept their respiratory muscles relaxed during

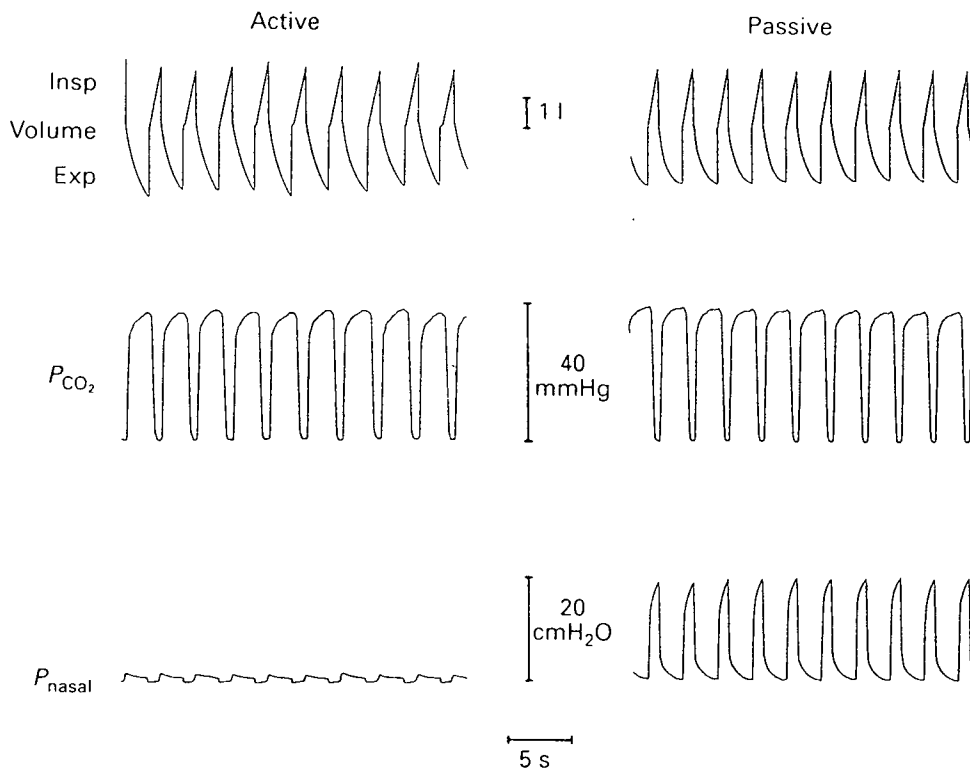


Fig. 2. Examples of original records of respiratory variables from one subject over 1 min infusion of  $H_2^{15}O$  during volitional breathing (active) and 'matched' positive pressure ventilation (passive). Note similarity of: inspired volumes (Insp), respiratory frequency, expiratory volume profiles (Exp) and end-tidal  $P_{CO_2}$  levels in the two conditions. In Passive, smooth and repeatable pressure profiles within the nasal mask ( $P_{nasal}$ ) indicate relaxed respiratory muscles.

positive pressure ventilation. Subjects did not report any particular concentration on their breathing during the 'passive' task other than being aware that they were being ventilated; by contrast, the 'active' task did require continuous attention to the rate and depth of breathing.

### *Pattern of breathing*

A record of the breathing pattern, tidal  $P_{\text{CO}_2}$  and pressure recorded within the nasal mask ( $P_{\text{nasal}}$ ) during the 'active' and matched 'passive' breathing tasks is

TABLE 1. Mean ventilatory variables during PET scans

| Subject<br>(scan no.) | $T_I$ (s) |      | $T_E$ (s) |      | $V_T$ (l) |      | $P_{\text{ET,CO}_2}$ (mmHg) |      |
|-----------------------|-----------|------|-----------|------|-----------|------|-----------------------------|------|
|                       | Act       | Pas  | Act       | Pas  | Act       | Pas  | Act                         | Pas  |
| AG (1)                | 2.1       | 1.7  | 4.0       | 3.4  | 1.83      | 1.60 | 34.9                        | 36.5 |
| AG (2)                | 2.1       | 1.8  | 3.9       | 3.7  | 1.62      | 1.70 | 35.5                        | 37.0 |
| RL (1)                | 2.0       | 1.6  | 3.3       | 3.4  | 1.72      | 1.63 | 37.0                        | 38.1 |
| RL (2)                | 1.9       | 1.8  | 3.6       | 3.5  | 1.41      | 1.42 | 36.6                        | 37.8 |
| KM (1)                | 1.8       | 1.6  | 2.9       | 2.9  | 1.32      | 1.50 | 39.4                        | 37.1 |
| KM (2)                | 2.2       | 2.0  | 3.9       | 3.3  | 1.81      | 1.65 | 37.3                        | 38.3 |
| LA (1)                | 1.9       | 1.5  | 3.4       | 3.0  | 1.72      | 1.82 | 38.0                        | 39.0 |
| LA (2)                | 1.9       | 1.5  | 3.5       | 3.2  | 1.74      | 1.70 | 37.2                        | 38.1 |
| LA (3)                | 1.6       | 1.5  | 3.1       | 3.2  | 1.51      | 1.55 | 40.6                        | 39.4 |
| AI (1)                | 1.4       | 1.4  | 3.2       | 3.1  | 1.32      | 1.40 | 43.0                        | 41.0 |
| AI (2)                | 1.4       | 1.4  | 3.2       | 3.1  | 1.45      | 1.45 | 38.5                        | 38.0 |
| AI (3)                | 1.6       | 1.6  | 3.6       | 3.1  | 1.71      | 1.70 | 35.5                        | 36.5 |
| RH (1)                | 1.6       | 1.6  | 4.8       | 3.4  | 1.60      | 1.45 | 35.0                        | 36.5 |
| RH (2)                | 2.5       | 1.6  | 4.7       | 3.4  | 1.83      | 1.54 | 33.0                        | 33.5 |
| RH (3)                | 1.6       | 1.6  | 3.9       | 4.2  | 1.70      | 1.55 | 32.2                        | 33.2 |
| $\bar{x}$             | 1.84      | 1.61 | 3.67      | 3.33 | 1.62      | 1.58 | 36.9                        | 37.3 |
| S.D.                  | 0.31      | 0.16 | 0.55      | 0.32 | 0.18      | 0.12 | 2.8                         | 2.0  |
| $P$                   | 0.004     |      | 0.02      |      | 0.16      |      | 0.23                        |      |

Mean values, for each of six subjects, of inspiratory time ( $T_I$ ), expiratory time ( $T_E$ ), tidal volume ( $V_T$ ) and end-tidal  $P_{\text{CO}_2}$  ( $P_{\text{ET,CO}_2}$ ) over the 1 min of  $\text{H}_2^{15}\text{O}$  infusion (9–14 breaths) during a PET scan. Each subject underwent two or three pairs of scans during 'active' voluntary inspiration (Act) and 'passive' positive pressure ventilation (Pas). The mean ( $\bar{x}$ ) and standard deviation (S.D.) of these average values for the fifteen paired scans comprising the rCBF analysis are shown.  $P$  gives the level of statistical significance for differences in the means of each variable for Active *vs.* Passive (paired  $t$  test).

shown for one subject in Fig. 2. A breath by breath analysis of inspiratory time ( $T_I$ ), expiratory time ( $T_E$ ), tidal volume ( $V_T$ ) and  $P_{\text{ET,CO}_2}$  corresponding to the 1 min of  $\text{H}_2^{15}\text{O}$  infusion (taken as representative of the scan period), was performed for each of the 'active' and 'passive' runs; the number of breaths taken during this period ranged from nine to fourteen between subjects but never differed by more than three between a matched pair of runs. The mean values of these ventilatory variables are given in Table 1. Paired  $t$  test analysis on each variable for the 'active' *vs.* 'passive' conditions in the fifteen matched runs revealed significantly lower values for both  $T_I$  (1.61 *vs.* 1.84 s) and  $T_E$  (3.33 *vs.* 3.67 s) in 'passive' compared with 'active' breathing but  $V_T$  and  $P_{\text{ET,CO}_2}$  were not significantly different between the two conditions.

All subjects showed a smooth and repeatable  $P_{\text{nasal}}$  trace during 'passive' inspiration (Fig. 2) for virtually all breaths occurring during scanning. Over the 1 min of  $\text{H}_2^{15}\text{O}$  infusion, the mean peak  $P_{\text{nasal}}$  ranged between 14.1 and 30.8  $\text{cmH}_2\text{O}$  for different subjects, and for individual scans the coefficient of variation of  $P_{\text{nasal}}$  in

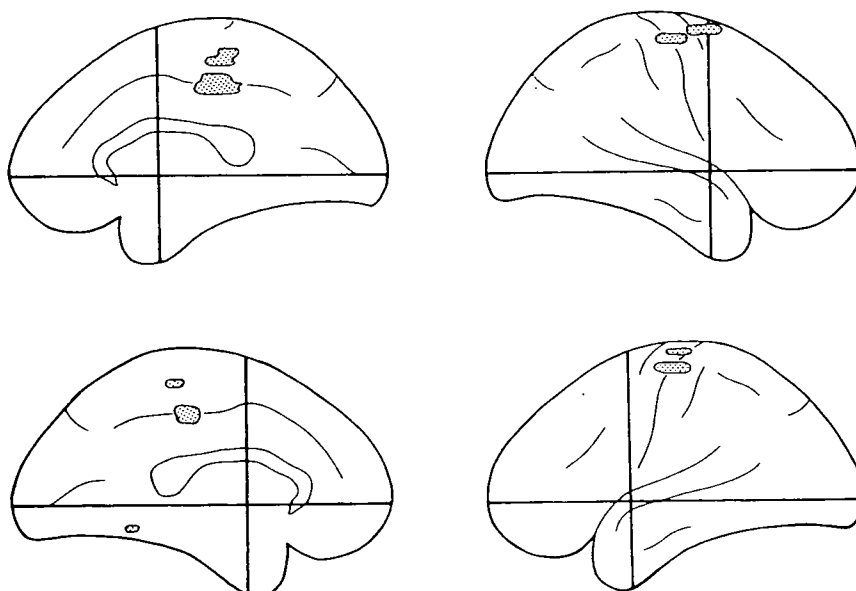


Fig. 3. Diagram summarizing the sites of significantly increased rCBF occurring with active inspiration in pooled data from 6 subjects. Medial (left-hand) and lateral (right-hand) views of the right (upper-half) and left (lower-half) hemispheres are shown. The horizontal lines indicate the position of the intercommissural (AC-PC) plane and the vertical line the location of Vca, a line passing through the anterior commissure (Talairach & Tournoux, 1988). The shaded areas indicate the regions, projected onto these views, at which significant ( $P < 0.05$ ) increases in rCBF occurred during the 'active' task. The right medial view shows two regions (comprising four foci) and the left medial view two regions (three foci) of increased flow within the SMA, which, due to their proximity to the mid-line, could not be allocated to either hemisphere with certainty. The medial view of the left hemisphere also shows, below the AC-PC plane, the focus of increased rCBF occurring within the cerebellum which overlay the cerebral cortex at this level. The lateral projections, show, on the right, a focus within the motor cortex and, anteriorly, one within the pre-motor cortex. On the lateral view of the left hemisphere are 2 foci of increased flow within the motor cortex. The brain outlines were copied from a drawing by R. Passingham taken from the atlas of Talairach & Tournoux (1988).

nine to fourteen successive breaths ranged between 0.9% and 6.5% (median 1.9%). During 'active' inspiration, peak  $P_{\text{nasal}}$  never exceeded  $-2.5 \text{ cmH}_2\text{O}$ , consistent with the relatively low resistance of the external breathing circuit (Fig. 1).

Minimal or no EMG activity was recorded in the sternomastoid muscles in the three subjects studied.

#### *Regional cerebral blood flow*

The brain volume successfully imaged in all subjects extended from the vertex to the upper cerebellum. Global cerebral blood flow was not significantly different ( $P = 0.4$ , paired  $t$  test) between the 'active' and 'passive' conditions. Significantly

increased flows ( $P < 0.05$ ) in association with the 'active' state occurred within five brain regions: the right pre-motor cortex, the supplementary motor area (SMA), the cerebellum and both the right and left motor cortices (Fig. 3). Given that the peri-Rolandic foci were located anterior to the fissure and that our experiment was designed to subtract out any change due to afferent activity, we felt justified in using

TABLE 2. Sites of significantly increased blood flow in active inspiration

| Region          | Average flow increase (%) | Co-ordinates (mm) |
|-----------------|---------------------------|-------------------|
| Left Motor (2)  | 5.4                       | -18, -23, 65      |
| Right Motor     | 4.3                       | +18, -18, 60      |
| Right Pre-motor | 7.6                       | +18, -2, 64       |
| SMA (4)         | 3.1                       | +4, -29, 48       |
| Cerebellum      | 4.9                       | -6, -52, -8       |

Degree of flow increase and co-ordinates for sites of significantly ( $P < 0.05$ ) increased blood flow in the 'active' compared to the 'passive' task. The flow changes represent the average values from the six subjects. Co-ordinates are given in order of sagittal, coronal and vertical locations and refer to the stereotaxic atlas of Talairach & Tournoux (1988). The sagittal reference is the mid-line (displacements to the right are positive), the coronal reference is a plane vertical to the intercommissural plane, passing through the anterior commissure with distances anterior being positive; the vertical is the distance above the intercommissural plane. The figures given in brackets indicate the number of foci (on different transverse planes) lying within the same anatomical region. All the regions except the cerebellum included some foci which were also significant at the  $P < 0.01$  level.

the term 'motor' rather than 'sensorimotor' cortex. All but the cerebellar region included foci which remained significant at the  $P < 0.01$  level. Foci of significantly increased flow occurred in four transverse planes within the SMA (two at the  $P < 0.01$  level). Within the left motor cortex, foci of significantly increased flow occurred in two transverse planes (one at the  $P < 0.01$  level). Average flow increases ranged from 3.1 to 7.6% within the different regions and are given with their co-ordinates in Table 2. The laterality of the cerebellar and SMA sites of activation is uncertain because of the proximity of the sagittal coordinates to the mid-line.

#### DISCUSSION

Respiratory motor control is characterized by the presence of rhythmical motor outflow that proceeds in the absence of conscious awareness, as well as by the capacity for a high degree of volitional modification of this basic rhythm. Physiological observations, as well as the effects of disease in man in which the automatic and 'voluntary' aspects of breathing can sometimes be dissociated (Plum & Leigh, 1981), suggest that these two functions reflect differences in the relative contribution from cortical and brain stem structures.

The present study was designed to examine the neural control of breathing in its 'most voluntary' form. The subjects were required to generate accurately a specific inspiratory volume and respiratory frequency, a task that could not be achieved without training. Anxiety, a potential influence on breathing (Howell, 1990) was minimized by training sessions, and by explanation and reassurance during the study. The target tidal volume was roughly three times normal and end-tidal  $P_{CO_2}$

was kept at low normal levels to ensure that chemical drives to breathe were minimized. The level of  $P_{\text{CO}_2}$ , however, was not low enough to cause a significant alteration in overall cerebral blood flow (Kety & Schmidt, 1948).

The 'passive' task was used as a control for the afferent activity evoked in the 'active' task. Inflation of the lungs excites afferents within the lung and airways, as well as in the skin and muscles of the chest wall, and there is direct evidence that afferents within intercostal nerves at least, project to the cerebral cortex (Gandevia & Macefield, 1989). In order that any cortical activation resulting from the 'passive' task reflect only the effects of peripheral afferent discharge, it was clearly necessary that the subjects' inspiratory muscles did indeed remain relaxed during this control condition; the recordings of pressure from within the nasal mask indicated that this was so. Although end-tidal  $P_{\text{CO}_2}$  levels showed that the overall levels of ventilation were well matched, there were small differences in the duration of the inspiratory and expiratory phases in the 'active' and 'passive' tasks. Furthermore, although afferent activity from all sources should have been very similar in the expiratory phases of both tasks, the afferent discharge generated during the inspiratory phases would not have been perfectly matched. While intrathoracic, cutaneous and joint receptor discharge should have been similar in the two conditions throughout the respiratory cycle, extrathoracic airway pressure receptors, facial afferents innervating skin within the mask and respiratory muscle afferents would have been expected to discharge differently during the inspiratory phase of the two tasks. Indeed perfect matching of afferent activity is probably impossible for this and most motor tasks.

Previous measurements of rCBF during movements of the eyes and limbs (Roland, Larsen, Lassen & Skinhøj, 1980; Fox, Fox, Raichle & Burde, 1985*a*; Colebatch *et al.* 1991*b*) have measured increases against a resting state. Due to the limited temporal resolution possible with PET, the problem arises of whether the increases seen are the cause, or the result of the movement. It has been shown, for example, that both muscle and cutaneous afferents project to the pre- and post-central gyri, the SMA and the pre-motor cortex (Powell & Mountcastle, 1959; Lemon & Porter, 1976; Hummelsheim, Bianchetti, Wiesendanger & Wiesendanger, 1988) and could have contributed towards the increased blood flow recorded for these regions. By subtracting out the effects of a 'passive' control state, it is likely that the sites identified represent cortical areas dominated by 'centrally generated' neural activity in association with the volitional inspiratory task.

The size of the flow increases reported here are modest in comparison with increases seen in association with limb movements. Repetitive contraction of a single digit, for example, has been reported to result in a 13% increase in rCBF within the sensorimotor cortex (Colebatch *et al.* 1991*b*). Several factors could have contributed towards the modest blood flow increases found in the present study. Firstly, the experiment was designed to remove flow increases associated with the 'passive' task. Such peripheral afferent activity may have contributed significantly to increases in rCBF seen with limb movements; Colebatch, Findley, Frackowiak, Marsden & Brooks (1990) reported increases of 15% in sensorimotor cortex blood flow in response to passive wrist movements. Secondly, only about one-third of the 'active' task scan time was actually spent in generating inspiratory airflow, the remainder being spent in expiration which occurred passively in both tasks. Finally, the



repetition rate used here (12 breaths  $\text{min}^{-1}$ ) was much slower than that used in some studies of limb movement (e.g. 40 movements  $\text{min}^{-1}$ ; Colebatch *et al.* 1991*b*). Although the dynamic sensitivity of rCBF to different rates of movement is not known, Fox & Raichle (1984, 1985), who studied occipital cortex rCBF responses to visual stimuli, found a linear increase in flow with increasing repetition rates between 1  $\text{s}^{-1}$  and 8  $\text{s}^{-1}$ .

The motor cortex, SMA, pre-motor cortex and cerebellum, activated in this study, have also been reported to be active in association with simple, repetitive arm and hand movements (Fox *et al.* 1985*a*; Fox, Raichle & Thach, 1985*b*; Colebatch *et al.* 1991*b*). This suggests that these regions participate in the generation of all voluntary movements with clearly defined targets and disagrees with the claim by Roland *et al.* (1980) that only complex motor tasks require SMA and pre-motor activation. Furthermore, there would appear to be a fundamental similarity in the mechanism by which voluntary movements of both the limbs and respiratory muscles are generated. Anatomically, the motor and non-primary motor areas are closely inter-related (e.g. Ghosh, Brinkman & Porter, 1987) and, for different movements of the arm, the activation within sensorimotor cortex, SMA and premotor cortex change roughly in parallel (Colebatch *et al.* 1991*b*). In unilateral movement, increases in flow in the pre-motor cortex may occur either bilaterally or just contralateral to the limb moved (Roland, Meyer, Shibasaki, Yamamoto & Thompson, 1982). In the present study, where a symmetrical bilateral movement was performed, the unilateral pre-motor activation seen raises the possibility of hemispheric specialization for the 'active' task; this may be related to a greater degree of attention associated with this manoeuvre (Pardo, Fox & Raichle, 1991).

The present study outlines the motor cortical areas involved in the inspiratory 'active' task but cannot define exactly which inspiratory muscles are 'represented' by these areas. Although inspiratory intercostal muscles could certainly have been activated during the 'active' task, the diaphragm would have been the principal muscle of inspiration. The locations in the motor cortical foci in each hemisphere correspond well to an earlier report of an optimal site for direct stimulation of the cortex to activate the diaphragm in man (Foerster, 1936). The use of transcranial magnetic stimulation in man (Maskill *et al.* 1991) has indicated an optimal site for activating the contralateral diaphragm, approximately 3 cm lateral to the vertex. With the assumption of a skull thickness of 0.8 mm, it is possible to calculate that the motor cortical sites identified in the present study correspond to locations approximately 2.5 cm lateral to the vertex.

The SMA, which in monkeys has been shown to have its own projection to the spinal cord (Macpherson, Marangoz, Miles & Wiesendanger, 1982) is nevertheless generally believed to have a role in planning movement 'upstream' of the motor cortex (Roland *et al.* 1980). Our results indicate a role for the SMA in voluntary respiratory movements and complement recent evidence for the presence of a Bereitschaftspotential (Deecke, 1987) preceding similar respiratory manoeuvres (Macefield & Gandevia, 1991). The location of the SMA focus in the present study lay at a similar vertical height above the intercommissural plane as reported in association with shoulder movements (Colebatch *et al.* 1991*b*) but 15 mm more posterior. Rostrocaudal somatotopy within the SMA has been reported for man, with

areas active in vocalization and eye movements lying progressively more rostral to that for arm movement (Talairach, Szlika, Tournoux, Prossalenti, Bordas-Ferrer, Corello, Iacob & Mempel, 1967; Fox *et al.* 1985*a*). Our data suggest that the SMA region, active in association with the inspiratory task used here, lies posterior to that for arm movement and is thus separated by it from the region involved in vocalization.

This study, using rCBF as an indicator of local neural activation (Raichle, 1987), has provided, for the first time, direct evidence for a functional role of specific cortical structures in the generation of a voluntary inspiratory task. The present methodology is potentially capable of enabling the study of respiratory motor control under a variety of different behavioural conditions in both normal volunteers and patients with defined deficits. Careful choice of experimental paradigms should allow conclusions to be drawn about the control of breathing not only in other overtly volitional tasks but also, for example, during speech and in states of anxiety. Such everyday examples of behavioural influences on breathing have, in the past, been extremely difficult to study in a rigorous fashion.

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## Recommendations for Fluorine-18 Production\*

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Data for the production of fluorine-18 ( $t_{1/2} = 109.6$  min;  $\beta^+ = 100\%$ ) are reviewed. The experience of some well-established European centres for positron emission tomography (PET), in the routine production of fluorine-18 for the purpose of labelling radiopharmaceuticals, is described. This experience is evaluated, along with published data, to give practical and technical recommendations to new PET groups entering this key area.

### Introduction

The labelling of radiopharmaceuticals with fluorine-18 ( $t_{1/2} = 109.6$  min;  $\beta^+ = 100\%$ ) will continue to play a major rôle in the development of positron emission tomography (PET) as a modality for clinical research, or even clinical diagnosis, in future years. For fluorine-18, perhaps for more than any other radionuclide, the ultimate success of any labelling reaction hinges on factors that are determined upstream by the conditions of radionuclide production. The challenge of fluorine-18 production for PET is to generate fluorine-18 in a well-characterized chemical form, that proves useful for subsequent labelling chemistry, ideally with minimal carrier, without contaminants that may bind the fluorine-18 into inert compounds and with sufficient activity to prepare useful activities (e.g. ca 10 mCi) of labelled radiopharmaceuticals for PET studies *in vivo*. These criteria are not always easily satisfied.

Several European centres have now gained extensive experience in the production of fluorine-18, specifically for labelling radiopharmaceuticals for PET. The aim of this report is to share this know-how with the increasing number of PET centres that are now being established. It includes a review of data on fluorine-18 production, a discussion of production techniques now developing in our own laboratories

and elsewhere, and recommendations, drawn from broad experience, that should be helpful to new PET groups entering this key area.

### Main Sources of Fluorine-18

Fluorine-18 can be reached by a wide variety of production channels (Table 1). The approach taken by a particular laboratory is mainly shaped by the facilities at hand. High activities are available from such diverse machines as cyclotrons, Van de Graaff generators and nuclear reactors. Cyclotrons classed (Wolf and Jones, 1983) as Level 1 (<10 MeV p or d) and Level 2 (<20 MeV p, plus perhaps other particles) have become the favoured machines for production at new PET centres.

Table 1 displays experimental data reported for the main reactions producing fluorine-18 on thick targets. Nuclear data for these reactions have been reviewed (Qaim, 1982) and recently compiled (Gandarias-Cruz and Okamoto, 1988). Of these reactions the  $^{20}\text{Ne}(d, \alpha)^{18}\text{F}$  and  $^{18}\text{O}(p, n)^{18}\text{F}$  reactions are of major practical interest, in view of their requirement for only a moderate projectile energy and a moderate beam current to give a useful yield. The  $^{20}\text{Ne}(d, \alpha)^{18}\text{F}$  and  $^{18}\text{O}(p, n)^{18}\text{F}$  reactions are mainly applied to prepare carrier-added (CA) molecular [ $^{18}\text{F}$ ]fluorine and no-carrier-added (NCA) [ $^{18}\text{F}$ ]fluoride, respectively. Nearly all fluorine-18 radiochemistry now stems from these products. These processes and their variations are therefore the main focus of the

\*A report from a task group organized within the framework of the EEC concerted action on "PET investigations of cellular regeneration and degeneration".

Table 1. Literature data for the production of fluorine-18 for medical use from different nuclear reactions on thick targets

| Reaction                                                              | Target                                 | E<br>(MeV)                                  | Thick<br>target yield <sup>a</sup>          | Main<br>form           | Reference                     |
|-----------------------------------------------------------------------|----------------------------------------|---------------------------------------------|---------------------------------------------|------------------------|-------------------------------|
| $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$                       | $^{18}\text{O}_2$                      | 14-0                                        | 216 mCi/ $\mu\text{A}^{\text{b},\text{c}}$  |                        | Ruth and Wolf (1979)          |
|                                                                       | $^{18}\text{O}_2$                      | 10-0                                        | 150 mCi/ $\mu\text{A}^{\text{c}}$           | $[\text{F}]\text{F}_2$ | Nickles <i>et al.</i> (1984)  |
|                                                                       | $\text{H}_2^{18}\text{O}$              | 16-0                                        | 110 mCi/ $\mu\text{A}^{\text{c}}$           | $[\text{F}]\text{F}^-$ | Kilbourn <i>et al.</i> (1985) |
| $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$                        | $^{20}\text{Ne}$                       | 14                                          | 91.9 mCi/ $\mu\text{A}^{\text{b},\text{c}}$ |                        | Casella <i>et al.</i> (1980)  |
|                                                                       | 0.1% $\text{F}_2/\text{Ne}$            | 14-2                                        | 12.2 mCi/ $\mu\text{Ah}^{\text{d}}$         | $[\text{F}]\text{F}_2$ | Casella <i>et al.</i> (1980)  |
|                                                                       | 0.18% $\text{F}_2/\text{Ne}$           | 11.2-0                                      | 10 mCi/ $\mu\text{Ah}$                      | $[\text{F}]\text{F}_2$ | Blessing <i>et al.</i> (1986) |
|                                                                       | 15% $\text{H}_2/\text{Ne}$             | 11.2-0                                      | 10 mCi/ $\mu\text{Ah}$                      | $[\text{F}]\text{HF}$  | Blessing <i>et al.</i> (1986) |
|                                                                       | 6.7% $\text{H}_2/\text{Ne}$            | 11.2-0                                      | 8 mCi/ $\mu\text{Ah}$                       | $[\text{F}]\text{F}^-$ | Blessing <i>et al.</i> (1986) |
| $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{Ne}^{\text{e}}$            | 10% $\text{H}_2/\text{Ne}$             | 6.3-0                                       | 11 mCi/ $\mu\text{A}^{\text{c}}$            | $[\text{F}]\text{HF}$  | Dahl <i>et al.</i> (1983)     |
| $^{16}\text{O}(\alpha,\text{d})^{18}\text{F}$                         | $\text{H}_2\text{O}$                   | 30                                          | 1.1 mCi/ $\mu\text{Ah}$                     | $[\text{F}]\text{F}^-$ | Clark and Silvester (1966)    |
|                                                                       |                                        | 48                                          | 7.0 mCi/ $\mu\text{Ah}$                     | $[\text{F}]\text{F}^-$ | Lindner <i>et al.</i> (1973)  |
| $^{16}\text{O}(\alpha,2\text{n})^{18}\text{Ne}^{\text{e}}$            | $\text{O}_2$                           | 40                                          | 14 mCi/ $\mu\text{Ah}$                      | $[\text{F}]\text{HF}$  | Nozaki <i>et al.</i> (1968)   |
| $^{16}\text{O}(^3\text{He},\text{p})^{18}\text{F}$                    | $\text{H}_2\text{O}$                   | 41-14                                       | 7 mCi/ $\mu\text{Ah}$                       | $[\text{F}]\text{F}^-$ | Fitschen <i>et al.</i> (1977) |
| $^{16}\text{O}(^3\text{He},\text{n})^{18}\text{Ne}^{\text{d}}$        | $\text{H}_2\text{O}$                   | 36                                          | 7.6 mCi/ $\mu\text{Ah}$                     | $[\text{F}]\text{F}^-$ | Knust and Machulla (1983)     |
| $^{20}\text{Ne}(^3\text{He},\alpha\text{n})^{18}\text{Ne}^{\text{e}}$ | 2% $\text{H}_2/\text{Ne}$              | 27.5                                        | 5-7 mCi/ $\mu\text{Ah}$                     | $[\text{F}]\text{HF}$  | Crouzel and Comar (1978)      |
|                                                                       |                                        | n Flux<br>( $\text{cm}^{-2}\text{s}^{-1}$ ) | Yield                                       |                        |                               |
| $^{16}\text{O}(^3\text{H},\text{n})^{18}\text{F}$                     | $\text{Li}_2\text{CO}_3$               | $3 \times 10^{13}$                          | 200 mCi/3 h                                 | $[\text{F}]\text{F}$   | Vera Ruiz (1988)              |
|                                                                       | $^6\text{LiOH}\cdot\text{H}_2\text{O}$ | $3 \times 10^{13}$                          | 250 mCi/h                                   | $[\text{F}]\text{F}$   | Vera Ruiz (1988)              |

<sup>a</sup>For 1 h experimental irradiation unless otherwise indicated by superscript.<sup>b</sup>Theoretical yield.<sup>c</sup>Saturation yield.<sup>d</sup>Yield per 2 h irradiation.<sup>e</sup> $^{18}\text{Ne}$  decays to fluorine-18 with a half-life of 1.67 s.

remainder of this report. Some other processes are considered for comparison or to indicate their future potential.

### Production of CA [ $^{18}\text{F}$ ]fluorine by the $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$ Reaction

The bombardment of high pressure neon (up to 25 bar) containing a low percentage (0.1-0.2%) of molecular fluorine with moderate energy deuterons (Lambrecht *et al.*, 1978; Casella *et al.*, 1980) is now a common and well-established method for producing CA molecular [ $^{18}\text{F}$ ]fluorine (Table 1). Table 2

shows experimental conditions and typical data for the production of [ $^{18}\text{F}$ ]fluorine via the  $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$  reaction at three European centres. Data reported (Casella *et al.*, 1980) from Brookhaven National Laboratory (BNL) are also shown for comparison. These centres use essentially the same method with small variations in some physical parameters. Several factors must be taken into account to achieve satisfactory routine production, as follows.

#### 1. Target design and construction

Target bodies and the necessary ancillary equipment (including air or electrically operated bellows

Table 2. Typical experimental conditions and production parameters for the production of [ $^{18}\text{F}$ ]fluorine by the  $\text{Ne}(\text{d},\alpha)$  reaction at different centres

|                        |                                               | Centre                       |                            |                               |                                |
|------------------------|-----------------------------------------------|------------------------------|----------------------------|-------------------------------|--------------------------------|
|                        |                                               | BNL                          | MRCCU                      | Jülich                        | Liège                          |
| Target body            | (type; mL)                                    | Honed Ni; 50                 | Polished Ni-201; 100       | Ni; 38                        | Ni; 206                        |
|                        | (internal size, cm)                           | 2.5 i.d. $\times$ 10         | 2.5 i.d. $\times$ 20       | 2.2 i.d. $\times$ 10          |                                |
| Target gas             | (% $\text{F}_2$ in $\text{Ne}$ ) <sup>a</sup> | 0.1                          | 0.12-0.15                  | 0.18                          | 0.17                           |
|                        | ( $\mu\text{mol F}_2$ ) <sup>b</sup>          | 50-60                        | 88 <sup>c</sup>            | 60                            | 220                            |
| Pressure               | (beam off, atm)                               | 25.8                         | 13.5                       | 18                            | 12.3                           |
|                        | (beam on, atm)                                | 32.5                         | 23                         | 30                            | 15                             |
| Window                 | (material, $\mu\text{m}$ )                    | Al, 810-Ni, 25               | Havar, 50-Ni, 25           | Nb, 25-Havar, 50              | Al, 80-Ni, 20                  |
|                        | (seals)                                       | Metal O-ring                 | Lead                       | Metal joints                  | Indium O-rings                 |
| E (incident)           | (MeV)                                         | 14-9.4                       | 13.8                       | 11.25                         | 12                             |
| Irradiation            | ( $\mu\text{A} \times \text{h}$ )             | 15 $\times$ 2                | 15 $\times$ 1.67           | 40 $\times$ 1                 | 10 $\times$ 1                  |
| Recovery of            | (mCi)                                         | 367                          | 250                        | 500                           | 120                            |
| $^{18}\text{F}$ at EOB | (% of theoretical) <sup>d</sup>               | 55                           | 43                         | 50                            | 51                             |
| Yield                  | (mCi/ $\mu\text{Ah}$ at EOB)                  | 12.5                         | 9-10                       | 12                            | 10-15                          |
| Specific activity      | (mCi/ $\mu\text{mol}$ at EOB)                 | 7-10                         | 1.1                        | 3.5                           | 0.8-1.2                        |
| Reference              |                                               | Casella <i>et al.</i> (1980) | Clark <i>et al.</i> (1990) | Blessing <i>et al.</i> (1986) | Guillaume <i>et al.</i> (1988) |

<sup>a</sup>Nominal value.<sup>b</sup>Calculated from target volume and nominal filling parameters.<sup>c</sup>Experimentally determined as 194-242  $\mu\text{mol}$  recovered, under conditions in which the fill-line contributes fluorine.<sup>d</sup>Theoretical yields were calculated according to Casella *et al.* (1980).

valves, pressure transducers and manometers) have been described formerly by various authors [e.g. Casella *et al.* (1980); Blessing *et al.* (1986)].

Nickel and Monel, machined or welded, appear to be the optimal metals for the target body. Stainless steel (316) is also convenient but needs longer passivation with fluorine (*vide infra*). Inconel also requires longer passivation and gives lower recovery of fluorine-18 (Casella *et al.*, 1980; Blessing *et al.*, 1986). Nickel plating on stainless steel deteriorates after a few runs (Blessing *et al.*, 1986). Generally, target bodies are 10–20 cm long and are fabricated from 1.5 to 2.5 cm i.d. tube with gas connections through stainless steel weld fittings. Swagelok fittings are recommended for this purpose. Experience now shows that honing of the target is unnecessary, but the target must be chemically clean.

The window is preferably made from 25 or 50  $\mu\text{m}$  Havar<sup>TM</sup> foil (Hamilton Precision Metals). Nickel, niobium and titanium foils have also been used. Aluminium or Haver, having a thick layer (e.g. 20–25  $\mu\text{m}$ ) of nickel on the side exposed to fluorine, has been used in some centres. Air, or preferably helium, cooling of the foil is recommended.

Several methods may be used to seal the beam entry window to the target body. Those that are satisfactory include the use of a copper ring (Leybold) a gold crush seal, an indium wire or a flat washer made from thick (0.5 mm) lead sheet.

## II. Target gas

The neon should be 99.998% pure as obtainable from Air Liquide or Messer Griesheim. In particular, it should be as free as possible from carbon oxides, nitrogen and fluorocarbons which can have a drastic effect on the chemical form of recovered radioactivity (Casella *et al.*, 1980; Bida *et al.*, 1980; Dahl and Schyler, 1985). These effects are discussed below. The pressure regulator should be of all metal construction and designed for use with high purity gases.

In the absence of added fluorine, nucleogenic fluorine-18 diffuses to the target wall and is chemically adsorbed (as nickel fluoride). In the presence of carrier fluorine, exchange of nucleogenic fluorine-18 competes with surface adsorption and recovery of radioactivity from the target becomes possible (Lambrecht *et al.*, 1978; Casella *et al.*, 1980; Bida *et al.*, 1980). Corrosion of target and ancillary components is not a significant problem if only dilute (e.g. < 2%) mixtures of fluorine in neon are used. A mixture of 2% fluorine in neon (with certificate of analysis) is supplied by Union Carbide, Air Liquide or Matheson. The pressure regulator is specially prepared for use with fluorine mixtures by the gas supplier. A mixture of 2% fluorine in helium, as prepared for lasers, can also be used as the source of fluorine gas. This is supplied by Matheson or Air Liquide with a full Monel delivery system.

## III. Target gas handling

Given the highly hazardous nature of fluorine, it is emphasized that a safe facility is required to dilute the stock fluorine mixture with further neon to achieve the composition desired within the target and to retrieve the fluorine-18 after irradiation.

Stainless steel and Swagelok fittings are recommended for transport lines, since surface fluorination (passivation) lasts longer at room temperature than for the alternatives of nickel and Monel. Teflon tubing, though chemically resistant, is unsuitable as it becomes permeable to fluorine with use (Casella *et al.*, 1980). Stainless steel bellows (Hoke, Nupro) valves have been found to be reliable in service. A piezoresistive, low dead-volume pressure transducer (Kistler) is recommended to monitor target pressure before and during irradiation. Great care should be taken to avoid any ingress of air into the gas handling facility and target. Flow purging rather than evacuation of air is preferred.

The use of the same line to load and unload the target with fluorine poses the risk that the amount of fluorine reaching the target before irradiation and that which is eventually recovered will depend critically on the fill/empty procedure (see footnote c, Table 2). In turn, control of the stoichiometry of radiofluorination reactions may be difficult, with an adverse effect on the reproducibility of yields. It is preferable to use a dedicated line for the filling of the target and also a low volume dedicated line for the recovery of radioactivity. The fill/empty stoichiometry of this arrangement can be highly reproducible.

## IV. Target operating conditions

The main factor that determines [<sup>18</sup>F]fluorine recovery is the chemical state of the target surface (Casella *et al.*, 1980; Bida *et al.*, 1980). Passivation (prefluorination) of the surface is necessary before bombardment to avoid a significant loss of fluorine-18 along with fluorine carrier. Two passivation methods have been proposed, namely "thermal" passivation and "beam-induced plasma" passivation. Thermal passivation requires the target to be heated for 1–3 h to between 100 and 200°C, when filled to a low pressure (a few bar) with dilute fluorine in neon. Beam-induced plasma passivation is achieved by deuteron bombardment of the target when filled to at least the same partial pressure of fluorine as used in production. Two or three irradiations, using ca 10  $\mu\text{A}$  of deuterons for 30 min, are carried out successively, with the contents of the target discarded after each irradiation. Beam-induced passivation is most easy to operate routinely. The frequency with which passivation is carried out varies between centres. Some centres, such as Liège, regard passivation as necessary before each bombardment. Others, such as the MRC Cyclotron Unit, find passivation to be necessary only after a target system has been opened and thereafter

only infrequently, if the target is in regular use for production. In general, passivation is more likely to be required the longer a target is not in use. It is considered that a good passivation is achieved when 75–80% of the fluorine-18 can be recovered in a production run.

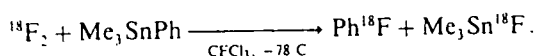
Cooling of the target during irradiation may not be necessary; metal targets can often be run under non-cooled conditions and are easier to build for air cooling.

Nickel targetry and the associated gas handling systems are still problematical to maintain in routine use. Small volume gold-plated targets seem to show promise for high yield and recovery of [ $^{18}\text{F}$ ]fluorine from the proton irradiation of [ $^{18}\text{O}$ ]oxygen (Wieland *et al.*, 1989), and might also offer advantages for neon targets. However, experience has shown that, where beam strikes cause local heating, gold plating of less than 50  $\mu\text{m}$  thickness, progressively peels off, deteriorates after a few runs, or diffuses into a copper backing. More experimental data are needed to assess the potential of gold-plated targets.

#### V. Chemical forms of the recovered fluorine-18

The chemical form of the recovered fluorine-18 depends critically on contaminants in the target gas (Casella *et al.*, 1980; Bida *et al.*, 1980; Dahl and Schlyer, 1985). The target neon gas should be as free as possible from nitrogen and carbon oxides, as these incorporate fluorine-18 into inert substances namely, nitrogen [ $^{18}\text{F}$ ]trifluoride and carbon [ $^{18}\text{F}$ ]tetrafluoride, respectively. Contamination by fluorocarbons should also be avoided (Table 3). Under such conditions, the fluorine-18 is mainly obtained as molecular [ $^{18}\text{F}$ ]fluorine (Bida *et al.*, 1980; Dahl and Schlyer, 1985).

The following reaction is useful to monitor the chemical activity of the available fluorine-18, as well as its specific activity (Blessing *et al.*, 1986):



The amount of [ $^{18}\text{F}$ ]fluorobenzene in the freon solution of labelled products is determined by gas chromatography, and its activity by  $\gamma$ -counting. From measurement of total recovered activity the reactive fraction of recovered fluorine-18 (presumed to be molecular [ $^{18}\text{F}$ ]fluorine), and its specific activity can be calculated. Blessing *et al.* (1986) found the

total organic radiochemical yield to be  $51 \pm 2\%$  and the fluorobenzene yield  $36 \pm 2\%$  over 30 runs under the conditions described in Table 2.

It should be noted that deuteron irradiation of neon containing a low percentage ( $<15\%$ ) of hydrogen has been used by several groups to produce NCA fluorine-18 (Clark *et al.*, 1973; Winchell *et al.*, 1976; Straatman and Welch, 1977; Lambrecht *et al.*, 1978; Helus *et al.*, 1979; Clark and Oberdorfer, 1982; Dahl *et al.*, 1983; Ehrenkaufer *et al.*, 1983; Blessing *et al.*, 1986). Various means have been used to recover the radioactivity, including removal from the heated target with a stream of hydrogen (Winchell *et al.*, 1976; Clark and Oberdorfer, 1982; Dahl *et al.*, 1983; Blessing *et al.*, 1986), recirculation of the irradiated neon over a basic trap (Clark *et al.*, 1973) and water-washing of the target after irradiation (Helus *et al.*, 1979; Blessing *et al.*, 1986). Removal from the heated target in a stream of hydrogen is invariably described as giving NCA hydrogen [ $^{18}\text{F}$ ]fluoride, though it has been argued that the true chemical form might be NCA ammonium [ $^{18}\text{F}$ ]fluorine [see Tewson (1989)]. Water washing of the Inconel target recovers a high proportion (90%) of the radioactivity as aqueous [ $^{18}\text{F}$ ]fluoride with high specific activity (10 Ci/ $\mu\text{mol}$ ), as determined by an ion selective electrode, and free of contamination by Co, Cr, Fe, Mo and Mn, as measured by optical emission spectroscopy in conjunction with inductively coupled plasma ( $<0.1$  ppm, detection limit) (Blessing *et al.*, 1986). Only 0.1 ppm levels of Ni and Cu were found (Blessing *et al.*, 1986). However, these neon/hydrogen targets are relatively inconvenient to operate and difficult to maintain and so are not in widespread use.

#### VI. Specific activity

The specific activity of molecular [ $^{18}\text{F}$ ]fluorine can be measured via titration of the iodine liberated on passing the target output into potassium iodide solution (Casella *et al.*, 1980). However, in principle, this method does not distinguish between the several oxidizing chemical forms of fluorine-18, such as [ $^{18}\text{F}$ ]labelled  $\text{OF}_2$ ,  $\text{HOF}$ ,  $\text{NFH}_2$  or  $\text{NF}_2\text{H}$ , that might arise in the target if there is any contamination by air. A misleading result might therefore be obtained. Preferably, specific activity is measured via the reaction with trimethylphenyl tin, which is expected to be more specific for fluorine-18 in the form of molecular fluorine (Blessing *et al.*, 1986).

The effect of systematically varying fluorine content between 0.006 and 0.18% ( $2\text{--}60 \mu\text{mol F}_2$ ) on activity recovery and specific activity has been examined (Blessing *et al.*, 1986). Between 0.10 and 0.18% of fluorine carrier, [ $^{18}\text{F}$ ]fluorine recovery increased but specific activity did not change. The optimal composition for recovery yield and specific activity seems to be 0.18% fluorine. There appears to be a trend in that the lower the incident energy of deuterons, the higher the fluorine concentration that

Table 3. Relative importance of impurities in neon gas on fluorine-18 recovery [according to Dahl and Schlyer (1985)]

| $\text{F}_2$<br>(%) | % Impurity in the<br>starting neon gas |              |               |               | % $^{18}\text{F}$<br>appearing as |               |               |
|---------------------|----------------------------------------|--------------|---------------|---------------|-----------------------------------|---------------|---------------|
|                     | $\text{N}_2$                           | $\text{O}_2$ | $\text{CF}_4$ | $\text{CO}_2$ | $\text{F}_2$                      | $\text{NF}_3$ | $\text{CF}_4$ |
| 1.00                | 0.49                                   | 0.60         | ND            | 0.13          | 29                                | 54.1          | 16.9          |
| 0.62                | 0.30                                   | 0.037        | ND            | 0.008         | 29                                | 50.7          | 20.3          |
| 0.047               | 0.002                                  | ND           | ND            | 0.001         | 98                                | —             | —             |
| 0.076               | 0.0002                                 | 0.009        | 0.008         | ND            | 15                                | —             | 85.0          |

ND, not detected.



is necessary for maximal recovery (Blessing *et al.*, 1986).

The volume of the target is an important factor. The smaller the size of the target, the more irreproducible are the results, both in terms of yield and specific activity. Plasma/wall interactions play an increasing rôle with decreasing diameter of the target body. The Jülich group have found that, for a collimated (1 cm  $\phi$ ), defocussed and wobbled beam, the optimal target size is 2.2 cm  $\phi \times 10$  cm giving a total fluorine content of 60  $\mu$ mol for the target, leading to a specific activity of 1.5–3.0 Ci/mmol in production (Blessing *et al.*, 1986).

Production of NCA [<sup>18</sup>F]Fluoride by the <sup>18</sup>O(p,n)<sup>18</sup>F Reaction on <sup>18</sup>O-enriched Water

The use of the <sup>18</sup>O(p,n)<sup>18</sup>F reaction on <sup>18</sup>O-enriched water is now the most effective method for the production of NCA [<sup>18</sup>F]fluoride (Table 1).

I. Calculated Production Yields

The excitation function for the <sup>18</sup>O(p,n)<sup>18</sup>F reaction has been determined for proton energies from 2.3 to 14.7 MeV on molecular [<sup>18</sup>O]oxygen (Ruth and Wolf, 1979). Saturation activities for fluorine-18 production are available and serve as reference data for the evaluation and extrapolation of experimental production capabilities using a low energy (Level I or II) cyclotron and <sup>18</sup>O-enriched water targets. From these data, the thick target rate of production of fluorine-18 from 100% <sup>18</sup>O-enriched water targets can be easily calculated as a function of proton energy (Table 4).

Under optimal conditions, several hundreds of millicuries of aqueous [<sup>18</sup>F]fluoride are easily available from a low energy cyclotron, in a bombardment time of less than 1 h. Table 5 summarizes typical experimental data on the production of [<sup>18</sup>F]fluoride

Table 4. Proton range in water and production rate of fluorine-18 from a target of 100% <sup>18</sup>O-enriched water as a function of proton energy

| $E_p$<br>(MeV) | Range in<br>water<br>(mg/cm <sup>2</sup> ) | Production<br>rate<br>(mCi/ $\mu$ Ah) |
|----------------|--------------------------------------------|---------------------------------------|
| 4              | 23.3                                       | 2.2                                   |
| 5              | 34.5                                       | 6.4                                   |
| 6              | 47.6                                       | 16.2                                  |
| 7              | 62.7                                       | 23.2                                  |
| 8              | 79.6                                       | 28.9                                  |
| 9              | 98.3                                       | 33.8                                  |
| 10             | 119                                        | 39.1                                  |
| 11             | 140                                        | 43.8                                  |
| 12             | 165                                        | 48.4                                  |
| 13             | 190                                        | 52.2                                  |
| 14             | 216                                        | 54.5                                  |
| 15             | 246                                        | 56.4                                  |
| 16             | 277                                        | 58.0                                  |
| 17             | 312                                        | 59.5                                  |
| 18             | 345                                        | 60.5                                  |
| 19             | 378                                        | 61.4                                  |
| 20             | 415                                        | 62.1                                  |
| 21             | 445                                        | 62.5                                  |
| 22             | 480                                        | 62.7                                  |

Table 5. Typical experimental data for [<sup>18</sup>F]fluoride produced at different centres from <sup>18</sup>O-enriched targets

|                                                    | Centre                        |                            |                                                        |                            |                               | Liege                          |
|----------------------------------------------------|-------------------------------|----------------------------|--------------------------------------------------------|----------------------------|-------------------------------|--------------------------------|
|                                                    | St Louis                      | Sendai                     | Villigen                                               | Turku                      | Jülich                        |                                |
| Material insert                                    | SS & Ti                       | Ti                         | Ag                                                     | Ag                         | Ti                            | Ni                             |
| Water width (mm)                                   | 3, 5, 7                       | 3, 4, 5                    | 5                                                      | 1.5                        | 3.5                           | 3, 4                           |
| Foils                                              | Havar or Ti                   | Al/Ti & Ag                 | Ag                                                     | Ni                         | Ti                            | Ti                             |
| Seal                                               | O-ring                        | O-ring                     | Ag                                                     | Metal                      | Weld & Ag                     | O-ring                         |
| Cooling (°C)                                       | Water (10)                    | Coolant (l)                | Water (30)                                             | Coolant (10)               | Water (10)                    | Water (10)                     |
| <sup>18</sup> O-Enrichment of H <sub>2</sub> O (%) | 97                            | 20                         | 98                                                     | 98                         | 97                            | 5                              |
| Volume (mL)                                        | 1.2, 2.0, 2.8                 | 2.5                        | 4.5                                                    | 0.195                      | 1.3                           | 1.8                            |
| Pressure (atm)                                     | 1                             | Open or circulated (Pd)    | Open                                                   | Open                       | 5-7                           | 2-3                            |
| E <sub>p</sub> (MeV)                               | 15                            | 16                         | 16                                                     | 12                         | 16.5                          | 22.6                           |
| Irradiation ( $\mu$ A $\times$ min)                | 15-20 $\times$ 80             | 20 $\times$ 60             | 20 $\times$ 60                                         | 10 $\times$ 60             | 25-35 $\times$ 60             | 10 $\times$ 60                 |
| Yield (mCi/ $\mu$ Ah at EOB)                       | 60                            | 9                          | 56                                                     | 30                         | 65                            | 3-3.2                          |
| Specific (Ci/ $\mu$ mol at EOB) activity           | ca 50                         | 4                          | NCA                                                    | 140                        | 200                           | 10                             |
| Reference                                          | Kilbourn <i>et al.</i> (1985) | Iwata <i>et al.</i> (1987) | Huszar and Weinreich (1985); Vogt <i>et al.</i> (1986) | Solin <i>et al.</i> (1988) | Nebeling <i>et al.</i> (1990) | Guillaume <i>et al.</i> (1990) |

from  $^{18}\text{O}$ -enriched water targets, taken from the literature and our own experience.

## II. Target Systems

Many different [ $^{18}\text{O}$ ]water targets have been experimentally tested and described (Wieland and Wolf, 1983; Kilbourn *et al.*, 1984, 1985; Huszar and Weinreich, 1985; De Jesus *et al.*, 1986; Keinonen *et al.*, 1987; Vogt *et al.*, 1986; Berridge and Tewson, 1986; Berridge and Kjellstrom, 1989; Iwata *et al.*, 1987; Mulholland *et al.*, 1989). The primary consideration in design has been to consume as little of the costly  $^{18}\text{O}$ -enriched water as possible during production. The requirement for small target volume in turn means that target thickness has to be selected carefully for effective use of the beam. Also radiolysis and boiling of the water [see Steinbach *et al.* (1990)] become important considerations. These can cause significant loss of target water in open targets (Kilbourn *et al.*, 1984; Berridge and Tewson 1986a, b; Iwata *et al.*, 1987) or unacceptable pressure build up or bubble formation in closed targets, with adverse effect on yield. Cavitation and even complete voiding of the target can occur during high current irradiations. To reduce these effects, the proton beam should be uniformly defocussed over the whole area of the target (Iwata *et al.*, 1987). Effective target cooling is mandatory.

The decision to operate a low pressure or high pressure target is fundamental, since it bears on target construction and the strategy for coping with the adverse effects of radiolysis and heat. Experience has shown that the most efficient water target is simply composed of a cavity for the target water, bounded by two rigid metallic foils, the back one being efficiently cooled by a suitable fluid. Targets requiring only 0.4–2.5 mL of water can be constructed on this basis. Other parameters of importance are the type of target seals, the chemical nature of the metallic insert and foils; the type of transfer tubing (Iwata *et al.*, 1987) and the facility for recovery of the  $^{18}\text{O}$ -enriched water, as these potentially bear on the reactivity and the specific activity of the generated [ $^{18}\text{F}$ ]fluoride.

Below we describe and discuss examples of reliable target assemblies designed for low and high pressure operation.

### (A) An example of a reliable low pressure target system

In the low pressure mode of operation, without suitable precautions, radiolysis and boiling can cause a significant loss of  $^{18}\text{O}$ -enriched water, and render the use of high beam currents impractical for high yields. Provision for effective venting (Wieland and Wolf, 1983; Berridge and Tewson, 1986a, b; Iwata *et al.*, 1987) or for the catalytic recombination of radiolytically generated oxygen and hydrogen (Iwata *et al.*, 1987) must therefore be considered. Recirculation of the water during irradiation has also been

found to offer some advantage for some targets (Keinonen *et al.*, 1987; Iwata *et al.*, 1987), but results are not necessarily consistent (Iwata *et al.*, 1987). A low pressure target in use at the MRC Cyclotron Unit, is described here as an example of a reliable system.

1. *Target hardware.* The target (Fig. 1) is fabricated from 25 mm dia stainless steel (316) and titanium for the proton irradiation of 20%  $^{18}\text{O}$ -enriched water. The front window is aluminium (155 mg/cm<sup>2</sup>, 0.57 mm thick) backed on the water side by a titanium foil (12.4 mg/cm<sup>2</sup>, 25  $\mu\text{m}$  thick). This maintains a flat front surface to the target and degrades the 19 MeV beam to 16 MeV. The water thickness is determined by a 3 mm thick stainless steel spacer. The back of the target is made from 125  $\mu\text{m}$  thick stainless steel (316) foil which is water-cooled. The foils and spacer are clamped together metal to metal. Hence, there are no O rings in the target water cell. The target cell has three  $\frac{1}{16}$ " stainless steel tubes inserted through the spacer, fixed and sealed with Locktite retainer 601, the joints having been pretreated with Locktite Activator T. A small pellet of palladium catalyst (Engelhard Model D) is supported in an external gas space to recombine any radiolytically-generated hydrogen and oxygen that might otherwise cause excessive internal pressure.

2. *Target water.*  $^{18}\text{O}$ -enriched water (97%, Isotec) is diluted to 20% enrichment with sterile HPLC grade water, and used as target material.

3. *Target operation.* The target is normally operated with an incident beam of 19 MeV protons (20  $\mu\text{A}$ , 20 mm dia, spun at 45–50 Hz). Table 5 gives performance data.

4. *Liquid transfer system.* The target is 45 m from the nearest hot cell in which the fluorine-18 can be used. Several types and sizes of tube have been tried to effect this transfer. Some (e.g. polythene) have eventually developed restriction to liquid flow. At present Teflon lines ( $\frac{1}{16}$ " o.d., 0.030" bore) are used with a neon drive pressure of 2 bar and flow regulator (Fig. 2). The transfer yield for 1–2 mL target water is currently >95% while the recovery of fluorine-18 is typically between 95 and 98%, within 5 min from EOB. At EOB ca 65% of the radioactivity is nitrogen-13 ( $t_{1/2} = 9.96$  min).

5. *Recovery of NCA [ $^{18}\text{F}$ ]fluoride and recycling of  $^{18}\text{O}$ -enriched water.* A procedure, based on that described by Schlyer *et al.* (1987, 1990) and by Hamacher *et al.* (1990) has been adopted for the recovery of [ $^{18}\text{F}$ ]fluoride and for the recycling of  $^{18}\text{O}$ -enriched water. A 90 mg bed of carbonate form ion exchange resin (100–200 mesh AG 1X 8) is made into a column (28  $\times$  3 mm).  $^{18}\text{O}$ -Enriched water is forced from the target through the column by low neon pressure (2 bar). Finally, neon is blown through the column to extract the last traces of liquid. The recovered water is pooled into 10–20 mL batches and distilled under reduced pressure using a micro rotary evaporator. The degree of  $^{18}\text{O}$ -enrichment

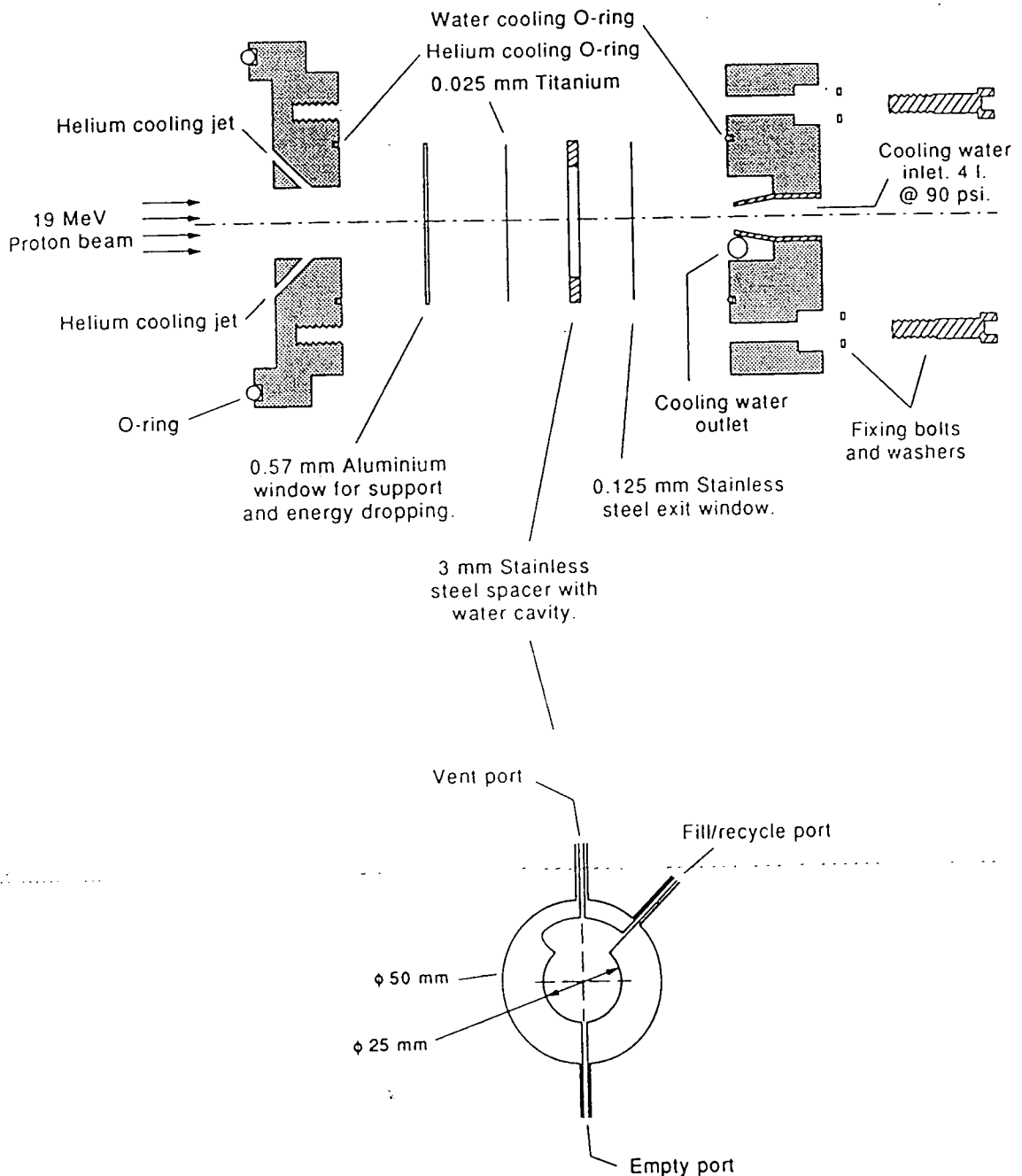


Fig. 1. An exploded schematic view of the target (internal volume, 2 mL) in use at the MRC Cyclotron Unit (London) for [ $^{18}\text{F}$ ]fluoride production via the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reaction on  $^{18}\text{O}$ -enriched water. Operational parameters are described in Table 5. See also the text.

is measured by mass spectrometry (Waters, 1990). Bacterial contamination of the  $^{18}\text{O}$ -enriched water must be avoided by Millipore filtration into clean sterile glass vials (Pierce). The [ $^{18}\text{F}$ ]fluoride is recovered from the anion exchange column by elution with potassium carbonate solution.

The procedure is highly efficient with respect to recovery of [ $^{18}\text{F}$ ]fluoride and to recycling of the  $^{18}\text{O}$ -enriched water. Great care must be taken in the preparation of the ion exchange resin to ensure that it is obtained fully in the carbonate form and free of carbonate solution. Otherwise, if prepared from the chloride form, residual chloride can compete with

[ $^{18}\text{F}$ ]fluoride in subsequent labelling chemistry [see, for example, Alexoff *et al.* (1990)].

6. *Reactivity of the NCA [ $^{18}\text{F}$ ]fluoride.* The [ $^{18}\text{F}$ ]fluoride is used routinely for the production of 2-[ $^{18}\text{F}$ ]fluoro-2-deoxy-D-glucose, according to Hamacher *et al.* (1986). Generally, over 90% of the radioactivity reacts in the first stage of the radiosynthesis, the displacement of the triflyl group, if performed in a glassy carbon vessel. If the reaction is performed in a glass vessel, incorporation is typically 60%.

7. *Specific activity.* The specific activity of the [ $^{18}\text{F}$ ]fluoride has not been measured directly.

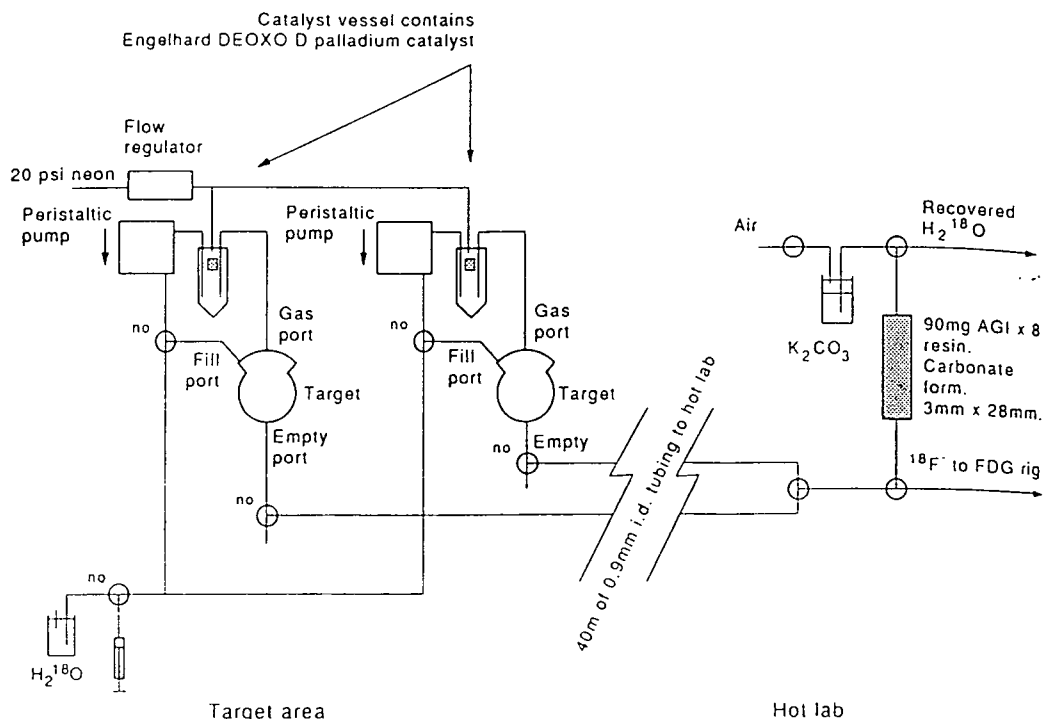


Fig. 2. Schematic representation of the unit for remotely filling the  $^{18}\text{O}$ -enriched water target at the MRC Cyclotron Unit. The principles of its operation are described in the text

However, the amounts of carrier arising from single irradiations are  $< 1 \mu\text{mol}$  as assessed indirectly by specific activity measurements on derived radiopharmaceuticals, such as  $[^{18}\text{F}]\text{PK 14105}$  (Pascali *et al.*, 1990). This corresponds to a specific activity at EOB of  $300 \text{ mCi}/\mu\text{mol}$  at EOB. Currently the target and recovery system are used to produce  $[^{18}\text{F}]\text{fluoride}$  for applications that do not demand high specific activity. It is expected that much higher specific activity could be achieved by avoiding Teflon lines for recovery (see Part B4, below), by producing higher activity from 98%  $^{18}\text{O}$ -enriched water, and by rigorously excluding work with CA molecular  $[^{18}\text{F}]\text{fluorine}$  from the production area.

(B) An example of a reliable high pressure target system

High pressure targets are operable at beam currents up to  $30 \mu\text{A}$ , due to the higher boiling point of the water under a higher pressure (Wieland *et al.*, 1986) which, as a diagnostic, can be monitored during irradiation. A target (Fig. 3) in use at Jülich is described here as an example of a system, which has functioned reliably, even after reaching an integrated beam current of  $3000 \mu\text{Ah}$ .

1. *Target hardware.* The target consists of a titanium body electron beam-welded to two titanium foils ( $75 \mu\text{m}$  thick), which act as front and back window. (Generally, depending on the target material used, electron beam welding or diffusion welding of the target foils is preferred to sealing by Viton or silver O-rings). It is a modified form of the targets

described earlier (Wolf *et al.*, 1985; Qaim *et al.*, 1987). The inner diameter of the present target is 21 mm with a water thickness of 3.5 mm. The target takes 1.3 mL of  $^{18}\text{O}$ -enriched water with no expansion space. Two  $\frac{1}{16}$ " stainless steel tubes, leading to a multi-port valve (Fig. 4) are connected via screws that are sealed with silver washers. During irradiation, the back window is water-cooled, typically to  $10$ – $12^\circ\text{C}$ , and the front window is helium-cooled to  $-5^\circ\text{C}$ .

2. *Purity of  $^{18}\text{O}$ -enriched water.* The purity of the  $^{18}\text{O}$ -enriched water used in high pressure targets is of major concern. It is found that high chemical purity is needed to avoid excessive pressure build-up. For large-scale production,  $> 90\%$   $^{18}\text{O}$ -enrichment is needed to avoid the generation of excessive  $[^{13}\text{N}]\text{nitrogen}$  by the  $^{16}\text{O}(p,\alpha)^{13}\text{N}$  reaction, which may pose radiation protection problems. Organic impurities must be absent as these can prevent recombination of radiolytically generated oxygen and hydrogen atoms, causing the target to burst. The  $^{18}\text{O}$ -enriched water is purified by treatment with active charcoal, followed by refluxing with potassium permanganate and potassium hydroxide, and finally triple distillation. Purity is assessed by capillary gas chromatography with an FID detector operating at highest sensitivity.

3. *Target operating conditions.* The target is normally operated with a  $25 \mu\text{A}$  beam of 17 MeV protons. Performance data are given in Table 3. The maximum current tested was  $35 \mu\text{A}$ . The pressure in a routine production run is between 5 and 7 bar.

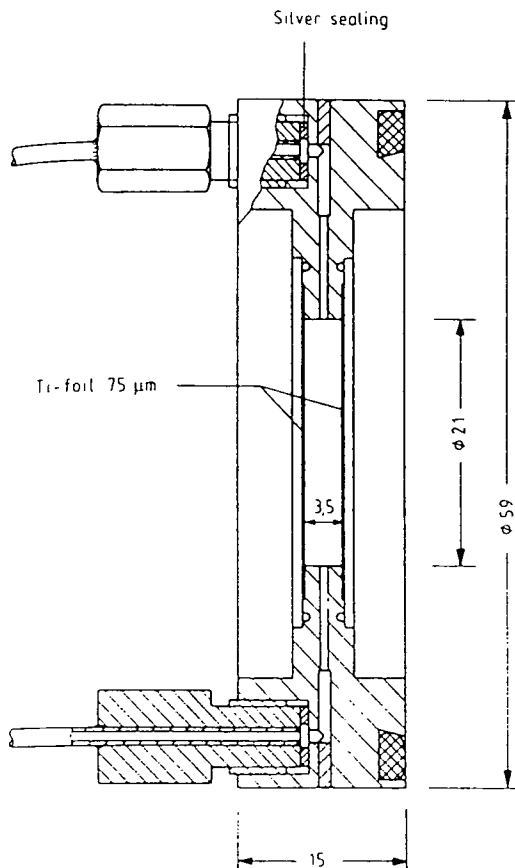


Fig. 3. Sectional view of the electron-welded titanium target (internal volume 1.3 mL) in use at KFA (Jülich) for  $^{18}\text{F}$  fluoride production via the  $^{18}\text{O}(p,n)^{18}\text{F}$  reaction on  $^{18}\text{O}$ -enriched water. Operational parameters are described in Table 5. See also the text.

(Fig. 5). Figure 6 shows the dependence of aqueous  $^{18}\text{F}$  fluoride yield on beam intensity and irradiation time.

4. *Liquid handling and transfer system.* The target can be remotely loaded with  $^{18}\text{O}$ -enriched water using the filling unit depicted in Fig. 4. Filling of the target with  $^{18}\text{O}$ -enriched water is achieved with a motor-driven syringe through 3-way valves (Asco 368) and 6-way valves (Valco AC6W), which are connected by polyethylene-polypropylene tube. The target is locked by the 6-way valve and the pressure is monitored by a piezoresistive pressure transducer with very low dead volume. A custom made polyethylene-polypropylene copolymer tube with an i.d. of 0.8 mm and helium drive pressure of 1.3 bar is considered a reliable transfer system. Transfer over 40 m through this tubing from the target to the nearest hot-cell by a helium drive pressure of 1.3 bar takes only 2 min. Teflon tubes are not recommended for the recovery of the  $^{18}\text{F}$  fluoride if high specific activity is required, since radiolysis and radical stripping can give rise to fluorine carrier.

5. *Recovery of NCA  $^{18}\text{F}$  fluoride and recycling of  $^{18}\text{O}$ -enriched water.* The  $^{18}\text{O}$ -enriched water is recovered by retrieving the  $^{18}\text{F}$  fluoride on a carbonate form ion exchange resin (Hamacher *et al.*, 1990). The water can be recycled 4 or 5 times, unless the enrichment of water falls below 90%, as measured by mass spectrometry and by measuring the amount of nitrogen-13 from a production run.

6. *Reactivity of the NCA  $^{18}\text{F}$  fluoride.* Cation and anion contaminants (Solin *et al.*, 1988) have generally been considered to influence the reactivity of NCA  $^{18}\text{F}$  fluoride. If Havar foils are used for the target the contaminants are mainly Fe, Cr and Co ions in concentrations ranging from 0.2 to 3 ppm as assessed by atomic absorption spectroscopy. Use of titanium foils results in a greater than 10-fold decrease in these ions and therefore can be recommended as the least contaminating metal to use as insert and window foil. Contamination of the  $^{18}\text{O}$ -enriched water with vanadium-48 was found to

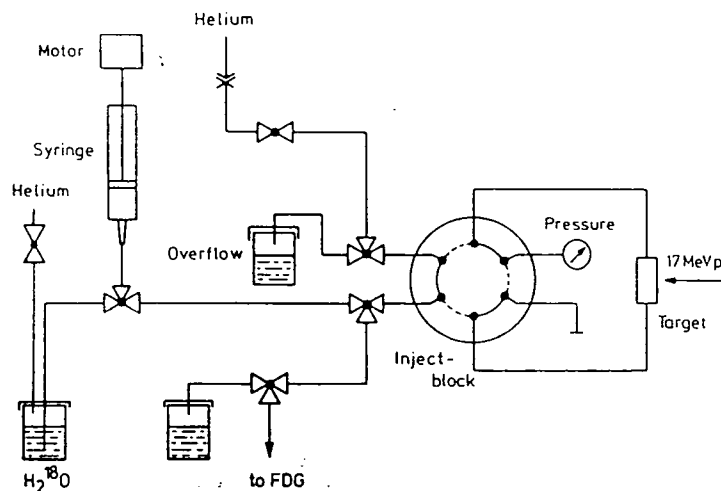


Fig. 4. Schematic representation of the unit for remotely filling the  $^{18}\text{O}$ -enriched water target at KFA (Jülich). The principles of its operation are described in the text.

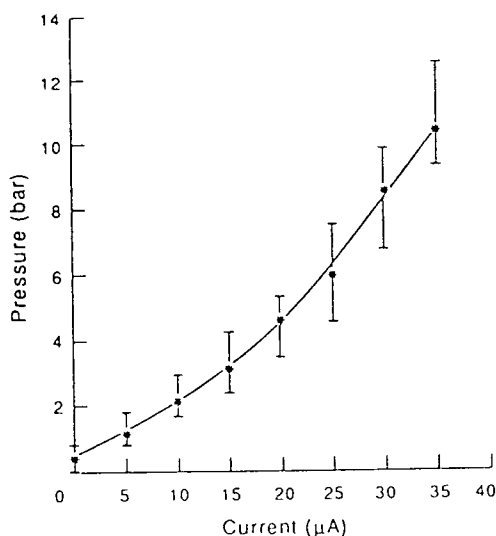


Fig. 5. The dependence of target pressure in a closed target (see Fig. 3) on beam intensity, as measured at KFA (Jülich). See Table 5 for target operational parameters.

be  $0.187 \mu\text{Ci}$  after irradiation for 10 min with a  $20 \mu\text{A}$  beam producing 270 mCi of fluorine-18. An advantage of the  $^{18}\text{F}$ fluoride recovery system is that cationic species are not co-recovered.

Over 90% of the recovered  $^{18}\text{F}$ fluoride reacts in the first stage of the synthesis of 2- $^{18}\text{F}$ fluoro-2-deoxy-D-glucose described by Hamacher *et al.* (1986), when a glassy carbon vessel is used.

7. *Specific activity.* The target delivers  $^{18}\text{F}$ fluoride with high specific activities (up to  $200 \text{ Ci}/\mu\text{mol}$ ) as measured by a fluoride selective electrode.

#### Fluorine-18 from the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ Reaction on $^{18}\text{O}$ Oxygen Gas

The  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reaction is of considerable interest for producing  $^{18}\text{F}$ fluorine in centres having a proton-only cyclotron. Nickles *et al.* (1984) first described the production of this important precursor from an oxygen-18 gas target, using a *two-step* irradiation process. Nickel is used as material for the target body, and nickel and Havar foils for the windows. The target is conically shaped and has a volume of 15 mL. After irradiating pressurized  $^{18}\text{O}$ -enriched (98%) oxygen gas, fluorine-18 becomes deposited on the target walls, and the oxygen-18 is recovered cryogenically. A second and short irradiation of 1% fluorine ( $75 \mu\text{mol}$ ) in krypton is needed for isotopic exchange of the adsorbed fluorine-18 and the recovery of more than 50% of the total fluorine-18 activity (Sunderland *et al.*, 1989). Target performance is determined by the level of fluorine passivation. Passivation with molecular fluorine at  $200^\circ\text{C}$  for several hours gives the best results. Up to 50% of the total radioactivity has been recovered as  $^{18}\text{F}$ fluorine. The  $^{18}\text{F}$ fluorine can be titrated by well known methods (Casella *et*

*al.*, 1980) and has been converted into acetyl  $^{18}\text{F}$ hypofluorite, which was then used for the synthesis of L-6- $^{18}\text{F}$ fluoro-Dopa (Sunderland *et al.*, 1990). Solin and Bergman (1986) used a similar process, but substituted fluorine in neon for fluorine in krypton. They recovered up to 25% of the total radioactivity. Half of the radioactivity was recovered in the  $^{18}\text{O}$ -enriched oxygen. Given the experience with nickel targets, such variations in recovered activity are not unexpected.

Wieland *et al.* (1989) described a *one step* proton irradiation of a mixture of helium, fluorine and  $^{18}\text{O}$ -enriched oxygen. A gold-plated copper body with a conical bore was used as target and no attempt was made to recover the  $^{18}\text{O}$ -enriched oxygen. Also, no aggressive passivation was used to precondition the target. Preliminary results are very encouraging. For example, Wieland *et al.* (1989) were able to produce more than 1 Ci of fluorine-18 radioactivity from a 1 h irradiation with  $30 \mu\text{A}$  beam of 10.4 MeV protons on a target containing only  $19 \mu\text{mol}$  of carrier fluorine. The achieved specific activity is close to  $50 \text{ mCi}/\mu\text{mol}$ , much higher than that usually achieved via the deuteron irradiation of neon/fluorine mixture (Table 2).

These results have to be reproduced routinely, and especially the radioactive product has to be tested regularly for its suitability in, for example, the preparation of acetyl  $^{18}\text{F}$ hypofluorite, and in turn of a radiopharmaceutical such as L-6- $^{18}\text{F}$ fluoro-Dopa, before either a one or a two step process with the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reaction can be recommended for the production of  $^{18}\text{F}$ fluorine.

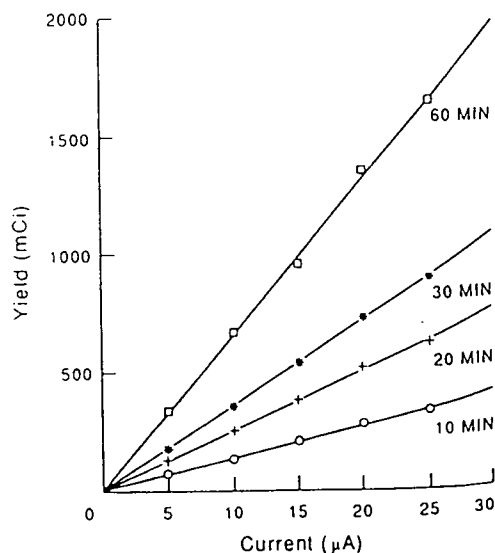


Fig. 6. Yield of  $^{18}\text{F}$ fluoride from the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reaction on 97.5%  $^{18}\text{O}$ -enriched water (1.3 mL) in a titanium target (Fig. 3), under various conditions of beam current and irradiation time, as measured at KFA (Jülich). Yield measurements were taken after transport of the irradiated water through 40 m of polyethylene-polypropylene tube (0.8 mm i.d.).

## Other Reactions for the Production of Fluorine-18

### I. $^3\text{He}$ - and $^4\text{He}$ -induced reactions

Large-scale production of fluorine-18 in high specific activity is possible with helion-induced reactions on oxygen, using water as target material (Clark and Silvester, 1966; Tilbury *et al.*, 1970; Lindner *et al.*, 1973; Fitschen *et al.*, 1977; Knust and Machulla, 1983; Solin *et al.*, 1988), or neon, using a neon hydrogen mixture (Crouzel and Comar, 1978) (Table 1). A critical comparison of these production routes, with an exhaustive bibliography, is given by Qaim and Stöcklin (1983). Due to the high energy needed ( $E > 40$  MeV) for substantial yield (Table 2) these routes are not widely used. They are not recommended for fluorine-18 production, unless compelled by lack of facility for the  $^{18}\text{O}(p,n)^{18}\text{F}$  process.

### II. Reactor-induced fluorine-18

The production of fluorine-18 in nuclear reactors via the  $^{16}\text{O}(t,n)^{18}\text{F}$  reaction has been extensively reviewed by an IAEA consultants meeting (Vera Ruiz, 1988). The consultants concluded that reactors with neutron thermal fluxes of  $1.0 \times 10^{13} \text{ cm}^{-2} \text{ s}^{-1}$  can produce sufficient fluorine-18 (30–100 mCi) for the preparation of useful quantities (3–10 mCi) of fluorine-18 labelled radiopharmaceuticals by nucleophilic substitution reactions. Generally, a lithium salt containing an oxy anion, such as hydroxide or carbonate, is irradiated with neutrons to enact the  $^6\text{Li}(n,^3\text{H})^4\text{He}$  reaction. The emitted tritons then perform the  $^{16}\text{O}(^3\text{H},n)^{18}\text{F}$  reaction. The practical difficulties are significant, not least because of the need to isolate the product from tritium and lithium.

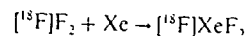
### $^{18}\text{F}$ Precursors for Labelling Procedures

Routes for the introduction of fluorine-18 into an organic structure mainly fall into the following categories:

- aliphatic electrophilic addition to double bonds;
- aromatic electrophilic substitution;
- aliphatic nucleophilic substitution;
- aromatic nucleophilic substitution.

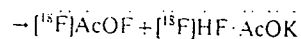
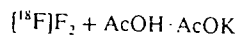
For electrophilic processes (a and b) the fluorine-18 has to be in a suitable chemical form. CA molecular  $^{18}\text{F}$ fluorine has found some useful application as a routine labelling agent, notably for L-6- $^{18}\text{F}$ fluoro-Dopa (Firnau *et al.*, 1984). However, molecular fluorine-18 is invariably non-selective as a fluorinating agent, because of its high reactivity and because of its participation not only in electrophilic processes but also in oxidation and free radical reactions. Hence, there is considerable interest in developing milder and more regioselective agents for "electrophilic"  $^{18}\text{F}$ fluorination.

Xenon  $^{18}\text{F}$ difluoride is a somewhat milder reagent, but is not always regioselective and can also fluorinate non-activated rings. So far, two procedures have been adapted to the routine synthesis of xenon  $^{18}\text{F}$ difluoride. The first involves isotopic exchange between xenon difluoride and hydrogen  $^{18}\text{F}$ fluoride. The radiochemical yield reaches 30% with a final specific activity of several tens of mCi/mmol (Schrobilgen *et al.*, 1981). The second procedure (Chirakal *et al.*, 1984) leads to ten-fold higher specific activities and is based on the reaction of xenon with  $^{18}\text{F}$ fluorine.



A chemical yield of  $65 \pm 15\%$  is obtained within 40 min giving access to a final production yield (EOB) of 11 mCi/ $\mu\text{Ah}$  and a sp. act. of 450 mCi/mmol.

Acetyl  $^{18}\text{F}$ hypofluorite possesses a number of advantages over molecular  $^{18}\text{F}$ fluorine as an electrophilic labelling agent. Compared to  $^{18}\text{F}$ fluorine it is considerably less violent, and has a much greater solubility in a wider range of solvents. Its regioselectivity is not necessarily better than that of  $^{18}\text{F}$ fluorine, but differs according to reaction circumstances [see, for example, Coenen *et al.* (1988)]. It can be prepared according to a microchemical method (Jewett *et al.*, 1984a, b) by passing dilute  $^{18}\text{F}$ fluorine through columns containing a complex of alkali metal acetate with acetic acid.



Among the various acetates which have been tested, the ammonium, potassium and caesium salts give better yields than the corresponding sodium salt (Fowler *et al.*, 1982). However, commercially available sodium acetate trihydrate gives similar results to acetic acid complexes (Bida *et al.*, 1984). The practical radiochemical yield of acetyl  $^{18}\text{F}$ hypofluorite from  $^{18}\text{F}$ fluorine is near theoretical (50%).

These few examples serve to show the scope for converting CA  $^{18}\text{F}$ fluorine into more manageable and more useful 'electrophilic' labelling agents. Many other agents are in development [e.g. Satyamurthy *et al.* (1990b, c)]. Useful NCA electrophilic agents, particularly  $^{18}\text{F}$ fluoroalkylating agents, can be prepared from NCA  $^{18}\text{F}$ fluoride by, for example, nucleophilic substitution on alkyl dihalides (Coenen *et al.*, 1986; Block *et al.*, 1987) or alkyl ditosylates (Block *et al.*, 1987).

For nucleophilic processes the fluorine-18 is required as 'naked' NCA  $^{18}\text{F}$ fluoride (i.e. in the absence of water). Several procedures have been described for the recovery of NCA  $^{18}\text{F}$ fluoride in a reactive form from  $^{18}\text{O}$ -enriched water (e.g. Brodack *et al.* (1986); Schlyer *et al.* (1987, 1990); Jewett *et al.* (1988, 1990); Alexoff *et al.* (1989); Hamacher *et al.* (1990)). These deliver the  $^{18}\text{F}$ fluoride in natural enrichment water or organic solvent. The strategy for

generation of reactive [ $^{18}\text{F}$ ]fluoride, is generally dissolution of the [ $^{18}\text{F}$ ]fluoride with a large counterion (e.g.  $\text{K}^+$ ,  $\text{Cs}^+$ ,  $\text{Rb}^+$ ,  $\text{Bu}_4\text{N}^+$  or  $\text{K}^+/\text{Kryptofix}$ ) in a polar aprotic solvent. Where required, water (or other solvent) is removed by distillation from an added base (e.g.  $\text{KOH}$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{Rb}_2\text{CO}_3$ ,  $\text{Et}_4\text{N}^+\text{OH}^-$ ,  $\text{Bu}_4\text{N}^+\text{OH}^-$  or  $\text{K}_2\text{CO}_3\text{-Kryptofix}$ ) and the [ $^{18}\text{F}$ ]fluoride salt dried by, for example, azeotropic distillation with acetonitrile or even by microwaves. This salt is then solubilized in the solvent containing the substrate for nucleophilic attack. Resolubilization efficiency is affected by the reaction vessel, solvent and perhaps other factors such as metal ion contamination (Brodack *et al.*, 1986). The intrinsic reactivity of the [ $^{18}\text{F}$ ]fluoride is affected by several factors, including cation and anion contaminants whose level are largely determined by the materials of target construction and operation (Nickles *et al.*, 1986). Cations, especially  $\text{Al}^{3+}$ , and  $\text{Ca}^{2+}$ , are probably most detrimental to successful chemistry. These contaminants can be avoided, if necessary, by purifying the [ $^{18}\text{F}$ ]fluoride by conversion into [ $^{18}\text{F}$ ]fluorotrimethylsilane, evaporation and hydrolysis (Gatley, 1989). However, it is not usually necessary to adopt this procedure routinely.

Fluorine-18 as hydrogen [ $^{18}\text{F}$ ]fluoride can be used for labelling aromatic systems by the Schiemann or triazene decomposition reactions (De Kleijn, 1977; Ng *et al.*, 1981; Barrio *et al.*, 1983; Berridge *et al.*, 1985; Satyamurthy *et al.*, 1990a). These methods are now seldom used because the Schiemann reaction requires carrier and the triazene decomposition reaction is generally low yielding. Reviews of fluorine-18 chemistry have been published recently (Berridge and Tewson, 1986a, b; Coenen, 1989; Kilbourn, 1990).

### Conclusion

Satisfactory routine production of CA [ $^{18}\text{F}$ ]fluorine is attainable with a suitably constructed, operated and maintained nickel target. The scope for improving recovery yield by, for example, the use of gold-plated targets needs to be addressed. Also the scope for exploiting the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reaction for producing CA [ $^{18}\text{F}$ ]fluorine in higher activity and specific activity is worthy of further attention. Careful attention to target design and operation now allows high activities of NCA [ $^{18}\text{F}$ ]fluoride to be produced in high activity, high specific activity and with excellent reactivity from the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reaction on  $^{18}\text{O}$ -enriched water. This is now clearly the method of choice for routine production. The recommended methods depend greatly on the purity of the target substances for success. Industry should be stimulated to ensure that these materials, particularly  $^{18}\text{O}$ -enriched oxygen gas and water, fluorine and inert gases, can continue to be available in adequate purity and at reasonable cost.

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# Asymmetric Synthesis of a Precursor for the Automated Radiosynthesis of *S*-(3'-*t*-Butylamino-2'-hydroxypropoxy)- benzimidazol-2-[<sup>11</sup>C]one (*S*-[<sup>11</sup>C]CGP 12177) as a Preferred Radioligand for $\beta$ -Adrenergic Receptors

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*S*-[1-(2,3-Diaminophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol has been synthesized in three steps from 2,3-dinitro-phenol and the chiral auxiliary, *S*-glycidyl-3-nitrobenzenesulphonate, to provide a precursor for labelling *S*-(3'-*t*-butylamino-2'-hydroxypropoxy)-benzimidazol-2-one (*S*-CGP 12177) with the short-lived positron-emitting radionuclide, carbon-11 ( $t_{1/2} = 20.4$  min;  $\beta^+ = 99.8\%$ ). Reaction of the diamine with [<sup>11</sup>C]phosgene, itself derived from no-carrier-added cyclotron-produced [<sup>11</sup>C]methane, provides radiochemically and chemically pure *S*-[carbonyl-<sup>11</sup>C]CGP 12177 in >95% enantiomeric excess after HPLC. Automated apparatus is described for safely producing up to 5.9 GBq (160 mCi) of *S*-[<sup>11</sup>C]CGP 12177 with high sp. act. (20–40 GBq/ $\mu$ mol or 0.54–1.08 Ci/ $\mu$ mol) in a form suitable for human intravenous injection at only 30 min from the end of radionuclide production. *S*-[<sup>11</sup>C]CGP 12177 is preferred to the formerly described racemate as a radioligand for the study of  $\beta$ -adrenergic receptors *in vivo* by positron emission tomography.

## Introduction

Several antagonists have been labelled with carbon-11 ( $t_{1/2} = 20.4$  min;  $\beta^+ = 99.8\%$ ) for the study of  $\beta$ -adrenergic receptors in heart *in vivo* by positron emission tomography (PET) [see Syrota (1988)], namely *R,S*-propranolol (Berger *et al.*, 1982), *R,S*-practolol (Berger *et al.*, 1983), *S*-pindolol (Prenant *et al.*, 1986) and *R,S*-CGP 12177 (Boullais *et al.*, 1985).<sup>‡</sup> These radioligands differ in affinity [see Bree

*et al.* (1986)], octanol–water partition coefficient (*P*) [see Main and Tucker (1985)] and  $\beta$ -adrenergic receptor sub-type selectivity [see Bree *et al.* (1986)].

*R,S*-[<sup>11</sup>C]Propranolol is highly lipophilic ( $\text{Log}_{10} P = 3.65$ ) with high affinity ( $K_D$ , 1.1 nM) but very low sub-type selectivity. Following i.v. injection into man, this radioligand is almost completely (98%) taken up by lung on first pass; however, this uptake is non-saturable. Furthermore, the myocardium cannot be visualized by PET, even at 75 min after injection of the radioligand (Syrota, 1988).

*R,S*-[<sup>11</sup>C]Practolol is much more hydrophilic ( $\text{Log}_{10} P = 0.79$ ) with selectivity for the  $\beta_1$  sub-type, which predominates in myocardium. Following i.v. injection into man this radioligand shows rapid uptake into myocardium enabling its visualization by PET. However, clearance of radioactivity from the

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‡Several racemic fluoroalkyl derivatives of the high affinity  $\beta$ -adrenergic receptor antagonist, carazalol, have also been labelled recently with fluorine-18 ( $t_{1/2} = 109.6$  min;  $\beta^+ = 96.9\%$ ) (Kinsey and Tewson, 1991).

myocardium is fast and bound radioligand cannot be displaced by large saturating doses of any of a range of  $\beta$ -receptor antagonists (Dormont *et al.*, 1983). On a simple theoretical basis the maximal binding potential of a radioligand for its receptors is given by  $B_{\max}/K_D$  *in vitro*, where  $B_{\max}$  is the receptor concentration, and should exceed 10 for a real possibility of using the radioligand to measure receptors *in vivo* (Eckelman, 1982). Hence the failure to observe specific receptor binding is consistent with the relatively low affinity ( $K_D$ , 110 nM) of *R,S*-practolol for  $\beta$ -receptors.

*S*-[ $^{11}\text{C}$ ]Pindolol has a high receptor affinity ( $K_D$ , 1.3 nM), similar to that of *R,S*-propranolol, but is much less lipophilic ( $\text{Log}_{10} P = 1.75$ ) and again has very low sub-type selectivity. However, an evaluation of its behaviour as a PET radioligand has been reported only briefly and not in man. Uptake in dog myocardium and displacement by excess pindolol has been reported but not in detail (Seto *et al.*, 1986).

*R,S*-CGP 12177 (Fig. 1) is a potent ( $K_D$ , 0.3 nM), hydrophilic ( $\text{Log}_{10} P = 1.81$ ) antagonist for both  $\beta_1$ - and  $\beta_2$ -adrenergic receptors. It shows low non-specific binding to membranes and low cellular uptake (Staehelin and Hertel, 1983; Staehelin *et al.*, 1983) and does not bind to internalized receptors (Staehelin and Hertel, 1983). *R,S*-[ $^{11}\text{C}$ ]CGP 12177 has been reported as a promising radioligand for PET studies of  $\beta$ -receptors in heart (Seto *et al.*, 1986). On this basis, *R,S*-[ $^{11}\text{C}$ ]CGP 12177 appears best suited of the known radioligands to specific measurements of cell-surface 'functionally active'  $\beta$ -adrenergic receptors *in vivo*.

However, studies *in vitro* with *R,S*- and *R*-[ $^3\text{H}$ ]CGP 12177 show that the *S*-enantiomer has approximately 80-fold greater affinity than the *R*-enantiomer for  $\beta$ -adrenergic receptors (Affolter *et al.*, 1985). Also studies of *S*-, *R*- and *R,S*-[ $^3\text{H}$ ]CGP 12177, injected i.v. into rats, have shown that the *S*-enantiomer gives approximately two-fold greater specific signal as represented by the ratio of  $\beta$ -receptor-bound radioligand to non-specifically bound radioligand, both in heart and in lung (Law and Burgin, 1989). It is well known that relatively inactive enantiomers of stereospecific receptor ligands may exhibit differential metabolism [see Testa (1986)], protein binding [see Simonyi *et al.* (1986)], biodistribution [see Testa *et al.* (1986)], toxicity [see

Ariens *et al.* (1986)], pharmacokinetics [see Walle and Walle (1986)] and pharmacological effect [see Lehmann (1986); Ariens (1986)]. Such differences are particularly well documented for  $\beta$ -receptor radioligands [see, for example, Lennard *et al.* (1983); Walle *et al.* (1983); Gut *et al.* (1984); Wilson and Thompson (1984); Sager *et al.* (1985); Richards and Tattersfield (1985)]. On this basis, it would clearly be preferable to use the *S*-enantiomer of [ $^{11}\text{C}$ ]CGP 12177, rather than the racemate, for PET studies of  $\beta$ -adrenergic receptors in both the heart and lung. One approach to obtaining the *S*-isomer is to replace the racemic diamine, [1-(2,3-diaminophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol, with the *S*-isomer (V) in the radiosynthesis from *nca* [ $^{11}\text{C}$ ]phosgene described by Boullais *et al.* (1985).

A 12-step synthesis of *S*-CGP 12177 from 2,3-diaminophenol is known, but does not proceed through the diamine enantiomer V (Affolter *et al.*, 1985) and is inapplicable to the preparation of  $^{11}\text{C}$ -labelled *S*-CGP 12177. We have previously described one approach to the synthesis of the diamine enantiomer V (Brady *et al.*, 1991), based on the known ability of enantiomerically pure *S*-glycidyl-3-nitrobenzenesulphonate (II) to react rapidly and efficiently with phenoxide ions (Klunder *et al.*, 1986, 1989) and of the epoxide rings of the resultant epoxy ethers to open by reaction with amines, giving products in which the configuration of the original glycidyl ester is retained (Klunder *et al.*, 1986). Problems with deprotection, due to the instability of the diamine V in basic media, led to very low yields (<1.5%). Here we report an alternative and more efficient approach to the asymmetric synthesis of the diamine V and describe its use in a safe automated radiosynthesis of *S*-[ $^{11}\text{C}$ ]CGP 12177 (VI) from *nca* [ $^{11}\text{C}$ ]phosgene (Fig. 2).

## Experimental

### Materials

*R,S*-[1-(2,3-diaminophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol and 2,3-dinitrophenol (I) were donated by Dr K. A. Jaeggi of Ciba-Geigy, Basel (Switzerland). Reference *R*- and *S*-(3'-*t*-butylamino-2'-hydroxypropoxy)benzimidazol-2-one (CGP 12177), that had been synthesized by routes independent to that described in this paper, were also donated by Dr K. A. Jaeggi. Other chemicals and solvents were purchased as follows: II, sodium hydride, *N,N*-dimethylformamide (DMF), *t*-butylamine, 5% palladium on carbon (Aldrich Chemical Co. Ltd); chloroform, ethanol, ethyl acetate and propan-2-ol were HPLC grade (Fisons); chlorine gas, research purity (Union Carbide). Other reagents were of 'Analar' quality.

### Methods

Mass spectrometry, with a quadrupole mass spectrometer (Nermag R10/10C), was used to investigate the content of crude reaction mixtures and partially

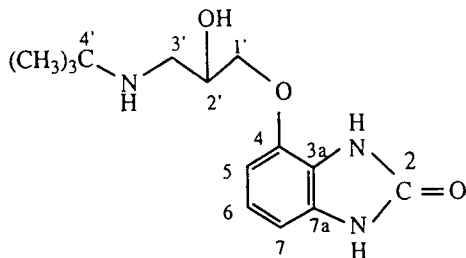


Fig. 1. Structure of CGP 12177 showing numbering system used in nomenclature and in assigning its  $^{13}\text{C}$ -NMR data.

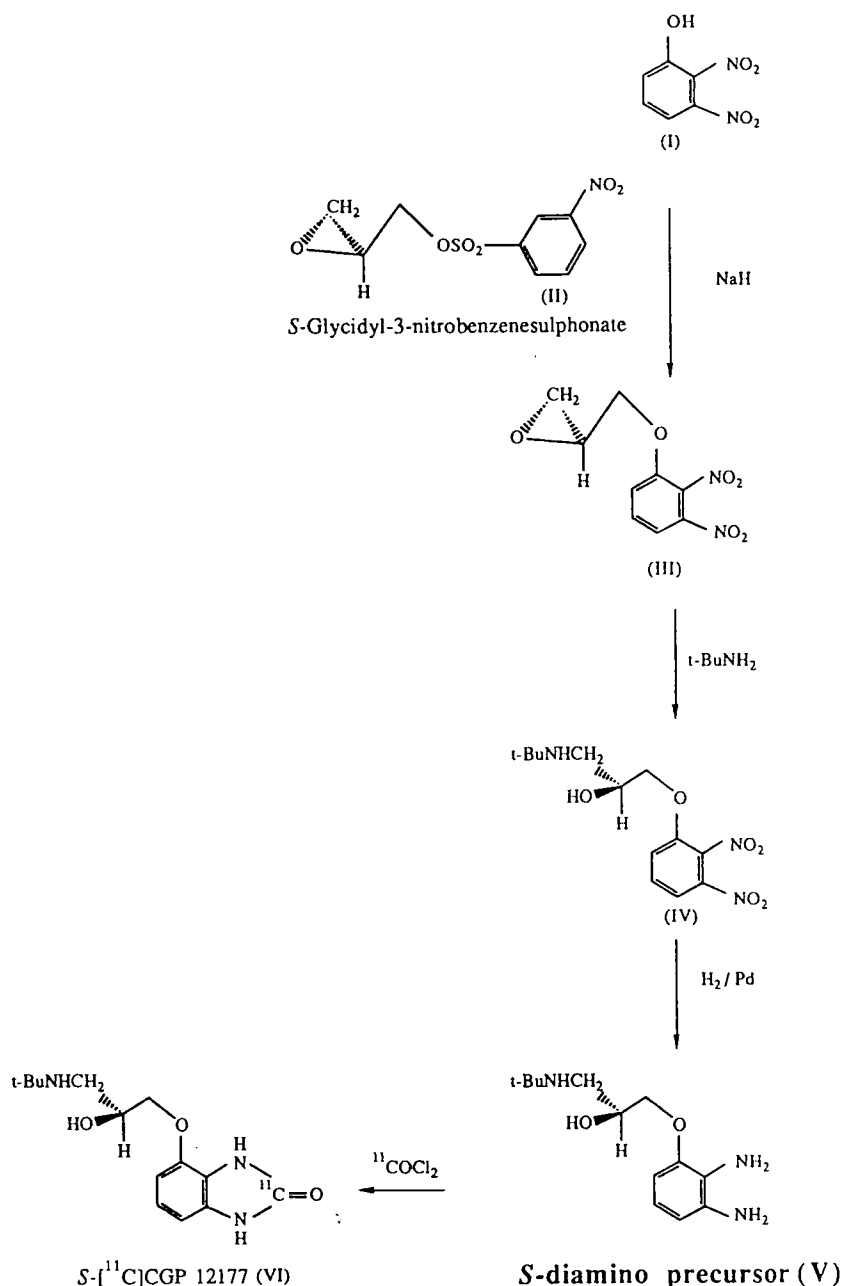


Fig. 2. Synthesis of *S*-diamino precursor **V** and its reaction with [<sup>11</sup>C]phosgene to give **VI**.

isolated products, as well as the structures of purified products. Samples were introduced into the ionization source of the spectrometer using the probe facility and vaporized by passing a current through the probe filament. The spectrometer was calibrated conventionally using FC-43 (perfluorotributylamine) and run in the electron impact (EI) mode or tuned for positive or negative ions in the chemical ionization (CI +ve or -ve) mode, using ammonia as reactant gas. Spectral data were collected using a PDP 11/23 (Digital Computers) and analysed using the Sidar software programme (Nermag).

Proton-decoupled <sup>13</sup>C-NMR spectroscopy was performed on a Bruker WM 250 spectrometer at 62.9 MHz, with DEPT editing for multiplicity at King's College NMR Service, University of London.

Circular dichroism was performed on a Jasco J-600 spectrophotometer at the National CD Service (SERC), Birkbeck College, University of London.

#### Preparation of *S*-[1-(2,3-dinitrophenoxy)]-2,3'-epoxypropane (**III**)

2,3-Dinitrophenol (**I**) (1.5 g, 8 mmol), containing 20% water, was dissolved in dry DMF (5 mL). Anhydrous magnesium sulphate (ca 3 g) was added and the suspension was allowed to stand for 1 h. The magnesium sulphate was removed by filtration and washed with dry DMF (3 mL). The orange solution of 2,3-dinitrophenol was cooled to ca -5°C. A suspension of sodium hydride (0.2 g, 8 mmol) in dry DMF (2 mL) was then added under nitrogen. The reaction mixture was stirred for 15 min. A solution of

**II** (1.75 g, 7 mmol) in dry DMF (3 mL) was then added dropwise and the reaction mixture stirred at room temperature for *ca* 4 h. Reaction progress was monitored by TLC on silica layers using ethyl acetate/ethanol (80/20, v/v) ( $R_f$  values: **I** = 0.87; **II** = 0.91; **III** = 0.17) and also by mass spectrometry (CI mode).

The reaction mixture was cooled in ice and a saturated aqueous solution of ammonium chloride (1 mL) was added slowly. The mixture was then extracted with chloroform (3  $\times$  20 mL). The chloroform extracts were combined, washed successively with saturated sodium bicarbonate solution (2  $\times$  40 mL) and water (2  $\times$  40 mL), dried over magnesium sulphate, filtered and evaporated under reduced pressure to give a pale orange solid. This was washed with pentane and dried under vacuum giving the pale yellow epoxypropane (**III**) (0.7 g, 43%).

**Characterization:**  $^{13}\text{C}$ -NMR (DMSO- $d_6$ ):  $\delta$  (ppm),  $C_1$ , 151.8 (C);  $C_2$ , 130.6 (C);  $C_3$ , 141.3 (C);  $C_4$ , 117.8 (CH);  $C_5$ , 132.8 (CH);  $C_6$ , 122.1 (CH);  $C_1$ , 72.2 (CH<sub>2</sub>);  $C_2$ , 50.2 (CH);  $C_3$ , 44.2 (epoxide-CH<sub>2</sub>). Small peaks were also observed at 125.0 and 121.4 ppm. For comparison **I** in CD<sub>3</sub>OD gave:  $\delta$  (ppm),  $C_1$ , 152.2 (C);  $C_2$ , 134.5 (C);  $C_3$ , 142.4 (C);  $C_4$ , 116.3 (CH);  $C_5$ , 132.5 (CH);  $C_6$ , 124.7 (CH) and the glycidyl compound **II** in DMSO- $d_6$  gave  $\delta$  (ppm)  $C_1$ , 138.5 (C);  $C_2$ , 123.6 (CH);  $C_3$ , 149.3 (C);  $C_4$ , 129.5 (CH);  $C_5$ , 132.4 (CH);  $C_6$ , 134.4 (CH);  $C_1$ , 79.7 (CH<sub>2</sub>);  $C_2$ , 49.5 (CH);  $C_3$ , 44.6 (epoxide-CH<sub>2</sub>).

**Mass spectrometry:** in CI +ve mode,  $m/z$  = 258 [ $M + \text{NH}_4$ ]<sup>+</sup>, 275 [ $M + \text{NH}_3 + \text{NH}_4$ ]<sup>+</sup> and 292 [ $M + 2\text{NH}_3 + \text{NH}_4$ ]<sup>+</sup>, and in EI mode,  $m/z$  = 240 [ $M$ ]<sup>+</sup> (12%), 184 (27%), 167 (4%), 164 (5%), 121 (4%), 107 (3%), 93 (13%) and 57 (100%). For comparison **I** gave: in CI +ve mode,  $m/z$  = 202 [ $M + \text{NH}_4$ ]<sup>+</sup>, 219 [ $M + \text{NH}_3 + \text{NH}_4$ ]<sup>+</sup> and 236 [ $M + 2\text{NH}_3 + \text{NH}_4$ ]<sup>+</sup>, and in EI mode,  $m/z$  = 184 [ $M$ ]<sup>+</sup> (56%), 126 (12%), 121 (5%), 109 (38%), 107 (4%), 96 (6%), 93 (36%) and 63 (100%). The glycidyl compound **II** gave in CI +ve mode,  $m/z$  = 277 [ $M + \text{NH}_4$ ]<sup>+</sup> and 294 [ $M + \text{NH}_3 + \text{NH}_4$ ]<sup>+</sup>, and in EI mode,  $m/z$  = 204 (15%), 186 (100%), 11 (14%), 170 (10%), 122 (59%), 92 (40%) and 76 (85%). A molecular ion at  $m/z$  = 259 was not observed in EI mode.

**Preparation of *S*-[1-(2,3-dinitrophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol (**VI**)**

A suspension of *S*-[1-(2,3-dinitrophenoxy)]-2',3'-epoxypropane (**III**) (0.38 g, 1.6 mmol) in propan-2-ol (5 mL) was added to freshly prepared *t*-butylammonium *t*-butylcarbamate. The latter was prepared by passing carbon dioxide through a solution of *t*-butylamine (0.3 g, 4 mmol) in diethyl ether (2 mL) at 0°C until only a white solid remained. The reaction mixture was stirred under nitrogen and heated on an oil bath at 80°C for *ca* 2 h. Reaction progress was monitored by TLC on silica layers using ethyl acetate/chloroform (70/30 v/v) ( $R_f$  values, **III** = 0.57; **IV** = 0.07) and also by mass spectrometry (CI mode).

Upon completion of the reaction the solvent was removed under reduced pressure giving the amine **IV** as an orange oil (0.4 g, 80%).

**Characterization:**  $^{13}\text{C}$ -NMR (CD<sub>3</sub>OD)  $\delta$  (ppm),  $C_1$ , 152.6 (C);  $C_2$ , signal not observed;  $C_3$ , 142.0 (C);  $C_4$ , 117.7 (CH);  $C_5$ , 132.8 (CH);  $C_6$ , 121.8 (CH);  $C_1$ , 74.1 (CH<sub>2</sub>);  $C_2$ , 69.9 (CH);  $C_3$ , 46.0 (amino-CH<sub>2</sub>);  $C_4$ , 51.3 (C);  $C_5$ , 28.8 (CH<sub>3</sub>).

**Mass spectrometry:** in CI +ve mode,  $m/z$  = 314 [ $M + \text{H}$ ]<sup>+</sup> and in EI mode,  $m/z$  = 298 (100%), 269 (5%), 116 (4%), 114 (5%), 100 (4%), 93 (5%) and 86 (3%). A molecular ion at  $m/z$  = 313 was not observed in EI mode.

**Preparation of *S*-[1-(2,3-diaminophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol (**V**)**

To a solution of *S*-[1-(2,3-dinitrophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol (**IV**) (0.4 g, 1.3 mmol) in ethanol (15 mL) was added 5% palladium/carbon (0.5 g) under nitrogen. Hydrogen was passed through the reaction mixture for *ca* 4 h at room temperature. Reaction progress was monitored by TLC on RP-C<sub>18</sub> layers (Merck) using ethanol as mobile phase ( $R_f$  values; **IV** = 0.55; **V** = 0.47) and also by mass spectrometry (CI mode). The catalyst was removed by filtration and washed with hot ethanol. The ethanol was removed under reduced pressure giving a brown oil (0.25 g, 77%). Portions of this crude product were purified by HPLC on a silica gel column ( $\mu$ -Porasil, 30 cm  $\times$  7.8 mm i.d., Waters Associates Inc.) eluted at 3.0 mL/min with a mobile phase of ethanol/ethyl acetate (80/20, v/v) giving the diamine **V** with a retention time of *ca* 20 min.

**Characterization:**  $^{13}\text{C}$ -NMR (CD<sub>3</sub>OD)  $\delta$  (ppm)  $C_1$ , 149.0 (C);  $C_2$ , 124.6 (C);  $C_3$ , 136.8 (C);  $C_4$ , 104.5 (CH);  $C_5$ , 120.1 (CH);  $C_6$ , 111.3 (CH);  $C_1$ , 72.7 (CH<sub>2</sub>);  $C_2$ , 70.6 (CH);  $C_3$ , 46.1 (amino-CH<sub>2</sub>);  $C_4$ , 51.4 (C);  $C_5$ , 28.7 (CH<sub>3</sub>).

**Mass spectrometry:** in CI +ve mode,  $m/z$  = 254 [ $M + \text{H}$ ]<sup>+</sup> and in EI mode,  $m/z$  = 253 [ $M$ ]<sup>+</sup> (22%), 238 (8%), 166 (5%), 163 (5%), 130 (10%), 124 (100%), 112 (27%) and 95 (41%).

#### Radiochemistry

##### (a) Production of *nca* [ $^{11}\text{C}$ ]methane

*nca* [ $^{11}\text{C}$ ]methane was produced using a Scanditronix MC 40 (Mark II) cyclotron by the  $^{14}\text{N}(\text{p}, \alpha)^{11}\text{C}$  nuclear reaction on nitrogen containing hydrogen (5%) at a total initial pressure of 1400 kN/m<sup>2</sup> (210 psi) in an aluminium gas target. Typically, bombardment was carried out for 20 min with a 30  $\mu\text{A}$  beam of 19 MeV protons. At the end of bombardment (EOB), the radioactivity (*ca* 74 GBq or 2 Ci) was transferred to the "hot-cell" (Fig. 3) through a pressure regulator set at 100 kN/m<sup>2</sup> (15 psi) at 1.5–2 L/min and then a dual trap containing soda lime and phosphorous pentoxide to remove all traces of carbon dioxide and water. All subsequent radiochemistry was performed within this hot-cell with automatic external control

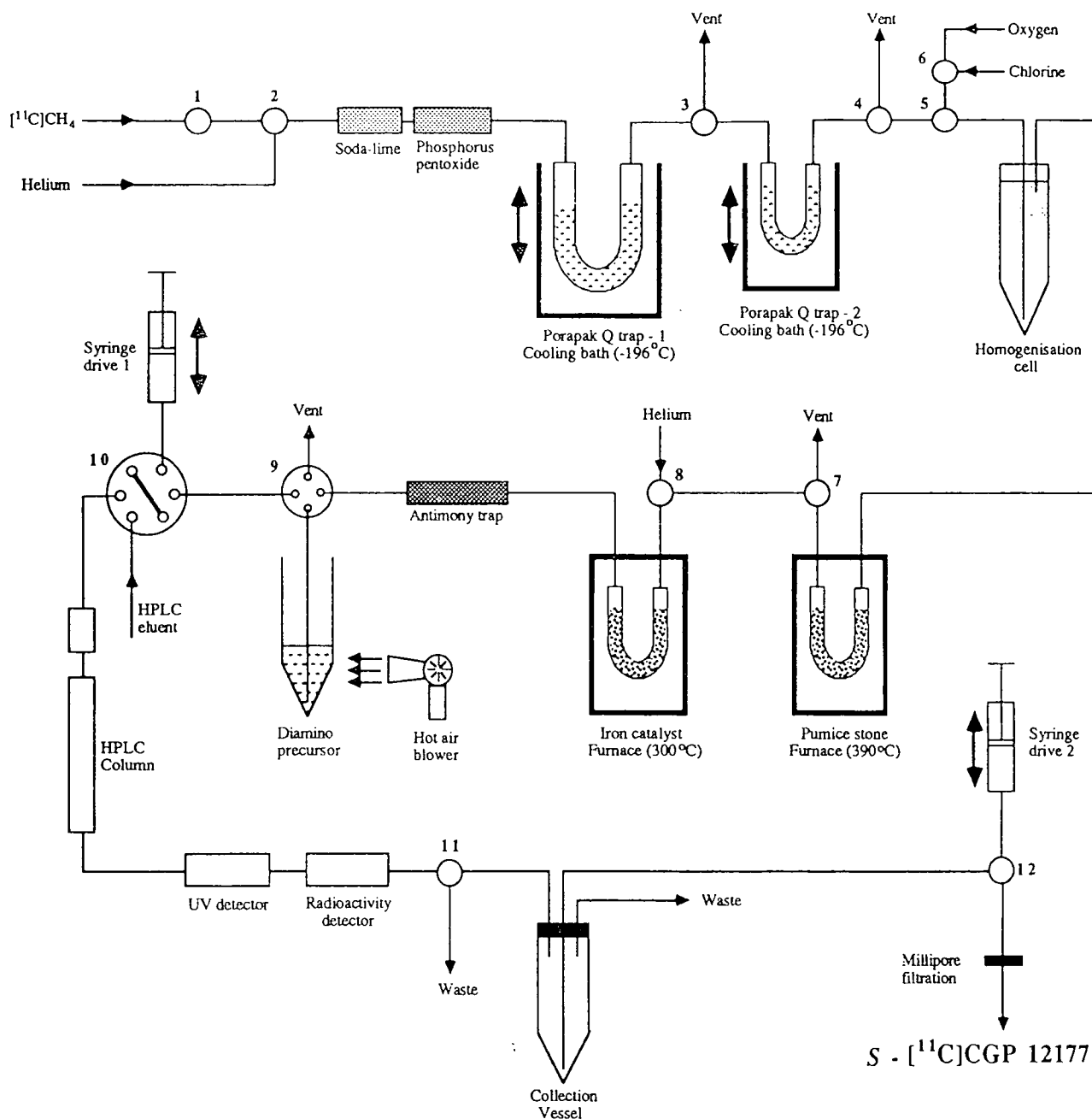


Fig. 3. Scheme for the automated radiosynthesis of VI. The apparatus is contained in a 'hot-cell' (85 mm thick lead shielding) and all operations are controlled externally. Cooling baths are raised and lowered from Porapak Q traps 1 and 2 by means of mechanized lab jacks (using pneumatically actuated valves). Valves 1–12 are of the following types: 1 and 2, stainless steel, solenoid; 3–8, 11 and 12, PFTE, solenoid; 9, four-port teflon rotary, pneumatic, Rheodyne; and 10, six-port stainless steel rotary, pneumatic, Rheodyne. In a typical procedure, the labelled [<sup>11</sup>C]methane is delivered to Porapak Q trap 1 and then concentrated into trap 2, by actuating valves 1 and 2. Valves 4 and 5 control the transfer of [<sup>11</sup>C]methane to the homogenization cell that has previously been filled with chlorine. At this stage, valve 6 controls the flow of oxygen which carried the mixture of [<sup>11</sup>C]methane and chlorine through the first furnace for conversion into [<sup>11</sup>C]carbon tetrachloride and then through the second furnace for conversion into [<sup>11</sup>C]phosgene. [<sup>11</sup>C]Phosgene is passed into a solution of the diamine V by opening valve 9 and the solvents are then removed using a hot air blower. Rotary valves 9 and 10 are used to recover the crude radioactive product and to inject onto the HPLC column. Valves 11 and 12 are used to collect the fraction containing pure product (VI).

for safety, as further described in the legend to Fig. 3. The [<sup>11</sup>C]methane was trapped in a stainless steel U-tube (140 × 4 mm i.d.) filled with Porapak Q (80–100 mesh, Water Associates Inc.) immersed in liquid nitrogen (–196°C). This trap was then flushed

with helium (15 mL/min) to discard any nitrogen and hydrogen, originating from the target, to waste. The [<sup>11</sup>C]methane was then transferred to a second smaller stainless steel U-tube (55 × 1.5 mm i.d.), also filled with Porapak Q, and immersed in liquid nitrogen to

concentrate the nca [ $^{11}\text{C}$ ]methane into a small volume (ca 1 mL).

(b) *Radiosynthesis of nca [ $^{11}\text{C}$ ]phosgene*

The second Porapak Q trap was allowed to warm to room temperature and the [ $^{11}\text{C}$ ]methane was transferred by helium flow (5 mL/min) to the homogenization cell, which had previously been loaded with chlorine (50 mL at 1 atm). The transfer of radioactivity was monitored by a radioactivity detector. When optimal transfer had been reached, a stream of oxygen (ca 15 mL/min) was used to carry the mixture of [ $^{11}\text{C}$ ]methane and chlorine out of the homogenization cell through a 1:5 mixture (3 g) of pumice stone impregnated with cupric chloride and neutral pumice stone, heated by an oven (Carbolyte, 150 mm length) set at 390°C. The generated [ $^{11}\text{C}$ ]carbon tetrachloride was then carried by the oxygen stream through iron filings (1.5 g), heated by a second identical oven set at 290°C. The generated nca [ $^{11}\text{C}$ ]phosgene was then passed through a glass tube (100  $\times$  5 mm i.d.) filled with a mixture of antimony powder (400 mg) and glass beads (1 mm dia) in order to remove any residual chlorine or chlorinated compounds.

(c) *Radiosynthesis of nca S-[ $^{11}\text{C}$ ]CGP 12177 (VI)*

[ $^{11}\text{C}$ ]Phosgene in oxygen was gently bubbled into an open conical glass vial containing a solution of V (0.2 mg, 0.8  $\mu\text{mol}$ ) in dichloromethane (100  $\mu\text{L}$ ) diluted with toluene (100  $\mu\text{L}$ ). [ $^{11}\text{C}$ ]Phosgene was led into the reaction vial by the stream of oxygen. The accumulation of radioactivity was monitored and continued until maximal (generally for ca 5 min). Solvents were then removed from the reaction mixture by heating the vial with a hot air blower, set to give temperatures of 95°C for 1 min followed by 125°C for 2 min. The radioactive residue was cooled to room temperature with cold air and taken up into 1.2 mL of the HPLC mobile phase [a mixture of disodium hydrogen phosphate solution (0.03 M, pH 6.0) and ethanol (85:15, v/v)]; and loaded onto a divinylbenzene column (RP1, Hamilton, 30.5  $\times$  0.7 cm i.d.), eluted at 2 mL/min. The eluate was monitored continuously for radioactivity and for absorbance at 254 nm. The radioactive fraction (ca 4 mL) containing the nca S-[ $^{11}\text{C}$ ]CGP 12177 (VI), having a retention time of 9.2 min, was collected and ethanol removed by heating if required. The solution was sterilized by millipore filtration (Millex GS, 0.22  $\mu\text{m}$ , Millipore). This solution was diluted to 8 mL with saline (1.8% w/v) and was ready for i.v. injection subject to satisfactory quality control. The final pH of this solution was 6.0. The radiosynthesis takes 30 min from EOB and typically provides 3.7–5.9 GBq (100–160 mCi) of VI ready for clinical use. The retention time of the S-diamino precursor V was 4.5 min. A stable impurity also eluted at 7 min.

*Characterization and analysis.* A sample of the formulated product (100  $\mu\text{L}$ ) was analysed on a column (Nucleosil-5 C<sub>18</sub>, 25  $\times$  0.46 cm) eluted at

0.7 mL/min with a mixture of dipotassium hydrogen phosphate solution (0.07 M, pH 4.5) and methanol (70/30, v/v). A single radioactive peak with the same retention time (7.8 min) as authentic CGP 12177 was observed. Radiochemical and chemical purity were greater than 99%. The specific activity of VI was found to be in the range 55–111 GBq/ $\mu\text{mol}$  (1.5–3 Ci/ $\mu\text{mol}$ ) decay-corrected to EOB, corresponding to the presence of 3–6  $\mu\text{g}$  of stable CGP 12177 (carrier) in the injected dose.

A sample for formulated VI was analysed during and after radioactive decay by mass spectrometry (EI and CI +ve mode). The spectra obtained were identical to those of reference CGP 12177.

*Further validation of the radiosynthesis.* The apparatus and procedure described for the radiosynthesis of VI were applied to prepare non-radioactive S-CGP 12177 from the S-diamino precursor V (0.2 mg, 0.8  $\mu\text{mol}$ ) and target gas in an identical manner, except that no proton irradiation was performed. It is known from the specific activity measurement on radioactive product that the radio-synthetic procedure also gives carrier (*vide supra*). The collected fraction was analysed by HPLC as described above and the chemical purity was found to be >99%. Solvent was removed from the collected HPLC fraction under reduced pressure.

Mass spectrometry of the residue gave spectra identical to authentic R,S-CGP 12177, i.e. in CI +ve mode,  $m/z = 280$  [ $\text{M} + \text{H}$ ] $^+$  and 297 [ $\text{M} + \text{NH}_4$ ] $^+$  and in EI mode  $m/z = 279$  [ $\text{M}$ ] $^+$  (20%), 264 [ $\text{M} - \text{CH}_3$ ] $^+$  (12%), 189 (8%), 150 (13%), 149 (8%), 121 (12%), 86 (100%) and 71 (27%).

$^{13}\text{C}$ -NMR of authentic R,S-CGP 12177 in DMSO- $d_6$  gave  $\delta$  (ppm) C<sub>4</sub>, 143.9 (C); C<sub>3a</sub>, 119.7 (C); C<sub>7a</sub>, 131.7 (C); C<sub>7</sub>, 106.1 (CH); C<sub>6</sub>, 122.7 (CH); C<sub>5</sub>, 104.1 (CH); C<sub>1'</sub>, 71.3 (CH<sub>2</sub>); C<sub>2'</sub>, 66.9 (CH); C<sub>3'</sub>, 45.3 (CH<sub>2</sub>); C<sub>4'</sub>, 58.0 (C); C<sub>5'</sub>, 25.7 (CH<sub>3</sub>); C<sub>2</sub>, 157.1 (C=O). Numbering which differs from components I–V is shown in Fig. 1.

*Determination of enantiomeric excess.* The circular dichroism of the residue (ca 2  $\mu\text{g}$ ) was measured in methanol and gave a value of +0.6 mdeg/absorbance unit at 270 nm. Authentic samples of S-CGP 12177 ( $[\alpha]_D = -13 \pm 1^\circ$ ,  $[\alpha]_{313} = -48^\circ$ ) and R-CGP 12177 ( $[\alpha]_D = +14 \pm 1^\circ$ ,  $[\alpha]_{313} = +49^\circ$ ) were measured under identical conditions and gave CD values of +0.68 and -0.68 mdeg/absorbance unit, respectively. The compound synthesized by the above procedure from the precursor V is unequivocally identified as containing >95% S(-)-enantiomer and <5% R(+)-enantiomer, corresponding to an e.e. (enantiomeric excess) of >90%.

## Discussion

Our aim was to radiolabel the S-enantiomer of CGP 12177 with the positron-emitting radionuclide, carbon-11, for evaluation as a radioligand for PET studies of  $\beta$ -adrenergic receptors in lung and heart.



*R,S*-CGP 12177 has previously been labelled with carbon-11 by reaction of [<sup>11</sup>C]phosgene with *R,S*-[1-(2,3-diaminophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol. We were unable to resolve this precursor, or its progenitors, by HPLC (Brady *et al.*, 1991) using any of a number of different chiral columns, including Chiralcel OD and Cyclobond I. These columns also failed to resolve racemic CGP 12177. We therefore sought a convenient synthetic route to the *S*-enantiomer of the precursor **V** for use in the radiosynthesis of *S*-[<sup>11</sup>C]CGP 12177.

That *S*-propranolol has been synthesized by the reaction of (2*S*)-glycidyl tosylate with sodium 1-naphthoxide, followed by ring opening with *iso*-propylamine (Klunder *et al.*, 1986), led us to consider the use of (2*S*)-glycidyl tosylate for the synthesis of the *S*-diamino precursor **V**. However, a recent report shows that the asymmetric synthesis of *S*- $\beta$ -receptor ligands is greatly improved by the use of *S*-glycidyl-3-nitrobenzenesulphonate in place of *S*-glycidyl tosylate (Klunder *et al.*, 1989). Under appropriate conditions the reactions of aryl oxides with the latter chiral auxiliary proceed not only faster than with *S*-glycidyl tosylate but also in some cases with almost complete retention of configuration. This rate enhancement is advantageous since the aryl epoxy ether is exposed to nucleophilic aryl oxide for a shorter period leading to higher yields. These considerations prompted us to use *S*-glycidyl-3-nitrobenzenesulphonate (**II**) in the synthesis of the *S*-diamino precursor **V** as a precursor to **VI**.

Our initial approach (Brady *et al.*, 1991) proceeded with the reduction of **I** to 2,3-diaminophenol followed by acetylation of the sensitive amino groups to give 2,3-diacetylaminophenol. Treatment of the latter with sodium hydride generated the phenoxide ion which was allowed to react with *S*-glycidyl-3-nitrobenzenesulphonate **II** to give *S*-[1-(2,3-diacetylaminophenoxy)]-2',3'-epoxypropane. Refluxing the latter epoxide with *t*-butylamine gave *S*-[1-(2,3-diacetylaminophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol. The acetyl protecting groups were removed by refluxing with potassium hydroxide to give the diamino precursor **V**. However, the instability of **V** in the basic media used for deprotection led to very low yields (<1.5%) [e.g. 3 g of **I** gave only 50 mg of **V**]. We sought therefore to circumvent these problems by building the side chain onto the di-nitro compound **I** first, and reducing the nitro groups in the last stage to give the di-amino precursor **V**, thus reducing the synthesis to three steps.

In our second approach, described here (Fig. 2), the phenoxide ion of **I** was generated by reaction of **I** with sodium hydride in DMF under nitrogen and is allowed to react, in the same pot with **II**, to give **III**. The epoxide ring of **III** opens by reaction with refluxing *t*-butylamine giving crude **IV** as an orange oil. The latter hydrogenates at room temperature over palladium on carbon in ethanol giving crude di-amino precursor **V** as a brown oil in an overall

yield of 24% from **I**. Final purification of **V** is achieved by preparative HPLC on a normal phase column (Lichrosorb Si-60) using methanol as eluent.

Compounds **I** to **VI** were characterized by mass spectrometry and <sup>13</sup>C-NMR spectroscopy. Mass spectrometry (CI mode) was also used to monitor the progress of all reactions (Waters *et al.*, 1990). The radiosynthesis of carbon-11 labelled *S*-CGP 12177 from **V** was performed in a fully automated system (Fig. 3) using *nc*a [<sup>11</sup>C]phosgene, itself prepared according to Landais and Crouzel (1987). Typically, this system delivers *ca* 5 GBq of radiochemically and chemically pure **VI** with a sp. act. up to 50 GBq/ $\mu$ mol at 40 min after EOB. By operating this system in a non-radioactive mode it was directly demonstrated that the enantiomeric excess of product is greater than 90%. *S*-[<sup>11</sup>C]CGP 12177, prepared in this apparatus from the precursor **V**, is now being applied to the study of  $\beta$ -receptors in heart and in lung with PET, and also in biological experiments. Results will be published elsewhere.

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## The on-line monitoring of continuously withdrawn arterial blood during PET studies using a single BGO/photomultiplier assembly and non-stick tubing

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**Key words:** BGO detector, continuous withdrawal, on-line monitoring, PTFE non-stick tubing, sum-peak detection

### Summary

A robust, highly sensitive system is described for monitoring the concentration of positron emitting radioisotopes contained within arterial blood continuously withdrawn during PET studies of the brain.

Utilizing a specially designed flow-through bismuth germanate detector, gammas are more effectively counted, replacing the less efficient method of positron detection with plastic detectors. A polytetrafluoroethylene flow path system has been developed to overcome the problem of highly cohesive tracers adhering to the tubing material.

Blood is drawn through the system from the radial artery by a medically approved peristaltic pump.

Syringe samples of blood are extracted periodically downstream of the detector, for calibrations, plasma assays, metabolic analysis and physiological measurements.

The complete system, including efficient heavy lead shielding is contained on a bedside trolley.

Blood activity is continuously recorded throughout the PET investigations, and stored directly by the scanning computer, and additionally backed up on disc by a P.C.

### Introduction

A flow-through detector has been developed which consists of 2 bismuth germanate (BGO) crystals mounted on a single photomultiplier tube. This affords the opportunity for measuring either the summed (coincidence) 1022 keV peak or, for maximum sensitivity, the combined 511 keV and 1022 keV peaks. The length of tubing monitored traverses a hair-pin pathway through the detector. This maximises the volume of blood detected without having to increase the internal diameter of the

tubing and hence the need for connectors which cause additional dispersion. Although background counts can be minimised by counting only the 1022 keV coincidence peak, in practice, through the use of heavy lead shielding, both the 511 keV and 1022 keV peaks are recorded to maximise sensitivity. The detection of annihilation photons is preferred to counting emitted positrons, using a beta counter, since larger volumes of blood can be monitored, and more reproducible calibrations can be realised. The count rate performance of the BGO assembly is found to be adequate for clinical

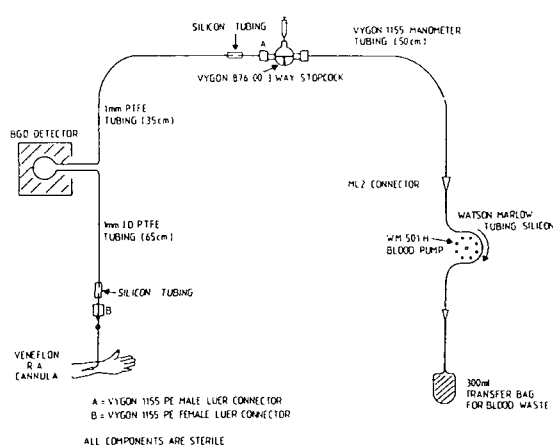


Fig. 1. Schematic diagram of on-line whole blood detection system.

PET studies. When counting the combined 511 and 1022 keV peaks, the deadtime at 10,000 counts per second is of the order of 1%.

Particular consideration has been given to the material from which the withdrawal tubing is made. Experience has shown that sterile polythene tubing, commercially available for blood with-

drawal, results in certain radiotracers sticking to the wall of the tubing. This causes incorrect concentrations being monitored due to pick up of the tracer between the patient and the detector and retention of the tracer within the length of tubing contained within the detector itself. To minimise these effects, polytetrafluoroethylene (PTFE) has been found to be the optimal material for the withdrawal tubing. This has resulted in the commercial manufacture of sterile lengths of PTFE tubing fitted with luer connectors specifically for PET studies.

A peristaltic roller pump has been used downstream from the detector for withdrawal. Experience has shown that most high grade pumps, as used in chemical laboratories, are not considered electrically safe for direct connection to patients. Hence care needs to be taken when selecting the pump to ensure that it has approved safety specifications for human use.

The blood withdrawal circuit includes a point from which samples may be withdrawn for: a) calibrating the BGO detector against a laboratory well counter, b) plasma assays from centrifuge samples, c) plasma metabolite analysis and d) physiological

Table 1. SUMMARY OF PHYSICAL CHARACTERISTICS

| BGO crystal assembly              | Number of BGO crystals | Number of PM tubes                      | Crystal dimensions (mm) |                                                                                                                                                                             |                                |                                         |
|-----------------------------------|------------------------|-----------------------------------------|-------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|-----------------------------------------|
|                                   |                        |                                         | length                  | width                                                                                                                                                                       | height                         | depth                                   |
|                                   | 2                      | 1                                       | 30                      | 30                                                                                                                                                                          | 60                             | 6                                       |
| Energy resolution of BGO detector | Isotope                | Energy keV                              | % FWHM                  | 68 Ge - measured as 1 mm ID tubing source located in the 'hairpin' shaped pathway between the crystals. 137 Cs - measured as 'point source' located midway between crystals |                                |                                         |
|                                   | 68 Ge                  | 511                                     | 17.7                    |                                                                                                                                                                             |                                |                                         |
|                                   | 68 Ge                  | 1022                                    | 11.9                    |                                                                                                                                                                             |                                |                                         |
|                                   | 137 Cs                 | 661.8                                   | 15.3                    |                                                                                                                                                                             |                                |                                         |
| Sensitivity and deadtime          | keV energy             | cps $\mu\text{Ci}^{-1} \text{ ml}^{-1}$ | cps $\mu\text{Ci}^{-1}$ | $\mu\text{sec/pulse}$ deadtime                                                                                                                                              |                                |                                         |
|                                   | sum peak               | ( $\pm 3\%$ )                           | ( $\pm 3\%$ )           |                                                                                                                                                                             |                                |                                         |
|                                   | 797 $\rightarrow$ 1290 | 787                                     | 10225                   |                                                                                                                                                                             |                                |                                         |
|                                   | 511 + sum pk           |                                         |                         |                                                                                                                                                                             |                                |                                         |
|                                   | 318 $\rightarrow$ 1290 | 2401                                    | 31187                   | 1.88                                                                                                                                                                        |                                |                                         |
| PTFE                              | mm                     | mm                                      | mm                      | ml                                                                                                                                                                          | ml/min                         | ml/min                                  |
| Blood withdrawal                  | ID                     | OD                                      | ID batch variation      | vol in FOV $\pm$ batch var                                                                                                                                                  | flow rate for 11C, 18F tracers | flow rate for $\text{H}_2^{18}\text{O}$ |
| Tubing                            | 1                      | 2                                       | $\pm 0.05$              | $0.077 \pm 9\%$                                                                                                                                                             | 2.5                            | 5.0                                     |

measurements for blood gases, haemoglobin and other parameters. This withdrawal point is placed downstream from the detector to avoid additional delay and dispersion being imposed on the blood flowing within the monitored length of tubing.

The system described has been integrated and assembled on a bedside trolley, the signal output of which feeds directly into the computer used for collecting the PET data.

### Method – general description

During clinical PET investigations, blood is continuously sampled from the radial artery cannula (RAC) through external tubing to enable continuous monitoring of the radiotracer content of whole blood. The arterial whole blood is pumped through tubing 1 mm ID, 2 mm OD, which passes through a BGO detector (see Fig. 1). The flow rate is set at 5 ml per minute for brain blood flow measurements using  $H_2^{15}O$ . This rate is also used for the first 10 min of  $^{11}C$  and  $^{18}F$ -labelled tracer studies and then reduced to 2.5 ml per minute. This ensures that the total volume of blood extracted from the patient does not exceed 300 ml during long (90 min) scans.

The 1 mm bore tubing passes between the two parallel blocks of BGO 6 mm apart. A special Teflon jig (see Fig. 2) has been designed into which the tubing is placed to describe an exposed hair-pin shaped flowpath, 98 mm in length, between the opposing crystal faces. This configuration provides increased exposure and minimum impedance to flow. In order to minimise delay and dispersion, the BGO detector is installed first in line within the withdrawal circuit. The distance from the patient's RAC to the mid point of the detector is 65 cm. Ten centimetres of lead shielding surrounds the detector allowing blood concentration levels less than 0.01 microcuries per ml to be accurately detected. The effective volume in the field of view of the BGO is approximately 0.08 ml.

To enable blood samples to be taken at pre-determined times during scanning, a 3 way tap is installed 35 cm downstream from the BGO detector (1 M from the RAC). These samples are taken

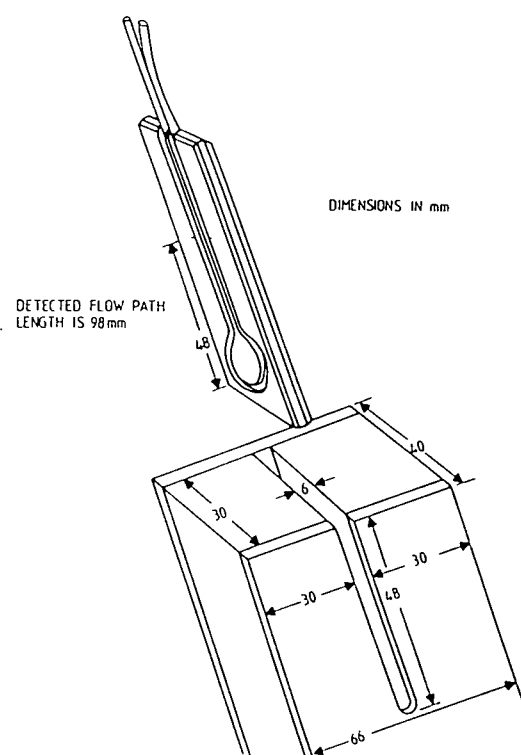
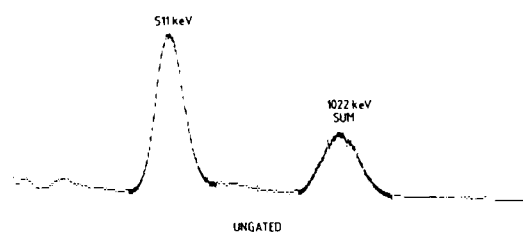


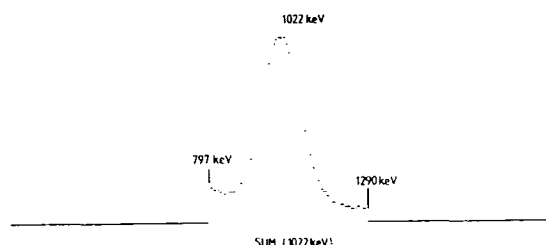
Fig. 2. Drawing of BGO detector assembly & jig.

into heparinised syringes and counted in a sodium iodide well counter to provide values of whole blood and plasma from which to calibrate the response of the BGO counter. Similarly, samples are extracted for metabolite analysis. Flushing is periodically carried out with heparinised saline introduced via the 3 way tap. This prevents the formation of clots which are especially susceptible within the RAC during the latter stages of the 90 min scans at the slower flow rate of 2.5 ml per min. At a point 50 cm further downstream of the tap is the blood pump [Watson Marlow WM 501H]. This eight roller, peristaltic pump, provides a smooth constant output utilising silicon manifold tubing. It has been approved for use with human subjects by The UK Department of Health. The waste blood and saline is pumped into a 300 ml 'transfer' bag, completing the closed system. Each component is sterile within this system. Fig. 1 shows diagrammatically the withdrawal and monitoring system.

(a)



(b)



(c)

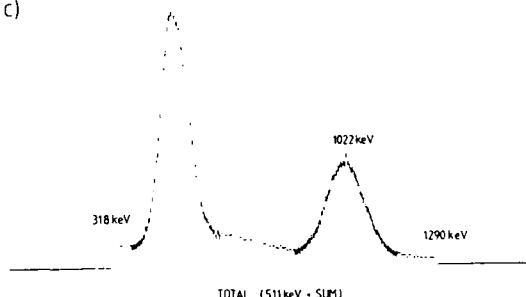


Fig. 3a.  $^{18}\text{F}$  spectrum (ungated) of activity filled tubing in the BGO detector. b.  $^{18}\text{F}$  spectrum gated sum-peak 797–1290 keV. c.  $^{18}\text{F}$  spectrum gated total (511 +  $\uparrow$  sum-peak) 318 keV–1290 keV.

### Instrumentation and electronics

The detection system is set up to measure the 511 keV and 1022 keV sum peak. The detector comprises two BGO crystals (30 mm  $\times$  30 mm  $\times$  60 mm) assembled on to a single photomultiplier (PM) tube. A light-guide connects the blocks of BGO to the PM tube. The assembly is sealed within an aluminium can presenting a neat, light-tight rectangular module 190  $\times$  65  $\times$  39 mm, the geometry of which is efficiently shielded. This detector

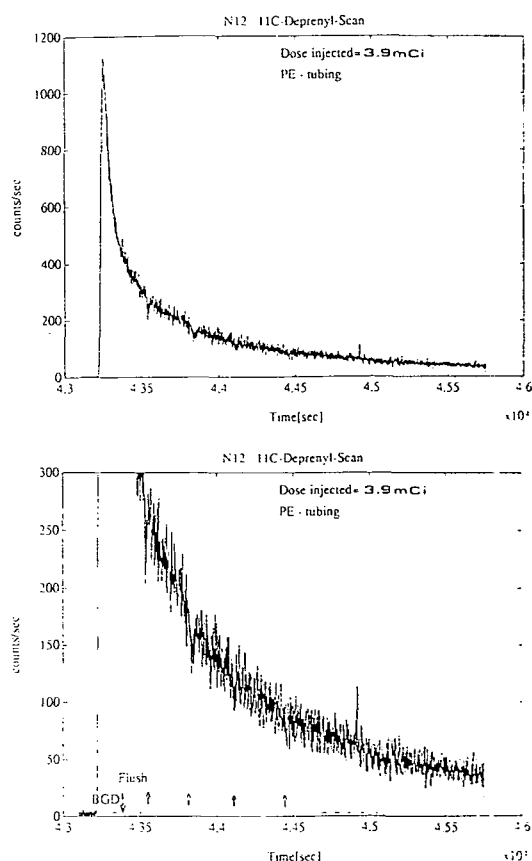


Fig. 4a, b. BGO whole blood curve (511 keV + sum) N12 shows  $^{11}\text{C}$ -deprenyl sticking to polyethylene tubing. No reduction of counts during flushing.

was constructed as a prototype by CTI Inc, Knoxville, USA.

The signal and high voltage are carried on separate leads brought out from the base of the assembly. The signal is fed into an EG & G Ortec preamp-amp single channel analyser mod. 4890 and the analogue pulse split into a second single channel analyser to enable 2 energy windows to be gated simultaneously (Fig. 3b and 3c). The digital outputs of both these analysers are fed into a quad scaler timer EG & G Ortec mod. 974. This acquires data continuously in one second integrated units throughout the scan and transmits them directly into a pre-defined count file in the PET scanner (CTI/Siemens ECAT 953B) system's 'SUN' computer. A separate PC is also used as an independent

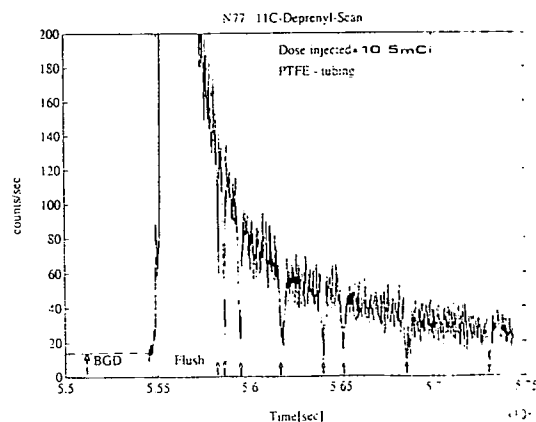
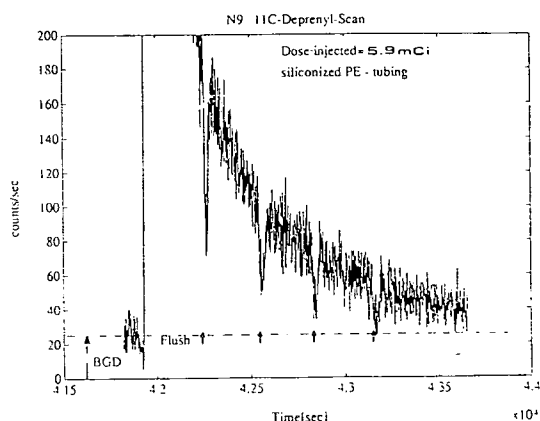
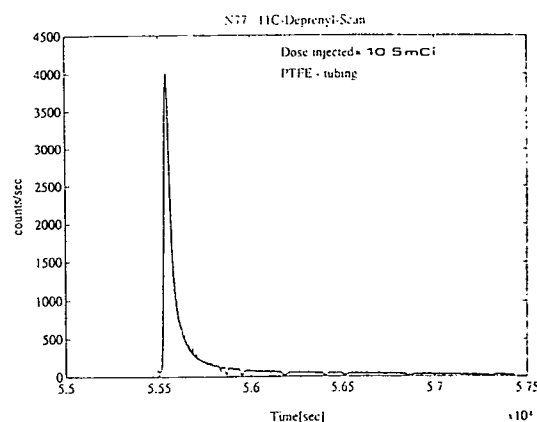
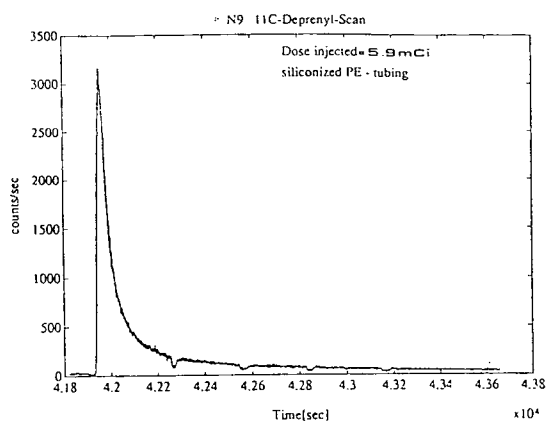


Fig. 5a, b. BGO whole blood curve (511 keV + sum) N9 shows reduced sticking of  $^{11}\text{C}$ -deprenyl to polyethylene tubing coated with silicon. During flushing, the count rate is still significantly above background.

Fig. 6a, b. BGO whole blood curve (511 keV + sum) N77 PTFE tubing shows no sticking of  $^{11}\text{C}$ -deprenyl in the tubing. During flushing, the count rate drops to almost background level.

back-up for data logging, using a Blue Chip DPC 10 counter-timer card. This acquires data which is continuously written to a  $5\frac{1}{4}$ " floppy disc. Each computer has its own internal clock. Hence all clocks have to be synchronised daily in order that data from any of the count files can be directly compared and related to absolute time. This is done to Greenwich Mean Time (GMT) using a radio receiver clock.

On-line whole blood data collected during scans can be formatted, and quantitatively analysed from calibrations supplied from accurately timed aliquot samples taken during emission data acquisition. Plasma and whole blood samples are counted in a well counter (WC), which is routinely cross-cal-

ibrated against the PET scanner's pixel element response. Since there is the possibility of  $\pm 9\%$  variation in detected volumes, due to batch variance in the manufacture of the PTFE withdrawal tubing (see below), cross calibrations for each scan are carried out (WC v BGO). However within production batches, variations of less than 3% are expected.

#### The selection of withdrawal tubing

Certain positron labelled compounds, (in particular  $^{11}\text{C}$ -deprenyl,  $^{11}\text{C}$ -PK11195 and  $^{11}\text{C}$ -raclopride) have been found to adhere very strongly to many types of commercially produced plastic medical

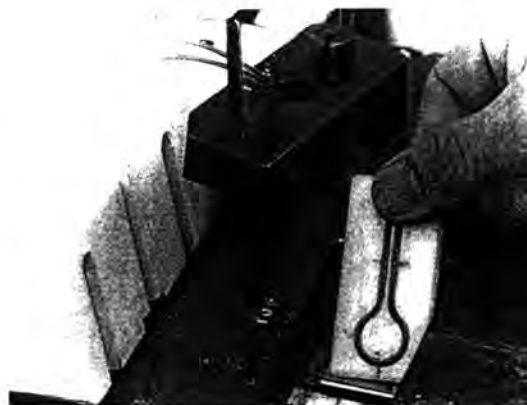
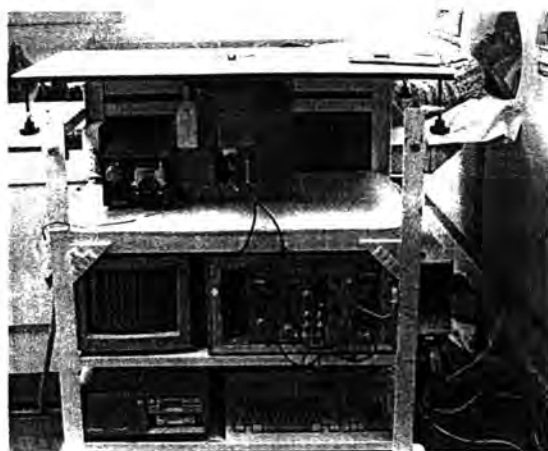


Fig. 7a. The on-line BGO detector trolley beside the CTI/Siemens ECAT 953B neuro PET scanner showing the shielded detector, blood pump, nucleonics, and back-up PC system. The three way sampling tap is mounted directly above the shielded detector on the trolley top which is manufactured from sterilizable 'Trespa' material. b. To minimise detection of extraneous gammas from tube containing radioactive blood outside the field of view, special lead inserts have been designed to surround the jig and tubing which extends from the top of the crystal assembly.

cannulae and catheters. In an attempt to overcome this problem, a variety of tubing types were investigated. These included polypropylene, polyethylene (PE) and PE tubing coated with silicon. However, only one product has been proved to have a near zero cohesion for all substrates investigated so far – namely PTFE. Previously, no sterile medical grade PTFE tubing of the desired dimensions with luer fittings has been available. The nearest acceptable tubing to this was 'chrom-line' – chromatographic grade tubing from PTFE Fabricators Ltd. This had to be connected to polyethylene luer connectors with silicon tubing and sterilised in house. Routine tests had to be frequently carried out for pyrogens and sterility. Because of the problems encountered in cleaning and sterilising PTFE tubing to create a pyrogen and

<sup>1</sup>PTFE Fabricators Ltd., Port Vale, Hertford, Herts SG14 3AB, UK

bacteria free system, Tony Davis Avon Medical Limited has produced a sterile PTFE extension tube with luer connectors specifically for PET studies. Prototypes from Avon have been successfully tested at Hammersmith. Commercial batches of this product in 50 cm and 100 cm lengths are now available. Figures 4–6 show examples when using withdrawal tubing of different materials for assaying <sup>11</sup>C-deprenyl, a typical highly adhesive compound.

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# Progress in Radiopharmacy

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# PRODUCTION AND APPLICATION OF OXYGEN-15; RADIOPHARMACY ASPECTS

J C CLARK BSc.

## ABSTRACT

Pioneering clinical research in the late '50s and early '60s established the value of Oxygen-15 studies in medicine (1-8). Imaging with a heavily collimated gamma camera in the mid '70s enabled estimates of blood flow and oxygen utilization to be made in brain and brain tumours (9,10). The application of PET technology soon enabled dramatic progress to provide quantitative measures of brain blood flow, blood volume and oxygen extraction (11,12) all using oxygen-15 labelled gaseous tracers administered by inhalation.

PET studies of organ blood flow have also been carried out using bolus injections of  $H_2O$  water (12) and more recently butanol (13) labelled with oxygen-15.

Clinically useful and often unique data (40) continue to be generated using PET-oxygen-15 methodology as a result of significant investment in scientific research in this area. Commercial exploitation of this research may soon enable a wider access to O-15 clinical diagnostic techniques when small simple oxygen-15 generators (41) become available and PET scanners can be made simpler and hopefully cheaper (42) !

The radiopharmaceutical aspects of this work will require some regulation for its ongoing safe application and efficacy but one hopes that the regulatory bodies will find ways of satisfactorily embodying the wide knowledge and expertise into manageable guidelines by close consultation and collaboration with the relevant experts in this field.

## PRODUCTION OF OXYGEN-15

Oxygen-15 may be produced with charged particle accelerators via several nuclear reactions (14). The long established  $^{14}N(d,n)^{15}O$  reaction is undoubtedly the most convenient (15,16,17), but special circumstances relating to specific types of particle accelerators

have led to the use of the  $^{15}\text{N}(\text{p},\text{n})^{15}\text{O}$  (18) and  $^{16}\text{O}(\text{p},\text{pn})^{15}\text{O}$  reactions (19,20,21).

#### Oxygen-15 via the $^{14}\text{N}(\text{d},\text{n})^{15}\text{O}$ reaction

Oxygen-15 has been produced using this reaction for many years (15,16,17). Providing the deuteron energy is not greater than 6 MeV no significant radionuclidic impurities are produced. If the nitrogen target gas contains some oxygen ( $\approx 1\%$ ) (16,22) oxygen-15 is recovered at the target output mainly as  $^{15}\text{O}_2$ . However, to ensure that the product is suitable for clinical application a flow-through purifier containing active carbon and soda lime is used to remove nitrogen oxides and carbon dioxide. If the nitrogen target gas contains carbon dioxide ( $\approx 1\%$ ) (16,22), oxygen-15 is recovered from the target output mainly as  $\text{C}^{15}\text{O}_2$ , here the main impurities are  $^{15}\text{O}_2$  and carbon monoxide generated by the radiolysis of the carbon dioxide in the target gas. Both are readily removed using a heated ( $400^\circ\text{C}$ ) oxidising reagent composed of a mixture of iron and copper oxides supported on kaolin. (22)

Another approach to the production of  $\text{C}^{15}\text{O}_2$  and  $^{15}\text{O}_2$  from a common target source is to carry out catalytic interconversion of  $\text{C}^{15}\text{O}_2$  to  $^{15}\text{O}_2$  in a flow system using metal oxide catalysts (eg Hopcalite-11) (23). Carbon monoxide labelled with oxygen-15 ( $\text{C}^{15}\text{O}$ ) may be produced from either  $^{15}\text{O}_2$  or  $\text{C}^{15}\text{O}_2$  by passing the primary product, produced and purified as described above, through a column of active carbon heated to  $\approx 900^\circ\text{C}$  (16,17,22,23). Any  $\text{C}^{15}\text{O}_2$  that remains after this reaction is removed by a soda lime trap. The outstanding problem relating to the clinical use of  $\text{C}^{15}\text{O}$  is the presence of stable carbon monoxide in the final product (24).

Methods are being developed which are directed towards reducing the stable carbon monoxide content in  $\text{C}^{15}\text{O}$  and hence ensuring patients' safety (25).

There are several ways by which oxygen-15 labelled water ( $\text{H}_2^{15}\text{O}$ ) can be prepared using the  $^{14}\text{N}(\text{d},\text{n})^{15}\text{O}$  reaction. The chemical synthesis of water by the palladium-catalysed reaction of oxygen ( $^{15}\text{O}_2$ ) with hydrogen is widely applied (16,26). Using this system it is possible that some ammonia is made due to the reaction of  $\text{N}_2$  and  $\text{H}_2$  over the

catalyst. Oxygen-15 labelled water can also be made from  $C^{15}O_2$  either by exchange labelling directly with water (8,17) or by its reaction with dilute sodium hydroxide and subsequent neutralisation with dilute hydrochloric acid to yield an isotonic sodium chloride solution (27). A further method of production of  $H_2^{15}O$  is to carry out an irradiation of nitrogen/hydrogen mixtures. Here the predominant product is  $H_2^{15}O$  vapour (28,29,30). Ammonia is also produced during the irradiation but the levels found in the infusion samples can be adequately controlled (31). With care it is possible to transport this vapour to a site remote from the target, usually in "Teflon" tubing, where the vapour may be extracted into a saline infusion medium. (29,30,31) (See below: "Oxygen-15 labelled water generators."

#### Oxygen-15 via the $^{14}N(p,n)^{15}O$ reaction

This reaction is used where an accelerator can only deliver protons of 8-10 MeV. A target gas of highly enriched  $^{15}N_2$  containing either 1%  $O_2$  or 1%  $CO_2$  is used and due to its expense care is taken to optimise target gas consumption (18). All the chemical processing techniques described for the  $^{14}N(d,n)^{15}O$  production route are employed to prepare and purify all the usual  $^{15}O$  labelled products.

#### Oxygen-15 via the $^{16}O(p,pn)^{15}O$ reaction

This reaction has a threshold energy of 16.6 MeV and consequently can only be employed using rather large cyclotrons. The tendency is only to use this method of production when other ones are not accessible (19,20,21). Pure oxygen is used as the target gas and copious amounts of ozone are produced during irradiation which are removed together with small amounts of  $^{13}N$  oxides and  $^{11}CO_2$  using active carbon and soda lime flow-through purifiers. Use of the purified product as  $^{15}O$ , suitably diluted to safe physiological levels is straight forward. However, the conversion of pure oxygen to water by reaction with hydrogen although feasible requires great care to control the highly exothermic reaction! A much more attractive route to  $H_2^{15}O$  using this reaction is to employ a  $H_2^{16}O$  water target (32). Here when pure water is irradiated the predominant  $^{15}O$  product is  $H_2^{15}O$ . Some nitrogen-13 is produced via the  $^{16}O(p,\alpha)^{13}N$  reaction together with

traces of  $^{18}\text{F}$  from the  $^{18}\text{O}$  (p,n) $^{18}\text{F}$  reaction on natural abundance  $\text{H}_2^{18}\text{O}$ . These impurities may be removed using mixed-bed ion-exchange columns (32).

The production of  $\text{C}^{15}\text{O}_2$  (19) and in particular  $\text{C}^{15}\text{O}$  using pure oxygen targets poses many practical problems and in the case of carbon monoxide insurmountable problems relating to toxic levels (21). Preliminary studies with water targets to produce high specific activity  $^{15}\text{O}_2$  and hence  $\text{C}^{15}\text{O}$  are encouraging (33)

#### OXYGEN-15 LABELLED WATER GENERATORS

As described above  $\text{H}_2^{15}\text{O}$  may be readily prepared by a variety of routes. However, several problems need to be addressed if safe and effective injections or infusions are to be prepared. Of primary concern is that the product should be free from any harmful impurities both chemical and radioactive and be sterile and pyrogen-free. Due to the short half-life of Oxygen-15 quality control of each sample or batch would be impractical. However, most groups (17, 26, 27, 30, 31, 32, 36) carrying out work in this area have evolved safe operating procedures (SOPS see appendix 1) which have been shown to be capable of maintaining the required standards. The measurement and delivery of the prepared doses of  $\text{H}_2^{15}\text{O}$  also requires careful attention both for the administrator and the recipient. As a typical injected bolus of  $\text{H}_2^{15}\text{O}$  would be 80-100 mCi the dose to the administrator is of great concern. Automated injectors and infusers are at an advanced stage of development in several laboratories and the commercial exploitation of these devices is anticipated. This transfer of technology will not of course be without some significant regulatory implications but it is hoped that close collaboration between the innovators, the commercial partners and the regulators will enable cost effective solutions to this challenge to be found.

#### OXYGEN-15 LABELLED BUTANOL GENERATORS

The production of  $\text{Bu}^{15}\text{OH}$  introduces another level of synthetic complexity into oxygen-15 clinical applications. Several groups are actively developing devices to produce  $\text{Bu}^{15}\text{OH}$  based on organoborane chemistry (34,13,35,36,37). The essential features of the procedure are as follows. Tri-n-Butylborane is immobilised on alumina SEP PAK (Waters) cartridges. Oxygen-15 labelled  $^{15}\text{O}_2$  is

reacted with the tri-n-butylborane, the oxidised intermediate is hydrolysed with water and the crude product is eluted onto C-18 SEP PAKS which retain the  $\text{Bu}^{15}\text{OH}$ . Further washing of this SEP PAK with water removes the water soluble impurities including boric acid and  $\text{H}_2^{15}\text{O}$ . Finally the  $\text{Bu}^{15}\text{OH}$  is eluted from the C-18 SEP PAK with 10% ethanol/saline. HPLC analysis of the product shows  $< 0.5\%$   $\text{H}_2^{15}\text{O}$  and 4-5% sec  $\text{Bu}^{15}\text{OH}$ . Boric acid derivatives resulting from the chemical processing of the primary tri-n-butyl borane are additionally controlled using anion exchange techniques (37). As with the  $\text{H}_2^{15}\text{O}$  generator systems, SOPs (see appendix 2) are being evolved for the routine production of this flow tracer for PET and it would seem likely that commercial  $\text{Bu}^{15}\text{OH}$  generators are being considered.

#### QUALITY CONTROL TECHNIQUES AND PROCEDURES

The gas phase products labelled with oxygen-15 are most readily analysed routinely using radio gas chromatography. The permanent gases are readily separated using columns of molecular sieve 5A and PORAPAK-Q. Specially designed concentric columns of these materials (Alltech CTRI) (17,38) allow a significant simplification of the analytical procedure and automated routine sampling can be applied (38). Chemical purity of the gas products is assessed using additional chemical gas analysis techniques for ozone, nitrogen oxides and carbon monoxide (22).

The oxygen-15 labelled infusion products undergo the usual screening test for sterility and apyrogenicity. Chromatographic analysis of  $\text{Bu}^{15}\text{OH}$  typically using a C-18 reverse phase HPLC column eluted with acetonitrile/water  $\text{NH}_4\text{OAc}$  (10 mMol) 10:90 (35) enables adequate rapid analytical separation of n $\text{Bu}^{15}\text{OH}$ , sec  $\text{Bu}^{15}\text{OH}$ ,  $\text{H}_2^{15}\text{O}$  and borane residues.

As in all short half-life radiopharmaceutical endeavour, much detailed analytical information is collected in the R & D phase of the work and it is widely acknowledged that for the design of practicable routine quality control procedures this data base is invaluable.

#### DISPENSING OF OXYGEN-15 PRODUCTS

Dispensing of the gas phase O-15 labelled products either as batches or for continuous inhalation can be readily regulated. With batch or bolus inhalation significant personal radiation exposure can be a

problem. With continuous inhalation personal exposure is readily minimised as the dispensing can be carried out on line using flow through radioactive concentration monitors and electronic flow meters. Systems for automatically regulating the radioactive dose level and duration for continuous administration for steady state PET studies have been developed (38,39). For the O-15 labelled infusates safe dispensing procedures are still evolving but remote dose monitoring and administration is imperative for the radiation safety of operating personnel .

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NUCLEAR MEDICINE DIVISION  
The University of Michigan  
MASTER FORMULA CARD

[ATTACHABLE LABEL]

PRODUCT: O-15 Water  
BATCH NO.:  
(xxxxxxH2Oyyy); X=Date; Y=Lot no.

| LOT NO. | INGREDIENT                      | SPECIFICATIONS                           | AMT SPECIFIED | AMT USED | INITIALS |  |
|---------|---------------------------------|------------------------------------------|---------------|----------|----------|--|
| 1       | Mixed bed resin column - 7mm ID | UM Cyclotron -PET Facility               | 1             |          |          |  |
| 2       | Deionized water                 | Sterile, pyrogen-free                    | 7 - 9 mL      |          |          |  |
| 3       | 20% Sodium chloride soln        | Sterile, pyrogen-free                    | 0.3 - 0.4 mL  |          |          |  |
| 4       | 0.22 µm vented syringe filter   | Millipore vented-GS                      | 1             |          |          |  |
| 5       | 10 cc multidose vial            | Sterile, pyrogen-free<br>Hollister-Stier | 1             |          |          |  |

CYCLOTRON

PRODUCT

EOB TIME:

PRODUCT (mCi): @ ET

CURRENT:

O.C. SAMPLE

DURATION:

O.C SAMPLE (mCi): @ min post-EOB (4min)

O.C. SAMPLE (mCi): @ min post-EOB (20 min)

QUALITY CONTROL

| TEST                   | METHOD                                                                               | RESULTS | DATE | INITIALS |  |
|------------------------|--------------------------------------------------------------------------------------|---------|------|----------|--|
| 1 Radionuclidic purity | QC sample @ 20 min should be < 0.8% of the activity of the sample @ 4 min (CapIntec) |         |      |          |  |
| 2 pH                   | pHion test paper - pH 4.5 - 8.0                                                      |         |      |          |  |
| 3 Visual               | Clear, colorless                                                                     |         |      |          |  |
| 4 Pyrogen test         | Limulus lysate assay                                                                 |         |      |          |  |
| 5 Sterility test       | FTM, TSB media incubation                                                            |         |      |          |  |
| 6                      |                                                                                      |         |      |          |  |
| 7                      |                                                                                      |         |      |          |  |

Product Approved for Human Use by: Date:

Original Master Formula Card Approved by: Date:

This batch record sheet is an accurate reproduction of the master formula card. Date

APPENDIX 1A

This document relates to the SOP used for the Oxygen-15 water production procedure described in Ref 32.

NUCLEAR MEDICINE DIVISION  
The University of Michigan  
MASTER FORMULA CARD

[ATTACH LABEL HERE]

PRODUCT: Resin Columns for O-15 Water

BATCH NO.:

(XXXXXXH2OCOLYYY); X=Date; Y=Lot no.

| LOT NO. | INGREDIENT                                                    | SPECIFICATIONS                      | AMT SPECIFIED | AMT USED | INITIALS |
|---------|---------------------------------------------------------------|-------------------------------------|---------------|----------|----------|
| 1       | AG1-X2 Anion exch. resin - 200-400 mesh, Cl <sup>-</sup> form | Biotechnology grade Biorad 140-1251 | 30 g          |          |          |
| 2       | AG50W-X2 Cation exch resin, 200-400mesh, H <sup>+</sup> form  | Biotechnology grade Biorad 142-1251 | 10 g          |          |          |
| 3       | Chelex 100 resin, 200-400 mesh, Na <sup>+</sup> form          | Biotechnology grade Biorad 142-2842 | 10 g          |          |          |
| 4       | Absolute ethanol                                              | USP                                 | ca. 300 mL    |          |          |
| 5       | Milli-Q water                                                 | Sterile, pyrogen-free               | ca. 400 mL    |          |          |
| 6       | Glass columns 7mm ID X 10 cm                                  | Sterile, pyrogen-free               | 6             |          |          |

INITIALS

DIRECTIONS (Use aseptic techniques. Perform all nonradioactive procedures in a laminar flow hood.)

1. Slurry together in 150 mL of 50% ethanol 30g of AG1-X2, 10g of AG50W-X2 and 10g of Chelex100. Filter the resin mixture and wash with an additional 300 mL of 50% ethanol. Resuspend the resin in 30% ethanol and slurry pack into 7 mm ID columns to a depth of 5 - 7 cm. Store refrigerated in 30% ethanol. Label the columns with lot number.

2. Before using a column, drain the 30% ethanol and equilibrate the column with Milli Q water

## QUALITY CONTROL

| TEST | METHOD | RESULTS | DATE | INITIALS |
|------|--------|---------|------|----------|
| 1    |        |         |      |          |
| 2    |        |         |      |          |
| 3    |        |         |      |          |
| 4    |        |         |      |          |
| 5    |        |         |      |          |
| 6    |        |         |      |          |
| 7    |        |         |      |          |

Product Approved for Human Use by: \_\_\_\_\_ Date: \_\_\_\_\_

Original Master Formula Card Approved by: \_\_\_\_\_ Date: \_\_\_\_\_

This batch record sheet is an accurate reproduction of the master formula card. \_\_\_\_\_

## APPENDIX 1B

This document relates to the SOP used for the Oxygen-15 water production procedure described in Ref 32.

PRODUCT: BATCH NO.: 

| INITIALS             | DIRECTIONS (Use aseptic techniques. Perform all nonradioactive procedures in a laminar flow hood.)                                                                                                                                                                        |
|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="text"/> | 1. Add 0.3 - 0.4 mL of sterile 20% saline to 10cc multidose vial.                                                                                                                                                                                                         |
| <input type="text"/> | 2. Connect target emptying line to top of mixed bed column. Connect sterile millipore filter to column outlet. A sterile needle on the millipore filter pierces the septum of the multidose vial which also has a sterile needle puncturing it.                           |
| <input type="text"/> | 3. Fill the target with 7 - 9 mL of sterile deionized water, then pressurize the target with 100 psi of helium overpressure.                                                                                                                                              |
| <input type="text"/> | 4. Irradiate target for 2 - 6 min with protons.                                                                                                                                                                                                                           |
| <input type="text"/> | 5. Close off helium overpressure at end of irradiation. Empty the target contents through the mixed bed column, millipore filter and into the multidose vial containing sterile 20% saline solution using the residual helium overpressure in the system as the push gas. |
| <input type="text"/> | 6. Note the amount of product in the vial and withdraw a small portion (0.1 - 0.3 mL) via sterile syringe for quality control tests.                                                                                                                                      |

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O-15 Water, Master Formula Card Dated 11-09-89, pg 2 of 2.

## APPENDIX 1C

This document relates to the GMP used for the Oxygen-15 water production procedure described in Ref 32.



## APPENDIX IIA

UNIVERSITY HOSPITALS OF CLEVELAND PET FACILITY  
SAFE OPERATING PROCEDURE FOR O-15 BUTANOL PRODUCTIONPreparation

1. Alumina SEP-PAK (No 1)  
If previously used, wash with EtOH, acetonitrile, flush with air, suck dry with vacuum. Dry in vacuum oven at 80°C at least hours. Flush with Argon, and put sleeve stoppers on both ends. Store in glove box. Inject 0.1 ml pure Bu<sub>3</sub>B (for 0.2% Oxygen in Nitrogen target gas). The prepared SEP-PAK is best used 5-hours after injection. Indefinite life span.  
For new Alumina SEP-PAK, flush with Argon and proceed as above
2. C-18 SEP PAK (No 2)  
Wash 2 C-18 SEP-PAKS with 3 ml pure EtOH, air bolus, followed by 6 ml Millipore water. Connect with short glass tubing. These may be reused many times.

Preparation

1. Flush lines with Helium (V13 open). He flow = 40-50.
2. For patient runs: sterilise all lines with EtOH, Millipore water. He flush.  
For add line 1: V2 open, V7 closed, V8 open  
For add line 2: V7 open, V8 open  
For collect line: V8 closed.
3. Beam on: 10  $\mu$  amp/10 min for 50-70 mCi Bu<sup>15</sup>OH dose  
V7, V8, V13 open
4. At EOB-1 min: Insert Alumina SEP-PAK. Helium flow on.
5. Empty target: Close V13, Open V17, V5.  
After 15 seconds, open V18.  
At 25 seconds, close V17, wait for pressure to drop below 1.  
Close V5, V7, V18, open V2.
6. 4ml wash of both SEP-PAKS through add line No 1 (2 portions)  
Add 1 ml millipore water and 2 ml air bolus. Then push 3ml through slowly, followed by a large air bolus. Watch the activity transfer to the lower C-18 SEP-PAK on the Francis meter
7. Three 1/2 ml washes of SEP-PAK No 2 (Millipore water) through add line No 2. Open Valve 7.
8. 5ml ethanol in sterile saline through SEP PAK No 2.  
(through add line No 2). V8 closed. Collect in syringe.
9. Measure on HPLC 15% Acetonitrile/0.01M NH<sub>4</sub>OAc  
Alltech ECONOSIL Column  
Flow = 2 ml min<sup>-1</sup>  
Scale = 2000 KCPM  

| Retention times | H <sub>2</sub> O | 1.45 min |
|-----------------|------------------|----------|
|                 | sec BuOH         | 4.4 min  |
|                 | nBuOH            | 5.7 min  |
10. Remove SEP-PAKS and flush dry system after all runs.  
Leave dry.

This document relates to the SOP used for the Oxygen-15 Butanol production procedure described in Ref 13. (Valve numbers refer to specific system and can be ignored for the present purposes).

## APPENDIX IIB

| Raw Materials<br>: Supplier              | Batch No.      | Expiry Date<br>month/year |
|------------------------------------------|----------------|---------------------------|
| N <sub>2</sub> /O.2%O <sub>2</sub> Linde | T44852         | 1/95                      |
| Helium Linde                             | UN1046         | 1/95                      |
| SEP-PAK-C18 Waters                       | P9324A1        | 1/95                      |
| SEP-PAK-Alumina Waters                   | P8148A2        | 1/92                      |
| Syringe 10ml<br>Beckton-Dickson          | OB530          | 9/93                      |
| Needle<br>25G5/8<br>Becton-Dickinson     | 9L023          | 1/95                      |
| TriButyl<br>Borane Alpha                 | E29G           | 1/91                      |
| Normal Saline                            | ZP033241       | 12/91                     |
| Sterile Baxter                           |                |                           |
| Filter 0.22μ<br>Sterile<br>Millipore     | H8Jk-<br>85499 | 1/92                      |

1. Open valves on compressed N2-O2 and He.
2. Open V13.
3. Rinse all lines with ethanol, Millipore Water, air and He.
4. Prepare 2 C-18 SEP-PAKS.
5. Prepare alumina SEP-PAK with .1ml tributylborane under Ar.
6. Assemble 10cc receiving syringe, cap and filter.
7. Load Target (V18).
8. Bombard for predetermined current and time.
9. At EOB-1 min, insert alumina SEP-PAK
10. At EOB, close V13, open V5, V17, V18.
11. At maximum activity close V17, V18 open V2, V8 close V7.
12. Two washes of both SEP-PAKS with water. (1ml, 3ml)
13. Three washes of SEP-PAK No 2 (.5ml/.5ml/.5ml) open V7.
14. Close V8. Add 5ml 10% ethanol in sterile saline.
15. Collect product.
16. Measure and send desired amount to clinic.

Procedure was followed as described above.

Signed ..... Approved .....

This document relates to the GMP used for the Oxygen-15 Butanol production procedure described in Ref 13. (Valve numbers refer to a specific system and can be ignored for the present purposes).

# Production of radiopharmaceuticals for PET

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## Abstract

A range of radionuclides suitable for use in PET is available for incorporation into Radiopharmaceuticals. The ones of principal interest are (half life shown in brackets).  $^{15}\text{O}$  (2.05 min),  $^{13}\text{N}$  (10 min),  $^{11}\text{C}$  (20 min),  $^{18}\text{F}$  (110 min) and those derived from the radionuclide generator systems  $^{82}\text{Sr}$  (25 day)/ $^{82}\text{Rb}$  (75 s),  $^{68}\text{Ge}$  (271 day)/ $^{68}\text{Ga}$  (68 min),  $^{62}\text{Zn}$  (9.3 hour)/ $^{62}\text{Cu}$  (9.8 min).

$^{15}\text{O}$ ,  $^{13}\text{N}$ ,  $^{11}\text{C}$  and  $^{18}\text{F}$  have to be produced using a dedicated accelerator close to the PET installation, whereas the radionuclide generators can be made using remote accelerator locations. In all cases, however, the synthesis of the radiopharmaceutical, its purification and quality control must of necessity take place at the PET facility.

These syntheses may range from a simple elution of a radionuclide generator, the eluted product being immediately suitable for administration to man for PET imaging, to a multi step rapid organic chemical radiosynthesis with high speed purification of the product to provide a highly selective marker for a specific biological process which can then be quantitatively studied using PET.

The paper will in no way attempt to be comprehensive in its coverage of this field, rather it will perhaps encourage the inquisitive reader to delve into the literature cited.

## 1. Nuclear reactions for the production of PET radionuclides.

### Oxygen-13

Three nuclear reactions are currently in use for the production of  $^{15}\text{O}$  for medical use they are:

|                                                  |                |       |
|--------------------------------------------------|----------------|-------|
| $^{14}\text{N}(\text{d},\text{n})^{15}\text{O}$  | $E_d$ 6-10 MeV | [1-4] |
| $^{15}\text{N}(\text{p},\text{n})^{15}\text{O}$  | $E_p$ 10 MeV   | [5]   |
| $^{16}\text{O}(\text{p},\text{pn})^{15}\text{O}$ | $E_p$ 17 MeV + | [6]   |

The  $^{16}\text{O}(\text{}^3\text{He},\alpha)^{15}\text{O}$  reaction with 8 MeV  $^3\text{He}$  is proposed for use with a new type of linear accelerator which is under development [23].

### Nitrogen-13

Nitrogen 13 is principally produced for medical use by the following reactions

|                                                 |                 |     |
|-------------------------------------------------|-----------------|-----|
| $^{16}\text{O}(\text{p},\alpha)^{13}\text{N}$   | $E_p$ 10-16 MeV | [7] |
| $^{13}\text{C}(\text{p},\text{n})^{13}\text{N}$ | $E_p$ 10-11 MeV | [8] |

### Carbon-11

Carbon-11 production for use in PET organic radiosynthesis is almost exclusively produced using the reaction  $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$   $E_p$  10-20 MeV [9].

Other possible reactions which are not without significant problems include:

|                                                         |                 |        |
|---------------------------------------------------------|-----------------|--------|
| $^{10}\text{B}(\text{d},\text{n})^{11}\text{C}$         | $E_d$ 5-15 MeV  | [1,10] |
| $^{11}\text{B}(\text{p},\text{n})^{11}\text{C}$         | $E_p$ 10-20 MeV |        |
| $^{11}\text{B}(\text{d},2\text{n})^{11}\text{C}$        | $E_d$ 15 MeV    | [10]   |
| $^{12}\text{C}(\text{}^3\text{He},\alpha)^{11}\text{C}$ | $E_{He}$ 8 MeV  | [23]   |

### Fluorine-18

Several nuclear reactions have been used to produce  $^{18}\text{F}$  for PET but the first two are now used almost exclusively.

|                                                            |                   |          |
|------------------------------------------------------------|-------------------|----------|
| $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$             | $E_d$ 10 MeV +    | [11, 13] |
| $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$            | $E_p$ 10 MeV +    | [12]     |
| $^{16}\text{O}(\text{}^3\text{He},\text{pn})^{18}\text{F}$ | $E_{He}$ 25 MeV + | [13]     |
| $^{16}\text{O}(\text{}^3\text{He},\text{p})^{18}\text{F}$  | $E_{He}$ 8 MeV +  | [13]     |

### Strontium 82/Rubidium 82

The production of large amounts of  $^{82}\text{Sr}$  relies on the high energy spallation of Molybdenum:

|                                              |               |      |
|----------------------------------------------|---------------|------|
| $\text{Mo}(\text{p},\text{x})^{82}\text{Sr}$ | $E_p$ 600 MeV | [14] |
|----------------------------------------------|---------------|------|

However practical quantities have been produced using the reaction

|                                                    |                |      |
|----------------------------------------------------|----------------|------|
| $^{85}\text{Rb}(\text{p},4\text{n})^{82}\text{Sr}$ | $E_p$ 60 MeV + | [15] |
|----------------------------------------------------|----------------|------|

A commercial  $^{82}\text{Rb}$  radionuclide generator is manufactured by Bristol-Myers-Squibb and is used principally in PET cardiac studies [16].

### Germanium-68/Gallium-68

Large scale production of  $^{68}\text{Ge}$  is based on the high energy proton spallation of Bromine containing targets:

|                                              |               |         |
|----------------------------------------------|---------------|---------|
| $\text{Br}(\text{p},\text{x})^{68}\text{Ge}$ | $E_p$ 600 MeV | [17,18] |
|----------------------------------------------|---------------|---------|

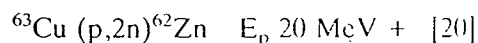
For lower energy accelerators the less productive reaction has been employed.

|                                                    |                |  |
|----------------------------------------------------|----------------|--|
| $^{69}\text{Ga}(\text{p},2\text{n})^{68}\text{Ge}$ | $E_p$ 20 MeV + |  |
|----------------------------------------------------|----------------|--|

A commercial  $^{68}\text{Ga}$  radionuclide generator is manufactured by NEN-Dupont which yields a product of use in radiopharmaceutical preparations [19]. A further and important use of  $^{68}\text{Ge}$  PET is its use as "Transmission sources" which are built into PET scanners to allow for attenuation correction of the emission data collected after the administration of the PET radiopharmaceutical.

### *Zinc-62/Copper 62*

This radionuclide is most commonly produced using the reaction



Radionuclide generators which can provide  $^{62}\text{Cu}$  suitable for incorporation into radiopharmaceuticals have been described [21] but none have as yet been produced on a commercial basis.

## **2. Particle accelerators for PET radionuclide production**

Consideration will be given here only to the dedicated machines that are intended solely to provide for the PET installation.

Although some PET facilities have made use of pre existing tandem Van Der Graaf accelerators the majority now make use of small cyclotrons. These are usually isochronous machines with proton energies of 10 to 17 MeV. They are commercially produced and in most cases are also able to accelerate deuterium ions, typically in the range 5 to 8 MeV. No provision is usually made for the heavier helium ions on these accelerators. The reader is recommended to refer to the suppliers

data sheets for full information about these accelerators. The principal suppliers being listed in Table I.

## **3. Recent developments in PET accelerators**

Early isochronous cyclotrons accelerated positive ions and relied on an electrostatic deflector to extract a useful beam. This component can be quite heavily stressed and many manufacturers are looking towards accelerating negative ions and achieving high efficiency extraction by "stripping" the particle's electrons to form a positive ion which will escape the accelerators magnetic field as the orbit curvature is instantly reversed. This advance may lead to cheaper and simpler to operate devices.

There is an upsurge in interest in two types of linear accelerators. One is based on the Tandem Cascade Accelerator (TCA) [22] which is effectively a tandem Van Der Graaf powered by a 1.8 MV electronic power supply. The resulting proton or deuteron energy can be as high as 3.7 MeV.

Another is based on device called a Radio Frequency Quadrupole (RFQ). Here the charged particles "ride" a radiofrequency wave set up between the quadrant vanes of a RF cavity. A device which is capable of accelerating  $^3\text{He}$  to 8 MeV has been described [23]. Finally the demonstration of the use of a very small deuteron cyclotron of 3.7 MeV solely to produce oxygen-15 has introduced a further possible way to support a PET operation [24].

## **4. Target systems for PET radionuclide production**

An essential interface between the accelerator charged particle beam and the radiopharmaceutical

**Table I – List of accelerator suppliers**

---

Ion Beam Applications, B1348, Louvaine - La Neuve, Belgium. FAX 32 10 47 58 10.  
Models: CYCLONE-3 (Oxygen-15 generator), CYCLON-10/5, CYCLON-18/9.

Japan Steelwork, Konigsallee 92a, 4000 Dusseldorf 1, Germany. FAX 49 211 32 8199.  
Models: BC 126, BC-1710.

Oxford Instruments/NKK, Osney Mead, Oxford OX2 ODX U.K., FAX 44 865 269690.  
Model: "OSCAR" (Super conducting magnet).

Scanditronix/GE Medical Systems, Husbyborg, S 75229 Uppsala, Sweden. FAX 46 185 37276.  
Model: S MC-17, "PET trace 200".

Siemens/CTI Medical Engineering Group Henkestrasse 127, D8520 Erlangen, Germany.  
Models: RDS-112 (Protons only).

Sumitomo/CGR, 1 Kand Mitoshiro-Cho, Chiyoda-Ku, Tokyo 101, Japan. FAX 3296 5391.  
Models: 325, 370.

---

synthesis is the Target. This is where the nuclear reactions outlined above are carried out in a controlled way in order to produce the desired radionuclide in an appropriate chemical form for the synthetic work which follows.

PET radionuclide targets operate predominantly in the gas or liquid phase and their geometry is determined principally by this. Both types of target have a beam entry window system that provides an essentially transparent aperture through which the high energy charged particles exit the accelerator vacuum and enter the target material containment vessel. These windows are invariably made of thin (10-50  $\mu\text{m}$ ) heat resistant metals or alloys and are cooled with Helium. The window is often under considerable stress especially in the case of high pressure gas targets. Gas targets are commonly used to produce Oxygen-15 from Nitrogen [1,2,5], Carbon-11 from Nitrogen [9] and Fluorine-18 ( $^{18}\text{F}_2$ ) from Neon [11]. Liquid targets are used for the production of Fluorine-18 ( $^{18}\text{F}$ -fluoride) from enriched water  $\text{H}_2^{18}\text{O}$  [25] and Nitrogen-13 from natural water ( $\text{H}_2^{16}\text{O}$ ) [7].

By control of the chemical conditions under which the irradiation takes place the chemical form of the product nuclide can be manipulated to achieve the form most appropriate for the next stage in the radiosynthesis. This is usually referred to as "in target chemistry". Examples include the production "in target" of  $^{15}\text{O}_2$ ,  $\text{C}^{15}\text{O}_2$ , [2]  $\text{H}_2^{15}\text{O}$  [3],  $\text{C}^{15}\text{O}$  [26],  $^{11}\text{CO}_2$ ,  $^{11}\text{CH}_4$  [27],  $^{18}\text{F}_2$  [11] and  $^{13}\text{NH}_3$  [28].

Where expensive enriched target materials are used, eg  $\text{H}_2^{18}\text{O}$ , it is usual to recover them by incorporating a chemical retrieval step in the radiosynthesis [29].

## 5. Radiolabelled precursor preparation

In some cases the radionuclide is produced in the target in a chemical form suitable for direct use. However, for organic radiosyntheses where fast reactions are essential to achieve useful end product specifications of radioactivity and specific radioactivity ( $\text{Ci}/\mu\text{M}$ ) it is usual to generate highly reactive radiolabelled intermediates or precursors.

Examples for carbon-11 would include the conversion of  $^{11}\text{CO}_2$  to  $^{11}\text{CH}_3\text{OH}$ ,  $\text{H}^{11}\text{CHO}$ ,  $^{11}\text{CH}_3\text{I}$  [30] and  $^{11}\text{CH}_4$  to  $^{11}\text{COCl}_2$  [31]. Fluorine-18  $^{18}\text{F}_2$  is converted to  $\text{CH}_3\text{COO}^{18}\text{F}$  (Acetyl hypofluoride) [32] and  $^{18}\text{F}$ -fluoride is prepared in "naked" form, all hydration water being removed, making the  $^{18}\text{F}$  of value in nucleophilic substitution reactions, eg the synthesis of  $^{18}\text{FDG}$  [33].

## 6. Radiopharmaceutical production for PET

Due to the short half-life of the most useful PET radionuclides the aim is to carry out the synthesis,

purification and formulation of the product as fast as possible. Typically a Carbon-11 product may be made available for injection some 30-40 minutes after the end of radionuclide production [35]. In the case of Fluorine-18, 90-120 mins would be typical, eg  $^{18}\text{FDG}$  [29].

However, it is often the case that the price one has to pay for speed is efficiency of incorporation of the radionuclide and the efficiency of the manipulative steps. Thus one typically will start a Carbon-11 synthesis with 2-3 Curies of  $^{11}\text{CO}_2$  and deliver 50-100 millicuries of formulated radiopharmaceutical. Even under these apparently adverse conditions many carbon-11 labelled radiopharmaceutical tracers of metabolic substrates and receptor ligands are now available to the PET clinical research with specifications that meet current needs. [34] The variety of synthetic techniques available in this field is still growing to meet the demand for new radiolabelled molecules to probe specific pathways using PET techniques. [35].

## 7. Hot cells and automated radiopharmaceutical production

The high levels of starting radioactivity make it mandatory that all the chemical processes be carried out in a lead shielded enclosure (HOT CELL) and that all manipulations are at least by remote control. Typically a Hot cell will have lead walls 50-100 mm thick and lead glass viewing windows of equivalent shielding performance for the positron annihilation radiations of 0.511 MeV.

The environment inside the hot cell should be controlled to avoid accidental escape of radioactivity and often it is also necessary to provide an environment within the cell suitable for radiopharmaceutical formulation. There is potential for conflict between these two requirements and no satisfactory solution has yet been demonstrated.

Although remote control achieves the required safety for the synthetic chemist, it is usually felt necessary to provide some degree of automation to the remote control devices [36]. This approach has led to faster more reliable procedures capable in some cases of repetitive operation. A variety of automation techniques are being employed ranging from dedicated compact synthetic units designed with one end product in mind to potentially flexible systems which make use of a Robotic arm device to "work" between an array of discrete workstations which when linked together function as a complete synthetic system [37]. Examples can now be found from commercial suppliers of both the dedicated unit, e.g. to make  $^{18}\text{FDG}$  and the Robot arm based system.

## 8. Quality assurance of PET radiopharmaceuticals

Before any radiopharmaceutical preparation for PET is administered it should be subjected to a

quality control procedure which has been designed to ensure that the product will not only be safe but also meet a specification that would enable valid interpretable data to be acquired in the PET study [38].

A vital part of these procedures is the establishment of a safe operating procedure (SOP) and a compliance to an established good manufacturing practice (GMP). This is essential as due to the short time available for analysis of PET radiopharmaceuticals. Some of the standard pharmaceutical tests e.g. sterility, cannot normally be carried out. The special consideration relating to the quantitative determination of purity and specific radioactivity for receptor radioligands in all cases is crucial to the ultimate goal of "in vivo" receptor density mapping [34, 35].

## Summary

The production of radiopharmaceuticals for PET has many challenges and involves several disciplines. Their use in applying the PET technique in aiding the understanding of *in vivo* metabolic and pharmacological processes is probably unrivalled in its picomolar sensitivity. Some clinical diagnostic exploitation of PET is now evolving using relatively simple Radiopharmaceuticals, e.g.  $\text{H}_2^{15}\text{O}$ ,  $^{13}\text{NH}_3$  and  $^{18}\text{FDG}$ . It would seem that due to complexities in the application Carbon-11 ligand Radiopharmaceuticals they will remain in the realm of research tools for some time to come.

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## An Oxygen-15 Generator: Early Experience with the IBA CYCLONE-3

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### Clinical Requirement

Oxygen-15 is widely acknowledged to perform a vital role in PET clinical applications, for example measurements of

- Blood volume by [ $^{15}\text{O}$ ]CO
- Blood flow by [ $^{15}\text{O}$ ]CO<sub>2</sub>, [ $^{15}\text{O}$ ]H<sub>2</sub>O, [ $^{15}\text{O}$ ]BuOH
- Metabolic rate by [ $^{15}\text{O}$ ]O<sub>2</sub>.

The Hammersmith PET programme exploits  $^{15}\text{O}$  tracer techniques extensively and it was appropriate to investigate potential dedicated  $^{15}\text{O}$  generator systems to allow the more flexible multi-particle 40 MeV cyclotron to be applied more efficiently to  $^{11}\text{C}$  and  $^{18}\text{F}$  PET radiopharmaceutical production.

### The Project

A collaborative programme drawn up with IBA, Louvain-la-Neuve. A performance specification was agreed that would meet the current demands for  $^{15}\text{O}$  products to support the Hammersmith PET programme. Thus, MRC propose to purchase the prototype CYCLONE-3.

### Acceptance tests

All products were produced at rates in excess of specification. CYCLONE-3 was shipped to Hammersmith in February 1991.

### Current status

Mechanical difficulties relating to insulators supporting dees have not yet allowed Hammersmith acceptance tests to be carried out. Corrective action is in progress.

A research phase has been initiated at Hammersmith with 50  $\mu\text{A}$  maximum to assess chemical processing, gas transfer losses and [ $^{15}\text{O}$ ]H<sub>2</sub>O generator integration.

Radiochemical purity of [ $^{15}\text{O}$ ]CO and [ $^{15}\text{O}$ ]CO<sub>2</sub> meets clinical requirements, however, the contamination of [ $^{15}\text{O}$ ]O<sub>2</sub> with 7.5 % [ $^{15}\text{O}$ ]N<sub>2</sub>O remains to be resolved. „In target“ production of [ $^{15}\text{O}$ ]H<sub>2</sub>O using N<sub>2</sub>/H<sub>2</sub> target gas mixtures gives very encouraging results.

Transport of [ $^{15}\text{O}$ ]H<sub>2</sub>O vapour with no added carrier has been achieved over 100 m of 1.5 mm inside diameter PTFE tube. About 50 % losses overall were found.

Table 1: Acceptance test data 8/9 January 1991

|                                                                                                                                            | Beam<br>( $\mu\text{A}$ ) | Flow<br>(ml/min) | Activity<br>(mCi/min) | Specific Activity<br>(mCi/min/ $\mu\text{A}$ ) | Performance specification<br>(mCi/min)* |
|--------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|------------------|-----------------------|------------------------------------------------|-----------------------------------------|
| [ $^{15}\text{O}$ ]O <sub>2</sub>                                                                                                          | 58                        | 430              | 140                   | 2.41                                           | 30                                      |
| [ $^{15}\text{O}$ ]CO <sub>2</sub>                                                                                                         | 83                        | 435              | 85                    | 1.02                                           | 80                                      |
| [ $^{15}\text{O}$ ]CO                                                                                                                      | 79                        | 475              | 115                   | 1.4                                            | 40                                      |
| * All data were measured 80 m distant from the target at a flow of 4 - 500 ml/min (to simulate transport to the most distant PET scanner). |                           |                  |                       |                                                |                                         |



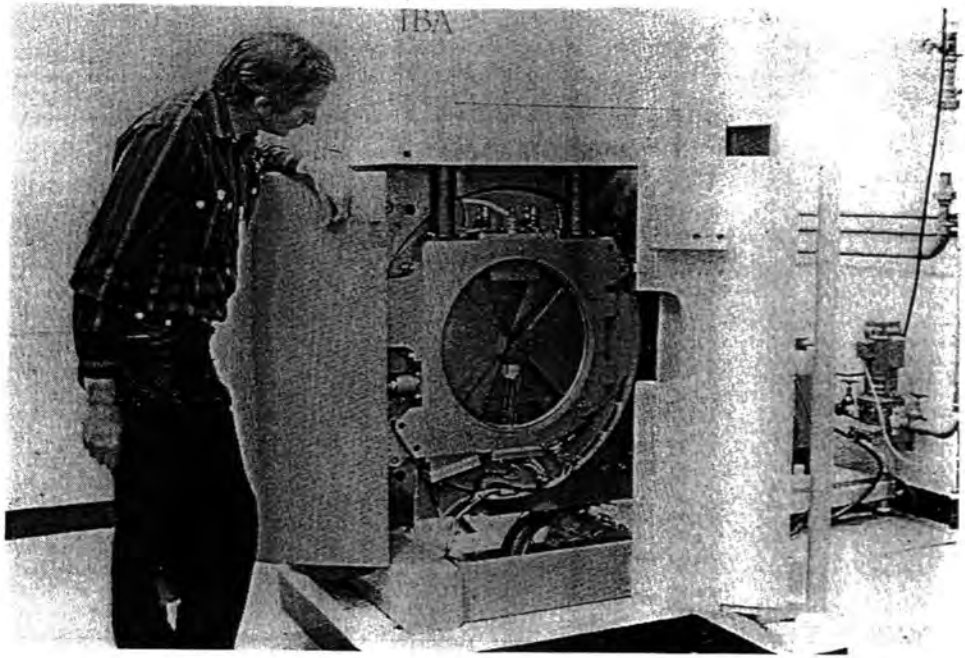


*Figure 1: The IBA CYCLONE-3*

## Status Report from Hammersmith

John C. Clark  
MRC Cyclotron Unit  
Hammersmith Hospital  
Ducane Road  
London W12 0HS, United Kingdom

### Hardware

- SCANDITRONIX MC 40 - Operated 24 hrs per day. PET in daytime. Isotope production overnight *e.g.*  $^{81}\text{Rb}/^{81\text{m}}\text{Kr}$ ,  $^{77}\text{Br}$ ,  $^{43}\text{K}$ ,  $^{203}\text{Pb}$ ,  $^{52}\text{Fe}$
- IBA CYCLONE-3  $^{15}\text{O}$  generator
- PET Scanner I: SIEMENS/CTI Model 931. Whole body machine used for Cardiology, Oncology and Neurology
- PET Scanner II: SIEMENS/CTI Model 953B. Brain machine used for Neurology. Retractable septa gives approximately 5 fold increase in sensitivity.  $\text{H}_2^{15}\text{O}$  infusion unit installed under the patient couch.

ing), all automated.

**Fluorine-18:** [ $^{18}\text{F}$ ]FDG automated, [ $^{18}\text{F}$ ]DOPA remote, [ $^{18}\text{F}$ ]Fluorouracil remote (FU).

**Oxygen-15:** [ $^{15}\text{O}$ ]CO $_2$ , [ $^{15}\text{O}$ ]O $_2$ , [ $^{15}\text{O}$ ]CO and [ $^{15}\text{O}$ ]H $_2$ O infusions automated (plus cyclotron schedule and PET schedule).

**Metabolite Analysis:** Many PET studies require plasma metabolite analysis to facilitate kinetic modelling. Analysis is mainly carried out using semiautomated radio-HPLC and RAYTEST PC integrator.

### Radiopharmaceuticals for PET

**Carbon-11:** Raclopride (RAC), Deprenyl (DEP), PK-11195, Diprenorphine (DPR), CGP-12177 (CGP), SCH-23390 (SCH), Flumazenil (FLU), Thymidine (develop-

### Scheduling

See Cyclotron Schedule (Table 1) and associated PET Scanner Schedules (Table 2). 20 - 25 clinical studies per week.

Table 1: MC 40 Cyclotron Operations Schedule, Week 4, 19-24 January 1992.

| <u>Date/time</u>            | <u>Beam/Posn</u>                                                 | <u>Particle/Energy</u> | <u>Target/Nuclide</u>                                     | <u>User Ericom</u> |
|-----------------------------|------------------------------------------------------------------|------------------------|-----------------------------------------------------------|--------------------|
| <u>Sunday 19 January</u>    |                                                                  |                        |                                                           |                    |
| 22:00 - 23:30               | Maintenance, select                                              | 25.5 MeV He4           |                                                           |                    |
| 23:30 - 24:00               | 5/11                                                             | He4/25.5               | A/K43                                                     | JM/150             |
| <u>Monday 20 January</u>    |                                                                  |                        |                                                           |                    |
| 00:00 - 00:30               | Select 33 MeV He4                                                |                        |                                                           |                    |
| 00:30 - 01:00               | 5/12                                                             | He4/33                 | As <sub>2</sub> O <sub>3</sub> /Br77                      | SAC/150            |
| 01:00 - 01:15               | Change targets                                                   |                        |                                                           |                    |
| 01:15 - 06:30               | 5/12                                                             | He4/33                 | NaBr/Rb81                                                 | HRK/MJM/150        |
| 06:30 - 10:00               | Maintenance/Engineering                                          |                        |                                                           |                    |
| 10:00 - 10:30               | Select 16.5 MeV d                                                |                        |                                                           |                    |
| 10:30 - 12:00               | 1                                                                | d/16.5                 | Neutron activation                                        | BCP/130            |
| 12:00 - 12:30               | Select 19 MeV p                                                  |                        |                                                           |                    |
| 12:30 - 12:45               | 5/8                                                              | p/19                   | H <sub>2</sub> O/F18(F <sup>-</sup> )PET II               | TJS/NS/150         |
| 12:45 - 13:15+++            | 5/4                                                              | p/19                   | N <sub>2</sub> /C11(FLU)PET II                            | DRT/168            |
| 13:15 - 13:30               |                                                                  |                        |                                                           |                    |
| 13:30 - 14:00o*             | 5/10                                                             | p/19                   | N <sub>2</sub> /C11(CGP) PET I                            | DJB/CJS/168        |
| 14:00 - 14:30               | Select 9.5 MeV d                                                 |                        |                                                           |                    |
| 14:30 - 15:00o*             | 5/6                                                              | d/9.5                  | N <sub>2</sub> /O15(CO)PET I                              | NS/170             |
| 15:00 - 15:30               | Select 16.5 MeV d                                                |                        |                                                           |                    |
| 15:30 - 16:30               | 5/1/2                                                            | d/16.5                 | Ne/F18 (condtn)                                           | NS/150             |
| 16:30 - 18:45               | PET Neurology study PET I CF/XXX                                 | 00:00 = 17:30          |                                                           | NS/170             |
| 18:45 - 19:15               | Study overrun                                                    |                        |                                                           |                    |
| 19:15 - 24:00               | Engineering/maintenance                                          |                        |                                                           |                    |
| <u>Tuesday 21 January</u>   |                                                                  |                        |                                                           |                    |
| 00:00 - 00:30               | Select 33 MeV He4                                                |                        |                                                           |                    |
| 00:30 - 05:15               | 5/12                                                             | He4/33                 | NaBr/Rb81                                                 | JM/MJM/150         |
| 05:15 - 05:45               | Select 19 MeV p                                                  |                        |                                                           |                    |
| 05:45 - 06:30***            | 5/9                                                              | p/19                   | H <sub>2</sub> O/F18(FDG)PET II                           | SAC/151            |
| 06:30 - 07:00               | Select 16.5 MeV d                                                |                        |                                                           |                    |
| 07:00 - 09:00**owbc5/1      | 5/1                                                              | d/16.5                 | Ne/F18(5 FU)PET I                                         | GB/151             |
| 09:00 - 11:00**             | 5/2                                                              | d/16.5                 | Ne/F18(DOPA)PET I                                         | MJM/151            |
| 11:00 - 11:30               | Select 9.5 MeV d                                                 |                        |                                                           |                    |
| 11:30 - 12:00**o            | 5/6                                                              | d/9.5                  | N <sub>2</sub> /O15(CO <sub>2</sub> )PET I                | JM/170             |
| 12:00 - 12:30               | Select 19 MeV p                                                  |                        |                                                           |                    |
| 12:30 - 13:00               |                                                                  |                        |                                                           |                    |
| 13:00 - 13:30+              | 5/4                                                              | p/19                   | N <sub>2</sub> /C11(PK)PET II                             | DJB/168            |
| 13:30 - 14:00               | Select 9.5 MeV d                                                 |                        |                                                           |                    |
| 14:00 - 14:30+              | 5/6                                                              | d/9.5                  | N <sub>2</sub> /O15(H <sub>2</sub> <sup>15</sup> O)PET II | NS/HRK/170/190     |
| 14:30 - 15:30               |                                                                  |                        |                                                           |                    |
| 15:30 - 17:15               | PET Cardiology study PET I NU/XXX                                | 00:00 = 16:15          |                                                           | HRK/170            |
| 17:15 - 19:00               | PET Neurology study PET I HR/XXXX                                | 00:00 = 17:45          |                                                           | HRK/170            |
| 19:00 - 19:30               | Study overrun                                                    |                        |                                                           |                    |
| 19:30 - 24:00               | Engineering/maintenance                                          |                        |                                                           |                    |
| <u>Wednesday 22 January</u> |                                                                  |                        |                                                           |                    |
| 00:00 - 00:30               |                                                                  |                        |                                                           |                    |
| 00:30 - 01:00               | Select 33 MeV He4                                                |                        |                                                           |                    |
| 01:00 - 06:30               | 5/12                                                             | He4/33                 | NaBr/Rb81                                                 | SAC/NS/150         |
| 06:30 - 07:00               | Select 16.5 MeV d                                                |                        |                                                           |                    |
| 07:00 - 09:00**             | 5/1                                                              | d/16.5                 | Ne/F18 (DOPA)PET I                                        | MJM/151            |
| 09:00 - 12:30               | PET Neurology H <sub>2</sub> <sup>15</sup> O study PET II JW/XXX | 00:00 = 10:00          |                                                           | JM/170             |
| 12:30 - 13:00               | Select 19 MeV p                                                  |                        |                                                           |                    |
| 13:00 - 13:15               | 5/4                                                              | p/19                   | N <sub>2</sub> /C11(NSC)                                  | GB/DRT/168         |
| 13:15 - 13:30               | 5/8                                                              | p/19                   | H <sub>2</sub> O/F18(F <sup>-</sup> )PET II               | TJS/NS/150         |
| 13:30 - 14:00*              | 5/4                                                              | p/19                   | N <sub>2</sub> /C11(RCP)PET I&BiolHRK/168                 |                    |
| 14:00 - 14:30               | Select 9.5 MeV d                                                 |                        |                                                           |                    |
| 14:30 - 15:00*              | 5/6                                                              | d/9.5                  | N <sub>2</sub> /O15(CO <sub>2</sub> )PET I                | DJB/170            |
| 15:00 - 16:00               |                                                                  |                        |                                                           |                    |
| 16:00 - 18:30               | PET Neurology study PET I AK/XXX                                 | 00:00 = 17:00          |                                                           | DJB/170            |
| 18:30 - 19:00               | Select 16.5 MeV d                                                |                        |                                                           |                    |
| 19:00 - 19:30               | 5/12                                                             | d/16.5                 | Fe/Co56                                                   | HRK/CSJF/150       |
| 19:30 - 24:00               |                                                                  |                        |                                                           |                    |

Table 1 (continued)

|                                                                                                                                                                                                                                                          |                                                                                |                     |                                                           |             |             |  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|---------------------|-----------------------------------------------------------|-------------|-------------|--|
| <b>Thursday 23 January</b>                                                                                                                                                                                                                               |                                                                                |                     |                                                           |             |             |  |
| 00:00 - 00:30                                                                                                                                                                                                                                            | Select 33 MeV He4                                                              |                     |                                                           |             |             |  |
| 00:30 - 06:30                                                                                                                                                                                                                                            | 5/12                                                                           | He4/33              | NaBr/Rb81                                                 |             | HRK/MJM/150 |  |
| 06:30 - 07:00                                                                                                                                                                                                                                            | Select 16.5 MeV d                                                              |                     |                                                           |             |             |  |
| 07:00 - 09:00**                                                                                                                                                                                                                                          | 5/2                                                                            | d/16.5              | Ne/F18(DOPA)PET I                                         | NS/151      |             |  |
| 09:00 - 12:30                                                                                                                                                                                                                                            | PET Neurology H <sub>2</sub> <sup>15</sup> O                                   | study PET II SR/XXX | 00:00 = 10:00                                             | SAC/170/190 |             |  |
| 12:30 - 13:00                                                                                                                                                                                                                                            | Select 19 MeV p                                                                |                     |                                                           |             |             |  |
| 13:00 - 13:30+++                                                                                                                                                                                                                                         | 5/4                                                                            | p/19                | N <sub>2</sub> /C11(FLU)PET II                            | DRT/168     |             |  |
| 13:30 - 14:00                                                                                                                                                                                                                                            | Select 9.5 MeV d                                                               |                     |                                                           |             |             |  |
| 14:00 - 14:30+++                                                                                                                                                                                                                                         | 5/6                                                                            | d/9.5               | N <sub>2</sub> /O15(H <sub>2</sub> <sup>15</sup> O)PET II | JM/170/190  |             |  |
| 14:30 - 15:00                                                                                                                                                                                                                                            | Select 19 MeV p                                                                |                     |                                                           |             |             |  |
| 15:00 - 15:30*                                                                                                                                                                                                                                           | 5/10                                                                           | p/19                | N <sub>2</sub> /C11(CGP)PET I                             | DJB/168     |             |  |
| 15:30 - 16:00                                                                                                                                                                                                                                            | Select 9.5 MeV d                                                               |                     |                                                           |             |             |  |
| 16:00 - 17:45*                                                                                                                                                                                                                                           | 5/6                                                                            | d/9.5               | N <sub>2</sub> /O15(CO <sub>2</sub> ,CO)PET I             | JM/170      |             |  |
| 17:45 - 18:30                                                                                                                                                                                                                                            | Select 52 MeV He3                                                              |                     |                                                           |             |             |  |
| 18:30 - 19:30                                                                                                                                                                                                                                            | 5/7                                                                            | He3/52              | Cr/Fe52                                                   | MLS/HRK/120 |             |  |
| 19:30 - 22:00                                                                                                                                                                                                                                            |                                                                                |                     |                                                           |             |             |  |
| 22:00 - 24:00                                                                                                                                                                                                                                            | No shift                                                                       |                     |                                                           |             |             |  |
| <b>Friday 24 January</b>                                                                                                                                                                                                                                 |                                                                                |                     |                                                           |             |             |  |
| 00:00 - 05:00                                                                                                                                                                                                                                            | No shift                                                                       |                     |                                                           |             |             |  |
| 05:00 - 05:30                                                                                                                                                                                                                                            | Select 33 MeV He4                                                              |                     |                                                           |             |             |  |
| 05:30 - 07:30                                                                                                                                                                                                                                            | 5/12                                                                           | He4/33              | NaBr/Rb81                                                 | SAC/JM/150  |             |  |
| 07:30 - 08:00                                                                                                                                                                                                                                            | Select 19 MeV p                                                                |                     |                                                           |             |             |  |
| 08:00 - 08:30***                                                                                                                                                                                                                                         | 5/9                                                                            | p/19                | H <sub>2</sub> O/F18(FDG)PET II                           | NS/150      |             |  |
| 08:30 - 10:15                                                                                                                                                                                                                                            | PET Neurology study PET I CB/XXX                                               | 00:00 = 09:15       |                                                           | SAC/170     |             |  |
| 10:15 - 12:15                                                                                                                                                                                                                                            | PET Cardiology study PET I NU/XXX                                              | 00:00 = 11:15       |                                                           | SAC/170     |             |  |
| 12:15 - 12:45                                                                                                                                                                                                                                            | Select 19 MeV p                                                                |                     |                                                           |             |             |  |
| 12:45 - 13:00                                                                                                                                                                                                                                            | 5/4                                                                            | p/19                | N <sub>2</sub> /C11(NSC)                                  | GB/DRT/168  |             |  |
| 13:00 - 13:30o                                                                                                                                                                                                                                           | 5/4                                                                            | p/19                | N <sub>2</sub> /C11(DPR)PET I                             | JM/168      |             |  |
| 13:30 - 14:00                                                                                                                                                                                                                                            | Select 9.5 MeV d                                                               |                     |                                                           |             |             |  |
| 14:00 - 14:30o                                                                                                                                                                                                                                           | 5/6                                                                            | d/9.5               | N <sub>2</sub> /O15(CO <sub>2</sub> )PET I                | MJM/170     |             |  |
| 14:30 - 15:45                                                                                                                                                                                                                                            |                                                                                |                     |                                                           |             |             |  |
| 15:45 - 18:00                                                                                                                                                                                                                                            | PET Neurology study PET I HJ/XXX                                               | 00:00 = 16:45       |                                                           | MJM/170     |             |  |
| 18:00 - 18:30                                                                                                                                                                                                                                            | Study overrun                                                                  |                     |                                                           |             |             |  |
| 18:30 - 19:45                                                                                                                                                                                                                                            |                                                                                |                     |                                                           |             |             |  |
| 19:45 - 24:00                                                                                                                                                                                                                                            | No shift                                                                       |                     |                                                           |             |             |  |
| Duty Engineer: MLR Operators Night: GCT/JG Early: NWM/JP Late: RGH/JC                                                                                                                                                                                    |                                                                                |                     |                                                           |             |             |  |
| * C11 for                                                                                                                                                                                                                                                | PET Raclopride study Wed PET I NT/XXX and Biology                              |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | 00:00 = 14:45 (CO <sub>2</sub> ) 15:00(RCP) [Met anal]                         |                     |                                                           |             |             |  |
| ** F18 for                                                                                                                                                                                                                                               | PET 6F DOPA Studies PET I Tues DBn, Wed NT, Thurs GS                           |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | [metabolites Wed & Thurs] 00:00 = EOB + 2½ hours                               |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | PLEASE NOTE CHANGE OF TIMING TUESDAY, 5 FU PRECEDES DOPA                       |                     |                                                           |             |             |  |
| *** F18 for                                                                                                                                                                                                                                              | PET FDG studies Tues & Fri                                                     |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | Tues PET II AK study 00:00 = 10:30 approx FDaG (no gases)                      |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | RMS & GYS despatch time 10:30 approx.                                          |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | Fri PET II AK study 00:00 = 11:00 approx. FDG (no gases)                       |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | PET I NU study 00:00 = 11:15 (gases) 12:15(FDG)                                |                     |                                                           |             |             |  |
| + C11 for                                                                                                                                                                                                                                                | PET PK 11195 study Tues PET II SR/XXX                                          |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | 00:00 = 14:15 (H <sub>2</sub> <sup>15</sup> O) 14:30 (PK)[Met anal]            |                     |                                                           |             |             |  |
| +++ C11 for                                                                                                                                                                                                                                              | PET Flumazenil studies Mon & Thurs PET II MP/XXX                               |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | Monday 00:00 = 14:15 (FLU) only [Met anal]                                     |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | Thursday 00:00 = 14:15 (H <sub>2</sub> <sup>15</sup> O) 14:30 (FLU) [Met anal] |                     |                                                           |             |             |  |
| o C11 for                                                                                                                                                                                                                                                | PET Diprenorphine study Fri. AKPJ                                              |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | Friday 00:00 = 14:15 (CO <sub>2</sub> ) 14:30 (DPR)[Met anal]                  |                     |                                                           |             |             |  |
| o* C11 for                                                                                                                                                                                                                                               | PET CGP studies PET I Mon JMBH/XXX and Thurs PC/XXX                            |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | Monday 00:00 = 14:45 (CO) 15:10 and 15:40 (CGP)                                |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | Thurs 00:00 = 16:15 (CO <sub>2</sub> ) 16:35 and 17:00(CGP) 17:30(CO)          |                     |                                                           |             |             |  |
| **o F18 for                                                                                                                                                                                                                                              | PET 5 FU study Tues PET I, OT/XXX                                              |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | 00:00 = 11:45 (CO <sub>2</sub> ) 12:00 (5FU) [met anal]                        |                     |                                                           |             |             |  |
| wbc F18 for                                                                                                                                                                                                                                              | 5 FU study on whole body counter Tues OT/XXX                                   |                     |                                                           |             |             |  |
|                                                                                                                                                                                                                                                          | 00:00 = 12:00 approx. or later.                                                |                     |                                                           |             |             |  |
| Circulation: KIG, CJP, JEC, RSJF, TJ, AAL, TJS, NU, CGR, OT, CAJF, Sun Rm, 114 NB, PET NB, CJVT, GCL, ADW, ASOR, 112M, KRB, CJP, PJNA, DBM, ENG NB, MLR, MLS, C ROOM, 15 X 2, JCC, SO KGPP, 244, Chem NB, KD, CJS, DJS, SLW, FB, VWP, SKL, DRT, Annex NB |                                                                                |                     |                                                           |             |             |  |

Table 2: PET Schedule. Week 4, 19-24 January, 1992

| Week<br>4                    | SCANNER ONE                                                                                                                                                                | Zero<br>time                                       | Chm                                  | Dr                   | SCANNER TWO                                                                              | Zero<br>time            | Chm              | Dr        |
|------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|--------------------------------------|----------------------|------------------------------------------------------------------------------------------|-------------------------|------------------|-----------|
| <b>MON<br/>20th<br/>JAN</b>  | <b>CYCLOTRON MAINTENANCE</b>                                                                                                                                               |                                                    |                                      |                      |                                                                                          |                         |                  |           |
|                              | Pulmonary.. $C^{15}O$<br>$^{11}C$ -CGP<br>Neuro(schizo).. $C^{15}O_2$ *                                                                                                    | 15:45<br>16:10<br>18:30                            | NS<br>DJB<br>NS                      | MH<br>CF             | Neuro(epilepsy).. $^{11}C$ -Flumazenil M                                                 | 14:15                   | DRT              | MP        |
| <b>TUE<br/>21st<br/>JAN</b>  | Oncology..... $C^{15}O_2$<br>$^{18}F$ -SFu M<br>Neuro(mve dis).. $^{18}F$ Dopa *<br>Cardiac..... $C^{15}O$ , $C^{15}O_2$<br>Neuro(dep P.D.).. $C^{15}O_2$                  | 11:45<br>12:00<br>13:30<br>16:15<br>17:45          | JM<br>GB<br>MJM<br>HRK<br>HRK        | OT<br>DB<br>NU<br>HR | Neuro(dementia).. $^{18}F$ F.D.G.<br>Neuro(M.S.).. $H_2^{15}O$<br>$^{11}C$ -PK11195 M    | 10:30<br>14:15<br>14:30 | SAC<br>NS<br>DJB | AK<br>SR  |
| <b>WED<br/>22nd<br/>JAN</b>  | Neuro(mve dis).. $^{18}F$ Dopa *<br>Neuro(mve dis).. $C^{15}O_2$<br>$^{11}C$ -Raclopride M<br>Neuro(dementia).. $C^{15}O_2$ , $^{15}O_2$ , $C^{15}O$                       | 11:30<br>14:45<br>15:00<br>17:00                   | MJM<br>DJB<br>HRK<br>DJB             | NT<br>NT<br>AK       | Neuro(normal).. $H_2^{15}O$ *<br>Biology.. $^{11}C$ -Raclopride                          | 10:00<br>15:00          | JM<br>HRK        | JW<br>AAL |
| <b>THUR<br/>23rd<br/>JAN</b> | Neuro(mve dis).. $^{18}F$ Dopa M<br>Cardiac.. $C^{15}O_2$<br>$^{11}C$ -CGP<br>$C^{15}O$                                                                                    | 11:30<br>16:15<br>16:35<br>17:00                   | NS<br>JM<br>DJB<br>JM                | CS<br>PC             | Neuro(normal).. $H_2^{15}O$ *<br>Neuro(epilepsy).. $H_2^{15}O$<br>$^{11}C$ -Flumazenil M | 10:00<br>14:15<br>14:30 | SAC<br>JM<br>DRT | SR<br>MP  |
| <b>FRI<br/>24th<br/>JAN</b>  | Neuro(normal).. $C^{15}O$ *<br>Cardiac.. $C^{15}O_2$ , $C^{15}O$<br>$^{18}F$ D.G.<br>Neuro(pain).. $C^{15}O_2$<br>$^{11}C$ -Diprenorphine M<br>Neuro(normal).. $C^{15}O_2$ | 09:15<br>11:15<br>12:15<br>14:15<br>14:30<br>16:45 | SAC<br>SAC<br>NS<br>MJM<br>JM<br>MJM | PG<br>NU<br>AJ<br>HJ | Neuro(dementia).. $^{18}F$ F.D.G.                                                        | 11:00                   | NS               | AK        |

M = METABOLITES

\* = NO SAMPLES

## "R2D2", A Bedside [ $^{15}\text{O}$ ]Water Infuser

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### Clinical Requirement

- "Hands off" infuser for flow studies in brain and heart.
- Must be user-friendly.
- Must be safe and effective for patient use.

### A Practical Solution

- Produce [ $^{15}\text{O}$ ]H<sub>2</sub>O vapour at the bedside.
- Introduce [ $^{15}\text{O}$ ]H<sub>2</sub>O into normal saline infusate using semi-permeable membrane exchanger (VISKING regenerated cellulose\*).
- Deliver normal saline using medically approved

pump IVAC 560.

- Two fluid handling valves for control of infusion parameters 24 Volt ANGAR/ASCO 368 3-port membrane valves.
- On-line radioactivity detector based on a loop of 1/16" PTFE around PHILIPS ZP-1300 GM tube.
- Counts acquired into MINI-INSTRUMENTS 6-90 scaler ratemeter modified with adjustable pre-scaler to allow direct calibration in "mCi infused".
- All controls and power supplies designed and built to comply with electromedical safety standards.
- Non-used product allowed to decay within the unit before discharge into sterile bag.

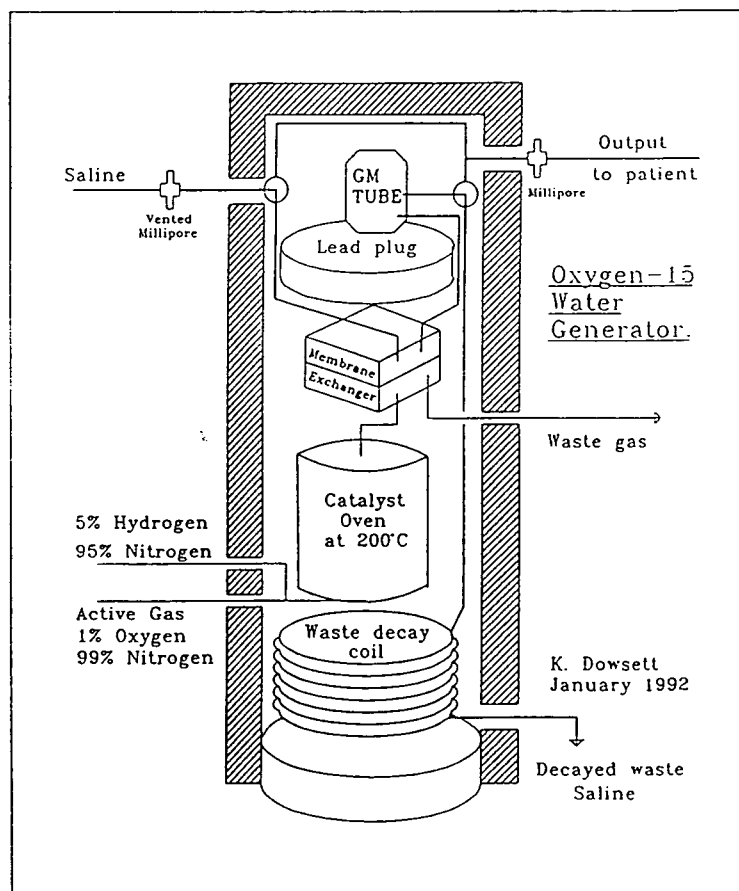


Figure 1: Schematic Drawing of the R2D2 Infuser.

\* VISKING - Mediceil International, Fax 071 700 4156, U.K.

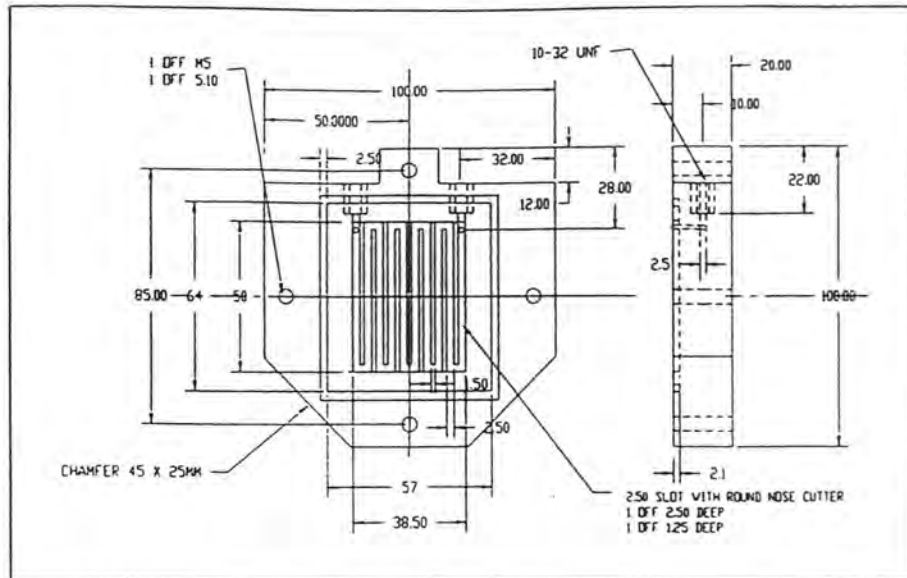


Figure 2: Membrane Exchanger in the R2D2 Infuser. Material: Perspex DWG No. 15.

### Typical Operating Parameters

Infusion for 2 minutes at flow rate of 10 ml/min. Infused radioactivity determined by PET scanner mode, typically:

SEPTA OUT - 15 mCi  
SEPTA IN - 80 mCi

GM counter calibration checked against standard ionization chamber routinely.

### Pharmaceutical Maintenance

Membrane exchanger cleaned and sterilised prior to

assembly. Fluid system washed with 0.9 % NaCl solution with Millipore filters in place. Pyrogen tests (LAL) and sterility tests carried out to validate assembly techniques. Routine operation involves replacement of disposables - pump tube - saline bag - Millipore filters and infusion catheters. Followed by LAL test.

### Current Status

R2D2 is in routine use in PET Brain Activation Studies and has been well received by the users.



Figure 3: Photographic View of the  $[^{15}\text{O}]\text{H}_2\text{O}$  Bedside Infuser R2D2.



Figure 4: Further Automation Trends at Hammersmith in the Planning Phase.

# Automated Carbon-11 Radiopharmaceutical Production

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## Control System

The Hammersmith methylation systems are controlled by a TOSHIBA EX40+ programmable controller with an EX40E expansion unit. This provides 48 inputs and 32 relay outputs. These devices have been programmed using ladder logic, a system based on relay type logic with open and closed contacts and relay coils. There is also a bi-directional shift register which sequences the operations. Programmable controllers were selected for reliability which at present is very good.

## Sequence of Operations (Fig. 1)

### „Set-up“ procedure.

- Purge apparatus with nitrogen, load lithium aluminium hydride, hydriodic acid, and precursor. Load syringes 17 and 26.
- Nitrogen flows through valves 2, 3, 4, 6 and 7 and also through 11 and 12. 200  $\mu$ l of  $\text{LiAlH}_4$  in THF is placed in the methyl iodide pot, and 200  $\mu$ l of HI

loaded into the loop controlled by valve 10.

- Approximately 2 mg of precursor in 500  $\mu$ l of DMSO + base is loaded into the methylation vial. Load syringe 17 with 9 ml of water and load syringe 26 with 10 ml of isotonic saline.

### „Run“ procedure.

- Trap activity. Trap is lowered into liquid argon, then valves 1 and 4 actuate to empty the target. Nitrogen flow continues through valves 2, 3, 6 and 7 and also through 11 and 12.
- Dispense activity. The trap is raised and nitrogen passed through it from valve 2. The carbon dioxide is carried through valve 6 and into the  $\text{LiAlH}_4$ . The nitrogen is vented through valve 7.
- Make methyl iodide. The THF is removed by heating, nitrogen flows through valves 2, 3, 4, 6 and 7. When the evaporation is finished the pot is cooled by air through valve 9. After cooling the HI is added through valve 10 under nitrogen pressure. The pot

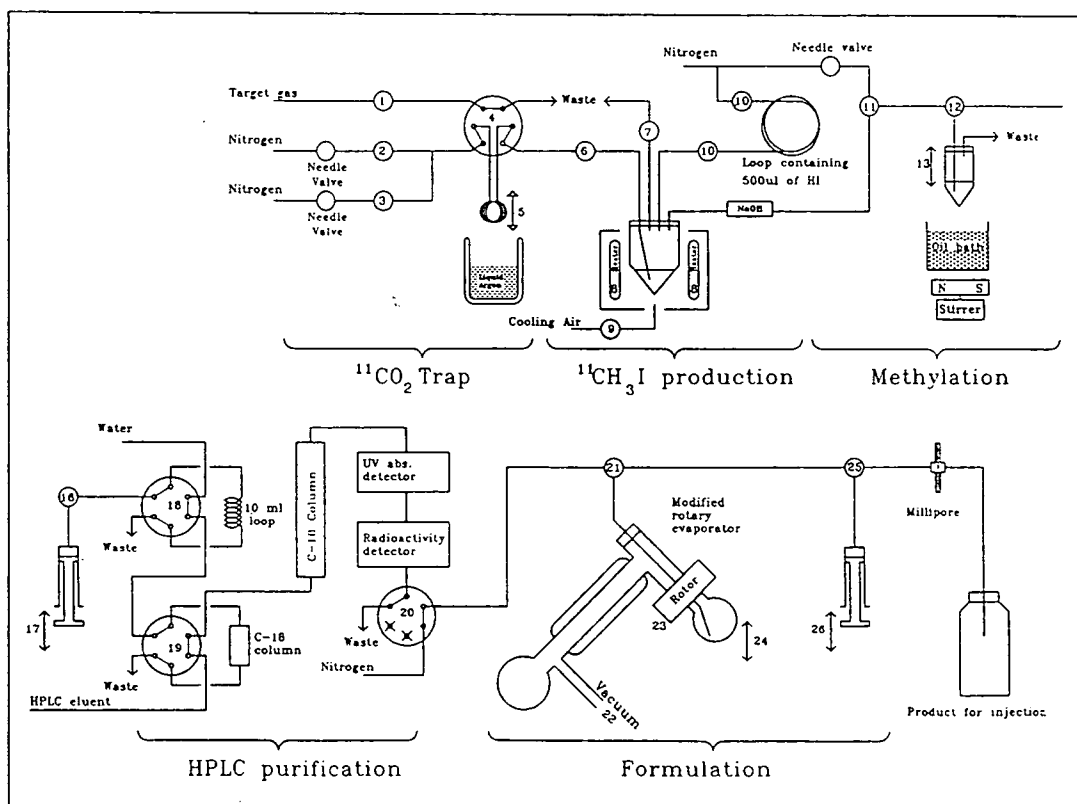


Figure 1: Schematic diagram of the automated radiopharmaceutical production.



- is then sealed and heated to 165°C for 2 minutes to convert any remaining methanol into methyl iodide.
- Methylate the precursor. Switch off nitrogen flow through valve 11 and distil methyl iodide through valves 11 and 12 into the precursor. After 5 seconds switch on nitrogen through valves 2, 4 and 6. After 1 minute lower vial into oil bath and heat to 90°C for 5 minutes. During this heating cool the methyl iodide pot with air through valve 9.
  - Purify precursor by HPLC. Raise the methylation vial and withdraw the contents through valves 12 and 16 into syringe 17. Wash the vial with water from syringe 17, then load the contents into the loop on valve 18. Switch valve 18 to transfer the contents on the loop onto the short C-18 column. Wash with water for 5 minutes. Switch valve 19 to wash the organic products onto a semi-prep C-18 column. The effluent from the C-18 column is monitored by UV and radioactivity detectors and valve 20 actuated by the operator to select the product.
  - Formulate the product. The eluent from the column is transferred through valves 20 and 21 into the rotary evaporator where the HPLC eluent is re-

moved. 10 ml of isotonic saline is then added from syringe 26. This is sucked out, then passed out of the cell and through a Millipore 0.22 µm filter into a sterile vial.

#### Labelled Compounds

This type of system has been used to methylate the following compounds:

- SCH 23390
- Deprenyl
- Raclopride
- PK 11195
- Flumazenil

A similar system with an additional deprotection stage (hydrolysis) has been used to label Diprenorphine.

#### Yield

A typical preparation will start with > 50 GBq of [<sup>14</sup>C]CO<sub>2</sub>. 45 minutes later this will yield around 2.7 GBq of labelled product. This typically has a specific activity in the region of 7-10 GBq/µmol @ 60 minutes EOB.

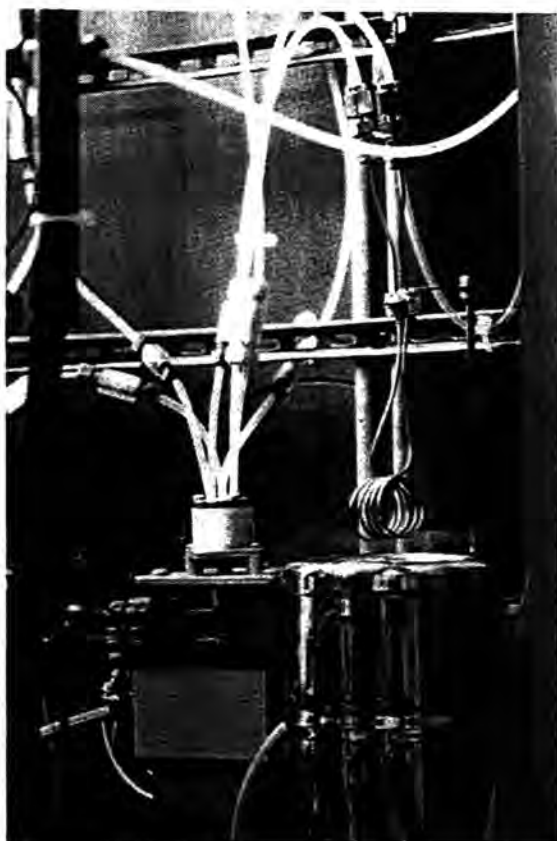


Figure 2: Carbon dioxide trap. This trap is constructed from 600 mm of 1/16" stainless steel tubing. This is cooled in liquid argon to prevent nitrogen condensation. Efficiency is > 99%.

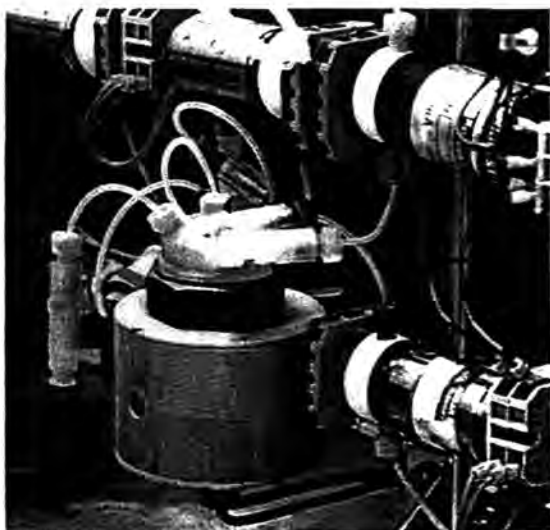


Figure 3: Methyl iodide reaction pot. This pot contains 200 µl of a saturated solution of lithium aluminium hydride in THF. This is prepared in a nitrogen glove box on a weekly basis. The complex formed by reaction with carbon dioxide is hydrolysed by addition of 200 µl of 55% hydriodic acid which is transferred under gas pressure from a loop above the pot.

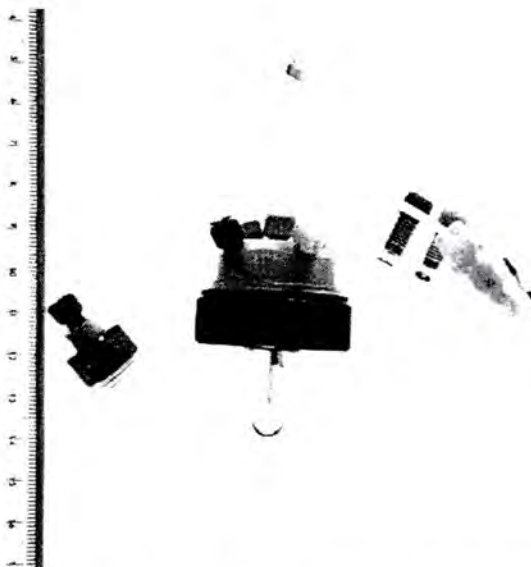


Figure 4: The methyl iodide pot is shown here life size. The lid is made of Kel-F with five 1/4-28 ports machined into it. It is sealed to the glass reaction pot by a teflon coated silicone rubber seal ring. Attached to the outlet is a trap which holds sodium hydroxide. This is used to prevent hydriodic acid distilling into the precursor with the methyl iodide.

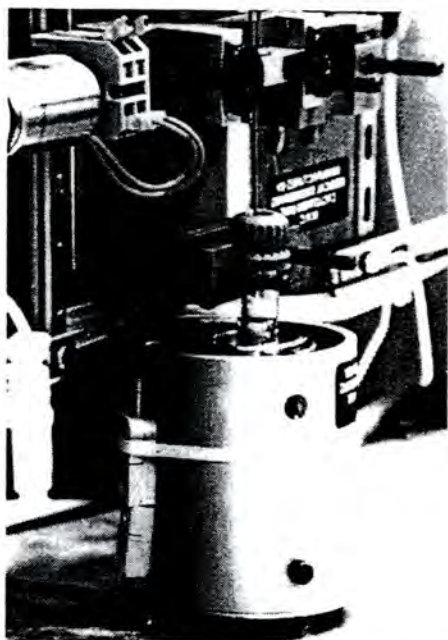


Figure 5: Methylation vial. The vial is sealed with a teflon faced septum which is pierced by two round nosed needles. Methyl iodide is distilled from the reaction pot into a vial containing the precursor in strong base. This is then lowered into the oil bath, and the needles withdrawn from the septum. The sealed vial is then heated to complete the reaction.

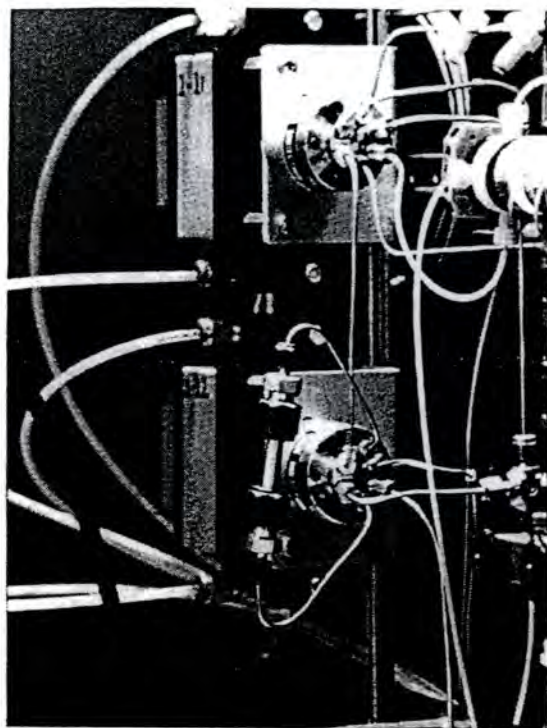


Figure 6: HPLC system. The reaction mixture is first loaded into a 10 ml loop then transferred to a short C-18 column. This retains organic compounds but ionic materials are washed to waste. The organic materials are then removed with a different solvent and transferred to an HPLC column.

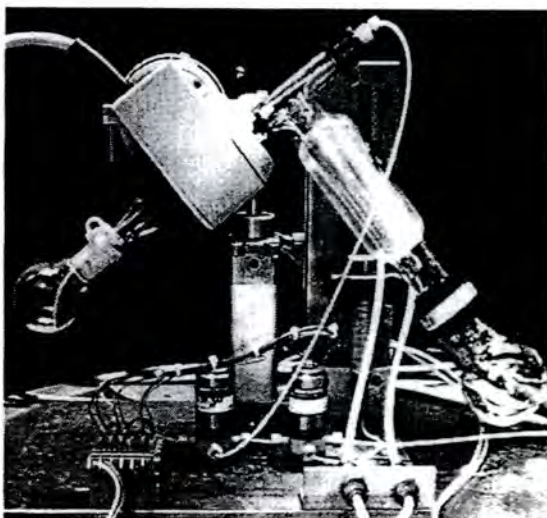


Figure 7: Formulation hardware. This is based on a modified rotary evaporator with a dip tube to allow liquids to be sucked out. It is mounted on an air cylinder with an oval piston to prevent rotation.

## Introduction: State of the Art in Automated Syntheses of Short-Lived Radiopharmaceuticals

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The goal of this session was to discuss automation as it currently exists in the field of radionuclide and radiochemical synthesis. Emphasis was placed on the problems participants have encountered in implementation of automation. Group participation was encouraged both to define the problems which require solutions and to present alternative solutions to those problems. In order to focus these efforts, the session began with a presentation of varying philosophies regarding arguments for and against automation. The session then worked through specific areas of radionuclide production and synthesis which are worth automating. These included target filling, transfer of materials to and from targets, additions, mixing, transfers into and out of reaction vessels, evaporations, incubations, and separations. The latter part of the session dealt with automation of quality control methods including formulations, set-up and clean-up.

### Approaches to Automation

John CLARK (Hammersmith Hospital, London) began the discussion by presenting his views of „What is automation, and why do we automate?“ He defined automation, very broadly, as anything that gets our hands out of the system. First and foremost, an automated system should be reliable. This includes both the mechanical system and the synthesis itself. Second, automation involves significant time and expense. It is not economically sensible to automate any radiosynthesis which won't be performed many times. One must decide if the clinical demands will be sufficient to justify the effort.

Jeanne LINK (Univ. of Washington, Seattle) categorized radiosyntheses in four ways:

- Manual syntheses where the chemist's hands perform transfers and additions;
- Remote syntheses where valves or manipulators are used to effect the transfers and additions;
- Remote automated syntheses where a computer is used to control the switching of valves of a remote process. The computer may be as simple as a sequence timer or as complex as a decision making computer. These systems can be dedicated to one synthesis or modular.
- Robotic syntheses which uses a mechanical arm to move the reaction vessel rather than just moving chemicals in and out of the reaction vessel. There can be significant complementarity between robotic and remote automated systems.

Manual radiosynthesis is undesirable, exposure to radiation should be avoided when possible. Yet almost all of our facilities still include some manual synthesis work. Why? It is a matter of resources; *i.e.* personnel time and skills, and equipment and supply costs. Manual synthesis is the fastest and least expensive method for implementing a radiosynthesis. If for example, a manual synthesis required \$15,000 in equipment and supply costs and a half-year of a chemist's time to develop the synthesis, the costs for remote systems would be at least as numbered in Table 1.

These are only baseline costs. The costs for remote systems typically are valves and tubing. Robert DAHL (North Shore Hospital, Manhasset) said one remote manipulator arm costs \$15,000. For a remote automated system or a robotic system more typical costs are \$70,000 to \$100,000. There are a few automated systems on the market (CTI, SCANDITRONIX, DANATEC, JSW, NKK, SUMITOMO *etc.*) and their costs are similar. However, there is more to be considered than initial capital costs. Each approach to synthesis has definite advantages and disadvantages.

LINK uses both a ZYMARK robotic system for  $^{11}\text{C}$  and  $^{18}\text{F}$  syntheses and dedicated remote systems for production of FDG and  $^{15}\text{O}$  compounds. She argued that the decision as to type of system to be used for a

Table 1: Comparison of Costs Between Different Levels of Automation

|                                           | Remote    | Remote Automated | Robotic   |
|-------------------------------------------|-----------|------------------|-----------|
| Initial Supplies Equipment                | >\$20,000 | >\$21,000        | >\$30,000 |
| Personnel (FTE years) Initial Development | 0.6       | 1.0              | 1.0       |
| Personnel (FTE years) Annual Support      | 0.1-0.2   | 0.25             | 0.25      |

Table 2: Advantages and Disadvantages of the Different Levels of Automation

|                  | Advantages                                                                                             | Disadvantages                                                                                               |
|------------------|--------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|
| Manual           | Flexibility is high<br>Cost is low<br>Cost to maintain is low                                          | High radiation exposure to chemists<br>Reliability can be low                                               |
| Remote           | Lower radiation exposure to chemists<br>Cost to maintain is still low                                  | Cost to develop is greater than for manual<br>Requires more space than a manual synthesis                   |
| Remote Automated | Low radiation exposure to the chemists<br>High reliability<br>Often useful for more than one synthesis | Initial cost to develop is greater than for manual or remote<br>Requires more space than a manual synthesis |
| Robotic          | Low radiation exposure to the chemist<br>High reliability<br>Useful for more than one synthesis        | Initial costs to develop are high<br>Requires more space than other systems                                 |

synthesis has to be matched to the resources and needs of an individual group and no single method is optimal for every situation.

DAHL offered additional views on automation: He defined an automated system as an „apparatus which, after a signal to perform a complex series of operations, does so with no further human intervention“. He gave two reasons to automate:

- to reduce personnel exposure to radioactivity and
- to reduce the time requirement for personnel to perform radiosynthesis.

In that light he felt that the only syntheses worth automating are high dose, well-defined procedures. You automate a synthesis by defining the process, selecting the appropriate apparatus and then developing the computer program and testing it. Automated systems should be simple to use and reduce exposure to radiation. DAHL uses a remote non-automated system for production of FDG. It takes no more or less time to do FDG by their system than by others; two hours. They use a plywood board with the synthesis vessels and tubing mounted on the front. They use stopcocks for valves and turn the stopcocks with remote manipulators. After the synthesis, they let the  $^{18}\text{F}$

decay and clean it out. With regard to the remote FDG synthesis used by DAHL, CLARK asked how many people are skilled enough to use manipulators? DAHL acknowledged that could be a disadvantage, many people do not have the experience or dexterity to operate them efficiently and reliably.

CLARK summarized his approach to automation. First, he figures out how to do the synthesis. Then he adds switches. He then programs the sequence of events, time, temperature and critical steps. He likes to use programmable logic controllers (PLC's) as controllers. His systems are built to be as space efficient as possible. For example, he packs at least two complete methylation systems in one hot cell; the only common point being the  $\text{CO}_2$  trap. He has independent PLC controlled set-up procedures for each synthesis. An operator stays close by to oversee HPLC product peak collection. His limitation is that he can't prepare the hot cell quickly for the next synthesis, due to radiation dose. Setting up an automated clean-up is an important future goal.

Greg GAEHLE (Washington University, St. Louis) presented some comparisons between two robotic systems, HUDSON and ZYMARK, for radiosynthesis [1,2].



GAEHLE prefers the HUDSON system, primarily for two reasons:

- The HUDSON robot software runs in a Windows environment and allows parallel syntheses. This is more convenient than the ZYMARK programming system. While the ZYMARK commands must be programmed in a linear fashion, programming of the HUDSON can follow branching paths.
- The HUDSON robot is roof mounted, thus saving floor space. They use their robot in a hot cell with 10 ft<sup>2</sup> of floor space.

He set up FDG automation in 1 month, although they had previous experience automating the synthesis of FDG using a ZYMARK robot. He learned the programming language in three days. He showed pictures of the robot in a mobile (on wheels) hot cell. GAEHLE also described the 18 stations which comprise the St. Louis robotic system: a robot arm, homing station, pipette unit, three dual liquid dispensing units (HAMILTON), balance (METTLER), capping station (HUDSON ROBOTICS), Vortex mixer (FISHER SCIENTIFIC), liquid/solid extraction station (ZYMARK), two HPLCs (SSI) with automated injectors, UV detectors, and two radiation detectors (BECKMAN), dose calibrator (ATOMIC PRODUCTS), two heating stations, a nitrogen purge device, fraction collection station (GILSON), filtration station, pipette shucker, waste receptacle, three vessel racks, cold bath, and shielded receiving and collection vessels.

Christian CROUZEL (Hôpital F. Joliot, Orsay) presented the Orsay philosophy regarding automation. They consider three situations when they automate systems:

- Routine hospital syntheses,
- Research syntheses, and
- Mixed routine / research syntheses.

A remote control system is used to produce CH<sub>3</sub>I. They have designed their system to be flexible so that other precursors, including acetone and acid chlorides, are made using one single remote system. They handle many mCi of FDG each day and they also use a remote automated system for this synthesis. They use a ZYMARK robot for production of other <sup>18</sup>F compounds, including fluorospiperone. CROUZEL stated that it is difficult to produce CH<sub>3</sub>I, because it is necessary to work with liquids. He recommended that if we are going to continue to rely on CH<sub>3</sub>I as a major precursor in <sup>11</sup>C synthesis then we should devise a method to make it directly from [<sup>11</sup>C]methane and then automate that synthesis as a gas.

Regin WEINREICH (PSI Villigen) gives priority to practical questions in automation, namely the frequency of expected production runs, and the finger doses to the personnel (The regulatory authorities also like automation as a kind of guarantee for unchanged production runs, but there is no fixed rule in Switzerland).

From this consideration, the PSI group automated 3 processes:

- [<sup>18</sup>F]2-FDG. It is believed that 2-FDG will be the main PET compound for the next decade. Further, they have delivery commitments to external FDG users. Thus, the philosophy was to construct a simple reliable compact apparatus for 2-FDG as a „working-horse“ with the option to change the reaction process by simple rearrangement of the valves and simple re-programming.
- [<sup>11</sup>C]CH<sub>3</sub>I. Methylation is the main process for introducing <sup>11</sup>C into biomolecules. Thus, it was decided to construct a synthesis apparatus with similar specifications as for 2-FDG, but with the option to extend to further labelling steps. This has been realized for [<sup>11</sup>C]raclopride.
- <sup>52</sup>Fe. The reason for construction of an automated apparatus for separation of longer-lived <sup>52</sup>Fe from irradiated Ni targets is the handling of very high activities of <sup>52</sup>Fe in order to increase its specific activity for some labelling purposes.

For realization of its ideas, the PSI group cooperates with DANATEC AG. PSI follows consequently the guideline that technical improvement must go along with a continuous improvement of quality control procedures.

There was considerable audience discussion regarding these approaches to automation and costs involved in different levels of automation. Bruno NEBELING (KFA Jülich) objected to generalizing the costs for automation; he felt that they were unnecessary and inaccurate. Jean-Luc MORELLE (IBA, Louvain-la-Neuve) thought that the cost estimates were reasonable. Karl ERDMAN (EBCO, Vancouver) suggested that one could use industrial robots which are less expensive than the ZYMARK, HUDSON or SCANDITRONIX robot systems. Kenneth KROHN (Univ. of Washington, Seattle) countered that while industrial robots cost less to purchase than the commercial laboratory robotic systems, their research group has both a ZYMARK system and an industrial robot (SCORBOT), and the latter has required much more work, *i.e.* time and money, to program and set up for radiopharmaceutical syntheses. CLARK concurred and recalled how much trouble SCANDITRONIX has had adopting the industrial ANATECH robot for radiopharmaceutical use. Johan ULIN (SCANDITRONIX, Uppsala) concurred. MORELLE had experience using robots early on in industrial electronics assembly and said using robotics for radiochemistry is quite different. There is much more than moving things involved in automation of a radiosynthesis. Industrial robots have been used at Karolinska Hospital in Stockholm [3]. Volker BECHTOLD (KfK Karlsruhe) and LINK stated that the cost of developing an individual synthesis would decrease when automation was developed as an assemblage of modules. In that situation, a single module operation such as liquid transfer or solid phase extraction could be used for multiple syntheses.

CLARK commented further on the advantages and disadvantages of different types of automation: „Many people have a wrong expectation of robots, if you want to do something lots of times then go to a dedicated system, if not go to a robot. Robots can in principle do anything, but they are very hard to automate.“ He agreed that the advantage of a robot is versatility, but asked if there wasn't a cross-contamination problem. Do you run the risk of cross-contaminating samples from one synthesis to another by using shared stations, hands, *etc.*? LINK agreed that the potential for contamination is real, but one should always be careful to never use the same transfer material, pipet tips, tubing, needles, *etc.* from one synthesis to another. Marc BERRIDGE (Univ. Hosp. of Cleveland) also felt that the flexibility available from robots is an advantage over dedicated systems.

Andrei SEBYAKIN (KURCHATOV Inst., Moscow) argued that one of the advantages of automated systems is that they require less sophisticated people to run them. An automated system should be „fool proof“, it should not require the same skill to operate as would a manual synthesis, or it is a waste of money. He was also concerned that it is difficult to standardize computer instruction from different sources. There are lots of costs in connecting to different source units (manufacturers). One should put intellect into a „really good“ system. Automation systems will not be cost effective if there is not a big demand and this will require standards in hardware and software. GAEHLE thought standardization was an unobtainable goal, manufacturers will be continually upgrading and changing their own products and are in competition with others. He felt that uniform hardware and software standards weren't necessary to meet SEBYAKIN's goals. He has set up and oversees a synthesis that he taught to a technician with no experience in chemistry for a cost of \$50,000 plus personnel time. SEBYAKIN countered with the argument that you pay now, then the procedure changes and you pay again. He restated the need to set standards for the industry. LINK said that this applied to both robots and automated boxes. One needs to pick a system which is the best fit to the number and type of syntheses and the resources available for the individual lab. Attempting to get everyone to use a single computer standard is akin to arguing religion. MORELLE agreed. A significant comment was offered by Rainer WAGNER (MAX-PLANCK Institute, Köln) who objected to referring to technicians in the laboratory as „inexperienced“ personnel. We need to hire and train people who know what they are doing in the laboratory; the personnel who work in his lab are competent to work with their syntheses.

BERRIDGE feels that a good system for performing the FDG synthesis by the Jülich method should also be useful for synthesizing other compounds made with fluoride using standard nucleophilic substitution reactions. Such a system under computer control could be programmed for the variations between such syntheses

with at least as much ease as a robotic system: Therefore, multi-synthesis use alone is not necessarily a factor which should move one to a robotic system. A similar „black box“ synthetic system has been constructed for the preparation and use of methyl iodide. It has been used not only to perform several methyl iodide methylations, but also GRIGNARD based procedures using labeled acetone. It might be used for other  $^{14}\text{C}$  syntheses. It seems that a general system could be designed for synthesis of several different preparations.

A general use system can be designed and built as long as the basic steps for the syntheses under consideration are the same, *i.e.* the reagents added, evaporations, and purifications via chromatography require the same number of reaction vessels. This had been demonstrated by the multiple syntheses performed with the CTI Chemistry Process Control Unit. However, there will always be trade-offs between efficiency, cost and general utility. One must always assess the function for which an automated system will be used and decide whether flexibility or routine use is the requirement.

The discussion returned to the comparison of two robot systems presented by GAEHLE. KROHN objected to his argument about the space advantage of the HUDSON robot. He commented that one can pack lots in a hot cell with either robot if you consider the full three-dimensional space. John SUNDERLAND (Univ. of Nebraska, Omaha) was curious about the mobile hot cell and asked if they bolted down the robot to maintain positioning. GAEHLE said not yet, but they would in the future. SUNDERLAND also asked how they did liquid transfers. They used pipets. GAEHLE was also asked how they maintain sterility? He said that they use sterile filtration for all of their products.

Tom RUTH (TRIUMF, Vancouver) asked; how many syntheses could be performed per hot cell „door opening“ using a robot? LINK said that they only programmed the ZYMARK to run one synthesis at a time, but more could be programmed. Two syntheses can be run concurrently using the ZYMARK robot at St. Louis and GAEHLE said they are working to automate clean-up after a synthesis and set-up for sequential syntheses so that the door doesn't need to be opened except to occasionally replenish supplies. DAHL was concerned that only one synthesis in one enclosure will be allowed by the FDA. There was a big protest from the audience that this didn't make sense. Michael HAKA (SUNY, Buffalo) said „the FDA doesn't think logically“. People agreed that it was more important to reduce exposure.

### Transfer In and Out of Targets

Richard FERRIERI (Brookhaven Natl. Lab) presented the automation work they have done with regard to making and delivering [ $^{18}\text{F}$ ]fluoride reliably to the labo-

ratory. In order to accomplish this they have automated filling of the fluoride target in a system which they presented as a poster as well as described in this meeting [4]. The system includes:

- A Pt wire conductivity sensor in the top target outlet which has direct feedback through conductivity response to insure that the target is filled.
- An automated resin / recovery system, located in the vault. This insures reliable [ $^{18}\text{F}$ ]fluoride production and delivery. The system maintains the chemical and isotopic integrity of [ $^{18}\text{O}$ ]water. It eliminates the transfer of irradiated [ $^{18}\text{O}$ ]water to the Hotlab. In the area of radiation safety, long-lived radionuclides residing in the irradiated target water, remain contained in the vault.

The conductivity sensor remains on during the filling and emptying of the target, but is turned off during irradiation. The sensor is a detector they had in the lab, platinum wire in polyethylene tubing, with a 25 VAC supply. FERRIERI preferred small wires that would fit within a 1/16 inch tube. The wire is kept out of the path of the beam. He also suggested that the conductivity detector could be used to show the degree of fullness of the target if the sensor were in a >> configuration.

There was considerable difference of opinion as to the usefulness of the conductivity detector. JÖRG STEINBACH (FZ Rossendorf, Dresden) uses a conductivity detector similar to that of FERRIERI, but also has an HPLC type valve attached to his target inlet which he closes during irradiation to hold the water in the target. CLARK argued that the fullness of the target was not difficult to ensure. One should just load the desired volume from a syringe and close the target when ready for irradiation. NEBELING agreed that a conductivity sensor isn't necessary. He monitors pressure on target to show that beam is on target and the target is full. There is 7 bar of resistance in his capillary tubing when water is going into the target; when the target is full this resistance decreases. On the other hand, several participants acknowledged that they had run beam on their [ $^{18}\text{F}$ ]fluoride targets when they were not full or dry, at least once. It is apparent that while some laboratories have avoided or solved the problem of keeping a water target full, simple feedback sensors, e.g. conductivity or pressure, are useful in the water target systems of many laboratories.

James MOSKWA (Univ. of Michigan, Ann Arbor) has had two unsuccessful experiences in using optical level sensors in the vault to monitor target fullness. Both detectors „died“ because they were neutron sensitive. He now uses a small ultrasound transducer and over fills the target intentionally. The sensor is clamp-mounted and works well, it is manufactured by MAGNETROL (120-C Jeffry Blvd. East, Deer Park, New York).

## Radiation Detectors

Radiation detectors have been discussed at previous targetry meetings [5]. However there is still a need for inexpensive radiation detectors which have small sensors, at least 3 decades of response, can be located in many positions around the hot cell and with low sensitivity to background. This may be useful for feedback in automation of syntheses. What types of detectors have people found for this purpose?

SUNDERLAND stated that NUCLEAR ASSOCIATES (PO Box 349, Carle Place, NY 11514-0349) has a radiation therapy monitoring device called „PC Rainbow“ which meets all of those requirements but cost: It's price is approximately \$10,000. The unit now handles 6 to 7 detectors which can be 10 to 20 meters away. He is trying to persuade the company to modify the unit to handle up to 12 detectors. The cost is ~ \$150 per semiconductor detector and they are about the size of the end of a Q-tip. They have a serial tie to the PC through an optical cable. SUNDERLAND is working with NUCLEAR ASSOCIATES and SIEMENS to get these commercialized for PET radiochemistry. The detectors are sensitive with reasonable accuracy from 5 mCi up to „lots more than you will ever make“. LINK asked what detector to use for a lower range of activity, for example 0.5 mCi to 2 mCi in 1 ml?

Jerry NICKLES (Univ. of Wisconsin, Madison) suggested that one of his students had solved this by mounting a BGO detector to a photo amplifier or photo resistors which then could either be read into a KEITHLEY electrometer or an acoustical transducer.

BERRIDGE uses a semiconductor detector called „the Posimeter“. The detector head is about the size of a film can, in aluminum from POLYTECH LAB, Houston (9407 Rowan, Houston, TX 77036, ask for Francis LING). The cost is around \$ 800 each. The detector head is connected by coaxial cable to a meter unit which mounts on the hot cell exterior in his lab. The unit is about 10x15x5 cm. The readout is a standard meter, with an associated scale adjustment wheel. It runs on 12 VDC, and comes with a calculator-type power supply which plugs into an ordinary outlet, and a small jumper cable to allow a series of meters to be run from one power supply. The lower detection limit is around 1 mCi, depending on geometry and the response is linear from 1 mCi to 5 Curies of  $^{11}\text{C}$ . There is an analog output which can be used to drive a chart recorder or computer input. He has used the devices as detectors in the hot cells with up to three of them per apparatus for the last four years. He also uses them successfully as chromatography detectors on the hot cell preparative LC's, and in a shielded syringe holder for dose dispensing. It is nothing fancy, but does the job while requiring no attention, little space, and offering no hazards. He has been satisfied with them.

FERRIERI uses probes similar to those described by SUNDERLAND. They come from THERADOS. He cautioned that these detectors are temperature-sensitive, but SUNDERLAND disagreed. The detectors he uses vary about 0.1% per °C. DAHL has also used small detectors from LND (3230 Lawson Blvd., Oceanside, NY 11572). LND has both small GM and ion chambers, called peanut detectors. These are for detecting  $\gamma$ -rays and so require shielding against background.

SUNDERLAND said that if people would be willing to pay \$3,000 to \$4,000 for a system like he described, he'd like to discuss it with the manufacturer. The consensus was yes. DAHL predicted that there will be several companies with useful, small radiation detection systems on the market in the next one or two years.

## Synthesis

### *Transferring Materials To and From Reactions.*

LINK has found that small volume, less than 100  $\mu$ l, water and air-sensitive transfers can be difficult to automate and described a few of the transfer methods they use at the Univ. of Washington. For example, the synthesis of [ $^{14}$ C]thymidine requires transfer of 100  $\mu$ l of oleum and 7  $\mu$ l diethyl malate as water free liquids into a reaction vessel. Both transfers are difficult because the liquids are viscous. In addition, it is necessary to set up these reagents in the hot cell between 1 and 2 hours before they are used. The oleum is placed in a Reactiware vessel under argon. A 1/16" teflon tube at the bottom of the oleum vessel connects to a dispense position to which the robot arm brings the reaction vessel. The oleum is transferred by pressurizing the vessel with argon. It is necessary to have 130  $\mu$ l of oleum in the vial to transfer 100  $\mu$ l to the reaction vessel. The diethyl malate is transferred using a 7  $\mu$ l HAMILTON syringe which their group built onto a robotic hand. They tried and abandoned using a micropump which can deliver 1-2  $\mu$ l aliquots. These pumps reliably deliver small volumes, but they have dead volumes of several hundreds of microliters because they are built with large pipe fittings on the inlet and outlet ports. The diethyl malate is made in limited quantities and cannot be wasted. A peristaltic pump also has a large dead volume.

For transfers, Ulrich SCHERER (Ludwigshafen, formerly PSI Villigen) uses 12-position rotary valves. The single rotary valve sequences through vials to deliver reagents to a common center port. He can use vacuum in between. He uses spring cap vials with TFE / rubber septa.

WAGNER uses a HAMILTON microlab dispenser connected to two six-way valves for the synthesis of DOPA. Organic reagents pass through one of the valves; aqueous reagents through the other. They are somewhat programmable and work fine. The system is reliable. He also uses stopcock style OMNIFIT valves

with home-made actuators from model airplanes, as do the researchers at Liège.

CLARK and NEBELING also use multiport valves. CLARK suggested that we should make a list of valves. A sign-up sheet for anyone who was interested was circulated. He has had trouble with membrane valves for phosgene synthesis. The valves were wrecked if chlorine got into them. He uses a loop that is filled with HI during set-up and then liquids are pushed with  $N_2$ . He doesn't need to change HI loop even if it looks brown. He worries about getting  $LiAlH_4$  and its hydrolysis debris into valve seats.

MORELLE likes stainless steel double 3-way RHEODYNE valves for his  $H_2O$  targets. He uses his own air-operated rotary actuator which allows for an all closed position. These valves have operated near a target for a long time. CLARK cautioned that the RHEODYNE valves have either Vespel or Tefzel rotors. The Vespel rotors fall apart with strong base. DAHL uses two 3-way valves with a loop between for microliter injections. He pressurizes the loop to transfer the liquids.

Ren IWATA (CYRIC, Sendai) doesn't use a mass flow meter anymore. He likes pinch valves on TFE tube so that there is no valve to clean after the synthesis. Bruce WIELAND (CTI West, Berkeley) also uses pinch valves, both double and single headed. He commented that tubing compatibility with the reagents is critical.

*Heating and Cooling.* SCHERER uses halogen lights for his syntheses and described his raclopride apparatus. He has found that when these lamps have quartz windows they can induce photoreactions. For production of methyl iodide, the  $^{14}C$  activity stuck to the vessel walls. He put a metallic cap on the heater and the problem disappeared. KROHN asked what the spectrum of the halogen lamp was. SCHERER said the lamps were 250 Watts but he didn't know the spectrum of light output. FERRIERI asked how long it took to cycle between hot and cold and what was the longevity of the lamp? SCHERER responded that the cycle was fast, he could go from -5 to +150°C in 30 seconds. He has never had a lamp to break in 1.5 years of use. FERRIERI was curious why SCHERER's yields of [ $^{14}C$ ]raclopride (45%) were higher than others (25% in the literature; 15% for DANNALS), he wondered if the halogen lights might have something to do with it. He asked if SCHERER had used these lights for other synthesis? Yes, methionine. KROHN suggested that they wrap the light with Al foil to see if photochemistry is contributing to their exceptionally high raclopride yields.

CROUZEL uses WOOD's metal baths for heating in his syntheses. It provides excellent heat transfer and is very simple to use.



NICKLES thinks that ultrasound has a role in heating syntheses and also likes WOOD's metal or oil to couple the reaction vessel to the heat bath for efficient heat transfer.

BERRIDGE commented that he has used WOOD's metal very successfully for heat transfer without the mess of oil. It is important to maintain constant heat transfer in processes which rely on heating for controlled times. Also, you must remove the vessel from the bath before cooling the WOOD's metal or it will break vessels! He drills out his heat blocks and then uses only a thin film of WOOD's metal to gain better heat conductivity. He feels that a machined aluminum block with a heating cartridge, thermocouple, temperature controller, and cooling channel works very well. The block is kept small for rapid heating, and water, CO<sub>2</sub>, and nitrogen can be used to cool it. Evaporations of solvents can be done conveniently under vacuum and with a gas stream, tuning the gas flow and temperature to meet the needs. Didier LeBARS (CERMEP, Lyon) uses WOOD's metal to 200°C.

NEBELING has a system for heating / cooling combined that operates from -200 to +200°C using 300 Watts. He uses a 1 mm thick film of oil to couple the reaction vessels to the heating block. If the Al block is already at the desired temperature, it takes only 1 1/2 min to change temperature in the reaction vessel. At Michigan they also combine heating and cooling. HAKA said that they had used an Al block to transfer heat. They blow CO<sub>2</sub> on the block to cool it and use a resistive cartridge to heat the block. The Michigan group has also used Vortex cooling on their reaction vessels.

WAGNER asked what people use for FDG heating? Does anyone use non-liquid cooling and/or heating? MORELLE uses glass reactor vials with Cu sheath heaters. NICKLES uses microwaves. He has not used it for heating but it is great for shaking things off the vessel walls. Martin ORBE (SCANDITRONIX, Uppsala) stated that Sharon STONE-ELANDER (Karolinska Hospital, Stockholm) uses microwaves for a lot of syntheses. The Liège group uses microwaves to synthesize altanserin. The group at Washington University, St. Louis also uses microwaves in syntheses.

CLARK asked if anyone measured temperature inside reaction vessels? No. LINK commented that temperature measurements of the heating bath are not ideal, particularly with short heating times. For example, she has found that 4 ml Reactiware vessels can vary significantly in wall thicknesses, enough to cause heat transfer into the reaction vessels to vary from one synthesis to another. MORELLE said that we shouldn't demand accurate temperature measurements for our reactions, we just need to make our (automated) syntheses reproducible.

David SCHLYER (Brookhaven Natl. Lab) uses thermal grease between a surface mounted thermocouple and

the reaction vessel to more accurately monitor the reaction temperature. David ALEXOFF calibrates the thermocouple before each run.

FERRIERI would like to see chemical sensors developed which would be in the reaction vessel to track starting reagents or tell something about the progress of a reaction. This is a tough goal with which they have no progress yet. The trouble is often competing reactions occur and interfere with the signals. It would be ideal to have specific sensors which monitor the reaction rate rather than the reaction conditions. While this is not yet feasible, the area of chemical microsensors is an emerging research area for analytical chemistry that we should watch carefully and we should be prepared to take advantage of new findings. See for example, Ref. [6].

**Evaporations.** WAGNER asked if anyone had tried Vortex evaporators. Specifically, he felt the „Turbo-Vap“ of ZYMARK was intriguing but too big, and he knew that there was another system on the market. He thought that Vortex mixing with convection to vacuum would have a big market for multi-vial work. LINK had borrowed a Vortex drying system from HNU Corp. and tried it. The maximum temperature of the instrument was 90°C and it took over 20 minutes to evaporate 5 ml of water. She said both the HNU and ZYMARK systems were too big and too slow. She also likes the concept but suggested that a home-made system which operated at higher temperature was worth further investigation. WAGNER thought that you should heat the system with light, hot air. FERRIERI commented that drying systems require considerable flexibility in mounting to the reaction system. This is particularly true for automated but not robotic systems.

BERRIDGE asked why are evaporations a problem? Isn't it a simple matter of gas in and vacuum out? He uses an Al block with WOOD's metal, a thermocouple probe, heat fast and then flows H<sub>2</sub>O through to cool the block. LeBARS replied it's not that easy, sometimes that approach just isn't fast enough for <sup>11</sup>C syntheses. The approach described by BERRIDGE can evaporate ~ 5 ml of water in 5 minutes without vacuum.

Rotary evaporators or systems such as that described by BERRIDGE are currently used for radiochemical syntheses, but often aren't optimal. Rotary evaporators have a large surface area, but it is difficult to quantitatively recover small volumes remotely. This has been accomplished to a certain degree by the chemists at TRIUMF [7].

**Solid Phase Chemistry.** IWATA described his system for solid phase methylation similar to that described by the University of Michigan group which he has incorporated into an automated system. He has submitted the full description of this system for publication to Applied Radiation and Isotopes. Basically [<sup>11</sup>C]methyl iodide is trapped in a short column, 55 µl,

containing Poropak Q or another appropriate adsorbent material at  $-42^{\circ}\text{C}$ , then solvent, base & substrate (precursor) introduced. The column is closed with valves and then heated to  $80^{\circ}\text{C}$  for the reaction. After the reaction is complete, the reaction mixture is washed off of the column, through a six-port valve and onto an HPLC column for separation of the product from the reactants. They are pleased with this system. They can reduce the amount of substrate which is used and yet get a greater concentration of substrate as well as an increase in radiochemical yield. There is some dead volume, but it is small. If he decreases the volume of adsorber used he obtains the same chemical yield but decreases his radiochemical trapping efficiency.

HAKA described the Michigan experience: Their philosophy is to do everything as simply as possible. Simple heating and evaporations. Solid phase has been a big focus at Michigan and solid phase systems are easily automated. A good example is their use of the trimethylammonium triflate for synthesis of FDG.

ORBE stated that SCANDITRONIX had two automated systems; one for production of methyl iodide and one for FDG. He went on to discuss the automated system for production of FDG, which utilizes the solid phase synthesis developed at Michigan [8]. The unit consists of a mechanical box which uses pinch valves and is controlled using a personal computer. The control unit has no user-adjustable variables. There is a disposable process kit used for each synthesis which consists of a panel which is  $\sim 30\text{ cm} \times 30\text{ cm}$  in area with holes for pinch valves. The system comes with the resin for FDG synthesis in a column. They do not use resin to remove acid at the end of the synthesis but use a neutralizing buffer. The box contains a heater. Greater than 70% of the [ $^{18}\text{O}$ ]water is recovered.

SUNDERLAND asked if the SCANDITRONIX chemists had experienced any pressure problems with the synthesis. HAKA said that they had experienced pressure problems at Michigan with the solid phase synthesis and asked what resin that ORBE used. ORBE said that they used the resin that Michigan had reported and that they did have some back-pressure problems. HAKA said they should have high back-pressure problems because that resin will decompose after a few minutes. It is necessary to push through the resin fast, i.e. within 1.5 minutes. The chemists at Michigan had found that the displacement was instantaneous and had found that dilution of the resin with high density polyethylene (Primax) from AIR PRODUCTS, 17 mg resin and 8 mg Primax, solved the back pressure problems and they controlled the flow rate of precursor through the column. SUNDERLAND asked how they put the tubing onto the pinch valves, and ORBE answered that the pinch valves fit nicely over the tubing in the process kit and the control system held the pinch valves open for placement during set-up. CLARK commented that the system looked heavy, and asked if it was self-shielding? ORBE replied that the full dimen-

sions were 55 cm high  $\times$  25 cm in diameter  $\times$  36 cm wide and it was not self-shielding. There is a lead container for shielding the product vial. CLARK also asked how they moved the syringes. ORBE replied that they used a stepper motor. NEBELING asked if the reagent vials came filled and ORBE replied that they were empty in the kit and had to be filled at synthesis time to a line marked on the vessel. ORBE stated that the tubing in the kit was polyethylene and the vessels were made of Tefzel. WIELAND commented that one needed the proper polyethylene history for a successful pinch valve-controlled radiochemical synthesis. MOSKWA asked what happens if you have an error, what kind of quality control is done to determine if there is unhydrolyzed material in the product and what radiochemical yields they obtained. ORBE replied that any error was a catastrophic failure which stopped the synthesis and the hard copy printout would tell one how far in the synthesis the error occurred. They did not worry about unhydrolyzed product because Sep-Paks were in the system to remove this material and the yields were  $40 \pm 10\%$ , not decay-corrected. BECHTOLD asked how many runs they had done and ORBE refused to reply, which was severely criticized by the participants as not in keeping with the spirit and purpose of the workshop. NEBELING asked what the radiochemical purity was and what quality control method was used to determine this? ORBE replied 98% radiochemical purity as measured by TLC. NEBELING stated that was impossible because the accuracy of TLC is  $\pm 5\%$ . MORELLE asked if SCANDITRONIX intended to design other similar synthesis units and ORBE replied that it should be possible.

**Separations.** LINK asked IWATA if they were still using the glass col extractor-phase separators for automation in their lab, IWATA replied that the product had been commercialized (HAMAMATSU, Inc.) and he had nothing further to add.

**Feedback of HPLC Loading/Injection.** RUTH automatically loads samples onto an HPLC by the system illustrated in Fig. 1. The system has worked well for him.

A similar system is used at the University of Washington. WAGNER works in the reverse direction to pull the sample rather than push it into the injector loop. CLARK injects his material first onto a short of high pressure C-18 concentration column which is then water-washed to get rid of unwanted aqueous, soluble materials and then eluted onto the preparative column with HPLC mobile phase.

**Assaying of Product Dose.** MOSKWA described the system he is currently testing for automating dose assay of product serum vials. He uses 3" long  $1\frac{1}{4}$  dia "rabbits" which contain the product multidose vial upside down. The rabbit, which has a hole drilled in the

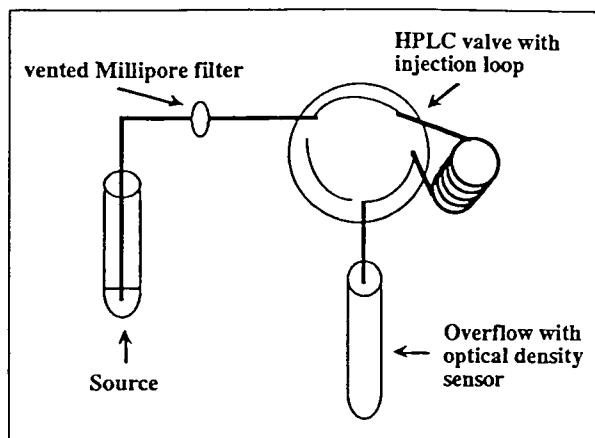


Figure 1: System for Loading Solutions into the HPLC (T.J. RUTH, Vancouver).

bottom, falls into a cup and a jig is used to push it into the dose calibrator. The rabbit ends up in the dose calibrator upside down, i.e. the hole and the multidose vial are right side up. The dose is recorded and a needle set is dropped directly into the multidose vial / rabbit combo for infusion into the patient.

BERRIDGE uses a small detector next to the dose syringe which he has calibrated for radiation dose units.

RUTH has home-made ion chambers similar to those developed by SOLIN and HESELIUS at Turku which have loops of 1/8" teflon tubing wound around between the anode and cathode and the sample flows through the tubing and is detected. These also have to be calibrated in dose units.

### Quality Control

**Formulations and Purity (radiochemical, chemical, sterility and apyrogenicity).** CLARK has a unique system for infusion of [ $^{15}\text{O}$ ] $\text{H}_2\text{O}$  which he presented as a poster and in discussion [9]. He produces the water at the bedside in a shielded unit and uses a membrane to transfer the water vapor into saline. For quality control, he sterilizes the membrane pack and puts a sterile filter at both ends. Routine tests of the infusate are carried out for sterility and apyrogenicity using LAL test kits.

CLARK asked how others keep their HPLC systems sterile and apyrogenic? NEBELING sterilizes his columns with acetonitrile and stores them in ethanol. LINK stores the columns in either 20% acetonitrile / 80% water or 50% ethanol / 50% water. She uses sterile apyrogenic 0.22  $\mu\text{m}$  filters on all openings and then changes to the correct mobile phase shortly prior to synthesis. DAHL warned that it is necessary to wash the column in  $\text{H}_2\text{O}$  before putting in organic, i.e. to remove all salts, if one has been using a buffer such as phosphate.

NICKLES uses Nylon-66 filters to remove pyrogens. While this may work, it is not obvious what the mechanism is for this removal. This certainly should be investigated in detail before it is used. PIERCE CHEMICAL Co. (PO Box 117, Rockford, IL 61105 U.S.A. or PO Box 1512, 3260 BA Oud-Beijerland, Netherlands) sells „Detoxigel“ for removal of pyrogens. For any methods of pyrogen removal, the chemists need to be certain that the federal and local regulatory agencies approve of these agents for pyrogen removal.

GAEHLE is evaluating the use of a pressure transducer on his robotic system to check for ruptured sterilization filters. This technique is not yet completely implemented but „looks promising.“ The SCANDITRONIX system also checks filter integrity using a pressure transducer.

BERRIDGE described his system for production of [ $^{15}\text{O}$ ]butanol. He has found that for his system the quantities of solutions added and the total time of synthesis are important. Control of solvent volumes is achieved using a single, controlled-pressure, gas supply as the driving force on solvent reservoirs and by adjusting the tubing length and diameter to provide convenient flow rates. Flow volumes are then measured by timing the opening of the valve for each solvent. It is a simple method which works well in this synthesis even though the yield and purity of the product are very sensitive to the solvent volumes used. Too great a volume of  $\text{H}_2\text{O}$  in the washes lowers the butanol yield from 60% down to 20%. The system is automatable without the use of feedback sensors, or with only one POLYTECH LAB radiation detector. At the moment it is run without a computer interface, using a „stopwatch-driven programmable bio-organic controller“ (human). WAGNER asked if he used an air bolus between each wash of his system, and BERRIDGE answered yes. GAEHLE mentioned that they have set up a system at St. Louis for delivery of sequential doses of [ $^{15}\text{O}$ ]butanol which uses a HAMILTON MICROLAB dual syringe injection station and a distribution valves to ten different set-ups for sequential syntheses of the [ $^{15}\text{O}$ ]butanol without clean-up [10].

LINK briefly discussed the poster on quality control to measure cryptand in FDG product by Ronald FINN (SLOAN-KETTERING, New York). She stated that the data appear to show that with the special resin column [11] there was little tritiated cryptand in the product. However, since a tritiated compound was used, the researchers still needed to show that the tritium label was not exchanged off of the molecule during the FDG synthesis and more work was needed.

**Clean-up.** FERRIERI presented some of the problems and automation solutions which they have implemented at Brookhaven regarding batch vs. serial syntheses. A single batch synthesis can satisfy PET needs for [ $^{18}\text{F}$ ]fluoride labelling of FDG. However for short-

lived radionuclides like  $^{11}\text{C}$ , single batch synthesis is not practical. There is usually not enough activity and the specific activity for later injections is a big concern. Specifically he worries about serial  $^{11}\text{C}$  syntheses. They do six cocaine runs per day and can't do batch syntheses. Their systems don't deal with failure mode and can't recover from errors. They need duplicate or replacement systems. How does one deal with things like contaminated reaction vessels (*etc.*) in serial syntheses? One can strip and clean vessels or use disposable components and replace them. If this is performed manually, these increase the radiation dose to the operator. One solution is to use robotics or manipulators, but then the costs in money and space are high vs. in-place self-cleaning. One of the solutions to these problems that is used at Brookhaven is to do in-place self-cleaning of the methyl iodide / HI vessel. The vessel is washed with acetone, then 2N HCl, then a series of cycles of acetone and vacuum + heat using an air bath reactor. By using this method, the specific activity is maintained. In fact specific activity increases with each procedure. They have built a multiport glass cow 1 port in with 4 ports out for synthesis of methyl iodide. WAGNER asked what happened to the  $\text{P}_2\text{O}_5$ ? FERRIERI replied that they don't use  $\text{P}_2\text{O}_5$ , they use a NaOH trap which they change every 1-2 weeks. The target gas and He pass through a tube of  $\text{MgClO}_4$  to dry the target gas. Other aspects of this problem are that they can't self-clean the alkylation vessel used for synthesis of raclopride at Brookhaven.

BERRIDGE had the following to add. Often, the cleaning of the apparatus is a minor variation on the synthetic manipulations. It generally requires that additional solvent reservoirs be attached and that the number of valves in the system be increased by a small percentage. Since cleaning does not generally require as strict control of temperatures, pressures *etc.* as a synthesis, it is really easier to automate in many respects than the synthesis. One must decide which parts of the apparatus should not be changed, but cleaned. Strategic location and shielding of waste reservoirs allow certain difficult components (resins, alumina) to be changed quickly after the system has been cleaned. This helps to reduce radiation exposure.

NEBELING was asked to comment on the Jülich automated FDG system which was presented at the last targetry meeting [12] which is self-cleaning. NEBELING stated that the system was working well but that the key to this success was the use of glassy carbon for their reaction vessel. LINK asked who supplies the glassy carbon and the list of suppliers is summarized as an Annex to these proceedings.

GAEHLE is using the robot for set-up and clean-up of supplies and vessels for the reactions. The Karolinska methyl iodide system uses valves to clean the components. They flush it several times with acetone then flush with ethanol 4-5 times, heat it, pass carrier gas

through it, and evaporate it dry. The cleaning takes 5 minutes per reactor and there are six methyl iodide syntheses done per set-up.

## Regulatory Issues Regarding Radiation Safety

MOSKWA raised one final issue. At the University of Michigan they produce  $^{11}\text{C}$  as a gas and have had radiation safety problems due to gaseous radionuclide emissions. Several hundred mCi of  $[^{13}\text{N}]\text{N}_2$  and  $[^{14}\text{O}]\text{O}_2$  go into their ducts from the  $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$  reaction. They have a 5 Ci release limit per year and a 10 mCi limit per irradiation. That value is easily exceeded, and has been very difficult for their group to achieve. They are concerned about methods to reduce emissions so that they can keep their laboratory working, and cautioned that others are likely to face similar problems as regulations on radionuclide emissions become more restrictive. WEINREICH said that the PSI group has installed two evacuated 60 l vessels to delay the emission of target gases into the air. Irradiation can start only after a good enough vacuum has been reached. The results in order to minimize the emitted radioactivity are excellent. It was agreed that we all begin thinking and working toward solutions now. It might be useful to use shielded delay / decay tanks of a suitable volume.

## Closing Comments

A distillation of the discussions on automation shows a remarkable amount of concurrence among such a diverse group of participants. While not all participants have automated their syntheses, there was a general positive attitude toward automation. The reasons given to automate were to reduce dose to personnel and to reduce personnel cost per synthesis. The reason not to automate was initial cost in time and personnel when resources are limited. There seem to be two principal categories of compounds which have been automated. First, precursors, particularly  $[^{11}\text{C}]$ methyl iodide and  $[^{18}\text{F}]$ fluoride recovery and drying. They are relatively simple to automate and have the greatest amounts of activity, therefore pose the greatest radiation hazard to personnel, during a synthesis. Second, the compounds most frequently synthesized by a group. This primarily means  $[^{18}\text{F}]$ FDG, but also  $[^{11}\text{C}]$ cocaine,  $[^{11}\text{C}]$ raclopride and  $[^{11}\text{C}]$ thymidine,  $[^{15}\text{O}]$ butanol and  $[^{15}\text{O}]$ water. Several of the participants (Jülich, BNL, Sendai, St. Louis, Hammersmith) have also set up these syntheses for either multiple syntheses or automated self-cleaning before one has to open the „hot cell“ to set up for another synthesis.

With regard to transfer to and from targets, most participants are using systems which are either automated or remote and which function well. It was apparent that feedback sensors could improve the reliability of water target radiochemical yields for many groups and possibly other targets as well. While, none of the radiation detectors proposed as radiation sensors for

target yields and synthesis transfers met all of the proposed criteria, there appear to be many detectors available if one is able to afford several thousand dollars, otherwise it appears that building one's own ion chambers is a viable alternative.

Very few participants described any problems with transfers into and out of reaction vessels and many alternative methods were presented. A remarkable number of people use multi-port, small volume distribution valves for their syntheses and are happy with the way they work. Maybe those of us who haven't used them should consider their use for our syntheses. With regard to heating and cooling, there was no consensus. There are many solutions, but none appears to be best for all situations at this time. The halogen heating introduced by DANATEC was intriguing. Their chemistry yields were very good and the idea that these lamps could improve or worsen the chemistry needs to be examined further. Evaporations are being performed the same way they have for years, but there is room for improvement with no obvious solution at this time. Separations are being carried out primarily using low or high pressure solid / liquid phases and the automation of these remains simple but in need of better feedback, such as the overflow level sensor of RUTH.

HAKA complained that PET chemists are unwilling to try new synthetic techniques when he discussed Michigan's commitment to develop solid phase chemistry. This is unfair, an unwillingness to try new synthetic methods was not in evidence at this meeting. It requires time and resources to develop a new synthesis and if one has a working synthesis, for example for FDG, it is difficult to justify the effort to make the change. Despite this, two groups in addition to Michigan presented their work with solid phase synthesis at this meeting. ORBE presented the SCANDITRONIX FDG system which uses the Michigan FDG method. IWATA presented a small volume column system for  $^{11}\text{C}$  methylations. Solid phase synthesis is much easier to automate than multiple liquid vial transfers, and with time it is likely that more solid-liquid phase syntheses will be introduced. The easiest to automate though, is gas transfers through solid catalysts and reactants. As CROUZEL stated, we should try to find a way to produce  $^{11}\text{CCH}_3\text{I}$  without liquids if we are going to continue to do methylations.

In general, it was good to see manufacturers coming forth with more automated radiochemistry synthesis units, interacting with the group, and for the most part freely exchanging information. CTI / SIEMENS has had their "black box" synthesis unit out for several years and published the details of the system fairly completely. It was disappointing that no one from that division of the company was available to participate in this session. The companies who did attend appear to be taking different approaches to the syntheses and it will be interesting to see how successful they are.

SCANDITRONIX has opted for two types of systems: They are continuing with the "ANATECH" robot for  $^{11}\text{C}$  syntheses; raclopride and methyl iodide, which are in use in St. Petersburg, and a black box with a completely disposable synthesis module for synthesis of  $^{18}\text{F}$ FDG. Both are operated using a personal computer. DANATEC, in conjunction with PSI, have developed relatively simple systems for production of  $^{18}\text{F}$ FDG and  $^{11}\text{C}$ methyl iodide and  $^{11}\text{C}$ raclopride using different heating technology and very simple microprocessor controls, which are easily accessible and reprogrammable by the user. EBCO is working with TRIUMF to commercially develop his dedicated automated  $^{18}\text{F}$ FDG and  $^{18}\text{F}$ -DOPA syntheses, which emphasizes feedback sensors and whose personal software concept was presented at the last targetry meeting by David MORRIS [13].

The greatest strength of this session was the tremendous group participation and discussion which will hopefully continue among the participants until the next meeting. One weakness was that all of the active participants were involved in PET radiosyntheses. There is a need for automation in other areas of radiosynthesis, particularly in high activity iodine production and syntheses. Some of these people were at the meeting and had participated before, perhaps we can better include them in the future.

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## PET RADIONUCLIDE PRODUCTION

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**ABSTRACT.** Several short-lived positron-emitters are routinely used in PET studies while a few others are of potential interest. A brief review of the decay data and production methods for several of these positron-emitters is given. Special attention is paid to the production of the four major positron-emitters, namely carbon-11, nitrogen-13, oxygen-15 and fluorine-18, which are widely applied in several European PET centres.

### 1. Introduction

The number of positron-emitting radionuclides is large (*cf.* Lederer and Shirley, 1978; Browne and Firestone, 1986). Some medically relevant short-lived positron-emitters and their decay data are listed in Table 1. Most of the PET studies to date are carried out with organic positron-emitters ( $^{11}\text{C}$ ,  $^{13}\text{N}$ ,  $^{15}\text{O}$  and  $^{18}\text{F}$ ). Several other radionuclides, for example potassium-38, selenium-73, bromine-75 and bromine-76, also find some applications. There is also considerable interest in copper-62, gallium-68 and rubidium-82, which are obtained via longer-lived generator systems.

The production of useful and potentially useful positron-emitters has been the subject of several review articles and books (Lambrecht, 1971; Clark and Buckingham, 1975; Welch, 1977; Stöcklin and Wolf, 1982, 1983; Vaalburg and Paans, 1983; Qaim, 1986a). Development of a production process involves a study of several aspects such as nuclear data, high current targetry, chemical processing, automation and quality control. A knowledge of nuclear data is essential for calculating thick target yields, estimating levels of radionuclidic impurities and designing suitable target systems. High current targets are needed to produce radionuclides in sufficient quantities. Due to the short half-lives of the useful positron-emitters the chemical separation and subsequent labelling procedures have to be fast. The high level of radioactivity calls for remotely controlled and, sometimes necessarily, automated methods. Quality control must be concerned with the radionuclidic, radiochemical, chemical and pharmaceutical quality of the products. This chapter deals mainly with the routine methods of production of commonly used positron-emitters. A brief discussion of less commonly used and generator-produced positron-emitters is also given.

The important production routes, the suitable energy ranges and the theoretically expected thick target yields over these energy ranges are given in Table 1. The yields are in

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| Radioisotope     | Decay     |                              |                     | Production                                 |                                                                                                                                                                                                                          |                                          |                                                                         |
|------------------|-----------|------------------------------|---------------------|--------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|-------------------------------------------------------------------------|
|                  | $T_{1/2}$ | Mode of decay (%)            | $E_{\beta^+}$ (keV) | Principal $\gamma$ -rays keV (% abundance) | Nuclear reaction                                                                                                                                                                                                         | Energy range (MeV)                       | Theoretical thick target yield MBq (mCi)/ $\mu$ Ah                      |
| $^{11}\text{C}$  | 20.4 min  | $\beta^+$ (99.8)<br>EC (0.2) | 960                 | 511(199.6)                                 | $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$<br>$^{11}\text{B}(\text{p},\text{n})^{11}\text{C}^*$<br>$^{10}\text{B}(\text{d},\text{n})^{11}\text{C}^*$                                                                  | 13 – 3<br>10 – 0<br>10 – 0               | 3820 (103)<br>3400 (92)<br>2479 (67)                                    |
| $^{13}\text{N}$  | 10.0 min  | $\beta^+$ (100)              | 1190                | 511(200)                                   | $^{12}\text{C}(\text{d},\text{n})^{13}\text{N}$<br>$^{13}\text{C}(\text{p},\text{n})^{13}\text{N}^*$<br>$^{16}\text{O}(\text{p},\alpha)^{13}\text{N}$                                                                    | 8 – 0<br>10 – 0<br>16 – 7                | 1961 (53)<br>4440 (120)<br>1665 (45)                                    |
| $^{15}\text{O}$  | 2.05 min  | $\beta^+$ (99.9)<br>EC (0.1) | 1720                | 511(199.8)                                 | $^{14}\text{N}(\text{d},\text{n})^{15}\text{O}$<br>$^{15}\text{N}(\text{p},\text{n})^{15}\text{O}^*$<br>$^{16}\text{O}(\text{p},\text{pn})^{15}\text{O}$                                                                 | 8 – 0<br>10 – 0<br>26 – 16               | 2368 (64)<br>2220 (60)<br>3700 (100)                                    |
| $^{18}\text{F}$  | 109.6 min | $\beta^+$ (97)<br>EC (3)     | 635                 | 511(194)                                   | $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$<br>$^{18}\text{O}(\text{p},\text{n})^{18}\text{F}^*$<br>$^{16}\text{O}(^3\text{He},\text{p})^{18}\text{F}$                                                                | 14 – 0<br>16 – 3<br>41 – 14              | 1110 (30)<br>2960 (80)<br>481 (13)                                      |
| $^{38}\text{K}$  | 7.6 min   | $\beta^+$ (100)              | 2680                | 511(200)<br>2168(99.9)                     | $^{35}\text{Cl}(\alpha,\text{n})^{38}\text{K}$<br>$^{38}\text{Ar}(\text{p},\text{n})^{38}\text{K}^*$<br>$^{40}\text{Ca}(\text{d},\alpha)^{38}\text{K}$<br>$^{40}\text{Ar}(\text{p},3\text{n})^{38}\text{K}$              | 22 – 7<br>16 – 12<br>12 – 0<br>32 – 30   | 259 (7)<br>777 (21)<br>207 (5.6) <sup>i</sup><br>192 (5.2) <sup>i</sup> |
| $^{73}\text{Se}$ | 7.1 h     | $\beta^+$ (65)<br>EC(35)     | 1320                | 511(130)<br>361(97)                        | $^{75}\text{As}(\text{p},3\text{n})^{73}\text{Se}$<br>$^{75}\text{As}(\text{d},4\text{n})^{73}\text{Se}$<br>$^{72}\text{Ge}(^3\text{He},2\text{n})^{73}\text{Se}^*$<br>$^{70}\text{Ge}(\alpha,\text{n})^{73}\text{Se}^*$ | 40 – 30<br>45 – 33<br>23 – 13<br>28 – 13 | 1406 (38)<br>651 (17.6)<br>55 (1.5)<br>126 (3.4)                        |

- Using highly enriched isotope as target material.

Experimental yield.



Table 1. Continued....

| Radioisotope                      | Decay            |                                    |                                  | Production                                 |                                                                                                                                                                                                                   |                                          |
|-----------------------------------|------------------|------------------------------------|----------------------------------|--------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|
|                                   | T <sub>1/2</sub> | Mode of decay (%)                  | E <sub>β<sup>+</sup></sub> (keV) | Principal γ-rays keV (% abundance)         | Nuclear reaction                                                                                                                                                                                                  | Energy range (MeV)                       |
| <sup>75</sup> Br                  | 1.6 h            | β <sup>+</sup> (75.5)<br>EC (24.5) | 1740                             | 511(151)<br>287(92)                        | <sup>76</sup> Se(p,2n) <sup>75</sup> Br <sup>*</sup><br><sup>75</sup> As( <sup>3</sup> He,3n) <sup>75</sup> Br<br><sup>76</sup> Se(d,3n) <sup>75</sup> Br <sup>*</sup><br><sup>75</sup> As(α,4n) <sup>75</sup> Br | 30 → 22<br>36 → 25<br>35 → 29<br>65 → 54 |
| <sup>76</sup> Br                  | 16.1 h           | β <sup>+</sup> (57)<br>EC (43)     | 3900                             | 511(114)<br>559(74)<br>657(16)<br>1854(15) | <sup>75</sup> As( <sup>3</sup> He,2n) <sup>76</sup> Br<br><sup>76</sup> Se(p,n) <sup>76</sup> Br <sup>*</sup><br><sup>77</sup> Se(p,2n) <sup>76</sup> Br <sup>*</sup>                                             | 18 → 10<br>16 → 10<br>25 → 16            |
| <sup>62</sup> Zn<br>↓ (generator) | 9.2 h            | β <sup>+</sup> (93)<br>EC (7)      | 660                              | 511(186)<br>41(25.2)<br>597 (25.7)         | <sup>63</sup> Cu(p,2n) <sup>62</sup> Zn<br><sup>60</sup> Ni(α,2n) <sup>62</sup> Zn                                                                                                                                | 26 → 21<br>30 → 15                       |
| <sup>62</sup> Cu<br>↓ (generator) | 9.7 min          | β <sup>+</sup> (98)<br>EC (2)      | 2930                             | 511(196)                                   |                                                                                                                                                                                                                   |                                          |
| <sup>68</sup> Ge<br>↓ (generator) | 271 d            | EC (100)                           |                                  |                                            | <sup>69</sup> Ga(p,2n) <sup>68</sup> Ge<br>RbBr (p.spall) <sup>68</sup> Ge                                                                                                                                        | 22 → 13<br>800, 500                      |
| <sup>68</sup> Ga                  | 68.3 min         | β <sup>+</sup> (90)<br>EC (10)     | 1900                             | 511(180)<br>1077(3)                        |                                                                                                                                                                                                                   |                                          |
| <sup>82</sup> Sr<br>↓ (generator) | 25 d             | EC (100)                           |                                  |                                            | Mo(p.spall) <sup>82</sup> Sr<br><sup>85</sup> Rb(p,4n) <sup>82</sup> Sr<br><sup>101</sup> Kr( <sup>3</sup> He,xn) <sup>82</sup> Sr<br><sup>101</sup> Kr(α,xn) <sup>82</sup> Sr                                    | 800<br>60 → 40<br>90 → 20<br>120 → 20    |
| <sup>82</sup> Rb                  | 1.3 min          | β <sup>+</sup> (96)<br>EC (4)      | 3350                             | 511(192)<br>776(13.4)                      |                                                                                                                                                                                                                   |                                          |

\* Using highly enriched isotope as target material.  
† Experimental yield.

a few cases experimental yields (Lambrecht *et al.*, 1978; Helus *et al.*, 1980; Pao *et al.*, 1981; Loc'h *et al.*, 1982; Robertson *et al.*, 1982; Thomas, 1987), since the excitation functions are either unknown or uncertain. In general, however, the yields were calculated from the reported excitation functions, as follows: carbon-11 (compilation by Keller *et al.*, 1973; Bida *et al.*, 1980b); nitrogen-13 (compilation by Keller *et al.*, 1973; Bair *et al.*, 1981; Sajjad *et al.*, 1986); oxygen-15 (Vera Ruiz and Wolf, 1977; Sajjad *et al.*, 1984, 1985); fluorine-18 (Nozaki *et al.*, 1974; Ruth and Wolf, 1979); potassium-38 (Qaim *et al.*, 1988; Tárkányi *et al.*, 1992); selenium-73 (Mushtaq *et al.*, 1988; Mushtaq and Qaim, 1990); bromine-75 (Paans *et al.*, 1980; Alfassi and Weinreich, 1982; Kovács *et al.*, 1985); bromine-76 (Janssen *et al.*, 1980; Alfassi and Weinreich, 1982; Kovács *et al.*, 1985); zinc-62 (Neirinckx, 1977; Kopecky, 1985); strontium-82 (Horiguchi *et al.*, 1980; Tárkányi *et al.*, 1988, 1990). Several other details of production methods are described below.

## 2. Commonly used Positron-emitters

The positron-emitters best suited to PET studies are the 'organic' radionuclides, carbon-11, nitrogen-13, oxygen-15 and fluorine-18. Whereas the former three radionuclides can be used only on site, fluorine-18 is suitable for longer lasting studies and for transport to PET centres without a cyclotron ('satellite' centres).

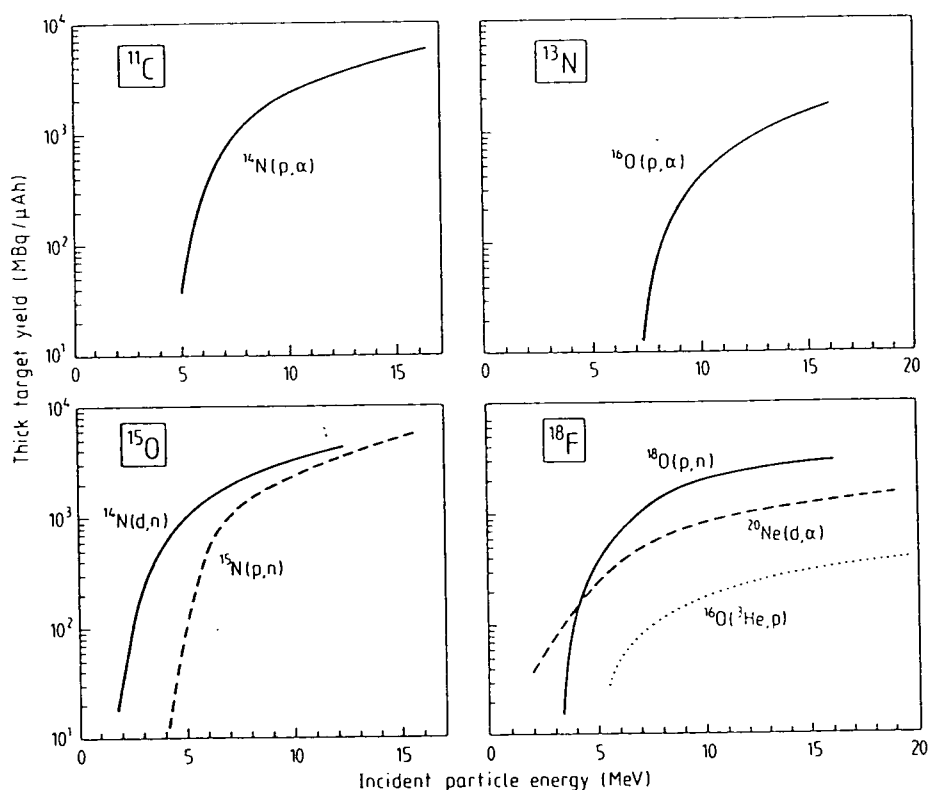


Figure 1. Integrated thick target yields of some commonly used positron-emitters expected from the most common production routes, plotted as a function of incident particle energy. Data were calculated using the measured excitation functions (for reference see text).

The thick target yields of the most common production routes, calculated from the measured excitation functions (Nozaki *et al.*, 1974; Vera Ruiz and Wolf, 1977; Ruth and Wolf, 1979; Bida *et al.*, 1980b; Sajjad *et al.*, 1984, 1985, 1986) are shown in Figure 1 as a function of incident particle energy. The values for nitrogen-13 and oxygen-15 give saturation yields; however, for carbon-11 and fluorine-18 an irradiation time of 1 h was assumed. It is emphasised that the curves should be regarded as approximations since the calculation of the thick target yield from an excitation function showing large fluctuations, as for the light mass nuclei discussed here, is rather uncertain. In Figure 1 no data are shown for the  $^{13}\text{C}(\text{p},\text{n})^{13}\text{N}$  reaction. Due to the very large uncertainty in the excitation function for this process, only the integrated thick target yield for the whole energy range is given in Table 1.

From the data presented in Figure 1 it is evident that, in principle, all four positron-emitters can be produced at a small cyclotron. In practice, however, several important considerations need to be addressed when embarking on a production scheme. State of the art information is summarised below.

## 2.1. CARBON-11

Over the last two decades a large number of compounds labelled with carbon-11 ( $t_{1/2} = 20.4$  min;  $I_{\beta^+} = 100\%$ ;  $E_{\beta^+} = 0.96$  MeV) have found application in biomedical and pharmacological studies using PET. With the increasing interest in investigations of receptor-ligand interactions it has become mandatory for radioligands to be prepared in high specific radioactivity. Generally, the starting precursor for radiosyntheses is high specific radioactivity [ $^{11}\text{C}$ ]carbon dioxide. For some radiosyntheses [ $^{11}\text{C}$ ]methane is prepared 'in target' as a precursor to the off-target synthesis of other labelling agents.

Several nuclear reactions can be used to produce carbon-11 (for reviews see Wolf and Redvanly, 1977; Ferrieri and Wolf, 1983). The common reactions are summarised in Table 1. Out of these processes the most convenient and commonly used is the  $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$  nuclear reaction on a target of nitrogen gas (Blaser *et al.*, 1952; Nozaki *et al.*, 1966; Epherre and Seide, 1971; Jacobs *et al.*, 1974; Casella *et al.*, 1978; Bida *et al.*, 1980b). The experimental production yields are of the order of 3.7–5.2 GBq/ $\mu\text{A}$  (100–140 mCi/ $\mu\text{A}$ ) for proton energies of 16 MeV. Useful activities (0.7–1.2 GBq/ $\mu\text{A}$ , 25–31 mCi/ $\mu\text{A}$ ) can also be produced using lower energies (8 MeV) (*cf.* Wolf and Jones, 1983).

Technical details with regard to achieving an efficient production of [ $^{11}\text{C}$ ]carbon dioxide of high specific radioactivity have been discussed in an EEC Task Group report (Crouzel *et al.*, 1987). Here we give an updated version of that report.

**2.1.1. Target construction.** Pure aluminium or aluminium alloy is a satisfactory material for target construction. Havar<sup>TM</sup>, titanium or stainless steel foils are suitable materials for target windows. Metal gaskets are preferable to rubber 'O' rings. The target should be made as small as possible with respect to beam profile and penetration, in order to achieve high specific radioactivity and fast emptying. For good recoveries of [ $^{11}\text{C}$ ]carbon dioxide, it is recommended that newly constructed targets be carefully washed with 0.1M-phosphoric acid followed by water, and dried under vacuum. It is advised that this process be carried out at each subsequent target maintenance.

**2.1.2. Target material.** The target gas should be of high purity and especially as free as possible from carbon-containing impurities. 'Nitrogen N60' (99.9999% pure, from Air Liquide), 'white spot' nitrogen (nominally 99% from BOC Ltd) and 'CO-free' nitrogen (with < 20 ppb CO and < 50 ppb CO<sub>2</sub>, from Linde) are known to be suitable. As a precaution against carbon dioxide contamination, a trap (3 mm x 2 cm) of supported lithium hydroxide, Ascarite<sup>®</sup>, molecular sieve (4 Å or 5 Å) or Porapak<sup>™</sup> (P or Q) can be inserted into the line that leads from the nitrogen supply to the target. Great care must be taken to prevent any contamination of the target by trap contents. To restrict the entry of carbon dioxide by diffusion, metal (stainless steel or copper) is preferred to nylon or other polymers as the material for the tubing between the gas supply and target. The internal diameter of the tubing should be kept as small as practicable with regard to achieving acceptable flow rates and minimising gas volume.

**2.1.3. Target operation.** Targets are operated only in the batch mode. Both cylindrical and conical targets are used, though the latter is preferred. A conical target is more difficult to construct but gives somewhat higher yields and much higher specific radioactivity, mainly due to the containment of the beam within the gas volume and the smaller volume of the target gas used.

A typical conical target used at the Baby Cyclotron (BC1710) at Jülich (Blessing *et al.*, 1988) is shown in Figure 2. It is 144 mm long, with front and rear parts having diameters

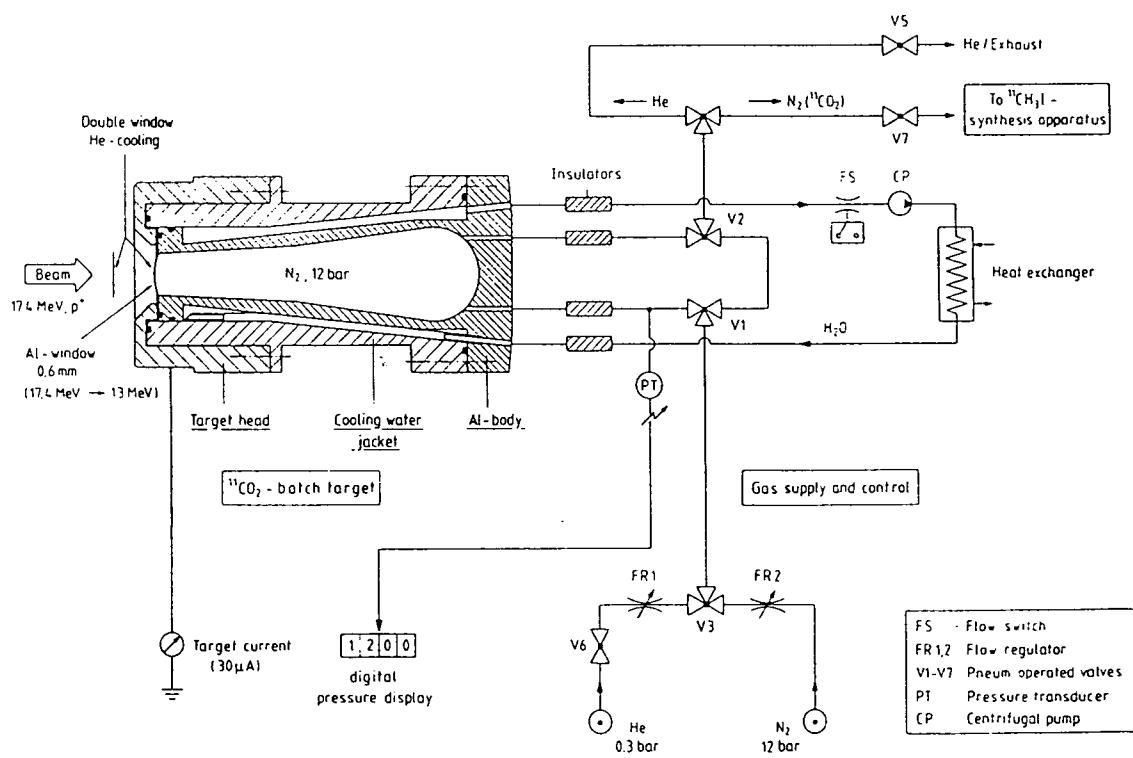


Figure 2. Batch target system used for production of high specific radioactivity [<sup>11</sup>C]carbon dioxide at KFA Jülich (after Blessing *et al.*, 1988)

of 18 and 44 mm, respectively. The target body is made of aluminium-magnesium alloy containing 3% magnesium (AlMg3). The double window in front of the target consists of a 0.6 mm thick aluminium sheet (closing the target) and a 50  $\mu\text{m}$  thick aluminium foil (separating the cyclotron vacuum). A helium stream (0.8 bar,  $-70^\circ\text{C}$ ) flows through the two foils and cools the window. The target body is cooled by flowing water. The target is filled with 'CO-free' nitrogen gas to a pressure of 12 bar. At a beam current of 45  $\mu\text{A}$  (in a typical production run) the pressure rises to 16 bar. The proton energy range within the gas corresponds to  $E_p = 13 \rightarrow 3$  MeV.

**2.1.4. Dose dependence of precursors.** The recoiling carbon-11 atoms undergo hot chemical and radiation chemical reactions in the gas phase, depending on the target fill and the radiation dose (Ache and Wolf, 1968). While using "pure" nitrogen containing traces of oxygen as target gas both [ $^{11}\text{C}$ ]carbon monoxide and [ $^{11}\text{C}$ ]carbon dioxide are formed; the former is favoured at very low doses and the latter at medium and high doses.

From the excitation functions of the competing nuclear reactions on nitrogen, namely  $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$  and  $^{14}\text{N}(\text{p},\text{pn})^{13}\text{N}$ , it is expected that over the optimal energy range for carbon-11 production ( $E_p = 13 \rightarrow 3$  MeV) the amount of nitrogen-13 impurity will be small. Experience has shown that the relative amount of [ $^{13}\text{N}$ ]nitrogen is strongly dose-dependent. Results of a radio-GC analysis are shown in Figure 3 (Nebeling *et al.*, 1990). Evidently, very short irradiations lead to relatively high amounts of [ $^{13}\text{N}$ ]nitrogen. Suitable irradiation times for [ $^{11}\text{C}$ ]carbon dioxide production appear to be 30 min or more.

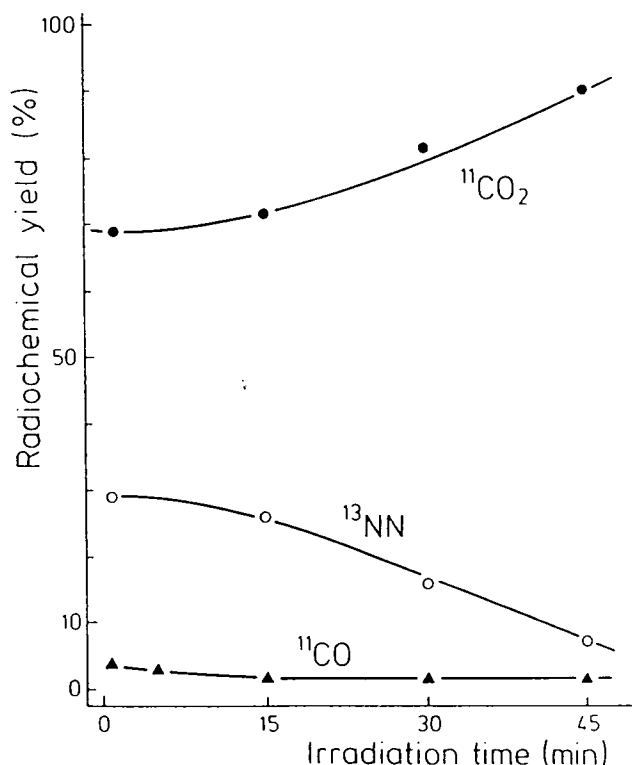


Figure 3. Relative radiochemical yields of some precursors produced by the proton irradiation of nitrogen in a batch target as a function of irradiation time. The proton energy range in the target was  $E_p = 13 \rightarrow 3$  MeV and the beam current 30  $\mu\text{A}$  (Nebeling *et al.*, 1990).

2.1.5. *[<sup>11</sup>C]Carbon dioxide recovery.* [<sup>11</sup>C]Carbon dioxide should be led from the target to a hot-cell in metal tubing (stainless steel, small diameter) and passed through a column of magnesium perchlorate (MgClO<sub>4</sub>·H<sub>2</sub>O) or granular phosphorus pentoxide to trap out any water from the nitrogen gas. To maintain high specific radioactivity, the [<sup>11</sup>C]carbon dioxide should be recovered from the target as quickly as possible. Generally, 2 min are required for releasing the target with a flow of 1–2 L/min. Two methods are recommended for [<sup>11</sup>C]carbon dioxide separation, namely the use of a cryogenic trap or the use of a molecular sieve trap.

*a. Use of a cryogenic trap.*

[<sup>11</sup>C]Carbon dioxide is trapped in a small stainless steel tube (40 cm i.d. x 0.2 mm) immersed in liquid nitrogen (b.p., –196°C) or preferably liquid argon (b.p., –186°C), the temperature being maintained using a temperature controller. The [<sup>11</sup>C]carbon dioxide is recovered simply by passing a slow stream of helium through the trap while it is warmed to room temperature. If liquid nitrogen is used care must be taken to avoid the entrapment of a large quantity of nitrogen which is released vigorously in only a few seconds during delivery. The efficiency of trapping is > 98% and recovery is quantitative. An alternative method uses a cryogenic trap which can be cooled down to –196°C and heated up to 200°C, each temperature in this range being set by a temperature controller.

*b. Use of a molecular sieve trap.*

[<sup>11</sup>C]Carbon dioxide is collected in a pre-activated column (6 cm x 9 mm i.d.) of molecular sieve (4 Å, 60–80 mesh). The column is pre-activated by heating to 400°C under vacuum, followed by cooling with a through flow of nitrogen. Entrapment of [<sup>11</sup>C]carbon dioxide is > 98% efficient. The [<sup>11</sup>C]carbon dioxide may be recovered in a small volume of nitrogen within 2–3 min by heating the trap to > 230°C, with a through flow of nitrogen (20 mL/min). The recovery of [<sup>11</sup>C]carbon dioxide is about 90%.

2.1.6. *Yield and purity.* For the target system shown in Figure 2 an irradiation at 45 μA for 0.7 h leads typically to a [<sup>11</sup>C]carbon dioxide batch yield of > 2 Ci (> 74 GBq). Analysis of the decay curve showed that the activity is more than 99.9% carbon-11 for E<sub>p</sub> = 13→3 MeV. The specific radioactivity of [<sup>11</sup>C]carbon dioxide was found to be about 1100 GBq/μmol (30 Ci/μmol).

2.1.7. *Considerations for the production of [<sup>11</sup>C]methane.* [<sup>11</sup>C]Methane can be produced in targetry identical to that used for [<sup>11</sup>C]carbon dioxide and under similar irradiation conditions except that the target is filled with 5% hydrogen in high purity nitrogen. The yield and specific radioactivity are also similar. The [<sup>11</sup>C]methane may be trapped from the target gas in a Porapak™ Q trap cooled with liquid argon. [<sup>11</sup>C]Methane is recovered by passing helium or nitrogen at about 100 mL/min through the trap while warmed to room temperature. This process may be repeated to obtain the [<sup>11</sup>C]methane in a small volume (ca 2 ml) of inert gas (see Landais and Crouzel, 1987).

## 2.2. NITROGEN-13

Nitrogen-13 (*t*<sub>1/2</sub> = 10 min; I<sub>β+</sub> = 100%; E<sub>β+</sub> = 1.19 MeV) has found limited application in biological studies (for an earlier comprehensive review see Cooper *et al.*, 1985). The most commonly used <sup>13</sup>N-labelled tracer in PET is [<sup>13</sup>N]ammonia; however, [<sup>13</sup>N]nitrate, for example, has also found application in plant physiology (*e.g.* Wieneke and Nebeling, 1990).

Both these chemical species can be produced easily using a water target.

Out of several nuclear processes which lead to the formation of nitrogen-13 (see Ferrieri and Wolf, 1983; Table 1), the  $^{13}\text{C}(p,n)^{13}\text{N}$  and  $^{16}\text{O}(p,\alpha)^{13}\text{N}$  reactions are most useful. The optimal energy range for the  $^{16}\text{O}(p,\alpha)^{13}\text{N}$  reaction is  $E_p = 16 \rightarrow 7$  MeV, and this process is thus ideally suited to a 'baby' cyclotron. This route offers the advantage of the use of a water target, though the yield is the lowest of the three reactions listed in Table 1. At a small cyclotron (with  $E_p \approx 11$  MeV) the nitrogen-13 yield from the  $^{16}\text{O}(p,\alpha)^{13}\text{N}$  reaction is rather low; the alternative at such a low energy cyclotron is the  $^{13}\text{C}(p,n)^{13}\text{N}$  process (Bida *et al.*, 1986) using highly  $^{13}\text{C}$ -enriched powder saturated with natural water (*i.e.* a slurry target). Since a natural water target constitutes the standard method for nitrogen-13 production (*cf.* Vaalburg *et al.*, 1975; Parks and Krohn, 1978; Lindner *et al.*, 1979; Slegers *et al.*, 1980), we describe it below in detail.

**2.2.1. Target hardware.** Target bodies made of nickel or titanium have been used. A typical target, as used at Jülich, is shown in Figure 4. It is exactly the same as that employed in [ $^{18}\text{F}$ ]fluoride production via the  $^{18}\text{O}(p,n)^{18}\text{F}$  reaction on  $^{18}\text{O}$ -enriched water (see below).

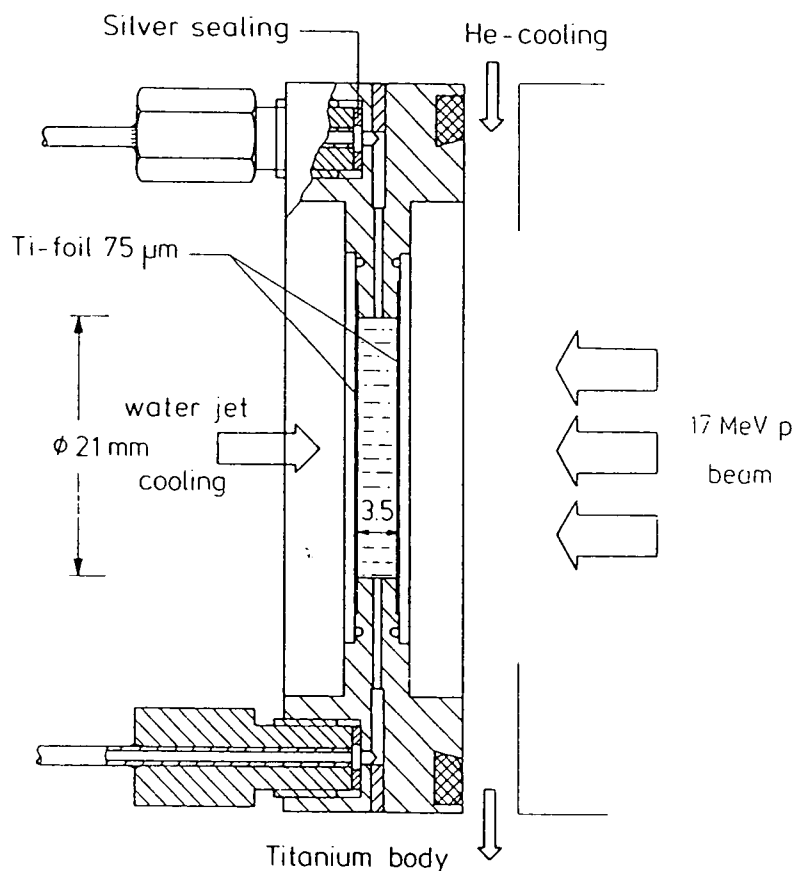


Figure 4. Sectional view of the electron-welded titanium target (internal volume, 1.3 mL) in use at KFA Jülich for nitrogen-13 production via the  $^{16}\text{O}(p,\alpha)^{13}\text{N}$  reaction on natural water. An identical target is used for fluorine-18 production via the  $^{18}\text{O}(p,n)^{18}\text{F}$  reaction using  $^{18}\text{O}$ -enriched water.

The target consists of a titanium body, electron beam-welded to two titanium foils (75  $\mu\text{m}$  thick), which act as front and back window. The inner diameter of the target is 21 mm with a water thickness of 3.5 mm. The target takes 1.3 mL of water with no expansion space. Two 1/16" stainless steel tubes, leading to a multi-port valve are connected via screws that are sealed with silver washers. During irradiation, the back window is water-cooled, typically to 8–10°C, and the front window is helium-cooled to  $-7^\circ\text{C}$ .

2.2.2. *Target operating conditions.* The target is normally operated with a 30  $\mu\text{A}$  beam of 17 MeV protons. The pressure in a routine production run is between 10 and 15 bar.

2.2.3. *Liquid handling and transfer system.* The target can be remotely loaded with water using the filling unit described below for [ $^{18}\text{F}$ ]fluoride production. A polyethylene-polypropylene copolymer tube with an i.d. of 0.8 mm and a helium drive pressure of 1.3 bar is considered a reliable transfer system. Transfer over 40 m through this tubing from the target to the nearest hot-cell by a helium drive pressure of 1.3 bar takes only 2 min.

2.2.4. *Dose-dependence of  $^{13}\text{N}$ -labelled precursors.* The major chemical species observed in the irradiated water are [ $^{13}\text{N}$ ]nitrate, [ $^{13}\text{N}$ ]nitrite, [ $^{13}\text{N}$ ]ammonium ion and [ $^{13}\text{N}$ ]hydroxylamine. Dose effects were observed in several studies (Parks and Krohn, 1978; Tilbury and Dahl, 1979; Root and Krohn, 1981). In general, with increasing dose, the proportion of the higher oxidation state increases at the expense of the lower oxidation states. A typical result is shown in Figure 5 (*cf.* Patt *et al.*, 1991). Effects of dose rate are observed only in the low dose region. The highest [ $^{13}\text{N}$ ]nitrate yield in pure water is  $85 \pm 5\%$  in the high dose region.

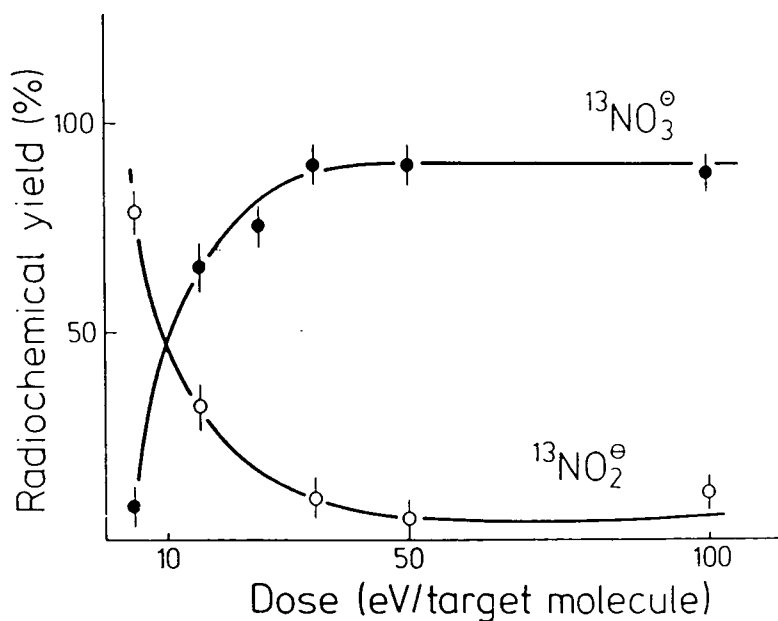


Figure 5. Dose dependence of  $^{13}\text{N}$ -labelled precursors in a natural water target. The proton energy range in the target was  $E_p = 16 \rightarrow 3$  MeV and the beam current 15  $\mu\text{A}$  (Patt *et al.*, 1991).

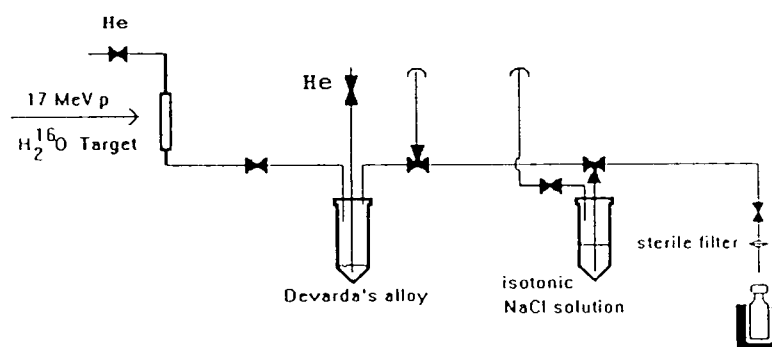


**2.2.5. Production of [ $^{13}\text{N}$ ]nitrate.** The irradiated water (1.3 mL, 99.99%  $^{16}\text{O}$ -enriched to avoid  $^{18}\text{F}$  impurity) is passed through an ion exchange column containing 200 mg of Dowex 50W-X8 (50–80 mesh) and connected to the end of the polyethylene-polypropylene tube. The by-products, [ $^{13}\text{N}$ ]ammonium ion and vanadium-48 (from the activation of titanium), are adsorbed on the column. Sulphuric acid (30%; 100  $\mu\text{L}$ ) is then added to the eluted target water and the mixture boiled to decompose nitrous acid and to expel it as nitrogen oxides. Quality control on the residual [ $^{13}\text{N}$ ]nitrate solution is performed by radio-HPLC on a Nucleosil<sup>TM</sup> 5SB column (250 x 4 mm) eluted with 50mM-perchloric acid adjusted to pH 3.8 with potassium hydroxide (Wieneke and Nebeling, 1990).

A 10 min irradiation with 17 MeV protons at 30  $\mu\text{A}$  leads to about 20.4 GBq (550 mCi) of [ $^{13}\text{N}$ ]nitrate. The radiochemical purity of the [ $^{13}\text{N}$ ]nitrate is > 99.8%. An alternative procedure consists of separation of the [ $^{13}\text{N}$ ]nitrate by preparative chromatography using a Nucleosil<sup>TM</sup> 10 SB column (250 x 10 mm) and the aforementioned eluent. The same results can be achieved by this method.

**2.2.6. Production of [ $^{13}\text{N}$ ]ammonia.** There are two methods for the production of [ $^{13}\text{N}$ ]ammonia (Figure 6).

#### A. Classical reduction with DeVarda's alloy



#### B. On-line reduction *in situ*

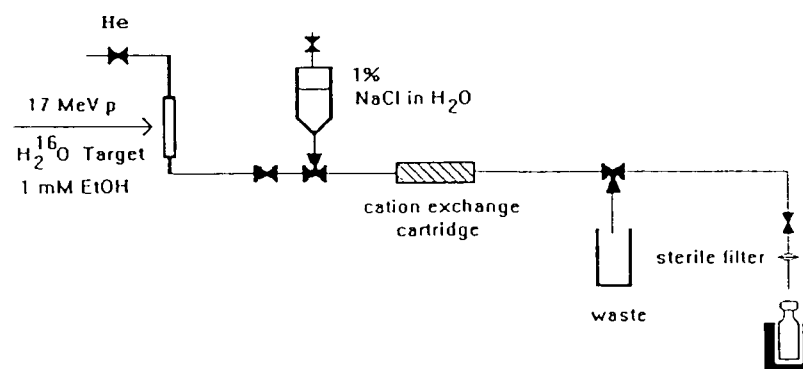


Figure 6. Schemes for the synthesis of [ $^{13}\text{N}$ ]ammonia (A) via classical reduction with DeVarda's alloy and (B) via on-line reduction *in situ*.

In the first method, which is the more classical, the irradiated water is transferred to a vessel containing DeVarda's alloy (*cf.* Vaalburg *et al.*, 1975; Slegers *et al.*, 1980). On reduction of the [ $^{13}\text{N}$ ]nitrate and [ $^{13}\text{N}$ ]nitrite to [ $^{13}\text{N}$ ]ammonia, the gaseous [ $^{13}\text{N}$ ]ammonia is swept by a stream of helium into saline solution. After sterile filtration the product is ready for use. In a new method oxidation is prevented *in situ* (Wieland *et al.*, 1991). Ethanol is added, as scavenger for oxidising radicals, to the distilled and degassed water to give a 1mM-solution. After an irradiation for 10 min at 20  $\mu\text{A}$ , the target volume is transferred to a cation exchange cartridge connected to the end of the transfer line to trap [ $^{13}\text{N}$ ]ammonium ion. The [ $^{13}\text{N}$ ]ammonia is then eluted with an isotonic solution of sodium chloride solution. After final sterile filtration the product is ready for use.

### 2.3. OXYGEN-15

Oxygen-15 ( $t_{1/2} = 2.05$  min;  $I_{\beta+} = 99.9\%$ ;  $E_{\beta+} = 1.72$  MeV) is extensively used in simple chemical forms to study oxygen metabolism, blood flow and blood volume in man with PET. Methods for the production of oxygen-15 and the preparation of [ $^{15}\text{O}$ ]oxygen, [ $^{15}\text{O}$ ]carbon dioxide, [ $^{15}\text{O}$ ]carbon monoxide, [ $^{15}\text{O}$ ]water and *n*-[ $^{15}\text{O}$ ]butanol are well established. Most of the production aspects were described earlier (Clark *et al.*, 1987). Here we give only a summary of the important features and discuss some of the recent developments regarding this radionuclide.

Methods for the production of oxygen-15 are summarised in Table 1 (*cf.* Clark and Buckingham, 1975; Beaver *et al.*, 1976; Vera Ruiz and Wolf, 1977; Sajjad *et al.*, 1984, 1985; Ruth, 1985; Strijckmans *et al.*, 1985; Welch and Kilbourn, 1985; Krohn *et al.*, 1986; Berridge *et al.*, 1990). The  $^{15}\text{N}(\text{p},\text{n})^{15}\text{O}$  reaction on highly enriched nitrogen-15 is used only when low energy protons are available ( $E_{\text{p}} \approx 11$  MeV). The use of  $^{15}\text{N}$ -enriched nitrogen gas to produce [ $^{15}\text{O}$ ]oxygen for both bolus and steady state metabolic studies has been demonstrated using a 4 mL gas target irradiated with a very well regulated 8–11 MeV proton beam (Wieland *et al.*, 1986b).

The  $^{16}\text{O}(\text{p},\text{pn})^{15}\text{O}$  process has a threshold energy of 16.6 MeV and is applied when a medium-sized proton only cyclotron is available. The tendency is to use this method of production only when others are inaccessible (Beaver *et al.*, 1976; Ruth, 1985; Krohn *et al.*, 1986). Pure oxygen is used as the target gas and copious amounts of ozone are produced during irradiation. This ozone is removed together with small amounts of [ $^{13}\text{N}$ ]nitrogen oxides and [ $^{11}\text{C}$ ]carbon dioxide using active carbon and soda lime flow-through purifiers. Use of the purified product as [ $^{15}\text{O}$ ]oxygen, suitably diluted to safe physiological levels, is straightforward. However, the conversion of pure oxygen into water by reaction with hydrogen, although feasible, requires great care to control the highly exothermic reaction! A much more attractive route to [ $^{15}\text{O}$ ]water, using this reaction, is to employ an [ $^{16}\text{O}$ ]water target (Mulholland *et al.*, 1990). Here, when pure water is irradiated, the predominant  $^{15}\text{O}$ -labelled product is [ $^{15}\text{O}$ ]water. Some nitrogen-13 is produced via the  $^{16}\text{O}(\text{p},\alpha)^{13}\text{N}$  reaction together with traces of fluorine-18 from the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reaction on natural abundance [ $^{18}\text{O}$ ]water. These impurities may be removed using mixed-bed ion exchange columns (Mulholland *et al.*, 1990).

The production of [ $^{15}\text{O}$ ]carbon dioxide (Beaver *et al.*, 1976) and in particular [ $^{15}\text{O}$ ]carbon monoxide using pure oxygen targets poses many practical problems and, in the case of carbon monoxide, insurmountable problems relating to toxic levels (Krohn *et al.*, 1986). Preliminary studies with water targets to produce high specific radioactivity

[ $^{15}\text{O}$ ]oxygen and hence [ $^{15}\text{O}$ ]carbon monoxide are encouraging (Link *et al.*, 1991).

The  $^{14}\text{N}(\text{d},\text{n})^{15}\text{O}$  reaction is the most widely used process. The energy of the deuterons incident on the nitrogen target gas should be adjusted to about 8–10 MeV. This energy should minimise the production of the longer lived radionuclidic impurities, nitrogen-13 ( $t_{1/2} = 10.0$  min) and carbon-11 ( $t_{1/2} = 20.4$  min) generated by the  $^{14}\text{N}(\text{d},\text{t})^{13}\text{N}$  (threshold energy, 4.9 MeV) and  $^{14}\text{N}(\text{d},\alpha\text{n})^{11}\text{C}$  (threshold energy, 5.8 MeV) reactions, respectively.

**2.3.1. Target design and gas delivery tubes.** The target vessel used to contain the nitrogen during irradiation is usually made of aluminium alloy. The pressure is usually determined by the diameter and length of the radioactive gas delivery pipe. Typically tubes with internal diameters from 1.5 to 2 mm and 50 m length are used; in exceptional cases tubes of a few hundred metres length have also been found to be practicable. Stainless steel, PTFE<sup>TM</sup> and polypropylene tubes have been used satisfactorily. A titanium beam entry window 25–50  $\mu\text{m}$  thick and 25–30 mm in diameter is fitted with a nitrile, Viton<sup>TM</sup> or metal sealing ring to a 30–50 mm diameter cylindrical vessel some 100–50 mm long, filled with nitrogen gas between 5 and 6 bar, respectively. As with all gas target and accelerator beam windows, provision must be made for their cooling. This is achieved with air or preferably helium jets with flow rates of 10–20 L/min at 1–1.5 bar, with recirculation in the case of helium.

In recent years conically shaped targets used in batch mode have found increasing application. The target system shown in Figure 2, for example, was adapted at Jülich to irradiations with 10 MeV deuterons.

### 2.3.2. Target gases.

#### a. [ $^{15}\text{O}$ ]Oxygen production.

In-target production of [ $^{15}\text{O}$ ]oxygen is achieved by irradiating nitrogen containing 0.2–0.5% (vol) oxygen. The lower level is quite critical as the recovered yield of [ $^{15}\text{O}$ ]oxygen falls dramatically when lower concentrations are used, but may be necessary when higher specific radioactivities are required as, for example, in some [ $^{15}\text{O}$ ]carbon monoxide labelling procedures. [ $^{15}\text{O}$ ]Water may also be prepared from [ $^{15}\text{O}$ ]oxygen. [ $^{15}\text{O}$ ]Oxygen production yields of 0.2–0.3 GBq/ $\mu\text{Amin}$  (5–8 mCi/ $\mu\text{Amin}$ ) using nitrogen-oxygen (0.5%) as target gas can be achieved at flow rates of 500 mL/min.

#### b. [ $^{15}\text{O}$ ]Carbon dioxide production.

In-target production of [ $^{15}\text{O}$ ]carbon dioxide is generally preferred to the less efficient chemical conversion of [ $^{15}\text{O}$ ]oxygen using hot activated charcoal. The target gas is nitrogen containing 2–2.5% carbon dioxide. [ $^{15}\text{O}$ ]Carbon dioxide production yields of 0.2–0.3 GBq/ $\mu\text{Amin}$  (5–8 mCi/ $\mu\text{Amin}$ ) using nitrogen-carbon dioxide (2.5%) as target gas can be achieved at flow rates of 500 mL/min.

**2.3.3. Target gas supply.** Target gas mixtures are generally purchased and are either certified mixtures, such as 0.2% oxygen in nitrogen 6.0, or made from medical grade gases [mixtures of N45 (99.995%)], depending on the requirements of the local regulating authorities.

**2.3.4. Target operation.** Deuteron beam currents of up to 50  $\mu\text{A}$  have been found to be practicable, the target windows being the principal limitation. It is possible to use the same target vessel for [ $^{15}\text{O}$ ]oxygen and [ $^{15}\text{O}$ ]carbon dioxide production provided that adequate

evacuation or flushing of the target and its supply pipes is achieved between irradiations. For  $[^{15}\text{O}]$ carbon monoxide production, the risk of accidental inclusion of oxygen or carbon dioxide with the inherent risk of producing high levels of carbon monoxide precludes the use of a common target in this case.

The batch targets are more suitable for bolus studies with  $[^{15}\text{O}]$ water and  $n\text{-}[^{15}\text{O}]$ butanol.

### 2.3.5. Processing and purification.

#### a. $[^{15}\text{O}]$ Oxygen, $[^{15}\text{O}]$ carbon monoxide and $[^{15}\text{O}]$ water.

The main chemical and radiochemical impurities ozone, nitrogen oxides and  $[^{15}\text{O}]$ carbon dioxide are removed using two adsorber columns in series. The first (25 mm diameter x 120 mm) is filled with sodalime and a second similarly dimensioned column is filled with granules (1.5 mm) of activated charcoal.

When  $[^{15}\text{O}]$ carbon monoxide is produced by passing previously purified  $[^{15}\text{O}]$ oxygen into a column of dried activated charcoal (60 mesh; 250 x 15 mm) at 1000°C, the presence of oxygen carrier inevitably leads to the production of potentially hazardous quantities of carbon monoxide. For example if a nitrogen target containing 0.25% oxygen is used, stable carbon monoxide at levels of at least 0.5% are produced. Under these conditions a yield of  $[^{15}\text{O}]$ carbon monoxide of 37 MBq/ $\mu\text{A min}$  (1 mCi/ $\mu\text{A min}$ ) can be achieved and by 20-fold dilution a carbon monoxide concentration of 0.025% can be administered safely for periods of up to 15 min. On the other hand methods are being developed which are directed towards reducing the stable carbon monoxide content in  $[^{15}\text{O}]$ carbon monoxide and hence ensuring patients' safety (*cf.* Votaw *et al.*, 1986).

$[^{15}\text{O}]$ Water is readily prepared by the palladium-catalysed reaction of  $[^{15}\text{O}]$ oxygen with hydrogen (*cf.* Clark and Buckingham, 1975; Meyer *et al.*, 1984; Clark and Tochon Danguy, 1992). A flow of purified  $[^{15}\text{O}]$ oxygen in nitrogen is mixed with hydrogen and passed over a few pellets of palladium-alumina catalyst, the resulting water vapour is trapped by bubbling the nitrogen carrier through sterile isotonic saline.

The catalyst also generates a small amount of ammonia which causes the pH of the saline to rise to 9. Consequently, the use of a physiological buffer may be desirable for some applications. Batches of over 3.7 GBq (100 mCi) can be readily prepared with deuteron beam currents of 20  $\mu\text{A}$ . Two alternative methods for the preparation of  $[^{15}\text{O}]$ water have been described, one involving the exchange reaction between  $[^{15}\text{O}]$ carbon dioxide and water (Welch and Kilbourn, 1985) and the other employing in-target reaction of oxygen-15 atoms with hydrogen in a nitrogen-5% hydrogen target gas (Vera Ruiz and Wolf, 1978). However, very little experience of these techniques exists.

#### b. $[^{15}\text{O}]$ Carbon dioxide.

During the irradiation of nitrogen-carbon dioxide (2–2.5%) the chemical and radiochemical impurities, carbon monoxide and  $[^{15}\text{O}]$ oxygen, are observed. Earlier attempts to reduce these have now been shown to be unreliable. The use of an oxidising column composed of a mixture of copper and iron oxides supported on kaolin reduces the  $[^{15}\text{O}]$ oxygen and carbon monoxide to < 0.02% and < 10  $\mu\text{L/L}$  respectively (Strijckmans *et al.*, 1985). Nitrogen-13 will inevitably be present in all systems that employ carbon dioxide in the target gas because of the  $^{12}\text{C}(\text{d},\text{n})^{13}\text{N}$  reaction (threshold energy, 0.4 MeV). For applications where > 0.5%  $[^{13}\text{N}]$ nitrogen cannot be tolerated the less efficient low temperature combustion of activated charcoal with  $[^{15}\text{O}]$ oxygen must be employed. The use of the copper-iron oxide column after the carbon furnace is recommended. Another approach to the production of

[ $^{15}\text{O}$ ]carbon dioxide and [ $^{15}\text{O}$ ]oxygen from a common target source is to carry out catalytic interconversion of [ $^{15}\text{O}$ ]carbon dioxide and [ $^{15}\text{O}$ ]oxygen in a flow system using metal oxide catalysts (e.g. Hopcalite<sup>TM</sup>-II) (Iwata *et al.*, 1988).

c. *n*-[ $^{15}\text{O}$ ]Butanol.

This appears to be a very promising agent for regional cerebral blood flow studies. Its production and quality control are described in the chapter on Automation (Crouzel *et al.*, this volume).

2.3.6. *Analysis of gaseous products.* A radio-GC is employed to analyse the chemical and radiochemical 'atmospheric gas' impurities (see Clark and Buckingham, 1975; Meyer, 1982). Typically a dual column instrument, equipped with a thermal conductivity detector for stable gas analysis plus a small sensitive volume flow-cell radioactivity detector for radioactive gas analysis, is used. The two columns most commonly used are:

(a) Porapak<sup>TM</sup>-Q (80–100 mesh, 1500 x 4 mm, pre-conditioned at 250°C)

(b) Molecular sieve 5Å (80–100 mesh 3000 x 4 mm, pre-conditioned at 350°C).

These columns when operated in combination between 25 and 80°C with a helium carrier gas flow rate of about 10 mL/min provide adequate separation of [ $^{15}\text{O}$ ]oxygen [ $^{13}\text{N}$ ]nitrogen, [ $^{15}\text{O}$ ]carbon dioxide, [ $^{15}\text{O}$ ]nitrous oxide and [ $^{13}\text{N}$ ]nitrous oxide.

The chemical analysis of the noxious gases, ozone, nitrogen dioxide, and carbon monoxide, down to the levels necessary for safety prior to the administration of a radioactive gas to man, cannot be carried out satisfactorily by GC. In the case of ozone classical iodometric analysis is recommended. For nitrogen dioxide colorimetric estimation is acceptable.

More details on the quality control of radioactive gases are given in the chapter on Quality Assurance and Quality Control (Meyer *et al.*, this volume).

## 2.4. FLUORINE-18

For fluorine-18 ( $t_{1/2} = 109.6$  min,  $I_{\beta+} = 97\%$ ;  $E_{\beta+} = 0.64$  MeV), perhaps for more than any other radionuclide, success in labelling depends on factors that are determined by radionuclide production. The following section of this chapter is mainly based on a report of a recently sponsored EEC Task Group (Guillaume *et al.*, 1991) and specifically discusses production.

2.4.1. *Main sources of fluorine-18.* Fluorine-18 can be reached by a variety of well-documented (Qaim 1982, 1986a; Qaim and Stöcklin, 1983; Gandarias-Cruz and Okamoto, 1988) routes (for a recent summary see Guillaume *et al.*, 1991). Of these, the  $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$  and  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reactions are of major practical interest, in view of their requirement for only a moderate projectile energy and a moderate beam current to give a useful yield (Figure 1). Hence, even simple cyclotrons, such as those classified by Wolf and Jones (1983) as Level I (< 10 MeV p or d) and Level II (< 20 MeV p, plus perhaps other particles), are capable of useful fluorine-18 production. The  $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$  and  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reactions are mainly applied to prepare c.a. molecular [ $^{18}\text{F}$ ]fluorine and n.c.a. [ $^{18}\text{F}$ ]fluoride, respectively. Currently, nearly all fluorine-18 radiochemistry stems from these cyclotron products, either through their direct use as labelling agents or via their conversion into others (for reviews see Berridge and Tewson, 1986b; Coenen, 1989; Kilbourn, 1990).

Table 2. Typical conditions and production parameters for the cyclotron production of [ $^{18}\text{F}$ ]fluorine by the  $^{20}\text{Ne}(\text{d},\alpha)$  reaction at different centres.  
(Data from Guillaume *et al.*, 1991, with permission).

| Centre                                | BNL                                                                                             | MRCCU<br>Hammersmith            | Jülich                         | Liège                          |
|---------------------------------------|-------------------------------------------------------------------------------------------------|---------------------------------|--------------------------------|--------------------------------|
| Target body                           | (type; mL)<br>(internal size, cm)                                                               | Honed Ni; 50                    | Ni; 38                         | Ni; 206                        |
| Target gas                            | (% $\text{F}_2$ in $\text{Ne}$ ) <sup>a</sup><br>( $\mu\text{mol F}_2$ ) <sup>b</sup>           | 2.5 i.d. x 10<br>0.1            | 2.2 i.d. x 10<br>0.18          | 0.17<br>220                    |
| Pressure                              | (beam off, atm)<br>(beam on, atm)                                                               | 50-60<br>25.8                   | 60<br>18                       | 12.3<br>15                     |
| Window                                | (material, $\mu\text{m}$ )                                                                      | 32.5                            | 30                             | Al; 80-Ni; 20                  |
| E (Incident)                          | (seals)                                                                                         | Al; 810-Ni; 25                  | Nb; 25-Havar; 50               | Indium O-rings                 |
| Irradiation                           | (MeV)                                                                                           | Metal O-ring                    | Metal joints                   | 12                             |
| Recovery of<br>$^{18}\text{F}$ at EOB | ( $\mu\text{A} \times \text{h}$ )<br>(GBq)<br>(mCi)                                             | 14-9.4<br>15 x 2<br>13.6<br>367 | 11.25<br>40 x 1<br>18.5<br>500 | 10 x 1<br>4.4<br>120           |
| Yield                                 | (% of theoretical) <sup>d</sup><br>(MBq/ $\mu\text{Ah}$ at EOB)<br>(mCi/ $\mu\text{Ah}$ at EOB) | 55<br>463<br>12.5               | 50<br>444<br>12                | 51<br>370-555<br>10-15         |
| Specific activity                     | (MBq/ $\mu\text{mol}$ at EOB)<br>(mCi/ $\mu\text{mol}$ at EOB)                                  | 259-370<br>7-10                 | 130<br>3.5                     | 29.6-44.4<br>0.8-1.2           |
| Reference                             | Casella <i>et al.</i> , 1980                                                                    | Clark <i>et al.</i> , 1990      | Blessing <i>et al.</i> , 1986  | Guillaume <i>et al.</i> , 1990 |

<sup>a</sup> Nominal value.

<sup>b</sup> Calculated from target volume and nominal filling parameters.

<sup>c</sup> Experimentally determined as 194-242  $\mu\text{mol}$  recovered, under conditions in which the fill-line contributes fluorine.

<sup>d</sup> Theoretical yields were calculated according to Casella *et al.* (1980).

The  $^{16}\text{O}(^3\text{He},\text{p})^{18}\text{F}$  reaction is also occasionally used for  $^{18}\text{F}$  fluoride production. The yield is low (Table 1 and Figure 1); the only advantage is that natural water rather than  $^{18}\text{O}$ -enriched water is used. However, the cost of the required helium-3 is also significant.

**2.4.2. Production of c.a.  $^{18}\text{F}$  fluorine by the  $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$  reaction.** The bombardment of high pressure neon (up to 25 bar) containing a low percentage (0.1–0.2%) of molecular fluorine with moderate energy deuterons (Lambrecht *et al.*, 1978a; Casella *et al.*, 1980) is a well-established method for producing c.a. molecular  $^{18}\text{F}$  fluorine. Table 2 shows experimental conditions and typical data for the production of  $^{18}\text{F}$  fluorine via the  $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$  reaction at a few centres. Several factors must be taken into account to achieve satisfactory routine production, as follows.

*a. Target design and construction.*

Target bodies have been described formerly (*e.g.* Casella *et al.*, 1980; Blessing *et al.*, 1986). Nickel and Monel™, machined or welded, appear to be the optimal metals for the target body. Stainless steel (316) is also convenient but needs longer passivation with fluorine (*vide infra*). Inconel™ also requires longer passivation and gives lower recovery of fluorine-18 (Casella *et al.*, 1980; Blessing *et al.*, 1986). Nickel plating on stainless steel deteriorates after a few runs (Blessing *et al.*, 1986). Generally, target bodies are 10 to 20 cm long and are fabricated from 1.5 to 2.5 cm i.d. tube with gas connections through stainless steel weld fittings. Swagelok™ fittings are recommended for this purpose. The target must be chemically clean before installation.

The window is preferably made from 25 or 50  $\mu\text{m}$  Havar™ foil (Hamilton Precision Metals). Aluminium or Havar™, having a thick layer (*e.g.* 20–25  $\mu\text{m}$ ) of nickel on the side exposed to fluorine, nickel, niobium and titanium have also been used. Several methods may be used to seal the beam-entry window to the target body. Those that are satisfactory include the use of a copper ring (Leybold) a gold crush seal, an indium wire or a flat washer made from thick (0.5 mm) lead sheet. Cooling of the foil, preferably with helium is recommended.

*b. Target gas.*

The neon should be 99.998% pure as obtainable from Air Liquide or Messer Griesheim, in order to recover the radioactivity mainly as molecular  $^{18}\text{F}$  fluorine (*vide infra*) (Bida *et al.*, 1980a; Casella *et al.*, 1980; Dahl and Schlyer, 1985). The pressure regulator should be all metal and designed for use with high purity gases.

In pure neon, nucleogenic fluorine-18 diffuses to the target wall and is chemically adsorbed. When a low (0.1–0.2%) proportion of carrier fluorine is present, exchange of nucleogenic fluorine-18 can compete with surface adsorption and recovery of most of the radioactivity from the target becomes possible (Lambrecht *et al.*, 1978a; Bida *et al.*, 1980a; Casella *et al.*, 1980). Corrosion of target and ancillary components is not a significant problem if only dilute (*e.g.* < 2%) mixtures of fluorine in neon are used. A mixture of 2% fluorine in neon, with certificate of analysis, can be obtained from Union Carbide, Air Liquide or Matheson. The pressure regulator is specially prepared for use with fluorine mixtures by the gas supplier. Alternatively, a mixture of 2% fluorine in helium, as prepared for lasers, can also be used as the source of fluorine gas. This is supplied by Matheson or Air Liquide with a full Monel™ delivery system.

*c. Target gas handling.*

Given the highly hazardous nature of fluorine, it is emphasised that a safe facility is required to dilute the stock fluorine mixture with further neon to achieve the composition desired within the target.

Stainless steel and Swagelok™ fittings are recommended for transport lines, since surface fluorination (passivation) lasts longer at room temperature than for the alternatives of nickel and Monel™. Teflon tubing, though chemically resistant, is unsuitable as it becomes permeable to fluorine with use (Casella *et al.*, 1980). Stainless steel bellows (Hoke, Nupro) valves are reliable in service. A piezoresistive, low dead-volume pressure transducer (Kistler) is recommended to monitor target pressure before and during irradiation. Great care should be taken to avoid any ingress of air into the gas handling facility and target. Flow purging rather than evacuation of air is thus preferred. It is preferable to dedicate a line for filling the target and to dedicate another low volume line for recovering radioactivity. The fill/empty stoichiometry of this arrangement can be highly reproducible.

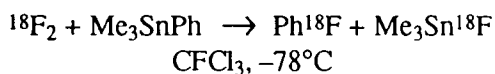
*d. Target operating conditions.*

The main factor that determines [<sup>18</sup>F]fluorine recovery is the chemical state of the target surface (Bida *et al.*, 1980a; Casella *et al.*, 1980). Passivation (prefluorination) of the surface is necessary before bombardment to avoid a significant loss of fluorine-18 along with fluorine carrier. Two passivation methods have been proposed, namely 'thermal' passivation and 'beam-induced plasma' passivation. Thermal passivation requires the target to be heated for 1–3 h to between 100 and 200°C, when filled to a low pressure (a few bar) with dilute fluorine in neon. Beam-induced plasma passivation is achieved by deuteron bombardment of the target when filled to at least the same partial pressure of fluorine as used in production. Two or three irradiations, using about 10 μA of deuterons for 30 min, are carried out successively, with the contents of the target discarded after each irradiation. Recoil passivation is most easy to operate routinely. In general, the requirement to repeat passivation is more likely the longer a target has not been in use. It is considered that a good passivation is achieved when 75 to 80% of the fluorine-18 can be recovered in a production run. Water-cooling of the target during irradiation is not usually necessary, so simplifying target construction.

*e. Chemical forms of the recovered fluorine-18.*

The chemical form of the recovered fluorine-18 depends critically on the purity of the target gas (Bida *et al.*, 1980a; Casella *et al.*, 1980; Dahl and Schlyer, 1985). The neon should be as free as possible from nitrogen and carbon oxides, as these incorporate substantial proportions of the fluorine-18 into inert substances namely, nitrogen [<sup>18</sup>F]trifluoride and carbon [<sup>18</sup>F]tetrafluoride, respectively. Contamination by fluorocarbons should also be avoided. Under such conditions the fluorine-18 is mainly obtained as molecular [<sup>18</sup>F]fluorine (Bida *et al.*, 1980a; Dahl and Schlyer, 1985).

The following reaction is useful to monitor the chemical activity of the available fluorine-18, as well as its specific radioactivity (Blessing *et al.*, 1986):



The amount of [<sup>18</sup>F]fluorobenzene in the freon solution of labelled products is determined by radio-GC. From measurement of the total recovered activity the reactive fraction of recovered fluorine-18 (presumed to be molecular [<sup>18</sup>F]fluorine), and its specific radioactivity



can be calculated. Blessing *et al.* (1986) found the total organic radiochemical yield to be  $51 \pm 2\%$  and the fluorobenzene yield to be  $36 \pm 2\%$  over 30 runs under the production conditions described in Table 2.

It should be noted that deuteron irradiation of neon containing a low percentage ( $< 15\%$ ) of hydrogen has been used by several groups to produce n.c.a. fluorine-18 (Clark *et al.*, 1973; Winchell *et al.*, 1976; Straatman and Welch, 1977; Lambrecht *et al.*, 1978a; Helus *et al.*, 1979; Clark and Oberdorfer, 1982; Dahl *et al.*, 1983; Ehrenkauf *et al.*, 1983; Blessing *et al.*, 1986). Various means have been used to recover the radioactivity, including removal from the heated target with a stream of hydrogen (Winchell *et al.*, 1976; Clark and Oberdorfer, 1982; Dahl *et al.*, 1983; Blessing *et al.*, 1986), recirculation of the irradiated neon-hydrogen mixture over a basic trap (Clark *et al.*, 1973) and water-washing of the target after irradiation (Helus *et al.*, 1979; Blessing *et al.*, 1986). Removal from the heated target in a stream of hydrogen is invariably described as giving n.c.a. hydrogen [ $^{18}\text{F}$ ]fluoride. Water washing of the target recovers a high proportion (90%) of the radioactivity as aqueous [ $^{18}\text{F}$ ]fluoride with a high specific radioactivity of 370 GBq/ $\mu\text{mol}$  (10 Ci/ $\mu\text{mol}$ ) (Blessing *et al.*, 1986). However, these neon-hydrogen targets are relatively inconvenient to operate and difficult to maintain and so are not widely used.

*f. Specific radioactivity.*

The effect of systematically varying fluorine content on activity recovery and specific radioactivity has been examined (Blessing *et al.*, 1986). Between 0.10 and 0.18% (60  $\mu\text{mol}$   $\text{F}_2$ ) of fluorine carrier, [ $^{18}\text{F}$ ]fluorine recovery increased but specific radioactivity did not change. The optimal composition for recovery yield and specific radioactivity seems to be 0.18% fluorine.

The volume of the target is an important factor. It was found that for a collimated (1 cm diameter), defocussed and wobbled beam, the optimal target size is 2.2 cm diameter  $\times$  10 cm length giving a total fluorine content of 60  $\mu\text{mol}$  for the target, leading to a specific radioactivity of 55.5–111 GBq/mmol (1.5–3.0 Ci/mmol) in production (Blessing *et al.*, 1986).

The specific radioactivity of molecular [ $^{18}\text{F}$ ]fluorine can be measured via titration of the iodine liberated on passing the target output into potassium iodide solution (Casella *et al.*, 1980). However, in principle, this method does not distinguish between the several oxidising chemical forms of fluorine-18 (such as  $^{18}\text{F}$ -labelled  $\text{OF}_2$ ,  $\text{HOF}$ ,  $\text{NFH}_2$  or  $\text{NF}_2\text{H}$ ) that might arise in the target if there is any contamination by air. A misleading result might therefore be obtained. Preferably, specific radioactivity is measured via the reaction with trimethylphenyl tin (*vide supra*), which is expected to be more selective for fluorine-18 in the form of molecular fluorine (Blessing *et al.*, 1986).

**2.4.3. Production of n.c.a. [ $^{18}\text{F}$ ]fluoride by the  $^{18}\text{O}(p,n)^{18}\text{F}$  reaction on  $^{18}\text{O}$ -enriched water.** The proton irradiation of  $^{18}\text{O}$ -enriched water is now the most effective method for the production of n.c.a. [ $^{18}\text{F}$ ]fluoride. Under optimal conditions, greater than 20 GBq (several hundreds of mCi) of aqueous [ $^{18}\text{F}$ ]fluoride are easily available from an 11 MeV proton cyclotron, in a bombardment time of less than 1 h. At a 'baby' cyclotron with  $E_p \approx 17$  MeV, batch yields of about 74 GBq (2 Ci) are available. Table 3 summarises typical experimental data on the production of [ $^{18}\text{F}$ ]fluoride from  $^{18}\text{O}$ -enriched water targets, as developed in several laboratories.

Table 3. Typical data for [ $^{18}\text{F}$ ]fluorine produced at different centres from  $^{18}\text{O}$ -enriched water targets.  
(Data reproduced from Guillaume *et al.*, 1991, with permission).

| Centre                                                  | St. Louis                        | Sendai                        | Villigen                                                     | Turku                         | Jülich                                                         | Hammersmith                   | Liège                             |
|---------------------------------------------------------|----------------------------------|-------------------------------|--------------------------------------------------------------|-------------------------------|----------------------------------------------------------------|-------------------------------|-----------------------------------|
| Material insert                                         | SS & Ti                          | Ti                            | Ag                                                           | Ag                            | Ti                                                             | SS316 & Ti                    | Ni                                |
| Water width (mm)                                        | 3, 5, 7                          | 3, 4, 5                       | 5                                                            | 1.5                           | 3.5                                                            | 3                             | 3.4                               |
| Foils                                                   | Havar or Ti                      | Al/Ti & Ag                    | Ag                                                           | Ni                            | Ti                                                             | Al/Ti & SS316                 | Ti                                |
| Seal                                                    | O-ring                           | O-ring                        | Ag                                                           | Metal                         | Weld & Ag                                                      | Metal to metal                | O-ring                            |
| Cooling ( $^{\circ}\text{C}$ )                          | Water (10)                       | Coolant (0)                   | Water (30)                                                   | Coolant (10)                  | Water (10)                                                     | Water (10)                    | Water (10)                        |
| $^{18}\text{O}$ -Enrichment of $\text{H}_2\text{O}$ (%) | 97                               | 20                            | 98                                                           | 98                            | 97                                                             | 20                            | 5                                 |
| Volume (mL)                                             | 1.2, 2.0, 2.8                    | 2.5                           | 4.5                                                          | 0.195                         | 1.3                                                            | 2.0                           | 1.8                               |
| Pressure (atm)                                          | 1                                | Open or<br>circulated (Pd)    | Open                                                         | Open                          | 15-25                                                          | 2                             | 2-3                               |
| E (MeV)                                                 | 15                               | 16                            | 16                                                           | 12                            | 16.5                                                           | 16                            | 22.6                              |
| Irradiation ( $\mu\text{A} \times \text{min}$ )         | 15-20x80                         | 20x60                         | 20x60                                                        | 10x60                         | 20-35x60                                                       | 20x60                         | 10x60                             |
| Yield (GBq/ $\mu\text{Ah}$ at EOB)                      | 2.22                             | 0.33                          | 2.07                                                         | 1.11                          | 2.41                                                           | 0.407                         | 0.11-0.12                         |
| (mCi/ $\mu\text{Ah}$ at EOB)                            | ca 60                            | 9                             | 56                                                           | 30                            | 70                                                             | 11                            | 3-3.2                             |
| Sp. Act. (TBq/ $\mu\text{mol}$ EOB)                     | 1.85                             | 0.148                         | nca                                                          | 5.18                          | 7.4                                                            | $11.1 \times 10^{-3}$         | 0.37                              |
| (Ci/ $\mu\text{mol}$ at EOB)                            | ca 50                            | 4                             | nca                                                          | 140                           | 200                                                            | 0.3                           | 10                                |
| Reference                                               | Kilbourn<br><i>et al.</i> , 1985 | Iwata <i>et al.</i> ,<br>1987 | Huszar &<br>Weinreich,<br>1985; Vogt<br><i>et al.</i> , 1986 | Solin <i>et al.</i> ,<br>1988 | Qaim <i>et al.</i> ,<br>1987; Nebeling<br><i>et al.</i> , 1990 | Clark <i>et al.</i> ,<br>1990 | Guillaume <i>et al.</i> ,<br>1990 |

### *a. Target systems.*

Many different [ $^{18}\text{O}$ ]water targets have been described (Wieland and Wolf, 1983; Kilbourn *et al.*, 1984, 1985; Huszár and Weinreich, 1985; Berridge and Tewson, 1986a; De Jesus *et al.*, 1986; Keinonen *et al.*, 1986; Vogt *et al.*, 1986; Wieland *et al.*, 1986a; Iwata *et al.*, 1987; Qaim *et al.*, 1987; Solin *et al.*, 1988; Berridge and Kjellstrom, 1989; Mulholland *et al.*, 1989; Steinbach *et al.*, 1990). The primary consideration in design has been to consume as little of the costly  $^{18}\text{O}$ -enriched water as possible during production. The requirement for small target volume in turn means that target thickness has to be selected carefully for effective use of the beam. Also radiolysis and boiling of the water become important considerations, if impure water is used for irradiation. These can cause significant loss of target water in open targets (Kilbourn *et al.*, 1984; Berridge and Tewson, 1986; Iwata *et al.*, 1987) or unacceptable pressure build up in closed targets, with adverse effect on yield. Effective target cooling of the front window by helium and the back side with water is mandatory.

The decision to operate a low pressure or high pressure target is fundamental, since it bears on target construction and the strategy for coping with the adverse effects of radiolysis and heat. Experience has shown that the most efficient water target is simply composed of a cavity for the target water, bounded by two rigid metallic foils, the rear foil being efficiently cooled by a suitable fluid. Targets requiring only 0.4–2.5 mL of water can be constructed on this basis. Other parameters of importance are the type of target seals, the chemical nature of the metallic insert and foils, the type of transfer tubing (Iwata *et al.*, 1987) and the facility for recovery of the  $^{18}\text{O}$ -enriched water, as these potentially bear on the reactivity and the specific radioactivity of the generated [ $^{18}\text{F}$ ]fluoride.

For labelling by nucleophilic substitutions the [ $^{18}\text{F}$ ]fluoride is required to be essentially free of water, *i.e.* to be 'naked' n.c.a. [ $^{18}\text{F}$ ]fluoride. Several procedures have been described for the recovery of n.c.a. [ $^{18}\text{F}$ ]fluoride in a reactive form from  $^{18}\text{O}$ -enriched water (*e.g.* Brodack *et al.*, 1986; Schlyer *et al.*, 1987, 1990; Jewett *et al.*, 1988, 1990, 1991a,b; Alexoff *et al.*, 1989; Hamacher *et al.*, 1990). These deliver the [ $^{18}\text{F}$ ]fluoride in natural enrichment water or organic solvent. Generally, the strategy for generating reactive [ $^{18}\text{F}$ ]fluoride is dissolution of the [ $^{18}\text{F}$ ]fluoride with a large counter-ion (*e.g.*  $\text{K}^+$ ,  $\text{Cs}^+$ ,  $\text{Rb}^+$ ,  $\text{Bu}_4\text{N}^+$  or  $\text{K}^+$ -Kryptofix<sup>TM</sup>) in a polar aprotic solvent. Where required, water (or other solvent) is removed by distillation from an added base (*e.g.*  $\text{KOH}$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{Rb}_2\text{CO}_3$ ,  $\text{Et}_4\text{NOH}$ ,  $\text{Bu}_4\text{NOH}$  or  $\text{K}_2\text{CO}_3$ -Kryptofix<sup>TM</sup>) and the [ $^{18}\text{F}$ ]fluoride salt dried by, for example, azeotropic distillation with acetonitrile, or even by microwaves. This salt is then resolubilised in the solvent containing the substrate for nucleophilic attack. Resolubilisation efficiency is affected by the reaction vessel, solvent and perhaps other factors such as metal ion contamination (Brodack *et al.*, 1986). The intrinsic reactivity of the [ $^{18}\text{F}$ ]fluoride is affected by several factors, including cation and anion contaminants whose level are largely determined by the materials of target construction and operation (Nickles *et al.*, 1986). Below we describe and discuss examples of reliable target assemblies designed for low and high pressure operation.

### *b. Example of a reliable low pressure target system.*

In the low pressure mode of operation, without taking appropriate precautions, radiolysis and boiling can cause a significant loss of  $^{18}\text{O}$ -enriched water, and render the use of high beam currents impractical for high yield. Provision for effective venting (Wieland and Wolf, 1983; Berridge and Tewson, 1986a; Iwata *et al.*, 1987) or for the catalytic recombination of radiolytically generated oxygen and hydrogen (Iwata *et al.*, 1987) must always therefore be

considered. Recirculation of the water during irradiation is also advantageous for some targets (Iwata *et al.*, 1987; Keinonen *et al.*, 1987), but results are not necessarily consistent (Iwata *et al.*, 1987). A low pressure target, in use at the MRC Cyclotron Unit at Hammersmith Hospital, is described here as an example of a reliable system.

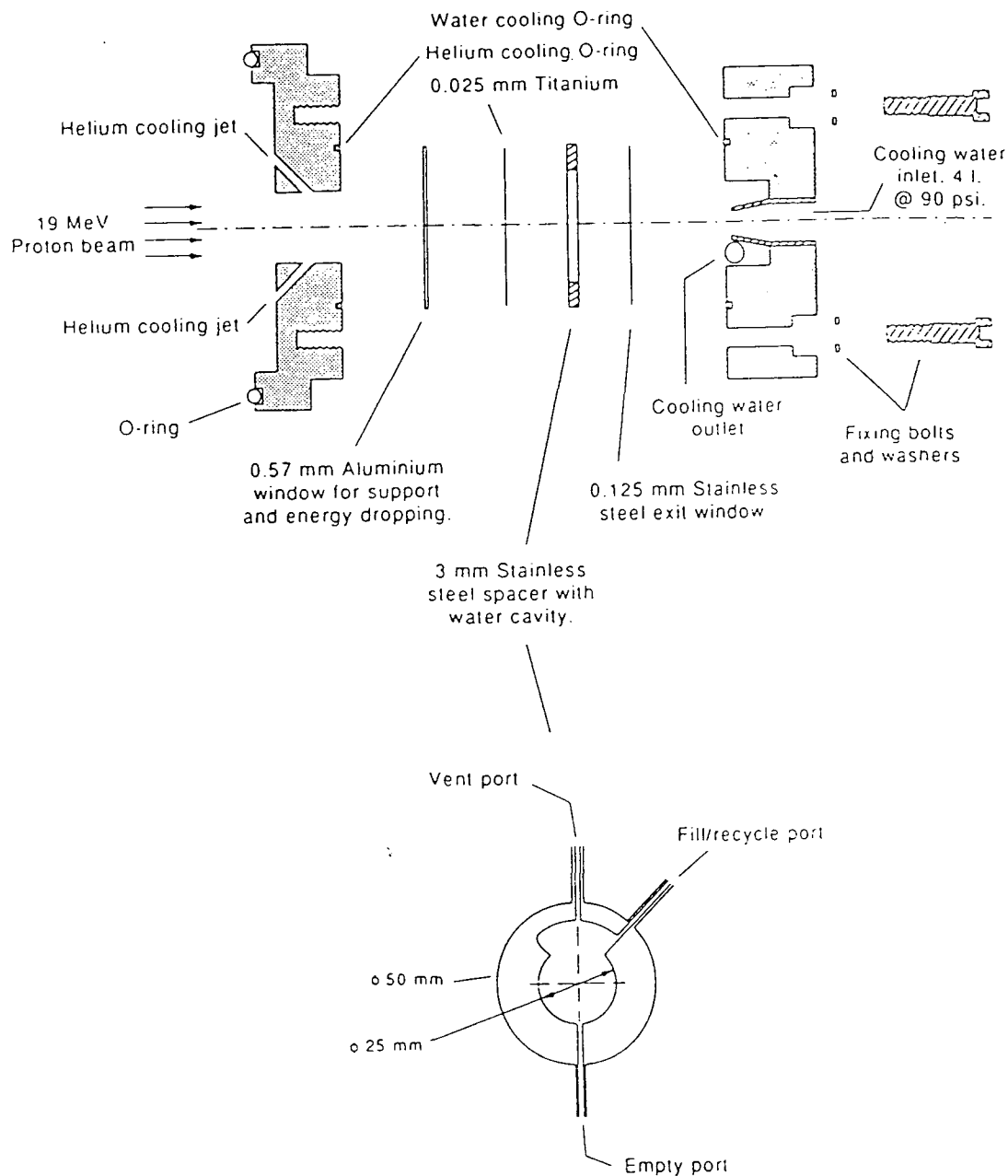


Figure 7. An exploded schematic view of the target (internal volume, 2 mL) in use at the MRC Cyclotron Unit (Hammersmith Hospital) for  $^{18}\text{F}$  fluoride production via the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reaction on  $^{18}\text{O}$ -enriched water.

The target (Figure 7) is fabricated from 25 mm diameter stainless steel (316) and titanium for the proton irradiation of 20%  $^{18}\text{O}$ -enriched water (97%  $^{18}\text{O}$ -enriched water from Isotec diluted with sterile HPLC grade water). The front window is aluminium ( $155\text{ mg/cm}^2$ , 0.57 mm thick) backed on the water side by a titanium foil ( $12.4\text{ mg/cm}^2$ , 25  $\mu\text{m}$  thick). This maintains a flat front surface to the target and degrades the 19 MeV beam to 16 MeV. The water thickness is determined by a 3 mm thick stainless steel spacer. The back of the target is made from 125  $\mu\text{m}$  thick stainless steel (316) foil which is water-cooled. The foils and spacer are clamped together metal to metal. Hence, no 'O' rings are in the target-water cell. The target cell has three 1/16" stainless steel tubes inserted through the spacer, fixed and sealed with Loctite retainer 601, the joints having been pretreated with Loctite Activator T (Cat. No 74736). A small pellet of palladium catalyst (Engelhard Model D) is supported in an external gas space to recombine any radiolytically-generated hydrogen and oxygen that might otherwise cause excessive internal pressure.

The target is normally operated with an incident beam of 19 MeV protons (20  $\mu\text{A}$ , 20 mm diameter, spun at 45–50 Hz). The target is 45 m from the nearest hot cell in which the fluorine-18 can be used. Teflon<sup>TM</sup> lines (1/16" o.d., 0.030" bore) are used with a neon drive pressure of 2 bar and flow regulator. The transfer yield for 1–2 mL target water is currently > 95% while the recovery of [ $^{18}\text{F}$ ]fluoride is typically between 95 and 98%, within 5 min from EOB. Though, at the end of irradiation approximately 65% of the radioactivity is nitrogen-13 ( $t_{1/2} = 9.96\text{ min}$ ) from the  $^{16}\text{O}(\text{p},\alpha)^{13}\text{N}$  reaction, this is not a problem for radiosyntheses with the [ $^{18}\text{F}$ ]fluoride which generally take more than one hour. Final products are devoid of nitrogen-13.

A procedure, based on those described by Schlyer *et al.* (1987, 1990) and Hamacher *et al.* (1990), has been adopted for the recovery of [ $^{18}\text{F}$ ]fluoride and for the recycling of  $^{18}\text{O}$ -enriched water. Carbonate form ion exchange resin (100–200 mesh AG 1X 8; 90 mg) is made into a column (28 mm x 3 mm).  $^{18}\text{O}$ -Enriched water is forced from the target through the column by low neon pressure (2 bar). Finally, neon is blown through the column to extract the last traces of liquid. The recovered water is pooled into 10 to 20 mL batches and distilled under reduced pressure using a microrotary evaporator. The degree of  $^{18}\text{O}$ -enrichment is measured with mass spectrometry (Waters, 1990). Bacterial contamination of the  $^{18}\text{O}$ -enriched water must be avoided by sterile millipore filtration into clean sterile glass vials (Pierce). The [ $^{18}\text{F}$ ]fluoride is recovered from the anion exchange column by elution with potassium carbonate solution. The procedure is highly efficient with respect to recovery of [ $^{18}\text{F}$ ]fluoride and to recycling of the  $^{18}\text{O}$ -enriched water.

The [ $^{18}\text{F}$ ]fluoride is used routinely for the production of 2- $^{18}\text{F}$  D G (2-[ $^{18}\text{F}$ ]fluoro-2-deoxy-D-glucose), according to Hamacher *et al.* (1986). Generally, over 90% of the radioactivity reacts in the first stage of the radiosynthesis, the displacement of the triflyl group, if performed in a glassy carbon vessel. If the reaction is performed in a glass vessel, incorporation is typically 60%.

#### c. Example of a reliable high pressure target system.

Targets without an expansion volume are operable at beam currents up to 30  $\mu\text{A}$ , due to the pressure build up in the target by radiolysis, depending on the purity of the water (Wieland *et al.*, 1986a) which, as a diagnostic, can be monitored during irradiation. A target (Figure 4) in use at Jülich is described here as an example of a system which has functioned reliably, even after reaching an integrated beam current of 3000  $\mu\text{Ah}$ .

The target consists of a titanium body electron beam-welded to two titanium foils (75  $\mu\text{m}$  thick), which act as front and back window. The dimensions of the target are exactly

the same as those described above for nitrogen-13 production. The target takes 1.3 mL of  $^{18}\text{O}$ -enriched water with no expansion space. Two 1/16" stainless steel tubes lead from each end of the target to a multi-port valve (Figure 8) and are connected via screws that are sealed with silver washers.

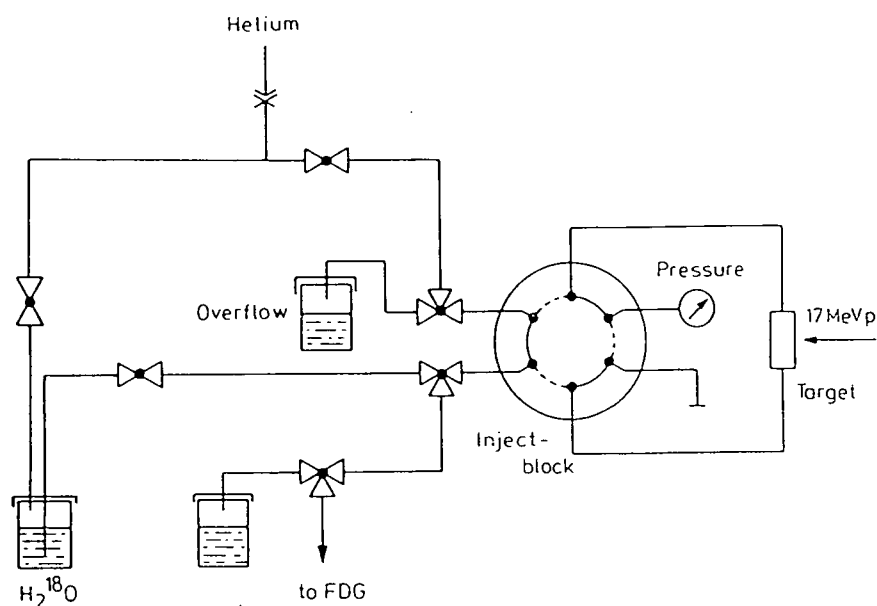


Figure 8. Scheme of the remotely loaded filling unit for the  $^{18}\text{O}$ -enriched water target used at KFA Jülich.

The purity of the  $^{18}\text{O}$ -enriched water used in high pressure targets is of major concern. It is found that high chemical purity is needed to avoid excessive pressure build-up. For large-scale production, greater than 90%  $^{18}\text{O}$ -enrichment is needed to avoid the generation of excessive [ $^{13}\text{N}$ ]nitrogen, which may pose radiation protection problems. Organic impurities must be absent as these can prevent recombination of radiolytically generated oxygen and hydrogen atoms, causing the target to burst. In order to eliminate traces of organic solvents after use, the  $^{18}\text{O}$ -enriched water is purified by reflux with potassium permanganate and potassium hydroxide, and finally double distillation. Purity is assessed by capillary GC with an FID detector operating at highest sensitivity.

The target is normally operated at a beam current of 20–30  $\mu\text{A}$  of 17 MeV protons, depending on the purity of water. The maximum current tested was 35  $\mu\text{A}$ . The pressure in a routine production run is in the range 15–25 bar. The yield of aqueous [ $^{18}\text{F}$ ]fluoride is linear with beam current up to 30  $\mu\text{A}$  and irradiation times up to 1 h.

Filling of the target with  $^{18}\text{O}$ -enriched water is achieved with a motor-driven syringe through 3-way valves (Asco 368) and 6-way valves (Valco AC6W), which are connected by polyethylene-polypropylene tube. The target is locked by the 6-way valve and the pressure is monitored by a piezoresistive pressure transducer with very low dead volume. A polyethylene-polypropylene copolymer tube with an inner diameter of 0.8 mm and helium drive pressure of 1.3 bar is considered a reliable transfer system. Transfer over 30 m through this tubing from the target to the nearest hot-cell takes only 2 min.

The  $^{18}\text{O}$ -enriched water is recovered by retrieving the  $^{18}\text{F}$ fluoride on a carbonate form ion exchange resin (Hamacher *et al.*, 1990). The water can be recycled 4 or 5 times, unless the enrichment of water falls below 90%, as measured by mass spectrometry and by measuring the amount of nitrogen-13 from a production run.

Cation and anion contaminants (Solin *et al.*, 1988) have generally been considered to influence the reactivity of n.c.a.  $^{18}\text{F}$ fluoride. If Havar<sup>TM</sup> foils are used for the target the contaminants are mainly Fe, Cr and Co ions in concentrations ranging from 0.2 to 3 ppm as assessed by atomic absorption spectroscopy. Use of titanium foils results in a decrease in these ions and therefore can be recommended as the least contaminating metal to use as insert and window foil. Contamination of the  $^{18}\text{O}$ -enriched water with vanadium-48 was found to be 6.9 kBq (0.187  $\mu\text{Ci}$ ) after irradiation for 10 min with a 20  $\mu\text{A}$  beam producing 10 GBq (270 mCi) of fluorine-18. An advantage of the  $^{18}\text{F}$ fluoride recovery system is that cationic species are not co-recovered. Over 90% of the recovered  $^{18}\text{F}$ fluoride reacts in the first stage of the synthesis of 2- $^{18}\text{F}$ FDG described by Hamacher *et al.* (1986), when a glassy carbon vessel is used. The target delivers  $^{18}\text{F}$ fluoride with specific radioactivities up to 7400 GBq/ $\mu\text{mol}$  (200 Ci/ $\mu\text{mol}$ ) as measured by a fluoride selective electrode.

2.4.4. *Fluorine-18 from the  $^{18}\text{O}(p,n)^{18}\text{F}$  reaction on  $^{18}\text{O}$  oxygen gas.* The  $^{18}\text{O}(p,n)^{18}\text{F}$  reaction is of considerable interest for producing  $^{18}\text{F}$ fluorine in centres having a proton-only cyclotron. Nickles *et al.* (1984) first described the production of  $^{18}\text{F}$ fluorine-18 from an oxygen-18 gas target using a two-step irradiation process. Nickel is used as material for the target body, and nickel and Havar<sup>TM</sup> foils for the windows. The target is conically shaped and has a volume of 15 mL. After irradiating pressurised  $^{18}\text{O}$ -enriched (98%) oxygen gas, fluorine-18 gets deposited on the target walls, and the oxygen-18 is recovered cryogenically. A second and short irradiation of 1% fluorine (75  $\mu\text{mol}$ ) in krypton is needed for isotopic exchange of the adsorbed fluorine-18 and the recovery of more than 50% of the total fluorine-18 activity (Sunderland *et al.*, 1989). Target performance is determined by the level of fluorine passivation. Passivation with molecular fluorine at 200°C for several hours gives the best results. The  $^{18}\text{F}$ fluorine can be titrated by well known methods (Casella *et al.*, 1980) and has been converted into acetyl  $^{18}\text{F}$ hypofluorite, which was then used for the synthesis of L-6- $^{18}\text{F}$ fluoro-DOPA (Sunderland *et al.*, 1990). Chirakal *et al.* (1992) have also produced fluorine-18 in this manner for the direct radiofluorination of dopamine. Solin and Bergman (1986) used a similar process, but substituted fluorine in neon for fluorine in krypton. They recovered up to 25% of the total radioactivity. Half of the radioactivity was recovered in the  $^{18}\text{O}$ -enriched oxygen. Given the experience with nickel targets, such variations in recovered activity are not unexpected.

Wieland *et al.* (1989) described a one step proton irradiation of a mixture of helium, fluorine and  $^{18}\text{O}$ -enriched oxygen. A gold-plated copper body with a conical bore was used as target and no attempt was made to recover the  $^{18}\text{O}$ -enriched oxygen. Also, no aggressive passivation was used to precondition the target. Preliminary results appeared encouraging. For example, Wieland *et al.* (1989) were able to produce more than 37 GBq (1 Ci) of fluorine-18 radioactivity from a 1 h irradiation with 30  $\mu\text{A}$  beam of 10.4 MeV protons on a target containing only 19  $\mu\text{mol}$  of carrier fluorine. The achieved specific radioactivity is close to 1.85 GBq/ $\mu\text{mol}$  (50 mCi/ $\mu\text{mol}$ ), and thus much higher than that usually achieved via the deuteron irradiation of a neon-fluorine mixture (Table 2).

These results have to be reproduced routinely. Especially the radioactive product has to be tested regularly for its suitability in, for example, the preparation of acetyl [ $^{18}\text{F}$ ]hypofluorite, and in turn of a radiopharmaceutical such as L-6- $^{18}\text{F}$ fluoro-DOPA, before either a one or a two step process with the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reaction on [ $^{18}\text{O}$ ]oxygen can be recommended for the production of fluorine-18.

### 3. Production Limits of Short-lived Organic Positron-Emitters at Low Energy Accelerators

In recent years considerable effort has been devoted to developing very high intensity small accelerators for the production of common positron-emitters, mainly on hospital sites [cf. the detailed presentations at Targetry Workshops in Vancouver (1989) and Villigen (1991)]. The major stipulated advantages of such accelerators include small size, a low activation of components, a low neutron background, and hence almost no requirement for shielding. In general three concepts and designs have been projected:

- Deuteron accelerating cyclotron, 3.6 MeV  $\text{d}^+$ , 70  $\mu\text{A}$ , for producing exclusively oxygen-15 via the  $^{14}\text{N}(\text{d},\text{n})^{15}\text{O}$  reaction on nitrogen gas
- Tandem cascade accelerator, 3.7 MeV  $\text{p}^-$  or  $\text{d}^-$ , 1 mA, for producing oxygen-15 via the  $^{14}\text{N}(\text{d},\text{n})^{15}\text{O}$  reaction and fluorine-18 via the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reaction
- $^3\text{He}$  Radiofrequency quadrupole accelerator, 8 MeV  $^3\text{He}$ , 15 mA, for producing nitrogen-13 via  $^{12}\text{C}(^3\text{He},\text{d})^{13}\text{N}$ , oxygen-15 via  $^{16}\text{O}(^3\text{He},\alpha)^{15}\text{O}$  and fluorine-18 via  $^{16}\text{O}(^3\text{He},\text{p})^{18}\text{F}$  reactions

In principle all three concepts appear to be promising, although the use of the helium-3 beam may be associated with more difficulties. In practice, however, only technology that withstands the stringent test of reliability and simplicity will be acceptable, especially in a hospital environment.

We consider the production of the two major PET radioisotopes oxygen-15 and fluorine-18 at a low energy accelerator in more detail (cf. Qaim, 1992). The excitation functions of the commonly used reactions,  $^{14}\text{N}(\text{d},\text{n})^{15}\text{O}$  and  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ , are shown in Figure 9 (reproduced from Vera Ruiz and Wolf, 1977; Ruth and Wolf, 1979; Sajjad *et al.*, 1984, 1985). A calculation of the integral oxygen-15 yield for an 'on-gas' deuteron energy of 3.2 MeV leads to a value of 222 MBq/ $\mu\text{A}$ , (6 mCi/ $\mu\text{A}$ ) which is 6.7% of the yield with 10 MeV deuterons. Evidently, to obtain an initial oxygen-15 activity of about 11 GBq (300 mCi), a beam current of about 50  $\mu\text{A}$  on the nitrogen gas target is mandatory. This is achievable with the present day technology; indeed the routine production of oxygen-15 with a small accelerator has now been demonstrated at Hammersmith Hospital. In the case of fluorine-18, on the other hand, the integral yield at  $E_p = 3.7$  MeV amounts to 33 MBq/ $\mu\text{Ah}$  (0.9 mCi/ $\mu\text{Ah}$ ) and corresponds to only 1.15% of the yield at 15 MeV. Beam currents of about 500  $\mu\text{A}$  are needed to obtain a batch of about 10 GBq (270 mCi) of fluorine-18. The production of intense beams is not a great problem; however, the construction of a target system capable of withstanding such intense beams involves a new dimension in targetry. A thin film of  $^{18}\text{O}$ -enriched water cooled at the back by liquid nitrogen, as suggested in one concept, may be promising but practical utility and cost-effective economics have yet to be demonstrated.



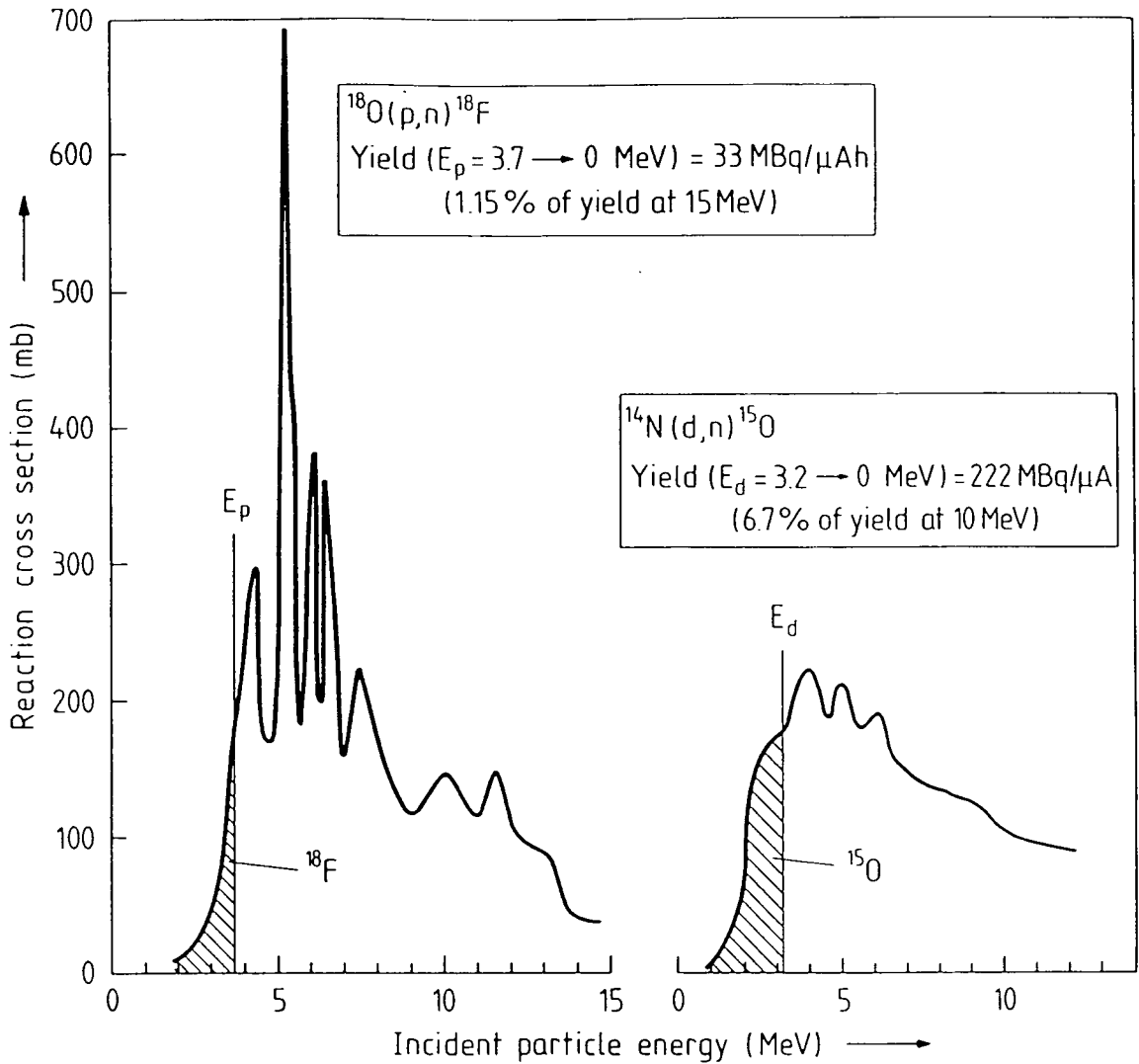


Figure 9. Production limits for fluorine-18 and oxygen-15 at low energy accelerators.

#### 4. Less Commonly used Positron-Emitters

The less commonly used positron-emitters for PET studies include potassium-38, selenium-73, bromine-75 and bromine-76. Furthermore, the positron-emitters, phosphorous-30, manganese-52<sup>m</sup>, iron-52, copper-64, krypton-77, rubidium-81 and rubidium-82<sup>m</sup> have also found limited applications. Rubidium-82<sup>m</sup> ( $t_{1/2} = 6.2$  h) may be of special interest as a longer-lived substitute for generator-produced rubidium-82 ( $t_{1/2} = 1.3$  min), although the radiation dose is somewhat high (see Kovács *et al.*, 1991). In this section we limit ourselves to a description of the production methods of the first four of the aforementioned positron-emitters (Table 1).

#### 4.1. POTASSIUM-38

Potassium-38 ( $t_{1/2} = 7.6$  min;  $I_{\beta+} = 100\%$ ;  $E_{\beta+} = 2.7$  MeV) has found application in myocardial blood flow studies using PET, although three other competing agents,  $[^{13}\text{N}]\text{ammonia}$ ,  $[^{15}\text{O}]\text{water}$  and  $^{82}\text{Rb}^+$ , are more widely used because they are easier to produce with low energy cyclotrons or via a generator system. Among the various methods suggested for the production of potassium-38 (Meyers, 1973; Lambrecht *et al.*, 1978b; Helus *et al.*, 1980; Tilbury *et al.*, 1980; Vandecasteele *et al.*, 1981; Yagi and Amano, 1981; Daube and Nickles, 1985; Guillaume *et al.*, 1988; Qaim *et al.*, 1988; Blessing and Qaim, 1990) the  $^{35}\text{Cl}(\alpha, n)$ -process at a medium energy cyclotron is most convenient, the optimal energy range being ( $E_{\alpha} = 22 \rightarrow 7$  MeV). Irradiation of a sodium chloride pellet with  $\alpha$ -particles at beam currents of about 10  $\mu\text{A}$  leads to sufficient quantities (500 MBq; 14 mCi) for one patient study. The irradiated pellet has simply to be dissolved in water and, after sterile filtration, is ready for human application. The level of long-lived radioactive impurities like sodium-22 ( $t_{1/2} = 2.6$  a) is very small ( $< 10^{-4}\%$ ).

In an attempt to produce potassium-38 at a small cyclotron, Tárkányi *et al.* (1992) recently investigated the  $^{38}\text{Ar}(p, n)$ -process using 95.7% enriched gas as target material. Determination of the excitation function and the measurement of production yields under high current irradiation conditions revealed that, in the optimal energy range of  $E_p = 16 \rightarrow 12$  MeV, the yield from this reaction is an order of magnitude higher than that from the  $^{35}\text{Cl}(\alpha, n)^{38}\text{K}$  process. The  $^{38}\text{Ar}(p, n)$ -process, however, has two drawbacks. Firstly, the highly enriched argon-38 is expensive (one target fill is approximately US \$10,000) and secondly, the recovery of argon-38 is more problematic as compared to krypton-82 or xenon-124 (used for producing of  $^{81}\text{Rb}/^{81\text{m}}\text{Kr}$  generators and  $^{123}\text{I}$ , respectively).

#### 4.2. SELENIUM-73

Selenium-73 ( $t_{1/2} = 7.1$  h;  $I_{\beta+} = 65\%$ ;  $E_{\beta+} = 1.32$  MeV) is a potentially interesting sulphur analogue for application in PET. It can be produced via several nuclear processes (Guillaume *et al.*, 1978; Nozaki *et al.*, 1979; Mushtaq *et al.*, 1988; Mushtaq and Qaim, 1990; Plenevaux *et al.*, 1990). Detailed comparative studies (Mushtaq *et al.*, 1988; Mushtaq and Qaim, 1990) have, however, revealed that only the  $^{75}\text{As}(p, 3n)^{73}\text{Se}$  and  $^{70}\text{Ge}(\alpha, n)^{73}\text{Se}$  reactions (the latter using highly enriched target material) are of practical interest. In the latter case a  $\text{Cu}_3^{70}\text{Ge}$  alloy was developed for irradiations with internal beams of 28 MeV  $\alpha$ -particles and selenium-73 was separated by thermo-chromatography (Blessing *et al.*, 1992). However, the need for very high beam currents, the necessity of recovering the expensive target material, and the low batch yield of selenium-73 make this process rather unattractive. The method of choice is therefore the  $^{75}\text{As}(p, 3n)^{73}\text{Se}$  process.

The excitation functions of  $^{75}\text{As}(p, xn)^{72,73,75}\text{Se}$  reactions are shown in Figure 10 (*cf.* Mushtaq *et al.*, 1988). Evidently the energy range  $E_p = 40 \rightarrow 30$  MeV is optimal for the production of selenium-73. The expected thick target yield of selenium-73 over this energy range is 1406 MBq/ $\mu\text{Ah}$  (38 mCi/ $\mu\text{Ah}$ ) and the level of selenium-72/75 impurities  $< 0.1\%$ . The major drawback of the process is the requirement of relatively high energy protons.

Only low current target materials like arsenic(III) oxide have been used so far for producing selenium-73 via the  $^{75}\text{As}(p, 3n)$ -process (Mushtaq *et al.*, 1988; Plenevaux *et al.*, 1990). The radioselenium is either separated by anion exchange chromatography or extracted directly into benzene. In the latter case the radioselenium is presumably in the

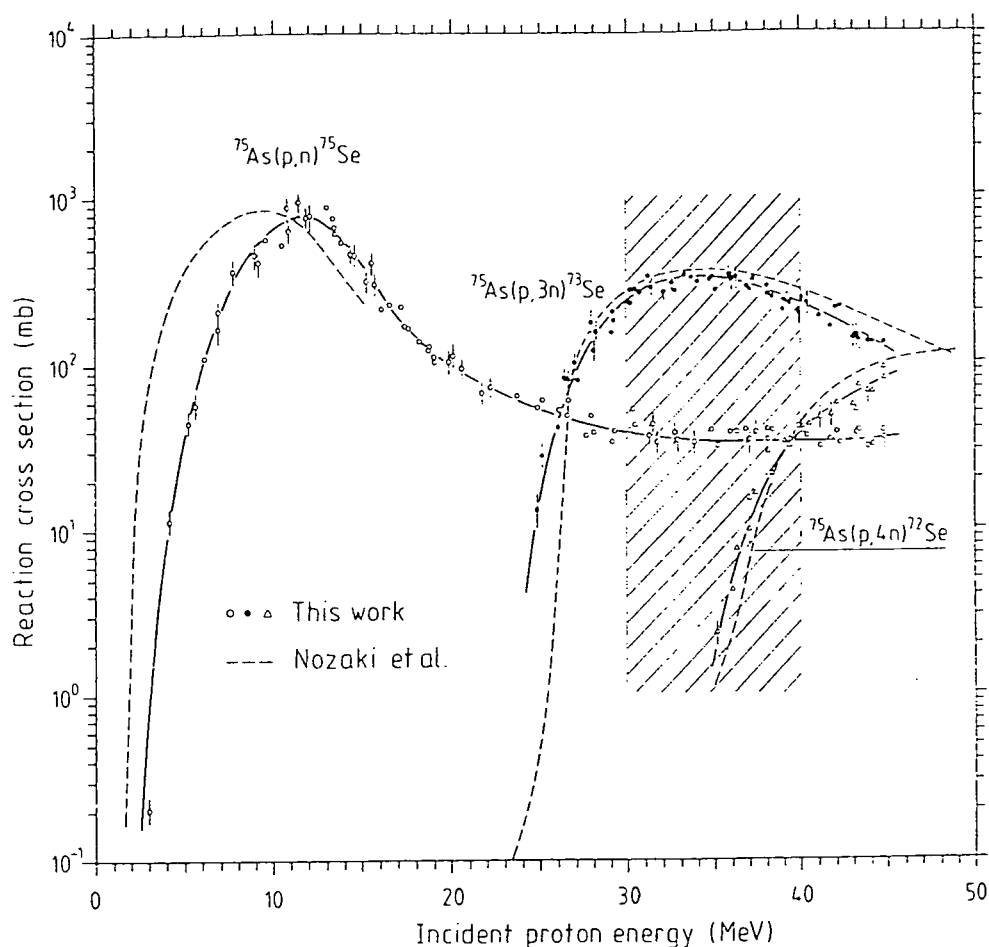


Figure 10. Excitation functions of  $^{75}\text{As}(p,xn)^{72,73,75}\text{Se}$  reactions. The optimal energy range for the production of selenium-73 is  $E_p = 40 \rightarrow 30$  MeV (after Mushtaq *et al.*, 1988).

elemental form and is very suitable for subsequent labelling work (Plenevaux *et al.*, 1990). Attempts to produce large quantities of selenium-73 via development of high current target materials and efficient chemical separation methods are presently underway in several laboratories.

#### 4.3. BROMINE-75

Bromine-75 ( $t_{1/2} = 1.6$  h;  $I_{\beta+} = 75.5\%$ ;  $E_{\beta+} = 1.74$  MeV) is the most useful radionuclide of bromine for PET studies. For its production several nuclear reactions have been suggested (for reviews see Qaim and Stöcklin, 1983; Qaim, 1986b), out of which two processes, namely  $^{75}\text{As}(^3\text{He},3n)^{75}\text{Br}$  and  $^{76}\text{Se}(p,2n)^{75}\text{Br}$ , have proved to be most suitable. The thick target yields of bromine-75 expected from those reactions are given in Figure 11 as a function of incident particle energy. The major impurity associated with both the processes is bromine-76; its contributions under the optimal energy ranges are also given in Figure 11. Evidently, the

$^{76}\text{Se}(p,2n)^{75}\text{Br}$  reaction is more advantageous provided that  $E_p \geq 25$  MeV and highly enriched target material are used. The  $^{75}\text{As}(^3\text{He},3n)^{75}\text{Br}$  reaction, on the other hand, makes use of natural arsenic. In both the cases solid targets are used.

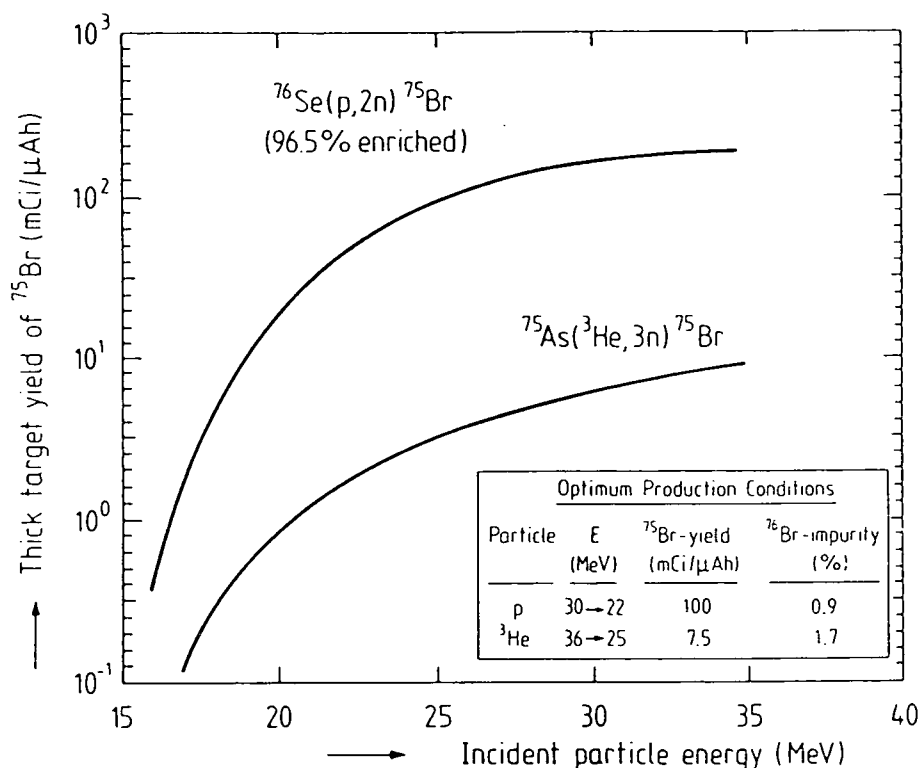


Figure 11. Thick target yields of bromine-75 calculated from the excitation functions (after Qaim *et al.*, 1986b).

In the case of  $^{76}\text{Se}(p,2n)^{75}\text{Br}$  process, a few selenides (like  $\text{Ag}_2\text{Se}$  and  $\text{Cu}_2\text{Se}$ ) were found to be suitable for irradiations with beam currents  $\leq 7 \mu\text{A}$  (Paans *et al.*, 1980; Vaalburg *et al.*, 1985). In another approach an external rotating target system was developed (Kovács *et al.*, 1985) where it is possible to irradiate low melting elemental selenium-76 with proton beam currents up to  $20 \mu\text{A}$ . The loss of selenium-76 after a 1 h irradiation amounts to  $< 1\%$ . The separation of radiobromine from irradiated selenium-76 is effected by thermo-chromatography at  $300^\circ\text{C}$ . Radiobromine is taken up in a small volume of hot water. The loss of selenium-76 during the process is about 1% and the target can be reused. The radiochemical yield of bromine-75, however, is only 40%. Due to this reason the process has not found wide application.

The more widely used method for the production of bromine-75 is the  $^{75}\text{As}(^3\text{He},3n)$ -reaction. A  $\text{Cu}_3\text{As}$ -alloy layer on a copper-backing is irradiated in the internal target system of a compact cyclotron with 36 MeV  $^3\text{He}$ -particles at beam currents of about  $100 \mu\text{A}$  (Blessing *et al.*, 1982; Blessing and Qaim, 1984). A sketch of the system is shown in Figure 12. The beam falls at an angle of  $6.2^\circ$  and only the back of the target holder is cooled. Radiobromine is separated from the irradiated target material via

thermo-chromatography (at 950°C) and is taken up in 1 mL of hot water (*cf.* Blessing *et al.*, 1982). The radiochemical yield of bromine-75 is > 90%. Radiochromatographic analysis shows that bromine-75 is present only as [<sup>75</sup>Br]bromide. The batch yield of bromine-75 is about 7 GBq (190 mCi). The main disadvantage of the process is the rather high level of bromine-76 impurity (6-8% at EOB).

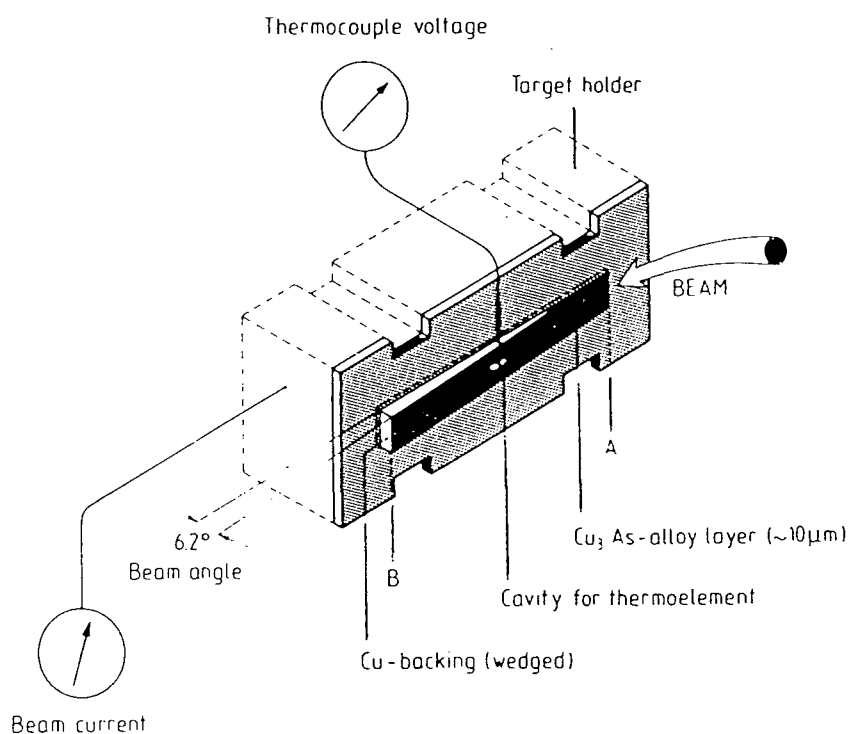


Figure 12. Sketch of the target and target holder used for irradiation with internal beams. The target consists of a thin layer of  $\text{Cu}_3\text{As}$ -alloy on a wedged copper backing. A thermoelement in a cavity in the middle of the target allows the adjustment of the beam (after Blessing and Qaim, 1984).

#### 4.4. BROMINE-76

Bromine-76 ( $t_{1/2} = 16.1$  h;  $I_{\beta+} = 57\%$ ;  $E_{\beta+} = 3.9$  MeV) has found very limited application because it causes a relatively high radiation dose. Nevertheless, this radioisotope seems to be suitable for longer-lasting PET studies, especially on animals (for a review see Mazière and Loc'h, 1986). Its production methods have been reviewed (Qaim, 1986b). The  $^{75}\text{As}(^3\text{He}, 2n)^{76}\text{Br}$ ,  $^{76}\text{Se}(p, n)^{76}\text{Br}$  and  $^{77}\text{Se}(p, 2n)^{76}\text{Br}$  processes have been suggested, although the latter two methods have not been used practically, mainly due to the difficulty in targetry with highly enriched selenium-76 and selenium-77. The method of choice is the  $^{75}\text{As}(^3\text{He}, 2n)^{76}\text{Br}$  reaction over the energy range of  $E_{^3\text{He}} = 18 \rightarrow 10$  MeV, although the yield is low. The production procedure for bromine-76 is the same as for bromine-75 (except for change in the energy range), and quantities up to 1 GBq (27 mCi) have been produced.

Somewhat lesser quantities have been produced via irradiation of elemental arsenic at medium currents, followed by separation of radiobromine via a wet chemical method (Mazière and Loc'h, 1986). The major impurity is bromine-77 ( $t_{1/2} = 56$  h) at a level of  $< 2\%$ .

## 5. Generator-produced Positron-emitters

Several generator produced short-lived positron-emitters find application in PET studies at centres without a cyclotron. Their production methods have been reviewed (Lambrecht, 1983; Knapp and Butler, 1984; Clark, 1986; Guillaume and Brihaye, 1986; Qaim, 1987). Of all the systems suggested the  $^{62}\text{Zn}(^{62}\text{Cu})$ ,  $^{68}\text{Ge}(^{68}\text{Ga})$  and  $^{82}\text{Sr}(^{82}\text{Rb})$  systems are widely used. The decay characteristics of the six radionuclides concerned are summarised in Table 1. Considerable effort has been devoted in recent years to the investigation of chemical aspects of generator preparation. Based on ion-exchange chromatography a large number of generators have been developed for each system. A consideration of all those generators is beyond the scope of this chapter. Here the discussion will be limited to the production of the three parent radionuclides concerned.

### 5.1. ZINC-62

Copper-62 ( $t_{1/2} = 9.7$  min;  $I_{\beta+} = 98\%$ ;  $E_{\beta+} = 2.93$  MeV) has found application in blood flow studies in heart and brain using PET (Green *et al.*, 1990), especially in chelated form. The parent zinc-62 ( $t_{1/2} = 9.2$  h) is generally produced via the  $^{63}\text{Cu}(p,2n)$ -process at a medium-sized cyclotron (see Robinson, 1976; Robinson *et al.*, 1980; Fujibayashi *et al.*, 1989; Green *et al.*, 1990). The optimal energy range for production is  $E_p = 26 \rightarrow 21$  MeV. A copper disc or copper-electroplated aluminium sheet is irradiated with protons at high beam currents. After the decay of short-lived radionuclides, copper is dissolved in hydrochloric acid and the solution transferred to an anion-exchange resin column (AG 1X8, 100–200 mesh,  $\text{Cl}^-$  form). Copper is eluted with 3M-hydrochloric acid, followed by zinc-62 with distilled water. The product is of high radionuclidic purity. The copper concentration in the zinc-62 eluate is  $< 2 \mu\text{g/mL}$  (Fujibayashi *et al.*, 1989). The eluate is generally evaporated to dryness and the residue containing zinc-62 is dissolved in 2M-hydrochloric acid. This solution is then loaded onto an anion-exchange generator column for periodic 'milking off' of the copper-62.

The  $^{63}\text{Cu}(p,2n)^{62}\text{Zn}$  reaction is a high yield process and 15 GBq (400 mCi) quantities of zinc-62 are easily produced (Green *et al.*, 1990). The  $^{60}\text{Ni}(\alpha,2n)^{62}\text{Zn}$  (Neirinckx, 1977) and  $^{64}\text{Zn}(\gamma,2n)^{62}\text{Zn}$  (Yagi and Kondo, 1979) processes give relatively low yields and have therefore not been used for routine production of zinc-62. A direct production route for copper-62 via the  $^{62}\text{Ni}(p,n)$ -reaction has also been suggested (Piel *et al.*, 1992). However, it could be of only limited application.

### 5.2. GERMANIUM-68

Gallium-68 ( $t_{1/2} = 68.3$  min;  $I_{\beta+} = 90\%$ ;  $E_{\beta+} = 1.9$  MeV) is applied for measuring blood-brain barrier integrity as well as for tumour localisation. Furthermore, it is widely used for the attenuation correction of positron tomographs. The parent germanium-68 ( $t_{1/2} =$

271 d) is long-lived and rather difficult to produce. The  $^{69}\text{Ga}(p,2n)^{68}\text{Ge}$  reaction has a high cross section but due to the long half-life of the product radioisotope the yield is low (Pao *et al.*, 1981; Loc'h *et al.*, 1982). Although a high current  $\text{Ga}_4\text{Ni}$  target capable of withstanding proton beams of up to 45  $\mu\text{A}$  was developed (Loc'h *et al.*, 1982), the method has not found wide application due to the very long irradiation times needed. The method of choice is thus the spallation process. This radionuclide is therefore exclusively produced at large accelerators where long irradiations in parasitic positions are possible (see Grant *et al.*, 1982; Robertson *et al.*, 1982). The purification of the product involves several multi-step wet chemical processes. The main impurity is germanium-71 ( $t_{1/2} = 11.2$  d) at a level of 3% at 60 d after EOB.

### 5.3. STRONTIUM-82

Rubidium-82 ( $t_{1/2} = 1.3$  min;  $I_{\beta+} = 96\%$ ;  $E_{\beta+} = 3.35$  MeV) is mainly used for myocardial blood flow studies and has found rather wide application. The production methods for the parent strontium-82 ( $t_{1/2} = 25$  d) have been reviewed (Waters and Coursey, 1987; Tárkányi *et al.*, 1988, 1990). In principle several processes can be used, for example helium-3- and  $\alpha$ -particle-induced reactions on natural krypton (Tárkányi *et al.*, 1988, 1990), (p,4n) reaction on rubidium-85 (Horiguchi *et al.*, 1980; Mausner *et al.*, 1987; Huszár *et al.*, 1989) and, above all, the spallation of molybdenum with high energy protons. In practice, however, the spallation process is the method of choice. The yields in  $^3\text{He}$ - and  $\alpha$ -particle-induced reactions are low and very long irradiations are needed. The (p,4n) reaction is a high yield process but targetry is rather difficult. The major quantities of strontium-82 are therefore presently produced via spallation of molybdenum at high energy accelerators where, similar to germanium-68 production, long parasitic irradiations are possible (see Robertson *et al.*, 1982; Thomas, 1987). Product is purified via multi-step wet chemical separations. The main impurity is strontium-85 ( $t_{1/2} = 65$  d) at a level of about 50% (EOS). On the other hand, the (p,4n) reaction has a great potential, both with regard to strontium-82 yield and strontium-85 impurity.

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## RADIOCHEMISTRY AUTOMATION FOR PET

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**ABSTRACT.** Methodological approaches to automation for PET radiochemistry are discussed. Detailed examples of automation using i) simple remote control, ii) PLC-operated devices and iii) computer-controlled devices are described for the syntheses of  $^{11}\text{C}$ -,  $^{15}\text{O}$ - and  $^{18}\text{F}$ -labelled radiopharmaceuticals. Approaches to robot-based automation are also discussed and exemplified.

### 1. Introduction

PET requires compounds labelled with short-lived positron-emitting radioisotopes, such as oxygen-15, nitrogen-13, carbon-11 and fluorine-18 that have half-lives of 2, 10, 20 and 110 minutes, respectively. In order to label the compounds, several steps must often be carried out, ranging from production of the radioisotope as a primary in-target precursor, sequentially through to synthesis of a secondary labelling agent by an on-line or batch procedure, radiolabelling, purification and quality control. This chapter aims to discuss the need to automate these processes and to describe the approaches and methodologies that have been applied.

### 2. Why Automate?

Only if radiopharmaceutical production is safe, reliable and efficient can PET become a routine clinical tool. In radiopharmaceutical production the short half-lives of the radioisotopes render the total time taken by all the processes a fundamental consideration; it is therefore essential for all steps in each radiosynthesis to be as fast and efficient as possible. The short half-lives also mean that many radiosyntheses are required repetitively. These radiosyntheses must be reliable and easily repeatable for the efficient use of costly PET facilities. Radiosyntheses must usually start from high initial radioactivity but must also be safe to personnel. Automation, within a shielded environment, is increasingly seen as a solution to these key logistical and safety requirements. Furthermore, it must be emphasised that each radiopharmaceutical batch must also meet appropriate quality assurance standards (see Meyer *et al.*, this volume). Significantly, automation can also assist in establishing and complying with all aspects of necessary 'good manufacturing practice' (GMP).

### 3. What to Automate?

In some cases it is preferable to have a simple remotely controlled system rather than a completely automated system, as for example when the radiosynthesis is not very often required or when the radiosynthesis is expected to undergo large modification to increase radiochemical yield. Put simply, full automation should only be applied to radiosyntheses that have reached an adequate degree of refinement and understanding.

For these radiosyntheses all the following processes should be automated or at least remotely controlled:

- Radionuclide production *i.e.*
  - positioning of the target-holder, target cooling and filling with target material.
- Intermediate chemistry *i.e.*
  - production of precursor in-target
  - production of a gaseous labelling agent 'on-line' or of a batch labelling agent.
- Labelling chemistry
- Radiopharmaceutical purification
- Formulation and dispensing.

The following may also be considered for automation:

- Quality control
- Metabolite analysis.

What types of functions can be automated? In order to perform radiosyntheses, the control of reaction temperatures, reaction times, gas flows and pressures is especially important. Furthermore, the automation of all operations, such as radioactivity measurement, reagent additions and extractions, must be considered. These are all amenable to automation of various kinds.

### 4. How to Automate?

When the radiosynthesis of a certain radiopharmaceutical is selected for automation all the chemical procedures have to be carefully defined with respect to reagents, catalysts, temperatures, feedback parameters, volumes, concentrations, materials and chemicals. This process should take into account the requirement for specific radioactivity. These are the chemical criteria. Two further requirements of automation are ease of operation and ease of maintenance. The setting up of an automated radiosynthesis for operation will involve cleaning and drying and also the changing of reagents, flasks and purification materials. It is particularly important for the system to be easily maintainable to allow a repeat radiosynthesis after a relatively short period.

The process of automation starts by selecting the types of workstation required and their combination. There are four main approaches to automation:

- Remote-control, which may be at various levels of sophistication
- Programmable logic control (PLC) with timed steps, perhaps with feedback control
- Computer-aided automation
- Robotics.

In order to automate, means must be found to measure and control temperatures, to measure radioactivity, to dispense solvents, reagents and to perform purifications, whether by extraction

(liquid-liquid, liquid-membrane or solid phase), selective affinity adsorption or chromatography, (such as HPLC). Furthermore, the materials constituting reactors or reaction flasks must be selected taking into account that most radiosyntheses need to be performed with large amounts of radioactivity at high specific radioactivity. Therefore, in any automation, attention must especially focus on:

- Materials in reactors, reaction flasks, tubings, transducers and valves
- Temperature controllers and regulators for reactors, reaction flasks and ovens
- Flow rate controllers and regulators
- Transducers
- Heaters, whether band heaters, heating wires, cartridge heaters (for use in block heaters), heating baths or air heaters (heat guns)
- Dispensing systems for reactors (reaction flasks)
- Extraction hardware (for solid-phase, liquid-liquid, liquid-membrane or gas-membrane extraction)
- Radioactivity detectors.

Specific radiosyntheses, which have been automated by the various non-robotic approaches mentioned above, will be presented. These will give an insight into the solutions that have been found to particular automation problems. This is followed by a general discussion of the robotic approach to automation, with a specific example of a multi-step synthesis. Finally, appendices deal with practical aspects of radioactivity detection (Appendix 1), valve selection (Appendix 2) and sources of glassy carbon reaction vessels (Appendix 3).

## 5. Examples of Remotely Controlled and Automated Radiosyntheses

### 5.1. THE REMOTE PRODUCTION OF [1-<sup>11</sup>C]ACETATE (AS AT HANNOVER)

[1-<sup>11</sup>C]Acetate is prepared by carboxylation of a Grignard reagent (methylmagnesium bromide) with cyclotron-produced [<sup>11</sup>C]carbon dioxide (Pike *et al.*, 1982, 1984; Brown *et al.*, 1987; Walsh *et al.*, 1989; Westera, 1991; Meyer *et al.*, 1993). In order to set up [1-<sup>11</sup>C]acetate production for routine clinical application in heart studies (Pike *et al.*, 1982, 1984; Buxton *et al.*, 1988, 1989; Walsh *et al.*, 1989; Kotzerke *et al.*, 1990), a system must be developed which allows repeated synthesis by an easy and remote assembly. The main obstacle to complete automation in the early published procedures (*e.g.* Pike *et al.*, 1982, 1984) is the liquid extraction step that is used to separate [1-<sup>11</sup>C]acetate from inorganic salts arising from the Grignard reagent. The modification described here is installed at Hannover and avoids this liquid extraction step. It was therefore easy to automate.

**5.1.1. Precursor handling.** After irradiation of nitrogen with protons the target gas is released into a vacuum system, and [<sup>11</sup>C]carbon dioxide is collected from the target gas in a trap that is cooled in liquid nitrogen. [<sup>11</sup>C]Carbon dioxide is then distilled into a special glass vessel, to which methylmagnesium bromide (0.1 mmol) in diethyl ether (1 mL) is added.

**5.1.2. Synthetic procedure.** The reaction vessel is connected to the synthesis apparatus shown in Figure 1. When in place the reaction mixture is warmed to 30–40°C for 5 min with a remotely controlled hot-air gun. The radioactive adduct is then hydrolysed by adding water (100 µL) from a pressure-driven dispenser. Organic solvents and decomposition products are removed by

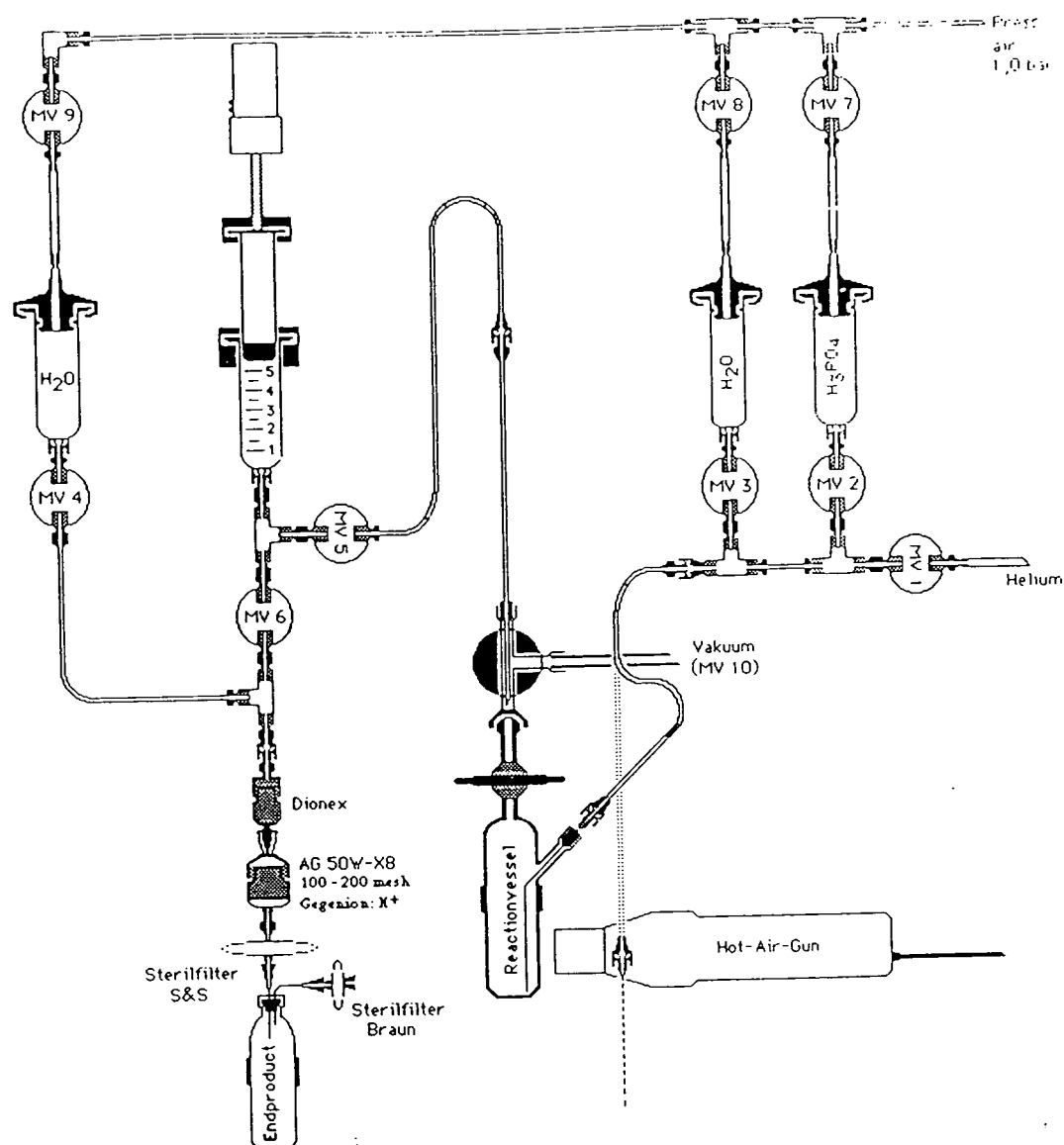


Figure 1. Apparatus for the production of  $[1-^{11}\text{C}]$ acetate (as at Hannover). MV signifies a solenoid valve.

warming the reaction mixture to  $120^\circ\text{C}$  under vacuum. The dry residue is redissolved in dilute phosphoric acid, added from another dispenser. Any free  $[^{11}\text{C}]$ carbon dioxide is then removed with a stream of helium and by warming to  $60^\circ\text{C}$  for 3 min.  $[1-^{11}\text{C}]$ Acetic acid, in dilute phosphoric acid, is then withdrawn from the reaction vessel into a motor-driven disposable syringe. Bromide and  $\text{Mg}^{2+}$  ions are removed from the product mixture by passage through a cartridge of silver oxide and cation exchange resin. Washing the purification line with water is performed to improve the radiochemical yield. The solution of purified product is passed through a sterile  $0.22\ \mu\text{m}$  filter and enters a sterile vessel containing sufficient sodium hydroxide

to neutralise the phosphoric acid. The preparation takes 20 min from EOB. The overall radiochemical yield of  $[1-^{11}\text{C}]\text{acetate}$  is 60%. The radiochemical purity of  $[1-^{11}\text{C}]\text{acetate}$  is found to be > 98% by HPLC.

## 5.2. REMOTELY CONTROLLED SYNTHESIS WITH CARBON-11 (AS AT ORSAY)

At Orsay remote-control is preferred to full automation for synthesis with carbon-11. This approach is used in several hot-cells and the main advantage is great flexibility. It can be used for many types of synthesis from different labelling agents, including  $[^{11}\text{C}]\text{iodomethane}$ ,  $[^{11}\text{C}]\text{formaldehyde}$ ,  $[2-^{11}\text{C}]\text{acetone}$  and  $[1-^{11}\text{C}]\text{acid chlorides}$ . The system is based on the use of small pneumatically-powered jacks to move reaction flasks in two dimensions, horizontal and vertical. The jacks are controlled by the operator from a panel located on the front of the hot-cell. The different vials are connected by catheters and can be isolated by remotely operated valves.

5.2.1.  *$[^{11}\text{C}]\text{Carbon dioxide production}$* .  $[^{11}\text{C}]\text{Carbon dioxide}$  is produced by the  $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$  reaction on nitrogen (99.9999% purity) and collected in a stainless steel trap immersed in liquid argon. The end of trapping is read from an ionisation chamber. The valves and the cryotrap movements are controlled by the operator.

5.2.2. *Production of labelling agents*. The synthesis of  $[^{11}\text{C}]\text{iodomethane}$  is based on the classical method (Comar *et al.*, 1974; Långström and Lundqvist, 1976), as recommended by Crouzel *et al.* (1987). A two-pot synthesis is used. The syntheses of  $[^{11}\text{C}]\text{formaldehyde}$ ,  $[2-^{11}\text{C}]\text{acetone}$  and  $[1-^{11}\text{C}]\text{acid chlorides}$  are based on the methods of Marazano *et al.* (1977), Berger *et al.* (1980) and Luthra *et al.* (1985, 1990), respectively. Heating, cooling and valve-switching are controlled by the operator.

5.2.3. *Labelling*. The labelling agent is distilled into the cold precursor solution with a flow of nitrogen. Maximal transfer of radioactivity is read from an ionisation chamber. For the labelling reaction, the vial is isolated by remotely controlled valves. All stages are controlled by the operator.

5.2.4. *HPLC purification*. The radiopharmaceutical is purified by HPLC. The loop of the injector is filled via a dip-tube from the diluted reaction mixture by applying gas overpressure within the reaction flask. The HPLC eluate containing the labelled product is identified by the operator by monitoring radioactivity and UV absorbance. The labelled product is collected in a flask by manual and remote switching of the fraction collect/waste valve and then heated to evaporate solvent.

5.2.5. *Components of the system*. All the used components are commercially available. The whole unit consists of five sub-units:

- Heating unit: 4 furnaces with fingers resistances (50–250°C)
- Power units and numerical temperature regulators (3 each, all *ex* Vulcanic Co.).
- Pneumatic unit: 7 jacks (6 for vertical displacement, 1 for horizontal displacement) operated by 7 electro-pneumatic units (*ex* Télémécanique Co.).
- Chemistry unit: 2 mL Reacti-vials™ each with a Teflon™-faced silicon septum (3 mm thick) retained by a Quickfit™ cap. The needles are by Becton

Dickinson. The 7 valves with pneumatic actuator are by Rheodyne. They are controlled by 4 electro-pneumatic units. Gas flows are measured by flow meters (Brooks). Radioactivities are monitored by ionisation chambers from Service Development Scientifique (SDS).

- HPLC unit: injection into the HPLC loop is carried out by pressure through a Rheodyne™ valve operated by an electro-pneumatic unit. The HPLC detectors are an ionisation chamber (SDS) for radioactivity and a UV absorbance detector for mass. After evaporation of the solvent, the radiopharmaceutical is dissolved in a physiologically acceptable medium (2–3 mL). This is then transferred into a small evacuated sterile vial using a recovery vial penetrator.

5.2.6. *Performance of the system.* The system is used for the routine production of [*S*-methyl-<sup>11</sup>C]methionine (Comar *et al.*, 1976), [*N*-methyl-<sup>11</sup>C]MQNB (Mazière *et al.*, 1982), [*N*-methyl-<sup>11</sup>C]flumazenil (Mazière *et al.*, 1984) and [*N*-methyl-<sup>11</sup>C]PK 11195 (Camsonne *et al.*, 1984) from [<sup>11</sup>C]iodomethane, and several other labelled compounds from other precursors in non-routine production. 3.7–7.4 GBq (100–200 mCi) of each radiopharmaceutical is produced with a specific radioactivity of about 37 GBq/μmol (1 Ci/μmol) at EOS.

### 5.3. AUTOMATION OF <sup>11</sup>C-METHYLATIONS USING A PLC (AS AT HAMMERSMITH HOSPITAL)

Many <sup>11</sup>C-labelled compounds of great interest to PET clinical science have now been prepared by the methylation of suitable precursors using [<sup>11</sup>C]iodomethane and the number continues to grow. At Hammersmith Hospital, in order to respond to the varied needs of a busy clinical research programme, a hot-cell has been successfully equipped with a <sup>11</sup>C-methylation facility that can be quickly set up and operated to produce radiopharmaceutical batches for clinical use. The sequence of operations that is needed to achieve each radiosynthesis is controlled in timed steps by a Programmable Logic Controller (PLC). This incorporates a 'plug-in' module that can be programmed to suit each individual radiosynthesis (Clark and Dowsett, 1992).

5.3.1. *[<sup>11</sup>C]Carbon dioxide production.* [<sup>11</sup>C]Carbon dioxide is produced by the proton irradiation of high purity nitrogen gas via the <sup>14</sup>N(p,α)<sup>11</sup>C reaction. For labelling operations it is advantageous to concentrate the [<sup>11</sup>C]carbon dioxide into a few millilitres from a volume of several litres of target gas. An automated cryotrap fabricated from a small coil of stainless steel tubing (20 cm x 1/16" o.d.) cooled in liquid argon is used. The use of liquid argon avoids the problematical co-trapping of significant volumes of nitrogen which occurs if liquid nitrogen is used as the coolant. The valves and cryotrap movement are controlled by the PLC's programme.

5.3.2. *Production of [<sup>11</sup>C]iodomethane.* The synthesis of [<sup>11</sup>C]iodomethane using the reaction of [<sup>11</sup>C]carbon dioxide with lithium aluminium hydride (LAH) and subsequent reaction with hydriodic acid is now widely applied (for a review see Crouzel *et al.*, 1987). The major problem in this radiosynthesis is maintenance of the high specific radioactivity required for <sup>11</sup>C-labelled receptor ligands. This can principally be achieved by the use of high quality LAH in small quantities under carefully controlled carbon dioxide-free atmospheres and by working quickly (for a review see Crouzel *et al.*, 1987). The heating, cooling and sequencing of the valves for [<sup>11</sup>C]iodomethane production are controlled by PLC programme.

5.3.3. *<sup>11</sup>C-Methylation of precursor.* The conditions under which the precursor is methylated depend critically on the class of compound of interest and the subsequent work-up and purification procedures. An automated reaction vial penetrator is used (see Figure 2). This device allows the relevant precursor to be introduced together with the reaction solvent and base in a septum-sealed vial. [<sup>11</sup>C]Iodomethane can then be distilled into the precursor solution via a round-tipped side-hole needle, which can be motor-driven through the septum under programme control. The needles can then be withdrawn and the vial lowered into the heating bath. The <sup>11</sup>C-methylation takes place for a pre-programmed time. The reaction mixture is extracted by re-inserting the needles and transferred to the loop of an HPLC injection valve. Again this sequence of events is controlled by the PLC.

5.3.4. *HPLC purification.* For some compounds, where the base and/or solvent are detrimental to the performance of the HPLC, sample-enrichment can be included in the programme of operations (see Figure 2). Here an injection loop (10 mL) is loaded with an aqueous wash of the reaction vial and the contents of this loop are then loaded onto a silica C-18 cartridge. This cartridge is washed with water and then back-eluted onto the main silica C-18 column for purification using the chosen eluent. The PLC controls the sequence of operations for the multi-port valves necessary to accomplish these steps. The HPLC eluate that contains the labelled product is identified by the operator by monitoring radioactivity and UV absorbance, and collected in the flask of a rotary evaporator by manual remote operation of the fraction collect/waste valve (see Figure 2).

5.3.5. *Product formulation.* Solvent is removed from the product by evaporation under reduced pressure using a micro-rotary evaporator, modified to allow it to be raised or lowered under the control of the PLC. Finally, under programme control, the formulation solvent is introduced into the flask and the resultant radioactive solution transferred via a millipore filter into a shielded shipping vial outside the hot-cell.

5.3.6. *Components of the system.* Wherever possible commercial components are used with little or no modification. A Toshiba EX40 industrial programmable logic controller, which uses ladder logic was chosen. The programme information is stored on a plug in Electronically Erasable Programmable Read Only Memory (EEPROM) module which can be interchanged readily. The controller contains relays that are suitable for low power (100–240 V a.c.; 2 A) and low voltage (24 V d.c.; 2 A) operations. Most of the valves and motorised drives are controlled directly by these relay outputs. The 240 V operated devices, such as heaters and pumps, are operated via isolating solid state relays. The two- and three-port Teflon™ diaphragm valves for liquid and vapour handling are by Angar Scientific [part nos 3682NC2430 and 368232430 for 24 V and up to 2 bar (30 psi) operation]. They have 1/4" x 28 threads in flat-bottomed ports suitable for use with low pressure chromatography fittings (e.g. 'Altex™', 'Omnifit™'). For gas handling the two- and three-port stainless steel valves are by Skinner [Honeywell Lucifer, part nos B2 RX 127 DC-2 and B14 DK 1075 DC-2 operated at 24 V for pressures up to 12 bar (330 psi) and 5.3 bar (80 psi), respectively]. The multi-port rotary valves are by Rheodyne. The low pressure Teflon™/Kel-F™ valve with 1/4" x 28 fittings is a six-port distribution valve with pneumatic actuator (part no 5011P). The HPLC valves are also by Rheodyne and are pneumatically-actuated stainless steel and Vespel™ (Dupont polyimide) rotary valves (part no 7010P). The rotary evaporator is a UV Micro (Heidolph) and has been modified to be raised and lowered pneumatically. The reaction vial for methylation is a Reacti-vial™ (Pierce, 1 mL) with a

Teflon<sup>TM</sup>-faced silicon septum 3 mm thick retained by a Quickfit<sup>TM</sup> (Corning) SQ 13 cap. The penetration needles are by Hamilton with rounded tips and side holes (part no 100.090.218). The syringe drives and septum penetrator drives are of 'in house' design and manufacture and are 'screw and nut' devices driven by electric motor. The HPLC detectors are by Mini Instruments for radioactivity detection (model 7-10C with a MC10 G-M tube) and by Severn Analytical for UV absorbance detection (model SA 6506).

5.3.7. *Performance of the system.* The system has been used routinely (see Pike *et al.*, 1990) to produce clinical batches of *S*-[*N*-methyl-<sup>11</sup>C]nomifensine (Ulin *et al.*, 1989) and [*O*-methyl-<sup>11</sup>C]raclopride (Ehrin *et al.*, 1987). It was also set up to make [*N*-methyl-<sup>11</sup>C]SCH 23990 (Halldin *et al.*, 1986) for a short period. The production of [*O*-methyl-<sup>11</sup>C]raclopride in particular benefitted from the sample-enrichment facility. The production parameters for these radiosyntheses are listed below.

a. *S*-[*N*-methyl-<sup>11</sup>C]Nomifensine.

[<sup>11</sup>C]Iodomethane is trapped in a solution of *desmethyl-S*-nomifensine (5 mg) in ethanol (400 µL). Then the reaction pot is sealed and heated at 90°C for 5 min. The reaction mixture is purified by HPLC on a µ-Porasil<sup>TM</sup> column (30 x 0.8 cm i.d.; Waters Associates Inc.) eluted with chloroform-methanol (90:10 v/v) at a flow rate of 3 mL/min. The product is formulated in isotonic saline which is sterilised by millipore filtration. A decay-corrected radiochemical yield of 23% from [<sup>11</sup>C]iodomethane provides 370–555 MBq (10–15 mCi) of *S*-[*N*-methyl-<sup>11</sup>C]nomifensine ready for injection with a specific radioactivity of 15 GBq/µmol (405 mCi/µmol at EOS).

b. [*O*-methyl-<sup>11</sup>C]Raclopride.

[<sup>11</sup>C]Iodomethane is trapped in DMSO (400 µL) containing *desmethyl*-raclopride (2 mg) and sodium hydroxide solution (5M; 10 µL). The reaction pot is sealed and heated at 90°C for 5 min. The reaction mixture is purified by sample enrichment followed by reverse phase HPLC using a µ-Bondapak<sup>TM</sup> C-18 column (30 x 0.8 cm i.d.; Waters Associates Inc.) eluted with methanol-10mM ammonium dihydrogen phosphate solution (60:40 v/v) at 3 mL/min. The product is formulated in isotonic saline which is sterilised by millipore filtration. A decay-corrected radiochemical yield of 25% from [<sup>11</sup>C]iodomethane provides 370–555 MBq (10–15 mCi) of [*O*-methyl-<sup>11</sup>C]raclopride ready for injection with a specific radioactivity of 10 GBq/µmol (370 mCi/µmol at EOS).

c. [*N*-methyl-<sup>11</sup>C]SCH 23390.

[<sup>11</sup>C]Iodomethane is trapped in acetone (900 µL) containing *desmethyl*-SCH 23390 (1 mg). The reaction pot is sealed and heated at 90°C for 10 min. The acetone is then evaporated off. The reaction mixture is purified by HPLC on µ-Porasil<sup>TM</sup> column (30 x 0.8 cm i.d.; Waters Associates Inc.) eluted with chloroform-methanol-*aq.* ammonia (90:1:0.1 by vol.) at 3 mL/min. The product is formulated in isotonic saline which is sterilised by millipore filtration. A decay-corrected radiochemical yield of 40% from [<sup>11</sup>C]iodomethane provides 0.37–1.11 GBq (10–30 mCi) of [*N*-methyl-<sup>11</sup>C]SCH 23390 ready for injection.

Subsequently a second hot-cell has been equipped with a very similar system to extend the range of routinely available <sup>11</sup>C-labelled compounds within each working day, for example, to [*N*-methyl-<sup>11</sup>C]PK 11195 (Camsonne *et al.*, 1984; Cremer *et al.*, 1992), [*N*-methyl-<sup>11</sup>C]flumazenil (Mazière *et al.*, 1984) and L-[*N*-methyl-<sup>11</sup>C]deprenyl (Fowler *et al.*, 1987).



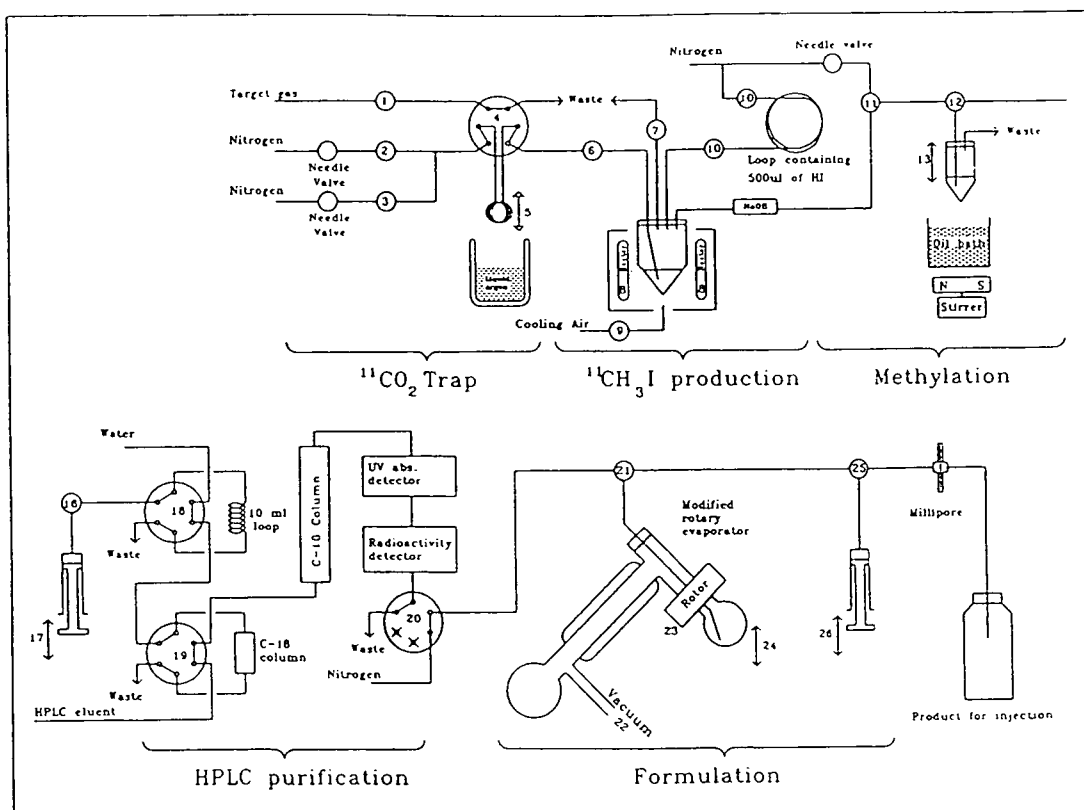


Figure 2. Scheme of PLC-controlled apparatus for labelling radiopharmaceuticals by methylation with  $[^{11}\text{C}]\text{iodomethane}$  (as at Hammersmith Hospital).

#### 5.4. ON-LINE PREPARATION OF $[^{11}\text{C}]\text{Iodomethane}$ AND $[^{11}\text{C}]\text{Methyl Triflate}$ (AS AT KFA JÜLICH).

**5.4.1. Introduction.** The reliable preparation of  $[^{11}\text{C}]\text{iodomethane}$  with high specific radioactivity and with a significantly reduced requirement for maintenance of the synthesis unit, by avoiding concentrated hydriodic acid as an iodination agent, are major objectives in the construction of a general methylation unit. A very important improvement in the synthesis of  $[^{11}\text{C}]\text{iodomethane}$  is the use of a column of triphenylphosphine di-iodide adsorbed on  $\gamma$ -alumina as the iodination agent (see Holschbach and Schüller, 1993a). An additional on-line column technique, the passage of  $[^{11}\text{C}]\text{iodomethane}$  through an alumina column which is charged with silver triflate, gives n.c.a.  $[^{11}\text{C}]\text{methyl triflate}$  as a highly reactive methylation agent (see Holschbach and Schüller, 1993b). Based on these column techniques, a fully automated unit for methylations with  $[^{11}\text{C}]\text{iodomethane}$  or  $[^{11}\text{C}]\text{methyl triflate}$ , incorporating feedback control and radiochromatography, has been developed in compliance with GMP. The set-up of the system is shown in Figure 3.

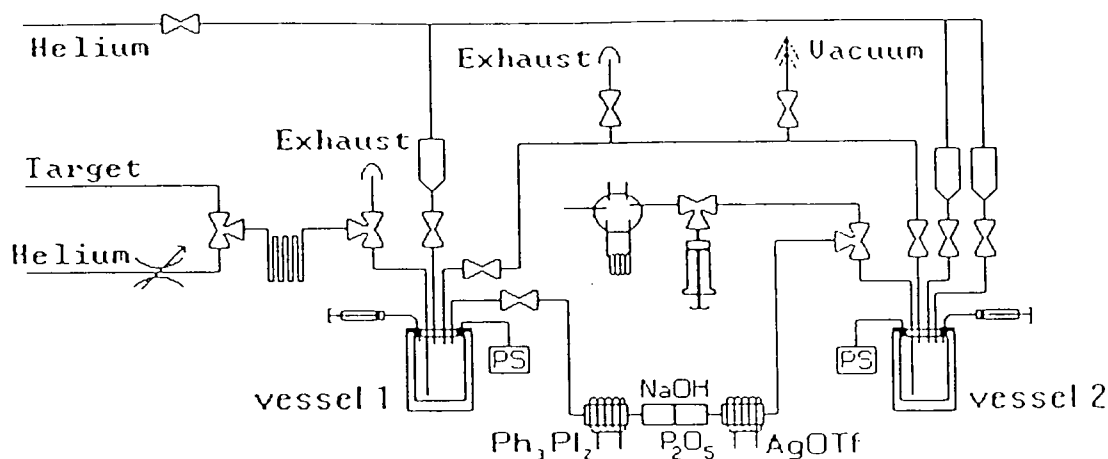


Figure 3. Scheme of the automated on-line preparation of [ $^{11}\text{C}$ ]iodomethane and [ $^{11}\text{C}$ ]methyl triflate (as at KFA Jülich).

**5.4.2. System operation.** The proton-irradiated nitrogen target gas (see Qaim *et al.*, this volume) is passed through a capillary tube at  $-193^{\circ}\text{C}$  to  $-185^{\circ}\text{C}$ , where the [ $^{11}\text{C}$ ]carbon dioxide is trapped. The trap is temperature controlled from  $-196^{\circ}\text{C}$  to  $200^{\circ}\text{C}$ . The [ $^{11}\text{C}$ ]carbon dioxide is then transferred by a flow of helium into reaction vessel 1 and reduced by lithium aluminum hydride. Subsequent addition of a mixture of diethyleneglycol monobutyl ether and water releases [ $^{11}\text{C}$ ]methanol, which is then converted into [ $^{11}\text{C}$ ]iodomethane or into [ $^{11}\text{C}$ ]methyl triflate by a column technique, as described above.  $^{11}\text{C}$ -Methylation is performed in reaction vessel 2 by transferring the methylating agent into the precursor solution. Both vessels are temperature controlled from  $-196^{\circ}\text{C}$  to  $200^{\circ}\text{C}$ . Also both reaction vessels have pneumatic drives to move the PEEK<sup>TM</sup> tubings in the vessels.

**5.4.3. System performance.** The preparation of [ $^{11}\text{C}$ ]iodomethane takes 12 min from the end of bombardment and has a radiochemical yield of 90% based on [ $^{11}\text{C}$ ]carbon dioxide. The mean specific radioactivity of the [ $^{11}\text{C}$ ]iodomethane is  $> 222 \text{ GBq}/\mu\text{mol}$  ( $6 \text{ Ci}/\mu\text{mol}$ ). HPLC purification of labelled products is performed on-line, including formulation to give an injectable solution.

## 5.5. AN ADVANCED SYSTEM FOR THE ADMINISTRATION OF [ $^{15}\text{O}$ ]WATER (AS AT HAMMERSMITH HOSPITAL)

**5.5.1. Introduction.** The important blood flow tracer, [ $^{15}\text{O}$ ]water, may be readily prepared by a variety of routes. However, several problems need to be addressed if safe and effective injections or infusions are to be prepared. Of primary concern is that the product should be free from any harmful impurities, both chemical and radioactive, and be sterile and apyrogenic. Due to the short half-life of oxygen-15, quality control of each sample or batch would be impractical. However, most groups carrying out work in this area have evolved safe operating procedures

(SOPs) which have been shown to be capable of maintaining the required standards (for recommendations on QA and QC see Meyer *et al.*, this volume). The measurement and delivery of the prepared doses of [ $^{15}\text{O}$ ]water also requires careful attention both for the administrator and for the recipient. As a typical injected bolus of [ $^{15}\text{O}$ ]water would be 3-3.7 GBq (80-100 mCi) the radiation dose to the administrator is of great concern. Automated injectors and infusers are at advanced stage of development in several laboratories and the commercial exploitation of these devices is anticipated.

5.5.2. *Design and operation of [ $^{15}\text{O}$ ]water infuser.* Figure 4 schematically describes a 'bedside' [ $^{15}\text{O}$ ]water infuser (dubbed 'R2D2') recently developed at the MRC Cyclotron Unit (Hammersmith Hospital) (Clark and Tochon Danguy, 1992). [ $^{15}\text{O}$ ]Water is synthesised in the lead-shielded bedside device by mixing cyclotron-produced [ $^{15}\text{O}$ ]oxygen (in 99% nitrogen-1% oxygen) with 5% hydrogen in nitrogen over a palladium catalyst in an oven at 200°C. The gaseous output is led to a semi-permeable membrane interface (Visking-Medicell International,

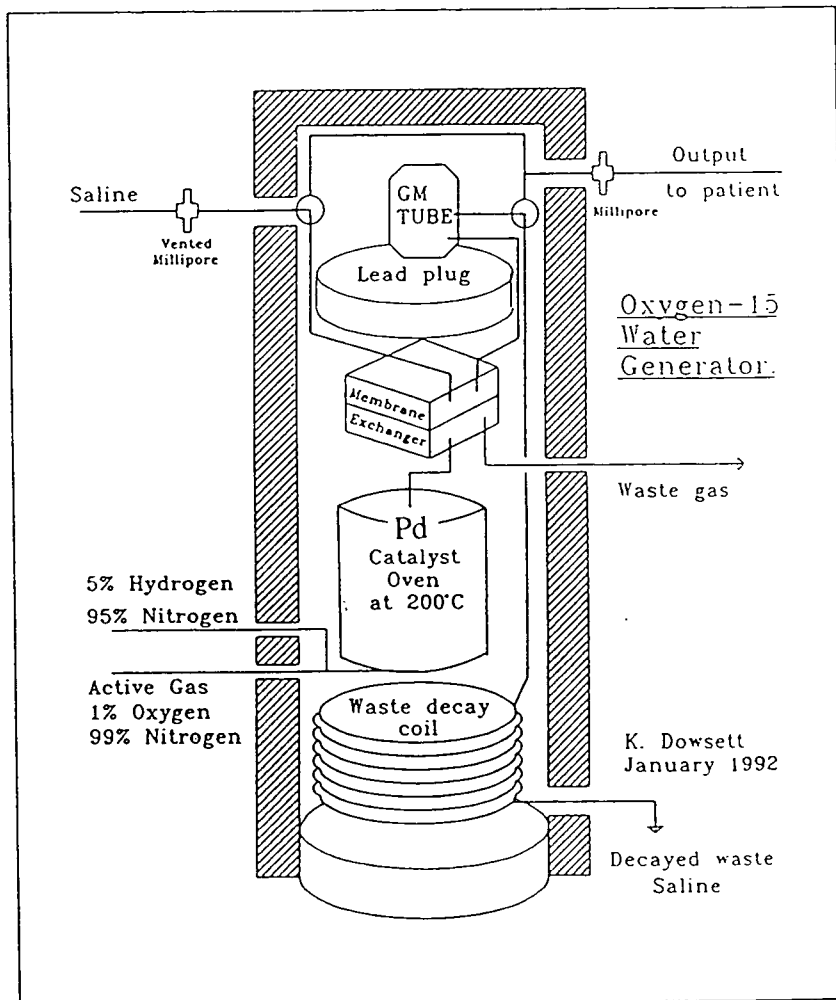


Figure 4. Schematic representation of the bedside [ $^{15}\text{O}$ ]water synthesis apparatus (as at Hammersmith Hospital).

U.K.) sandwiched between Perspex (DWG No 15) blocks. The other side of the interface is flushed with sterile saline. [ $^{15}\text{O}$ ]Water diffuses rapidly across the membrane and is taken up into the sterile saline. This is then infused into the subject for study via a shielded G-M tube and sterile millipore filter, using a pump (IVAC 560) approved for medical application. The fluid handling valves, for control of the infusion parameters, are three-port membrane valves (Angar/Asco, 368, 24 V). 'On-line' radioactivity detection is based on passing the radioactive saline through a loop of Teflon<sup>TM</sup> tubing (1/16" o.d.) wound around the G-M tube (Philips ZP-1300). The counts are taken into a scaler-ratemeter (Mini Instruments 6-90) modified with adjustable pre-scaler to allow direct calibration as 'mCi infused'. This calibration is routinely checked against a standard ionisation chamber. All controls and power supplies are designed and built to comply with electromedical safety standards. Non-used product is allowed to decay within the unit before discharge into a sterile bag. The device is typically operated to give an infusion for 2 min at a flow rate of 10 mL/min. The amount of radioactivity infused is determined by the mode of operation of the PET scanner (CTI-Siemens Neuro-PET) and is typically 560 MBq (15 mCi) for 'septa out' and 3 GBq (80 mCi) for 'septa in'.

**5.5.3. Infuser maintenance.** Pharmaceutical maintenance involves cleaning and sterilising the membrane exchanger prior to assembly and washing the fluid system with isotonic saline solution while the millipore filters are in place. Pyrogen tests (LAL) and sterility tests are carried out to validate the assembly techniques. Routine operation requires replacement of the disposable components, such as the pump tube, saline bag, millipore filters and infusion catheters, followed by pyrogen tests (LAL).

## 5.6. AN ADVANCED SYSTEM FOR THE ADMINISTRATION OF [ $^{15}\text{O}$ ]WATER (AS AT HANNOVER)

**5.6.1. Introduction.** Depending upon the clinical question, [ $^{15}\text{O}$ ]water for blood flow studies may be administered by different protocols:

- Bolus injection of up to 4 GBq (108 mCi) with strictly defined and repeatable input function.
- Steady state infusion in the range of 50–300 MBq/min (1.35–8.1 mCi/min) at a flow rate of 2.5 mL/min for 30 min.
- Ramp infusion with increasing or decreasing specific radioactivity in the range of 0.2–1 GBq (5.4–27 mCi) for 5–10 min.

In order to minimise radiation dose to personnel, a system for the fully automated production of [ $^{15}\text{O}$ ]water (Meyer *et al.*, 1986; Matzke *et al.*, 1993) and its infusion or injection by remote control has been installed at Hannover and this is now described.

**5.6.2. [ $^{15}\text{O}$ ]Oxygen production.** [ $^{15}\text{O}$ ]Oxygen is produced by the  $^{14}\text{N}(\text{d},\text{n})^{15}\text{O}$  reaction in a nitrogen gas target. The target gas contains 0.5% oxygen as carrier and its mean transit time from the target is 2.2 min. The maximal activity at the input of the [ $^{15}\text{O}$ ]water system is 6 GBq/min (160 mCi/min) at a target pressure of 14 bar (210 psi) and a beam current of 30  $\mu\text{A}$ .

**5.6.3. [ $^{15}\text{O}$ ]Water production.** The [ $^{15}\text{O}$ ]water production and infusion system is mounted on a laboratory trolley standing beside the PET camera. All radioactive parts of this system, except the calibration devices, are shielded within a lead box of 20 x 30 x 20 cm inner shape with a 15 x 13 cm lead glass window. The wall thickness of the box is 5 cm. The window has an

equivalent shielding factor (see Figure 5). Within the lead box the target gas enters a flow meter. Hydrogen (2 mL/min) is added downstream. The gas mixture passes through a tube oven, filled with about 0.2 g palladium (1%) catalyst on a charcoal support (4–8 mesh) which is held at a reaction temperature of 150–160°C. The resulting [ $^{15}\text{O}$ ]water vapour is trapped in a four-necked flask filled with isotonic saline solution. The level of the saline in the flask is monitored by a set of ten light barriers. With a small gear pump, the [ $^{15}\text{O}$ ]water is pumped out and into the calibration and infusion system. All devices within the lead box are fixed to a mounting plate, which can easily be lifted out of the box for maintenance. Outside the box is a flow meter with a needle valve for the control of the hydrogen gas flow, and also a second gear pump, which withdraws isotonic saline solution from an infusion bottle to fill the absorption flask inside the lead box. The radioactivity levels inside the absorption flask and in the waste line are monitored by two G-M counters and are registered by a pen-recorder.

The whole apparatus is operated under the command of an automatic system, which includes interlock devices in order to prevent mis-operations. For safety reasons all electrical parts which are in contact with the infusion system are driven by 12 V only.

Under maximal radioactivity load (bolus injection), the radiation dose at a distance of one metre from the apparatus is less than 30  $\mu\text{Sv/h}$ .

**5.6.4. Bolus injection.** The system for the bolus injection consists of a pneumatically-driven syringe (10 mL), placed in an ionisation chamber and a solenoid valve for switching flow direction. The connection to the patient is by an infusion tube and a sterile filter with a total void volume of 1.7 mL. The amount of [ $^{15}\text{O}$ ]water inside the syringe is monitored by a set of ten pilot lamps at the control unit.

The system is operated in the following manner. The absorption flask is filled to a level of 11 mL. Then [ $^{15}\text{O}$ ]oxygen production is started. When sufficient radioactivity (at least 1.7 times the desired injection radioactivity) is sampled, the labelled water is pumped into the injection syringe. This takes 15 s and gives approximately 1.3 times the injection dose within the syringe. Interlock circuits in the control unit prevent air from pumping into the syringe (minimum level in the absorption flask, 1 mL) and from mistakes in handling the system. Injection is started by pressing a push-button switch when the radioactivity has decayed to 1.2 times the injection dose. It may be stopped immediately at any time. The injection of 10 mL is completed within 7 s.

The whole procedure from start of bombardment to end of injection takes about 10 min, and the next run may be started immediately.

**5.6.5. Constant infusion and ramp infusion.** The radioactivity control unit for the constant infusion and ramp infusion methods is a G-M detector with a calibration loop (0.2 mL). It is connected to the patient by an infusion tube, a three-way valve and a sterilisation filter (void volume, 1.9 mL). In all cases a constant flow rate of 2.5 mL/min is maintained by pump 2.

For steady state infusion the saline volume in the absorption flask is kept constant to 13 mL by pump 1, which is under command of the level control system. The saline flow is started 4 min after the start of [ $^{15}\text{O}$ ]oxygen production. An equilibrium is reached about 10 min later, when the infusion may be started. The radioactivity level of the infusion solution is pre-defined by the beam current and the flow rate of the target gas. The relatively large volume in the absorption flask buffers short deviations in beam current, so that an additional control mechanism for the radioactivity level is unnecessary. A deviation from the saline level in the absorption flask causes an alarm to signal, and both pumps will be switched off by the control

unit at differences greater than 15%.

For the ramp infusion, the start procedure is the same as for constant infusion, except that the saline level is low (2 mL) for decreasing, and high (13 mL) for increasing specific radioactivities. When equilibrium is reached, the flow rate of pump 1 is set to a value greater or smaller than 2.5 mL/min. This will result in  $e$ -function shaped gradients. Linear gradients are realised by continuous variation of the flow rate of pump 1 under the control of an external ramp generator or a computer.

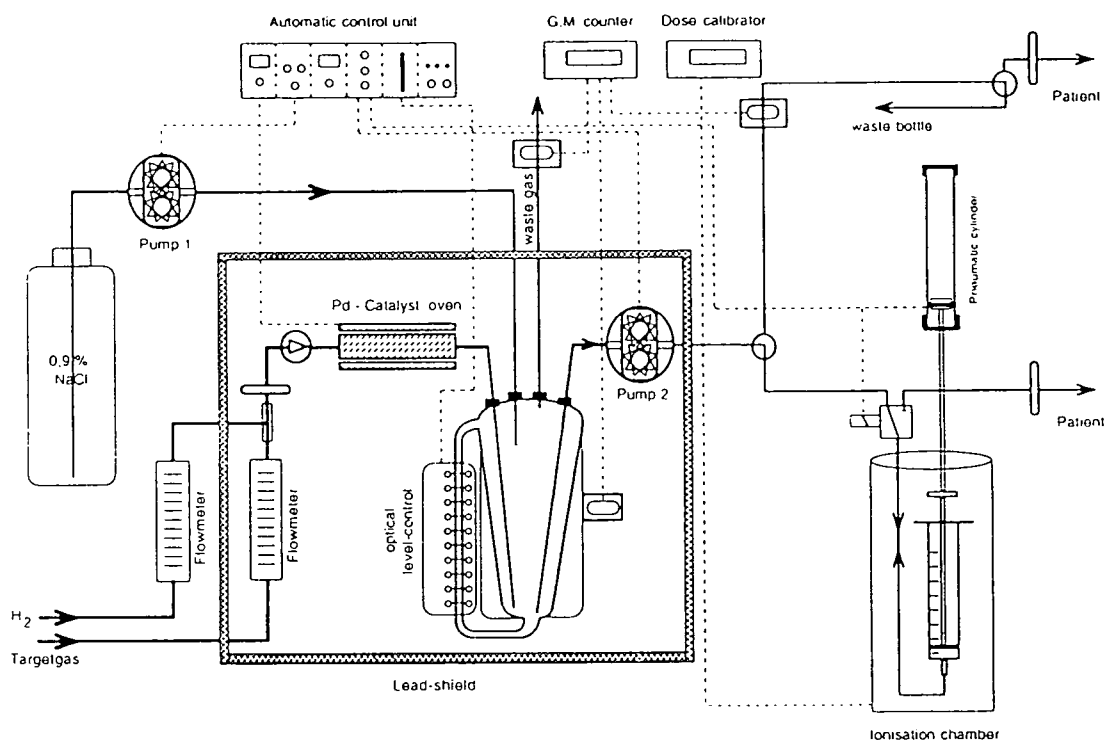


Figure 5. [ $^{15}\text{O}$ ]Water production system and infusion system, as at Hannover.

## 5.7. PRODUCTION AND QUALITY CONTROL OF $n$ -[ $^{15}\text{O}$ ]BUTANOL USING A PLC (AS AT KFA JÜLICH)

**5.7.1. Introduction.**  $n$ -[ $^{15}\text{O}$ ]Butanol, because of its optimal lipophilicity (Berridge *et al.*, 1991; Walter *et al.*, 1993), is of great interest for measuring regional cerebral blood flow (rCBF) by PET. Labelled  $n$ -butanol, compared to [ $^{15}\text{O}$ ]water, is a freely diffusible tracer over a wider range of rCBF (Herscovitch *et al.*, 1987); the use of [ $^{15}\text{O}$ ]water rather than  $n$ -[ $^{15}\text{O}$ ]butanol may underestimate cerebral blood flow in regions with high flow rate (Herscovitch *et al.*, 1983). The efficient and reliable production of  $n$ -[ $^{15}\text{O}$ ]butanol, by an automated device that can provide several batches in sterile vials from only one set-up, is a prerequisite for the use of this perfusion tracer in PET.

The synthesis of  $n$ -[ $^{15}\text{O}$ ]butanol by reaction of molecular oxygen with tri- $n$ -butylborane in tetrahydrofuran was first reported by Kabalka (1984) and by reaction with tri- $n$ -butylborane loaded onto an alumina Sep-Pak<sup>TM</sup> by Kabalka *et al.* (1985) and Takahashi *et al.*, (1986). A procedure to synthesise  $n$ -[ $^{15}\text{O}$ ]butanol rapidly and sequentially in sufficient activity and purity

for clinical use was soon developed (Berridge *et al.*, 1986). Remotely operated production systems have since been reported (Berridge *et al.*, 1990; Bauer and Wagner, 1991; Goodman *et al.*, 1991; Moerlein *et al.*, 1993).

**5.7.2. Design features.** At Jülich, in order to meet the clinical demand, there was a need for a device operated by a PLC that would be capable of producing up to eight batches of  $n$ -[ $^{15}\text{O}$ ]butanol, each in four minutes, with built-in quality control (for QA/QC recommendations see Meyer *et al.*, this volume). The schematic form of the device that was built to meet this demand is shown in Figure 6. It was assembled from two 2-position-8-way valves, two pneumatic syringe pumps, two flow controllers, four 2-way diaphragm and six 3-way diaphragm valves. The system contains eight alumina and C-18 cartridges. The [ $^{15}\text{O}$ ]oxygen activity trapped on the alumina cartridge and the activity of the final isotonic solution of the  $n$ -[ $^{15}\text{O}$ ]butanol are monitored with calibrated G-M tubes. A pneumatic device for dispensing the filtered solution of  $n$ -[ $^{15}\text{O}$ ]butanol into a shielded sterile and evacuated vial is also used. The system is controlled by a PLC with start, stop and delay push buttons. Up to eight productions of  $n$ -[ $^{15}\text{O}$ ]butanol in isotonic saline containing 5% ethanol can be performed within a turn-around time of 15 min. For a second set-up of the system, easy replacement of the alumina and the C-18 cartridges is all that is required.

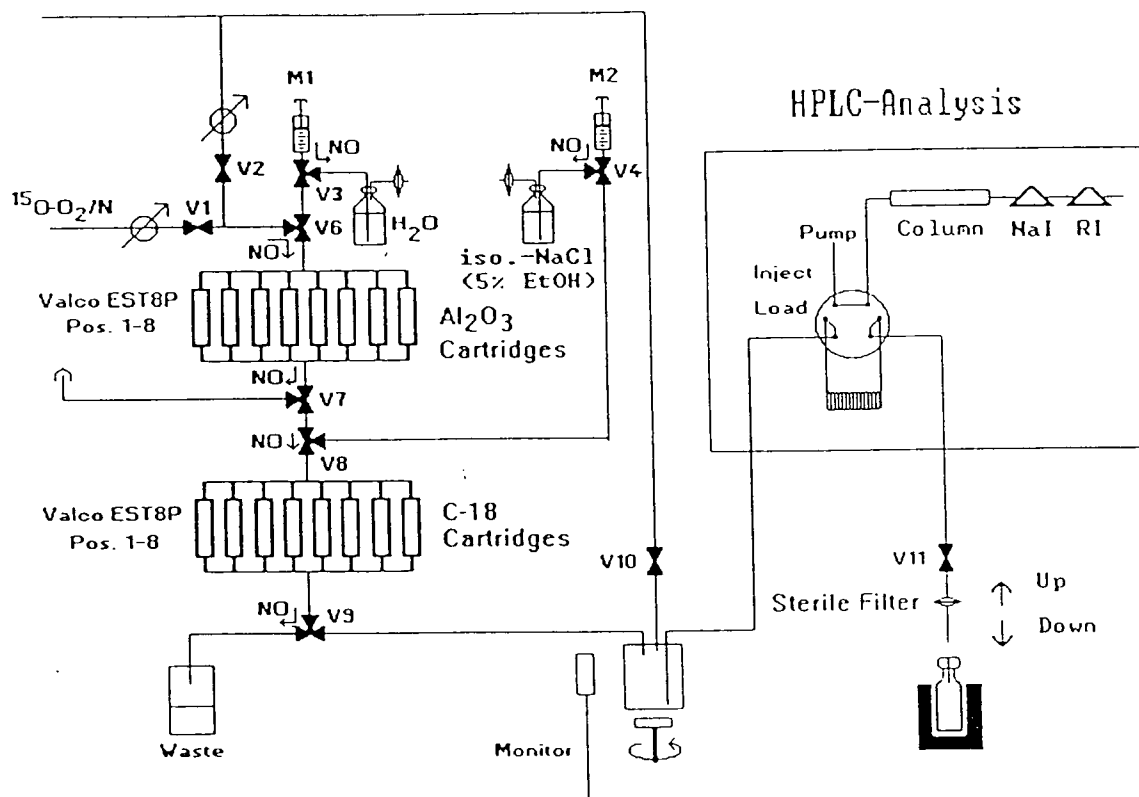
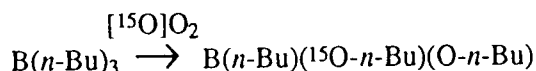
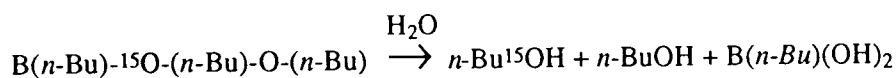


Figure 6. Schematic of the set-up for automated  $n$ -[ $^{15}\text{O}$ ]butanol synthesis with built-in quality control by HPLC, as at KFA Jülich. VI-VII are diaphragm valves. M1 and M2 are pneumatic syringe pumps.

5.7.3. *System operation.* The production of oxygen-15 via the  $^{14}\text{N}(\text{d},\text{n})^{15}\text{O}$  reaction is carried out at either of two cyclotrons, the JSW BC1710 or the CV28 in the Institute of Nuclear Chemistry in Jülich. The high current targets are constructed from aluminum and have a conical shape and a volume of 100 mL; they have as front window, a 50  $\mu\text{m}$  Havar™ foil in the case of the JSW 1710 and a 330  $\mu\text{m}$  aluminum plate to degrade the beam energy from 14 to 10 MeV in the case of the CV 28. The irradiated target gas (0.2% oxygen in nitrogen at a pressure of 5 bar, 75 psi), is typically bombarded with a 22  $\mu\text{A}$  beam of 10 MeV deuterons for 10 min. After irradiation, the target gas is transported over a distance of 40 m through a stainless steel tube of 2 mm inner diameter at a flow rate of 1 L/min and passed for 55 s through an alumina cartridge, pre-loaded with tri-*n*-butyl-borane (80  $\mu\text{L}$ ).



The hydrolysis of the  $^{15}\text{O}$ -labelled *n*-butyl-borane complex with 3 mL of sterile endotoxin-free water takes about 56 s.



The hydrolysed *n*-butyl-borane mixture is then passed into a C-18 cartridge for extraction of the *n*- $^{15}\text{O}$ ]butanol from boron compounds. The *n*- $^{15}\text{O}$ ]butanol is eluted from the C-18 cartridge with 5 mL of isotonic sodium chloride solution containing 5% ethanol. This procedure takes 73 s. In 45-50 s the eluted volume is mixed and filtered through a 0.2  $\mu\text{m}$  sterile filter into an evacuated vial, shielded by lead (3.5 cm thick). An average activity of 5.5 GBq (150 mCi) of *n*- $^{15}\text{O}$ ]butanol is obtained under these irradiation and synthesis conditions.

5.7.4. *Quality control.* Quality control by HPLC is performed automatically by analyzing 20  $\mu\text{L}$  of the solution taken just in front of the sterile filter (Figure 6). The analysis of the *n*- $^{15}\text{O}$ ]butanol by radio-HPLC is performed on a C-18 column (Hypersil™, 5  $\mu\text{m}$ , 60 mm x 4 mm) eluted with 15% acetonitrile in water. A sodium iodide [NaI(Tl)] detector and a refractive index detector are coupled in series. Using the short C-18 column, the analysis time is only 1.25 min, just 25 s longer than the time needed for sterile filtration.

Table 1. Retention times and content of the *n*- $^{15}\text{O}$ ]butanol solution determined by radio-HPLC, as at KFA Jülich.

|                                       | RT (s) | %               | Carrier (mg/mL) |
|---------------------------------------|--------|-----------------|-----------------|
| $^{15}\text{O}$ ]Water                | 25     | $0.05 \pm 0.02$ | -               |
| <i>sec</i> - $^{15}\text{O}$ ]Butanol | 40     | $0.70 \pm 0.2$  | $0.2 \pm 0.08$  |
| <i>n</i> - $^{15}\text{O}$ ]Butanol   | 49     | $98.5 \pm 0.7$  | $4.8 \pm 1.6$   |

Table 1 gives the radioactive components present in *n*- $^{15}\text{O}$ ]butanol solution, their HPLC retention times (RT), relative proportions and associated carrier. The radiochemical purity of *n*- $^{15}\text{O}$ ]butanol obtained by radio-HPLC is  $98.5 \pm 0.7\%$ . Only  $^{15}\text{O}$ ]water and *sec*- $^{15}\text{O}$ ]butanol



are detected as radioactive impurities. The amount of *n*-butanol carrier varies in the range of 22–28 mg per batch, which represents a chemical yield of about  $34 \pm 4\%$  based on the starting tri-*n*-butyl-borane. The average specific radioactivity of *n*-[ $^{15}\text{O}$ ]butanol is therefore about 13 GBq/mmol (350 mCi/mmol). The boron impurities, which cannot be determined by HPLC, are analyzed by atomic absorption spectroscopy. The average value is  $2.7 \pm 1.3 \mu\text{g/mL}$ . Figure 7 shows chromatograms for eight successive analyses, and their consistency.

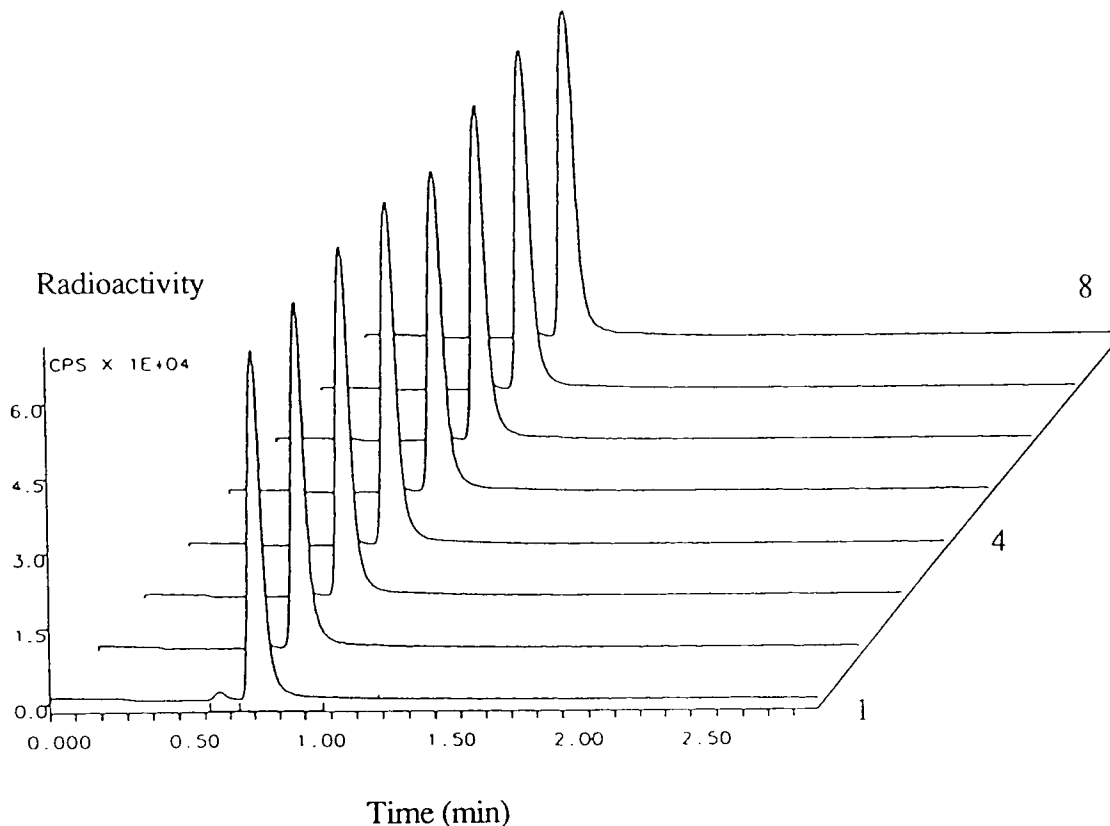


Figure 7. 3-D plot of the radioactivity channel of chromatograms from the quality control of eight successive productions of *n*-[ $^{15}\text{O}$ ]butanol, as at KFA Jülich.

#### 5.8. REMOTELY CONTROLLED SYNTHESIS OF L-6-[ $^{18}\text{F}$ ]FLUORO-DOPA (AS AT HAMMERSMITH HOSPITAL)

5.8.1. *Introduction.* L-6-[ $^{18}\text{F}$ ]Fluoro-DOPA is produced routinely as a marker for central dopaminergic neurons for clinical PET studies at the MRC Cyclotron Unit (Hammersmith Hospital). The radiochemistry used (see Pike *et al.*, 1990) is based on that originally described by Adam *et al.* (1986), as recommended by Luxen *et al.* (1992), namely electrophilic fluorination of a protected L-DOPA derivative [L-ethyl-*N*-acetyl-( $\beta$ -3-methoxy-4-acetoxyphenyl)-alaninate] with [ $^{18}\text{F}$ ]acetyl hypofluorite, followed by deprotection with hydriodic acid and HPLC separation of the 2- and 6-[ $^{18}\text{F}$ ]fluoro-derivatives of L-DOPA.

5.8.2. *[<sup>18</sup>F]Fluorine production.* [<sup>18</sup>F]Fluorine is produced by the deuteron (16.5 MeV; 13–16  $\mu$ A) irradiation of neon (research grade, BOC Ltd) containing 0.2% v/v fluorine (research grade, BOC Ltd), initially at 200 psi. Target parameters and operating conditions are described in Guillaume *et al.* (1991). At the end of irradiation (90–120 min; 20–32  $\mu$ Ah) the [<sup>18</sup>F]fluorine is introduced by stainless steel tubing (1/8" o.d.) into a lead-shielded hot-cell through a controllable needle valve (Nupro).

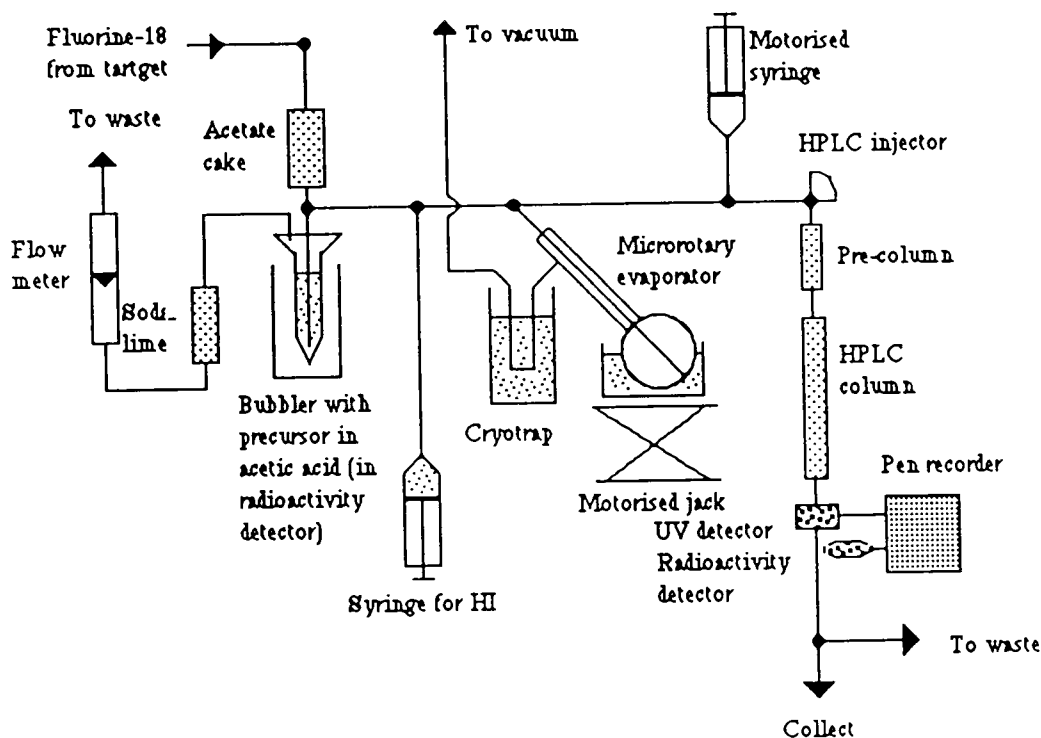


Figure 8. A schematic representation of apparatus for the remote production of L-6-[<sup>18</sup>F]fluoro-DOPA, as at Hammersmith Hospital.

5.8.3. *Radiochemistry.* The set up of the cell is shown schematically in Figure 8. A stainless steel gauge (Budenberg) is used to monitor gas pressure between the target and needle valve. The fluorine-18 is led in Teflon™ (1/8" o.d.) tubing through a stainless steel cartridge containing sodium-acetate 'cake' and then into a glass bubbler containing a solution of precursor (70–100 mg) in glacial acetic acid (12–15 mL). This sits in a well-counter, thereby enabling the accumulation of radioactivity to be monitored. The outlet of the bubbler is a Teflon™ tube leading the gas to waste through a soda lime trap and flow meter (Meterate OPE RS1, ruby float). Swagelok™ stainless steel fittings are used to connect the tubing to the needle valve and to the cartridge. Altex™ fittings are used to connect the tubing to the bubbler and to the flow meter. The eight three-way switching valves that are used in the system are Rheodyne™ slider valves (model 5301 or 5302) with remotely controlled pneumatic actuators (model 5300). The bubbler is connected to a mini rotary evaporator (Buchi) which has its flask in a silicone oil bath (Heidolph HB140-0) at 165°C. The rotary evaporator is modified to have a liquid nitrogen cooled trap. The oil bath is mounted on a motorised lab-jack which can be raised or lowered by remote control. The acetic acid is taken into the rotary evaporator under reduced pressure

through a Teflon™ dip-tube which for most of its length is held straight by a glass tube before finally terminating at the lowest part of the evaporation flask. Acetic acid is then removed from the radiofluorinated product by rotary evaporation. Then hydriodic acid (BDH, 'MAR', 1.5 mL plus hypophosphorous acid, 0.5 mL) is introduced into the rotary evaporator from a plastic syringe on the outside of the cell via Teflon™ tubing (1/16" o.d.). Hydrolysis is performed at 165°C for 20 min. Then the hydriodic acid is rotary evaporated off. Water (*ca* 3 mL) is three times added to the flask and evaporated. Finally 'neutralisation buffer' (KH<sub>2</sub>PO<sub>4</sub>, 71 g/L plus Na<sub>2</sub>HPO<sub>4</sub>, 69 g/L; pH 4; 1.5 mL) is added to the flask to dissolve the radioactive product. This is withdrawn into a plastic syringe mounted in a homemade syringe-drive (based on a remotely controlled motorised lead-screw).

5.8.4. *HPLC purification.* The contents of the syringe are injected into a remotely controlled pneumatically-operated HPLC injection valve (Rheodyne 7030, with 5701 actuator) through a vented millipore filter (Sterile Acrodisc, 0.2 µm; Gelman Sciences). The sample is remotely injected onto a preparative HPLC column (Nucleosil™ 5 C 18, 5 µm particle size, 25 cm x 20 mm i.d.) eluted at 6 mL/min with 70 mM-potassium dihydrogen phosphate solution. The eluate is monitored for absorbance at 280 nm and for radioactivity. The peak eluting after L-2-[<sup>18</sup>F]fluoro-DOPA and with the same retention time as reference L-6-fluoro-DOPA is collected by remote operation of a waste/collect valve and diluted to 10 mL with the same buffer. Finally, this solution is sterilised by millipore filtration (0.22 µm pore size, Millex GS).

5.8.5. *System performance.* The collected product (190–560 MBq; 5–15 mCi) has an average radiochemical purity of 93.8% and an average specific radioactivity of 4–8 MBq/µmol (110–220 mCi/µmol) corrected to EOS. The preparation takes about two hours from EOB.

## 5.9. COMPUTER-AIDED AUTOMATION OF NUCLEOPHILIC RADIOFLUORINATION (AS AT KFA JÜLICH)

5.9.1. *Introduction.* 2-[<sup>18</sup>F]Fluoro-2-deoxy-D-glucose (2-<sup>18</sup>FDG) is currently one of the most important radiopharmaceuticals (see Fowler and Wolf, 1986; Coenen *et al.*, 1987). The increasing demand for 2-<sup>18</sup>FDG has accelerated the design and construction of remotely controlled or automated procedures as well as laboratory robotic systems for its synthesis. In order to prepare 2-<sup>18</sup>FDG routinely at Jülich by the favoured method (Coenen *et al.*, 1987) based on Kryptofix™ 2.2.2. (A.P.E. 2.2.2.)-mediated nucleophilic fluorination (Hamacher *et al.*, 1986), a computer-controlled modular apparatus was built. This includes a module for the rapid separation of [<sup>18</sup>F]fluoride from [<sup>18</sup>O]water for other nucleophilic fluorinations (Hamacher *et al.*, 1990).

5.9.2. *Computer control unit.* All processes in the synthesis of 2-<sup>18</sup>FDG are electronically controlled by an IBM PC/AT clone equipped with standard peripherals. The microcomputer is allowed to communicate with an Optomux™ network via an RS 422/485 adapter card, which is plugged in directly and provides up to 4000 V isolation between the PC and the communication link. For standard input/output signals, three Optomux™ digital and one analog brain board with up to 16 I/O channels are used. Six lines are programmed to read status information from the system, such as temperature, pressure, the level of liquid in the purification unit, and the actual scale of three radioactivity detectors. The temperature is controlled by a 12-bit digital-to-analog converter. A total number of 28 solenoid valves can be operated directly. In the Optomux™ unit

The diagram illustrates a complex chemical synthesis and purification system. Key components include:

- Target Chamber:** Produces  $^{18}\text{F}_2$  from a target.
- Gas Handling:** Helium gas is filtered (0.2  $\mu\text{m}$ ) and used in the target chamber. A syringe and monitor are used for gas handling.
- Column Section:** A series of columns containing THF, HCl (2 mol/l), HCl (0.1 mol/l),  $\text{H}_2\text{O}$ , Ethanol, and Acetone.
- Reactor Section:** A reactor with a stirrer, heating, and a nozzle, cooled by a reactor cooling system.
- Purification Section:** A C-18 cartridge and a monitor are used for final purification.
- Waste and Exhaust:** Waste is collected in a container, and exhaust is filtered (0.2  $\mu\text{m}$ ) and released.

5.9.4. **[<sup>18</sup>F]Fluoride separation.** The fluoride separation unit is connected on-line to the [<sup>18</sup>O]water target via a polypropylene tube. Before the [<sup>18</sup>F]fluoride and the [<sup>18</sup>O]water are separated, the water phase (1.5 mL) is collected in a degassing vessel and the total radioactivity monitored by a System 414 Monitor (Genitron Instruments). This vessel is connected to an air-actuated six-way valve (Valco, AC6W) that is combined with a small ion exchange column (20 x 2 mm) having a G1 Pyrex™ frit and a loop containing potassium carbonate solution. The outlet

valve at the column connects to two vials: vial a) to collect the  $[^{18}\text{O}]$ water after fixation of the fluoride and vial b) to mix, using a motor-driven syringe, the  $[^{18}\text{F}]$ fluoride containing potassium carbonate solution with the appropriate amount of Kryptofix™ 2.2.2. solubilised in acetonitrile. The transport of the solution through the resin is accelerated by reducing the pressure to about 20 mbar at the end of the column using a vacuum pump.

5.9.5. *Reaction vessel.* The central part of the apparatus is a single unit reactor (Figure 10), for a

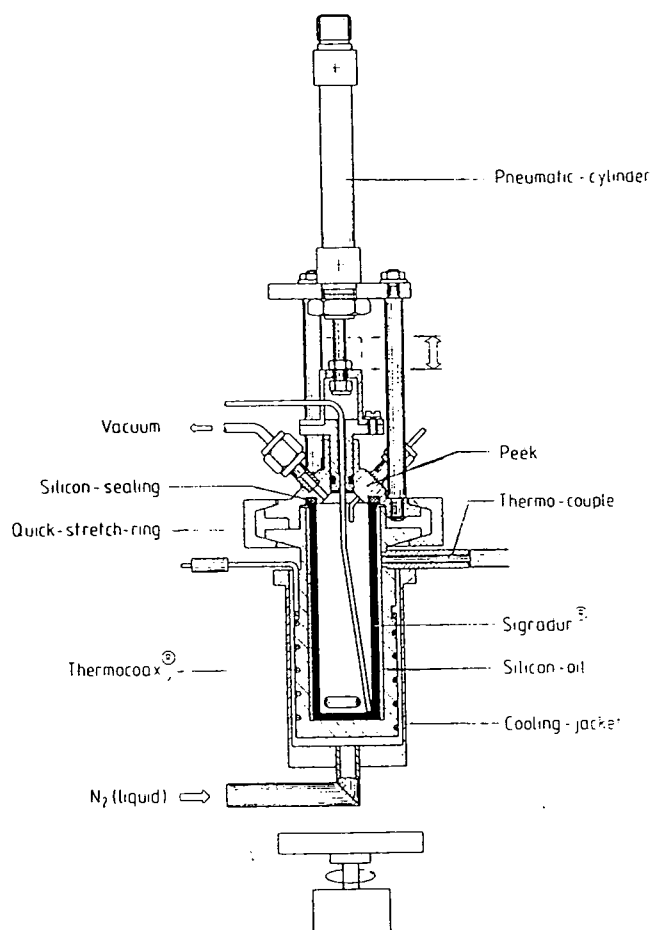


Figure 10. Sectional view of the single unit reactor with a cylindrical reaction vessel (17 x 70 mm) made of glassy carbon, as at KFA Jülich.

one-pot reaction system that contains a single heated reaction vessel combined with a rectangular arrangement of Teflon™ membrane valves and up to nine reagent reservoirs above the reactor. (For the 2- $^{18}\text{F}$ FDG synthesis only five reservoirs are used). The closed system allows reactions in isothermal and isometric conditions, in the range of ~ 0–2 bar (0–30 psi) as limited by the Teflon™ membrane valves. The cylindrical reaction vessel is made of glassy carbon (Sigradur™, 17 mm in diameter, 2 mm wall thickness and approximately 70 mm in height) surrounded by a copper cylinder with an integrated electric filament (Thermocoax™). The advantage of glassy carbon in comparison with glass is its extreme chemical resistance, low

capacity for adsorption and more efficient heat conduction. The top of the glassy carbon vessel is closed by a stainless steel cover which is connected to the cylinder by a 'quick-stretch ring'. The small space between the glassy carbon and the copper cylinder is filled with silicone oil (ca 1 mL). Accordingly, the heat capacity is relatively low. The cylinder is surrounded by a steel coat with an inlet for liquid nitrogen at the bottom. (The injection of liquid nitrogen in a closed cooling jacket allows a temperature to be set in the range from 77–500°K, which is of interest in syntheses with [ $^{11}\text{C}$ ]iodomethane).

**5.9.6. Purification unit.** The purification unit consists of a special glass column containing the AG11-A8 resin (Biorad) with a built in liquid level sensor (Honeywell, Dusseldorf) and an eluent reservoir at the top of the resin. The solenoid valves at the top and bottom of the purification column are switched automatically, depending on the signal of the liquid level sensor.

**5.9.7. Filling unit.** All the 2- $^{18}\text{F}$ FDG produced is collected in a glass vessel with lead shielding and measured by the System 414 Monitor in order to get the radioactivity concentration in MBq or mCi per mL via the computer. After sterile filtration through a millipore filter (0.22  $\mu\text{m}$ ) the isotonic 2- $^{18}\text{F}$ FDG solution is apportioned into evacuated ampoules via pneumatic piloting of a sterile tube cannula. The filling unit is installed below the synthesis device in a separate small lead-shielded box (40 x 40 cm).

**5.9.8. Performance of the system.** The routine production of 2- $^{18}\text{F}$ FDG has been accomplished several hundred times with excellent reliability. The integrated automatic cleaning programme at the end of radiosynthesis makes it possible to reuse the apparatus within one hour. Nevertheless, it is necessary after each third radiosynthesis to open the reactor and to clean the glassy carbon vessel with a soft tissue. In the course of several radiosyntheses, polymeric side products accumulate which are insoluble in the solvents used for the purification sequence, such as boiling water, ethanol and acetone. Besides the manual purification step, the only manual procedure which has to be performed before running a new radiosynthesis is to fill up the reservoirs and to change the column and the Sep-Pak<sup>TM</sup> cartridges.

Although some minor problems exist with respect to the decreasing reliability of some membrane valves, especially the three-way valves after 40 radiosyntheses, the automated system is a convenient apparatus for the routine production of high amounts of 2- $^{18}\text{F}$ FDG. The preparation takes 58 min and gives 2- $^{18}\text{F}$ FDG in  $50 \pm 10\%$  radiochemical yield with a radiochemical purity of  $98.5 \pm 0.5\%$  and a specific radioactivity of  $> 370 \text{ GBq}/\mu\text{mol}$  (10 Ci/ $\mu\text{mol}$ ). The same device, without any appreciable modification, has been used for the routine production of 2- $^{18}\text{F}$ fluoro-D-mannose from the epimeric form of the 2- $^{18}\text{F}$ FDG precursor, and also for the production of 3- $N$ - $^{18}\text{F}$ methylspiperone.

## 6. Robot-based Automation

### 6.1. INTRODUCTION

All automation requires standardisation of repetitive procedures. The equipment used is chosen for its compatibility with other components of the system. Personal computers are used to time the execution of pre-programmed operations. The one major feature distinguishing a robot-based system from a dedicated automatic system is that the mechanical operations are performed by a

multi-functional manipulator – the robot arm – instead of a series of mono-functional devices.

There are pros and cons to using robots in radiochemical procedures. Once the initial components are chosen and the operator is familiar with the system, a large degree of inherent flexibility is retained since the arm can be re-programmed to perform new operations. As in a dedicated system, the robot-based system can be used to execute a well-optimised procedure in exactly the same way every time. In contrast to dedicated systems, the robot can be programmed to remove contaminated vessels to a shielded waste area, thereby lowering the level of background radiation and increasing accessibility to a shielded work area in a given period. The robot may be used as a developmental tool to optimise the sequences of mechanical operations in a new procedure before building a dedicated system. The same manipulator can potentially perform several different procedures within the same set-up. In high-volume analytical procedures, such as in the analysis of biological samples, the robot always performs the analyses in the same manner, thereby eliminating errors from variations in laboratory techniques. No transcription errors are made since the data are automatically recorded and personnel are freed from time-consuming work.

On the other hand, the cost of the robot alone is often substantially higher than the combined costs of the equipment used in dedicated automated systems. Most glassware and disposable items must be redesigned to a more robot-friendly construction for optimal reliability. Either the shielded work area must be custom-designed to fit the operating and service requirements of the robot or the choice of robot must take into account the limitations of the available work area. Even for commercial robots, custom-made to perform specific tasks, considerable time is usually needed for set up and one or several persons needs to be completely familiar with the function and coding of the control system.

Once the decision has been made that investment in robotics is justified for present and/or future applications, the time required for implementation will depend on the expertise of the personnel working on the project and whether or not the system being considered has previously been optimised for the particular application. Some examples of this developmental procedure are surveyed below, following a specific example of robotic radiosynthesis from the group at Liège. Other laboratories in Europe have experience with robotics, as follows: *France*: Caen and Orsay; *Netherlands*: Groningen; *Russia*: St. Petersburg; *Sweden*: Stockholm and Uppsala. The three main robotic systems to be discussed are summarised in Table 2.

Table 2. Comparison of technical data for the main robots discussed.

|                      | Zymark      | Scanditronix       | Karolinska  |
|----------------------|-------------|--------------------|-------------|
| Robot type           | Zymate      | (RB-86)Anatech AB  | RTX (UMI)   |
| Coordinate system    | Cylindrical | Cartesian + linear | Cylindrical |
| Number of axes       | 4 + 2       | 5                  | 7           |
| Operation range      | 360°        | 180° - 6 m linear  | 180°        |
| Programmable speeds  | yes         | yes                | yes         |
| Multiple hands       | yes         | yes                | no          |
| Reach                | 64 cm       |                    | 68 cm       |
| Lift capacity        | 2 kg        | 10 kg              | 2 kg        |
| Positioning accuracy | 1 mm        | 0.1 mm             | 0.5 mm      |
| System controller    | Easylab™    | IBM PC             | IBM PC      |

## 6.2. A SPECIFIC EXAMPLE OF ROBOT-ASSISTED RADIOSYNTHESIS — 6-[<sup>18</sup>F]FLUORO-L-DOPA (AS AT LIÈGE)

6.2.1. *Introduction.* Although, the labelling of radiopharmaceuticals with fluorine-18 by remote-control is feasible, automatic robotic radiosynthesis is more desirable since it appears to be more versatile and therefore more useful for research. In order to demonstrate the potential of such an approach, which avoids excessive radiation exposure to operatives, a robotic system has been developed in the Cyclotron Research Centre of the University of Liège based on the Zymate™ Laboratory Automation System (Zymark Corp.) (Table 2). This was the first robotic system to be used in PET-related procedures and is probably still the most widely used. The robot arm has an operational range of 360°. EasyLab™ software and a variety of workstations are available for performing operations often used in laboratory procedures. These may be used as they are, or modified to fit the special vessels and techniques used in PET radiochemistry. It is configured and programmed to prepare a variety of radiopharmaceuticals, such as 6-[<sup>18</sup>F]fluoro-L-dopa, 2-[<sup>18</sup>F]fluoro-L-tyrosine, [<sup>18</sup>F]altanserin and 4-[<sup>18</sup>F]fluoro-tropapride, all by n.c.a. nucleophilic radiofluorination.

6-[<sup>18</sup>F]Fluoro-L-dopa and 2-[<sup>18</sup>F]fluoro-L-tyrosine are produced as tracers of presynaptic dopamine neurons and of cerebral protein synthesis rate, respectively. The n.c.a. enantioselective labelling of 6-[<sup>18</sup>F]fluoro-L-dopa is based on nucleophilic substitution in 2-trimethylammonium-3,4-dimethoxy benzaldehyde triflate (Lemaire *et al.*, 1992, Lemaire, 1993) and the labelling of 2-[<sup>18</sup>F]fluoro-L-tyrosine is similarly based on nucleophilic substitution in the corresponding 4-methoxy benzaldehyde. The remaining steps in these multi-step syntheses require conversion of the radiofluorinated aldehydes into the corresponding benzyl iodide [by treatment with di-iodosilane (DIS)], alkylation of *S*-Boc-BMI with this electrophilic agent, hydrolysis and finally HPLC purification (Lemaire *et al.*, 1993). [<sup>18</sup>F]Altanserin is labelled by nucleophilic substitution in the corresponding nitro compound and purified by HPLC (Lemaire *et al.*, 1991). Its suitability for mapping 5HT<sub>2</sub> receptors *in vivo* with PET has been demonstrated (Sadzot *et al.*, 1993). Tropapride has been labelled with fluorine-18 at the 4 position of its benzylic group by reductive amination in the presence of 4-[<sup>18</sup>F]fluorobenzaldehyde and norbenzyl precursor (Damhaut *et al.*, 1992). This molecule exhibits high selectivity and specificity for D<sub>2</sub> receptor sites and appears to be a potential radioligand for the study of the dopaminergic system with PET.

6.2.2. *Components of the system.* The robot used for these radiosyntheses is a Zymate™ II Laboratory Robot (Zymark Corp.), as specified in Table 2. The software control is EasyLab Plus™, and routines for several modules were written using this language. Several components were acquired from Zymark, and others modules, more specific to the particular radiosynthesis were designed and fabricated 'in house'. The components were arranged in a 360° arc around the robot arm and were mounted on sectors positioned around the Zymate™ II Core System. All the system was set up on a shielded bench top (150 x 175 cm). Located beneath the robot are the Power and Event Controller as well as the multiplexer, also developed 'in house', which allows the control of 64 outputs for the control of valves and others modules. The controller and the HPLC system are also located beneath the bench top. A block diagram of the Zymate™ system is shown in Figure 11. In the legend the modules marked Zymark were purchased from Zymark Corporation while the modules developed in Liège are marked CRC. Figure 12 shows a photograph of the configured system.



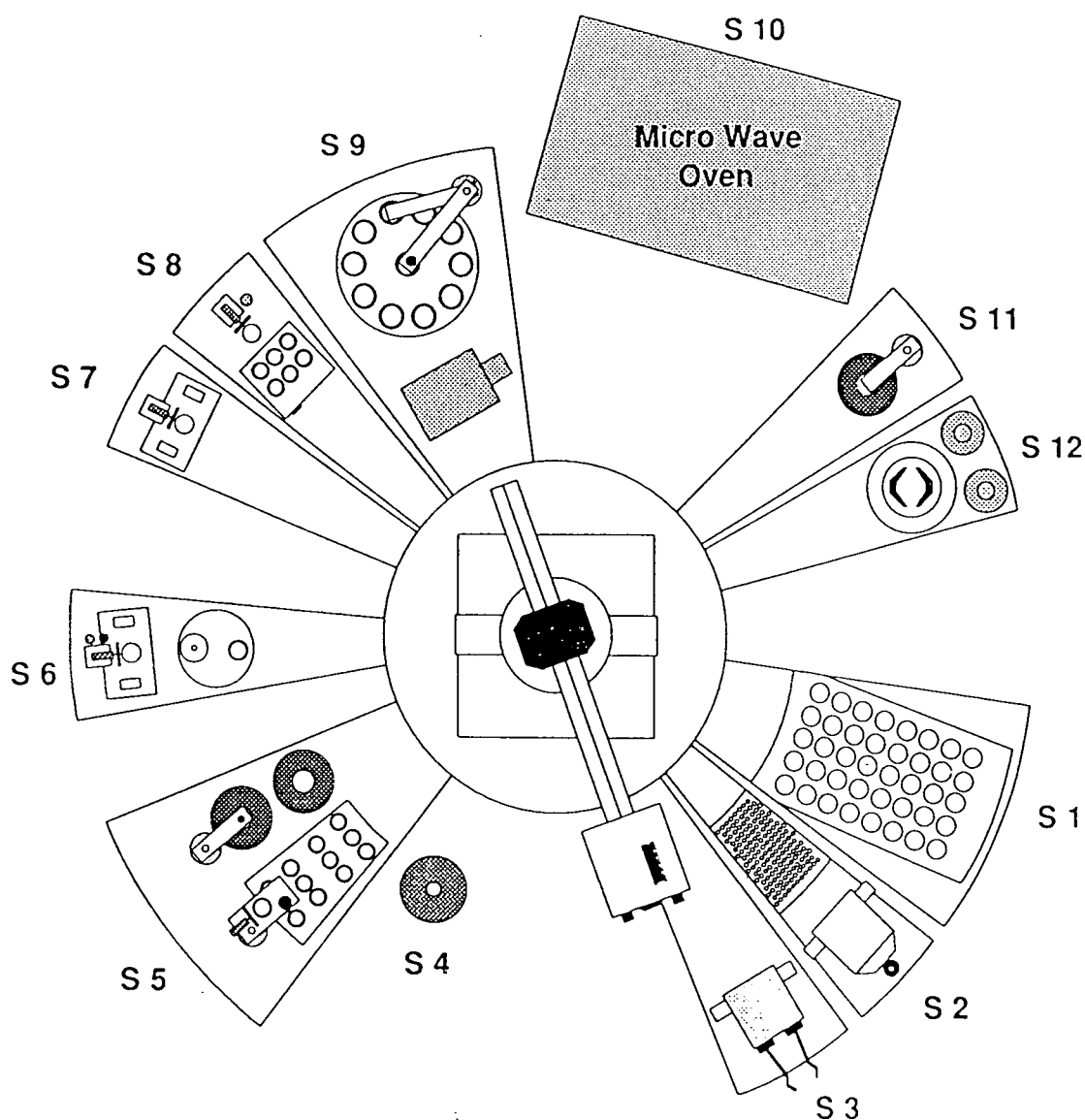


Figure 11. Description of the different sectors (S1–S12) around the Zymate II Core System. S1—Rack containing disposable glass tubes (Zymark). S2—Pipetting Hand and tips (Zymark). S3—General Purpose Seizing Hand (Zymark). S4—Lead pig (CRC). S5—Column for the separation of  $[^{18}\text{F}]$ fluoride from irradiated water, a rack with capped reagent vials, two ovens, one equipped with an optical level probe (CRC). S6—C-18 Sep-Pak chromatography system (CRC). S7—Sector for formulating the injectable solution (CRC). S8—Homemade silica chromatography system and rack for the preparation of DIS (L-6- $[^{18}\text{F}]$ fluoro-Dopa and L-2- $[^{18}\text{F}]$ fluoro-tyrosine) (CRC). S9—HPLC injector and fraction collector (CRC). S10—Microwave oven (CRC). S11—Oven for the hydrolysis step (L-6- $[^{18}\text{F}]$ fluoro-Dopa and L-2- $[^{18}\text{F}]$ fluoro-tyrosine) (CRC). S12—Capping pysection providing capping and uncapping of round, screw cap containers (Zymark).

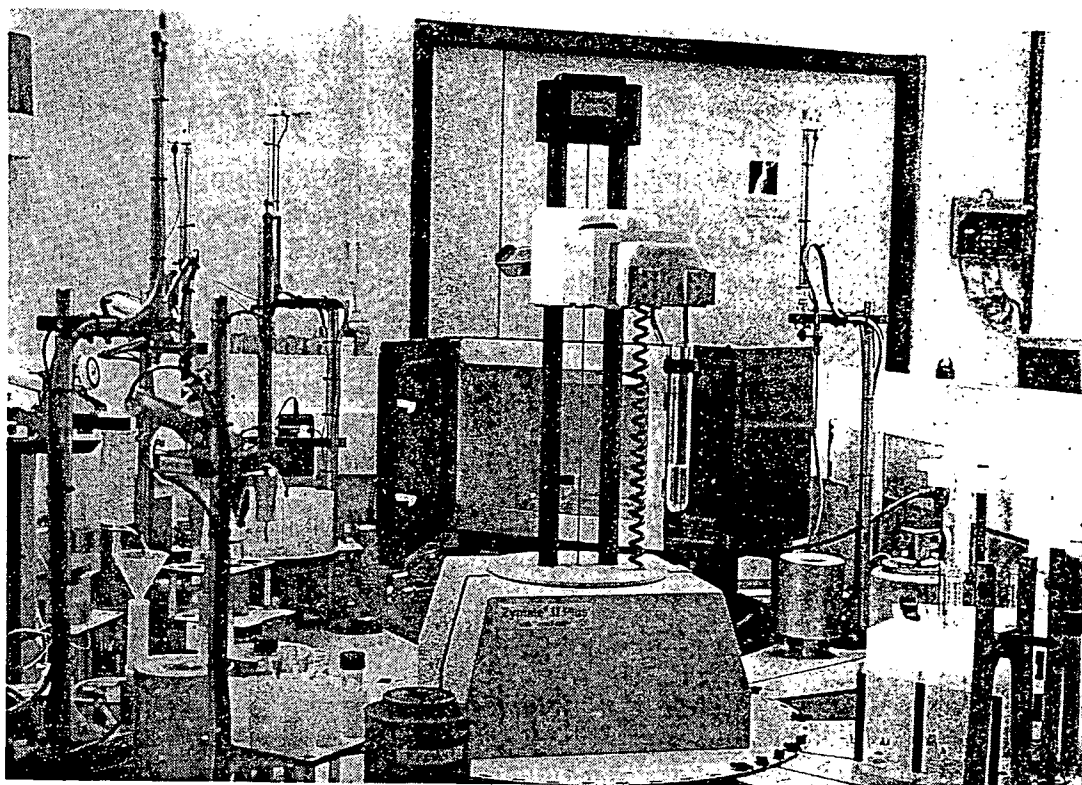


Figure 12. Zymate™ Laboratory System used for the routine production of four  $^{18}\text{F}$ -labelled radiopharmaceuticals.

6.2.3. *Labelling of L-6- $^{18}\text{F}$ fluoro-Dopa as an example.* The radiosynthesis of L-6- $^{18}\text{F}$ fluoro-Dopa requires many steps in common with the labelling of other radiopharmaceuticals, including production of  $^{18}\text{F}$ fluoride, recovery of  $^{18}\text{O}$ -enriched water, evaporation of natural abundance water, resolubilisation of the radioactivity in an aprotic dipolar solvent, labelling in a microwave oven or in an aluminium heating block, Sep-Pak™ pre-purification, HPLC injection and separation, and finally preparation of an injectable solution.

*a. Initialisation.*

During radionuclide production, the robot initialisation program is loaded into the robot controller and executed. It allows the modules of the robotic system to be loaded with all components (*e.g.* vials, tips, tubes), solvent and reagents necessary for the radiosynthesis. To continue, manual validation of each step of the program with the keyboard, is required. At the end of bombardment, the main program is loaded.

*b. Production of  $^{18}\text{F}$ fluoride ion.*

No-carrier-added  $^{18}\text{F}$ fluoride is produced in a nickel target by the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  nuclear reaction on  $^{18}\text{O}$ -enriched water (45%). The target is remotely loaded using a syringe pump through a fill entrance at the bottom of the target. A second port at the top of the target is used for venting during loading and irradiation. At the end of bombardment (1 h, 10  $\mu\text{A}$ ), the radioactivity (11–13 GBq; 300–350 mCi) is transferred under nitrogen gas pressure (0.67 bar; 10 psi) through Teflon™ tubing (30 m) to the robot room (sector 5, Figure 11).

*c. Recovery of n.c.a. [ $^{18}\text{F}$ ]fluoride and  $^{18}\text{O}$ -enriched water.*

The radioactivity is trapped on a Dowex<sup>TM</sup> 1X8 anion exchange resin and the  $^{18}\text{O}$ -enriched water recovered in a receiving vial placed in sector 5. The end of transfer is detected manually and the command is then entered by keyboard into the robot controller to continue the synthesis. A conical glass vessel containing Kryptofix<sup>TM</sup> 2.2.2. (22 mg) and potassium carbonate (4.2 mg) is then substituted for the  $^{18}\text{O}$ -enriched vial, which is capped in the capping station (sector 12). After distillation, this water is used again for further production. [ $^{18}\text{F}$ ]Fluoride is then eluted from the resin by potassium carbonate solution (7 mg/mL; 400  $\mu\text{L}$ ).

*d. Labelling reaction.*

Conversion of the [ $^{18}\text{F}$ ]fluoride into its potassium-Kryptofix 2.2.2. salt in an anhydrous organic solvent (DMSO) is achieved in the oven in sector 5. The water is evaporated to dryness under nitrogen in an aluminium heating block (120°C). The end of evaporation is detected automatically with an optical probe and a feedback signal is sent to the robot's computer which then allows the synthesis to continue. Therefore, this critical evaporation step can be achieved reproducibly and is highly independent of parameters such as water volume, temperature and nitrogen flow.

The Pipetting Hand, equipped with a tip, is then selected and the [K-Kryptofix 2.2.2.]+[ $^{18}\text{F}$ ]- salt dried by three successive additions and evaporations of acetonitrile (3 x 100  $\mu\text{L}$ ). The precursor (15 mg) in DMSO (900  $\mu\text{L}$ ), previously placed in the rack of sector 5, is aspirated in the tip and added to the dry residue. The probe is then moved up. The vial is capped in the capping pysection with the General Purpose Hand and introduced into the second oven in sector 5. After the labelling reaction (10 min, 140°C), the vial is placed either in a lead pig (sector 4, for assessment of radioactivity), or uncapped for subsequent Sep-Pak<sup>TM</sup> pre-purification.

*e. Sep-Pak<sup>TM</sup> pre-purification.*

By using the Pipetting Hand, the DMSO solution is diluted in glass tube number 1 in the rack in sector 1, which contains 20 mL of 0.5M-hydrochloric acid. The labelling vial is rinsed with this solution. With the General Purpose Hand the tube containing the radioactivity is moved from the rack on sector 1, and the solution poured into a pneumatically actuated 50 mL syringe (previously filled with 5 mL of 0.5M-hydrochloric acid). The solution is then pushed through the C-18 Sep-Pak<sup>TM</sup> cartridge with a slight flow of nitrogen. The level of liquid is detected automatically by measuring the conductivity between two platinum electrodes. Before dryness, the lid is moved up and the support washed with water. The [ $^{18}\text{F}$ ]fluorobenzaldehyde is finally eluted with dichloromethane and dried by passing through a magnesium sulphate column which is rinsed with additional dichloromethane. The final solution is collected in a conical vial and moved from sector 5 into the rack in sector 8.

*f. Preparation of DIS and reductive iodination.*

In a conical vial containing iodine, phenylsilane is added with the Pipetting Hand. With the General Purpose Hand a Teflon<sup>TM</sup> lid is placed on the vial and removed after 2 min. The Pipetting hand is selected again and the radiofluorinated aldehyde transferred to the DIS reagent. With the General Purpose Hand the vial containing the [ $^{18}\text{F}$ ]fluorobenzyl iodide is displaced so that the solution is poured onto a silica gel column and eluted with dichloromethane. The purified [ $^{18}\text{F}$ ]fluorobenzyl iodide is collected in another vial and the dichloromethane evaporated.

*g. Alkylation and hydrolysis reactions.*

After alkylation, which is not presently conducted with the robotic system (under development), the [ $^{18}\text{F}$ ]fluoroalkylated derivative is hydrolysed in the oven in sector 11.

#### *h. HPLC purification.*

A homemade fraction collector has been developed (sector 9). Presently robotic controlled injection onto the HPLC is under development. The robotic preparation of L-6-[ $^{18}\text{F}$ ]fluoro-Dopa takes a similar time (120 min) to a remotely controlled preparation and gives a similar radiochemical yield (25 % decay corrected to E.O.B.).

6.2.4. *Other radiopharmaceuticals.* The software written for the L-6-[ $^{18}\text{F}$ ]fluoro-Dopa radiosynthesis can be used for labelling other radiopharmaceuticals with only minor modification. Indeed, the flexibility of the Easilab Plus<sup>TM</sup> programming language easily allows any modification needed. For the radiosynthesis of L-2-[ $^{18}\text{F}$ ]fluoro-tyrosine all the same homemade workstations are used. For the radiosynthesis of [ $^{18}\text{F}$ ]altanserin the HPLC system is modified to accept an additional C-18 column. The application of robotics is not limited to the routine preparation of these four  $^{18}\text{F}$ -labelled radiopharmaceuticals for PET studies. Set up for the labelling and purification of several new radiopharmaceuticals is easily performed the same day with all the hardware presently available on our robotic system.

### 6.3. A SURVEY OF OTHER APPLICATIONS OF ROBOTICS IN RADIOSYNTHESIS

6.3.1. *At the University of Washington (St. Louis).* The PET group at the University of Washington (St. Louis), first reported the use of the Zymate system in the radiosynthesis of  $16\alpha$ -[ $^{18}\text{F}$ ]fluoroestradiol- $17\beta$  (Brodack *et al.*, 1985, 1986). This particular radiosynthesis was chosen because the half-life of the radioisotope is adequately long, the operations are compatible with robotic procedures and the long synthesis time and low radiochemical yield require large amounts of starting radioactivity. The procedures performed included conversion of [ $^{18}\text{F}$ ]fluoride into the tetra-*n*-butylammonium salt in anhydrous solvents, handling of air-sensitive solvents, evaporation of solvents to dryness, heating and cooling, liquid-liquid separations and HPLC purification. In addition to the commercially available equipment incorporated in the procedure a number of modifications were introduced by the St. Louis group. These included the use of radiosynthesis-specific reagent and vessel racks, methods for the reproducible placement of the reaction vessels in heating or cooling baths and of specially constructed nitrogen purge lines that allowed the robot to perform other tasks during the evaporation of solvents. The General Purpose Hand was modified for Luer<sup>TM</sup> adapters to accommodate disposable needles, injections onto the HPLC system, monitoring of the eluate and the collection of fractions. The robotic procedure requires less time than the manual procedure (80 vs 90 min) but the radiochemical yields are lower (5–6 vs 22%). The major problems reportedly encountered were control of the evaporation time needed for resolubilisation of the [ $^{18}\text{F}$ ]fluoride, lower efficiency in sample extractions than when performed manually and inefficient transfer for injection onto the HPLC system.

Based on their experiences with this first application, the St. Louis group subsequently modified the system to include smaller custom-made workstations and standardised the addition of reagents and the reaction vessels (Brodack *et al.*, 1988b). The robot workspace is more efficiently used. They demonstrated the flexibility of the procedure by performing several radiosyntheses within the same set-up, namely those of  $16\alpha$ -[ $^{18}\text{F}$ ]fluoroestradiol- $17\beta$  with improved yields, 3-*N*-( $\omega$ -[ $^{18}\text{F}$ ]fluoropropyl)siperone and *n*-[ $^{11}\text{C}$ ]butanol. In the new radiolabelling with [ $^{18}\text{F}$ ]fluoride, a rack is simply added for the reagents and for vessels specific to 3-*N*-( $\omega$ -[ $^{18}\text{F}$ ]fluoropropyl)siperone synthesis and the HPLC system is modified to accept an additional silica column. The radiochemical yields of 3-*N*-( $\omega$ -[ $^{18}\text{F}$ ]fluoropropyl)siperone are

slightly lower than for the manual procedure (15–18% vs 20–28%, EOS) due to the longer synthesis time required (70 vs 40 min). A third reagent rack in the same robotic set-up allowed two preparations of *n*-[1-<sup>11</sup>C]butanol within 25 min of each other without operator intervention (compared to at least 60 min in manual preparations). The time required for the robotic preparation is slightly longer than for the manual-remote preparation (25 vs 17 min), but the radioactivity yields are essentially the same.

Using the same reagent and vessel racks already implemented in the previous radiofluorinations, only minor modifications were necessary for the production of 2-<sup>18</sup>F DG in sufficient activities for clinical PET studies (Brodack *et al.*, 1988a). The resolubilisation station was modified to include a platinum crucible. A new lab-station allowed attachment of a reflux column to a Reacti-vial™. Extraction procedures were performed as previously with columns packed with the appropriate material (silica C-18 and AG11 A8 ion retardation resin). The radiochemical yields and time of preparation are comparable to the manual-remote results: 12–17% vs 12–23% and 70 vs 60 min, respectively. Since sub-routines already generated in the previous robotic procedures can be used, this new robotic synthesis reportedly requires about one week for implementation.

The St Louis group have recently described the use of an articulated arm produced by CRS PLUS (Hudson Robotics) for 2-<sup>18</sup>F DG synthesis (Gaehle and Welch, 1992). Total Control Software™ controls the system through an IBM or compatible PC. The system is a space-saving alternative to the Zymark™ system.

6.3.2. *At the University of Washington (Seattle).* Since robotic procedures are normally slower than manual procedures, their primary application has been in labelling with fluorine-18 rather than carbon-11. One additional application of the Zymark system in <sup>11</sup>C-labelling has, however, been reported by the PET group at the University of Washington in Seattle (Link *et al.*, 1989), namely the synthesis of the tracer [1-<sup>11</sup>C]D-glucose. This requires large activities of the labelling agent, [<sup>11</sup>C]cyanide, to be handled. In addition to manipulations and reagent additions, a sensitive titration of the pH of the reaction mixture is performed by the robot. The total time required for the robot-assisted radiosynthesis is less than 5 min more than the manual-remote procedure and the quantities of radiotracer produced are sufficient for PET studies. The same group has recently reported the use of Zymark robotics for the multi-stage synthesis of [2-<sup>11</sup>C]thymidine from hydrogen [<sup>11</sup>C]cyanide (Courter *et al.*, 1992).

6.3.3. *At Brookhaven National Laboratory.* The Zymark system has recently been used by the Brookhaven PET group for measuring the plasma kinetics of total radioactivity and fraction of unmetabolised tracer (Alexoff *et al.*, 1991, 1992), an otherwise time- and personnel-demanding procedure. As demonstrated with blood samples from a PET study with 3-*N*-[<sup>18</sup>F]methylspiperone, the robot centrifuged, counted and pipetted different samples simultaneously to determine the discrete time-input function. The throughput of the robot is half that of a highly trained technician. Metabolite assays for 3-*N*-[<sup>18</sup>F]methylspiperone, [*N*-methyl-<sup>11</sup>C]cocaine and [*O*-methyl-<sup>11</sup>C]raclopride, based on solid-phase extractions, can also be performed with excellent reproducibility for replicate samples. Not only might the total throughput be increased by this use of robots, but human exposure to radiation and potentially infectious biological materials are minimised. Possible errors due to variations in laboratory techniques are also reduced.

6.3.4. *At Stockholm University-Karolinska Hospital.* For a number of reasons, a PET group may wish to design and implement its own robotics. The reasons may include space limitations, economy, the flexibility of tailoring the system to fit its own needs, and the desire to do basic research in the technique as a whole. In this case, a manipulator with the best performance characteristics (reproducibility, flexibility, strength) is chosen to use the planned work area effectively. If the group of scientists designing the system are competent in computer programming and interfaces, mechanical and electrical engineering and chemistry, a versatile system can be developed.

The system designed by the Stockholm University-Karolinska PET group (Appelquist *et al.*, 1989, 1991, 1993) illustrates such a development. The chosen robot arm is an RTX from Universal Machine Intelligence (Table 2). It is vertically mounted on a rail and can be installed on the back wall of a hot-cell for operation in the semi-circle in front of it. A Multi-Function Editor (MFE) was developed for building and interpreting control sequences for the system. The computer program recognises commands for moving the robot and for operating the equipment used in the syntheses (electrovalves, flow regulators, heating elements, event waits, *etc.*). The user-interface of the MFE is based on mouse-activated pull-down menus to provide a user-friendly mode of operation. The robot can be guided through motions in the desired directions by moving the mouse or by coordinates entered from the keyboard. Since simple editing commands and mouse-driven input are used in building the command sequences, the time required for a new user to learn how to program the system is minimised.

Radiosynthesis-specific equipment is fixed on a removable Plexiglas™ tray connected to the base plate of the robot, allowing easy access for service or cleaning but still ensuring reproducible re-positioning. Supporting equipment is installed separately in or around the hot-cell. The glassware is, where possible, selected from common reaction vessels such as Reacti-vials™ or, for special applications, is designed to be robot-friendly and standardised. Flow-line connections via needle stations, heating units, electrovalves, radioactivity monitors, as well as storage stations for reaction vessels have been specifically designed for optimal reliability of the total system. The use of the system as a developmental tool has been demonstrated in the production and applications of [ $^{11}\text{C}$ ]iodomethane and in nucleophilic substitution reactions with [ $^{18}\text{F}$ ]fluoride and [ $^{11}\text{C}$ ]cyanide.

6.3.5. *At Uppsala.* Installation and the time required for the first successful use of robotics may be reduced by choosing a complete commercial radiosynthesis system. One such system, based on the Anatech RB-86 robot (Table 2), has been adapted for PET radiochemical procedures by Scanditronix AB (Kjellström and Lindbäck, 1988). For radiosyntheses, the robot arm is installed on a rail on the back wall of the shielded area. Since it can be moved linearly up to a distance of six metres, it can be used to service separate but adjacent hot-cells. However, to utilise this feature maximally, the working space must be custom-designed for installation of the rail through all the cells from the back side. The robot system is delivered with lab-stations for performing common operations (pipetting, liquid dispensing, solid phase extractions, vortexing, vial capping, heating, cooling, evaporations and millipore filtrations). Additional equipment specific to the radiosyntheses of 2- $^{18}\text{F}$ FDG and [ $^{11}\text{C}$ ]iodomethane is also available, thereby enabling application of the robotics soon after installation. However, as with all robotic systems, routine operation requires that at least one person is well-versed in the control characteristics of both the robot and the supporting equipment. The system is extendable to alkylations with [ $^{11}\text{C}$ ]iodomethane, other types of syntheses or even analytical procedures associated with PET. The vendor supplies a tool for performing such operations but the applications will require user-design.

6.3.6. *At St Petersburg.* The group at St. Petersburg, in collaboration with Scanditronix AB, have recently reported the use of the RB-86 robot (Anatech AB) with workstations from Scanditronix AB for the synthesis of [ $1\text{-}^{11}\text{C}$ ]acetate (Korsakov *et al.*, 1992a) and also for [ $S\text{-methyl-}^{11}\text{C}$ ]methionine from [ $^{11}\text{C}$ ]iodomethane (Korsakov *et al.*, 1992b).

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## CHANGES IN GLOBAL CEREBRAL BLOOD FLOW IN HUMANS: EFFECT ON REGIONAL CEREBRAL BLOOD FLOW DURING A NEURAL ACTIVATION TASK

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### SUMMARY

1. The primary objective of this study was to examine in man, how induced changes in global cerebral blood flow (gCBF) affected a regional cerebral blood flow (rCBF) increase resulting from a neural activation task (opening of eyes). A secondary objective was to quantify how such induced changes in gCBF were distributed between representative regions of either predominantly grey matter or white matter.

2. Positron emission tomography with intravenous infusion of  $H_2^{15}O$ , was used to measure gCBF in six normal males. Concomitant measures of rCBF were obtained in three different regions of interest (ROI): a representative area of predominantly grey matter, a representative area of predominantly white matter and an area of visual cortex.

3. Cerebral blood flow was altered by establishing steady-state changes in  $P_{CO_2}$  at a near constant ventilation of approximately  $30\text{ l min}^{-1}$ . The mean  $P_{ET,CO_2}$  ( $\pm$  s.d.) levels (mmHg) that resulted were: low,  $21.8 \pm 1.8$ ; normal,  $39.8 \pm 1.0$ , and high,  $54.8 \pm 1.2$ . The normal and high levels were obtained by adding appropriate amounts of  $CO_2$  to the inspire. The corresponding mean gCBF levels across all six subjects with eyes closed were: low,  $24.2 \pm 4.6$ ; normal,  $37.2 \pm 3.9$  and high,  $66.8 \pm 7.6\text{ ml min}^{-1}\text{ dl}^{-1}$ .

4. Blood flow in grey matter (insular cortex) and white matter (centrum semiovale) at normal levels of  $P_{CO_2}$  averaged  $56.8 \pm 10.1$  and  $20.3 \pm 3.4\text{ ml min dl}^{-1}$  respectively. As  $P_{CO_2}$  rose, the increase in rCBF to grey matter was approximately three times greater than that to white matter.

5. An activation state of eyes open in a brightly lit room was compared to a baseline state of eyes closed in a darkened room at the three levels of  $P_{CO_2}$  (and hence at three levels of gCBF). Over the whole gCBF range a significant ( $P = 0.028$ ) effect of increasing rCBF in the visual cortex ROI was found in response to opening

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the eyes; the effect of this activation on rCBF was not significantly dependent ( $P = 0.34$ ) on the  $P_{\text{CO}_2}$  (and hence gCBF) level. The effect of the activation on the rCBF was apparently 'additive' to the rise of rCBF associated with  $P_{\text{CO}_2}$ -related gCBF increase.

6. The results confirm the need to normalize for changes in gCBF during studies of rCBF in response to an activation protocol. They also provide support for the use of an 'additive' model to achieve such normalization provided that other cortical areas behave in a similar manner to that of the visual cortex.

#### INTRODUCTION

Regional blood flow in the brain is tightly coupled to local metabolic demand (Kuschinsky & Wahl, 1978; Siesjo, 1984) and hence is sensitive to regional neuronal activation; this was first shown in conscious man by Olesen (1971). Global cerebral blood flow (gCBF) does not, in general, reflect changes in regional cerebral blood flow (rCBF) unless the region activated is very large. The major factors that control gCBF are the perfusion pressure and the autoregulation mechanism, together with chemical and metabolic factors such as the  $P_{\text{CO}_2}$  in the capillaries, the  $\text{H}^+$  ion concentration and  $P_{\text{CO}_2}$  in the perivascular space and the cerebral tissue  $P_{\text{O}_2}$  (Witzleb, 1987; Guyton, 1991).

To our knowledge, the dependency of an rCBF increase resulting from a neural activation upon the prevailing gCBF has not been studied previously. However, this has been noted as a problem by workers using positron emission tomography (PET) to study regional brain functions (Horowitz, Duara & Rapoport, 1984; Metter, Reige, Kuhl & Phelps, 1984; Ford, 1986; Moeller, Strother, Sidtis & Rottenberg, 1987). In the absence of experimental data and with the need to take account of within- and between-subject variation in gCBF (with the particular requirement to identify sites of cerebral activation using *intersubject* averaging), statistical models have been developed to normalize for the possible confounding effects of changes in gCBF. A proportional model (i.e. rCBF change is linearly dependent on gCBF and the relationship goes through zero) has been used by some workers (Fox, Mintun, Reimen & Raichle, 1988). Alternatively, an additive model has been proposed (Friston, Frith, Liddle, Dolan, Lammertsma & Frackowiak, 1990) based on the concept that changes in rCBF resulting from activation are independent of gCBF. In a study of neural activation resulting from differing verbal fluency tasks in four subjects, Friston *et al.* (1990) concluded that activated brain regions were more sensitive to global changes than would have been predicted by a simple proportional model and furthermore that the findings were consistent with an independence of regional and global change.

The present study therefore seeks to clarify the relationship between the prevailing level of gCBF and the increase in rCBF in specific sites resulting from local regional activation. We chose to change baseline gCBF over a wide range using controlled steady-state alterations in arterial  $P_{\text{CO}_2}$  ( $P_{\text{a,CO}_2}$ ) at three levels. We designed our study such that breathing was kept constant. At each level we examined the rCBF response to a well-established activation protocol, visual cortical activation (opening of the eyes); this is known to produce a large increase in rCBF. During the course of these studies we also wished to compare the results of changing  $P_{\text{a,CO}_2}$  levels on the cerebral circulation and its distribution to

representative areas of grey and white matter, as elucidated by PET, with those obtained previously in animals and man using more invasive techniques.

Depending on the outcome of this study, we planned to further investigate whether hypercapnic stimulation of breathing activates any cerebral regions (Murphy, Meir, Adams & Guz, 1990) independently of the overall increase in gCBF, an inevitable consequence of an increase in  $P_{a,CO_2}$ .

## METHODS

### Subjects

Six normal right handed males (mean age 32; range 26–42 years) were studied; informed consent was obtained. The study was approved by the local ethical committee and approval to administer radioactive isotopes was given by the Administration of Radioactive Substance Approval Committee (ARSAC) UK. Two of the subjects were co-authors and the others were scientific colleagues.

### Experimental protocols and measurements

#### Induction of changes in global cerebral blood flow (gCBF)

Changes in global cerebral blood flow in each individual were produced by establishing different levels of arterial  $P_{CO_2}$  at rest. To establish hypocapnia, subjects volitionally hyperventilated, with a constant respiratory rate of 10–12 breaths  $\text{min}^{-1}$  and a tidal volume of 1.5–2.0 l, to produce a stable end-tidal  $P_{CO_2}$  ( $P_{ET,CO_2}$ ) of approximately 20 mmHg. To achieve normocapnia, this pattern of ventilation was maintained and dead space (corrugated plastic tubing; i.d. 3.5 cm) was added to establish a  $P_{ET,CO_2}$  of around 40 mmHg. Hypercapnia ( $P_{ET,CO_2}$  of around 55 mmHg) was established with the same ventilatory pattern but with additional dead space and supplementary inspired  $CO_2$ . In the presence of any dead space, normoxia was ensured by supplementary inspired oxygen sufficient to keep arterial oxygen saturation (finger probe oximetry; Ohmeda, Biox 3700e, Louisville, USA) at the subject's resting level (> 95%). In all conditions, subjects breathed through a nasal mask connected to an ultrasonic flowmeter (Branta, Birmingham, UK) and  $CO_2$  analyser (Hewlett Packard 47210A, USA).

Subjects required training to produce the 'standard breathing pattern' prior to PET scanning. Initially, they were provided with external cues by listening to a cycling ventilator, set at the desired respiratory frequency ( $f_R$ ) and inspiratory time ( $T_I$ ); the targeted tidal volume ( $V_T$ ) was learnt by providing verbal feedback. Training was given over a number of sessions until subjects could maintain a constant breathing pattern for periods of 6 min without external cues and irrespective of different  $P_{CO_2}$  levels.

#### Visual activation protocol

The visual cortex was activated by the subjects keeping their eyes open with the room brightly lit; the control state was with the room darkened and the eyes closed.

### PET scans

PET studies were performed using an ECAT 953B (CTI/Siemens, Knoxville, USA) dedicated head scanner; the performance characteristics have been described elsewhere (Spinks *et al.* 1992). The scanner was used in 2-dimensional mode with interplane tungsten septa in place (Townsend, 1991). Radiolabelled water ( $H_2^{15}O$ ), produced continuously by the catalytic reaction of  $^{15}O_2$  and hydrogen, was used as a tracer of cerebral blood flow. A venous line was inserted in the antecubital fossa on the left side to allow administration of the tracer. An arterial line was inserted into the left radial artery to allow monitoring of radioactive levels (for subsequent quantification of gCBF and rCBF values) and for analysis of blood gas levels. A polyurethane head mould was fitted to minimize head movement and the head was placed in the scanner so that the lowermost plane of scan acquisition was approximately parallel to and 15–18 mm above the orbito-meatal line. Subjects lay supine in the quiet darkened room with eyes closed while a transmission scan (using orbiting  $^{68}Ge$ / $^{68}Ga$  rods) was collected prior to tracer administration for the purpose of individual attenuation correction of emission data.

Cerebral blood flow studies were performed using a previously described method (Lammertsma *et al.* 1990; Colebatch *et al.* 1991). Briefly, between 1.8 and 2.9 GBq of  $H_2^{15}O$  was administered intravenously at a constant rate infusion of 10 ml  $\text{min}^{-1}$  over 1 min; this was followed by a 30 s

saline flush. A multi-frame dynamic scan was acquired over a 4 min period starting 30 s prior to the start of the intravenous infusion. Arterial levels of radioactivity were monitored continuously using an on-line detection system as described previously (Colebatch *et al.* 1991). Global and regional cerebral blood flow could then be calculated over the period of each scan.

#### *Protocol*

Six scans were collected for each subject with periods of at least 10–12 min between scans to allow for both decay of radioactivity and any required re-establishment of normocapnia from the previous measurement. Two scans were performed at each level of  $P_{ET,CO_2}$ ; one of each pair was in the rest condition with eyes closed while the other was in the neural activation condition of eyes open. The ordering of conditions was balanced within a study and across subjects. The stable ventilatory pattern and the  $P_{ET,CO_2}$  required were established for at least 2.5 min before the beginning of the scan and then maintained during the scanning period. A 2 ml sample of arterial blood was taken immediately at the end of the scanning period for analysis of pH,  $P_{CO_2}$  and  $P_{O_2}$  (Novastat Profile 5, Nova Biomedical, Waltham, MA, USA).

#### *Blood pressure measurement*

On a separate occasion, blood pressure was measured (Sphygmomanometry with Korotkov sounds) during identical experimental conditions, but without PET scanning, in five of the six subjects.

#### *Image analysis*

Image analysis was performed on a SPARC1 computer (Sun Microsystems Europe Inc., Surrey, UK) using an interactive image analysis software package (ANALYZE, Biodynamic Research Unit, Mayo Clinic, Rochester, MN, USA). Calculations and image matrix manipulations relating to the identification of sites of activation were performed in PRO-MATLAB (The Mathworks Inc., Sherborn, MA, USA). To increase the validity and precision of the 'regions of interest' (ROI) placement, all the images were stereotactically normalized as previously described (Friston, Frith, Liddle & Frackowiak, 1991). Following normalization, the data correspond to the standard brain dimensions used by Talairach & Tournoux (1988) in their stereotactic atlas. The resulting images consisted of twenty-six planes of voxels (i.e. slices) measuring 2 mm by 2 mm by 4 mm in the  $x$  (right and left of midline),  $y$  (rostral and caudal to the anterior commissural line) and  $z$  (dorsal and ventral to the intercommissural plane) directions respectively.

#### *Measurement of global cerebral blood flow*

A whole brain ROI was drawn directly on the raw data of the dynamic images; the scalp was excluded. This region was drawn in the middle third of the brain and global flows were then calculated using the technique of Lammertsma *et al.* (1990).

#### *Identification of regions of interest and calculation of their rCBF*

*Without activation – grey and white matter.* Grey and white matter ROIs were chosen from one of the cerebral hemispheres. The grey matter ROI was sited in the insula and its operculae from the frontal and temporal lobes (see inset of Fig. 2); this region was positioned on a number of planes (3–6) which varied between subjects to allow the same gyral anatomy to be included from brains of different size. The volume of brain identified was 10–15 ml with the central plane 4 mm below the intercommissural line. The white matter ROI was sited in the centrum semiovale region (see inset of Fig. 2) using three planes; the volume of brain identified was approximately 5 ml with the central plane 28 mm above the intercommissural line.

To obtain the highest possible degree of accuracy, these ROIs were projected on the original dynamic frames in order to obtain grey and white matter time-activity curves. These curves were then fitted to give both rCBF and the volume of distribution of water, taking into account delay and dispersion of the arterial input function as described previously (Lammertsma *et al.* 1990); this allowed the computation of absolute blood flow.

*With visual activation task.* The sites of neuronal activation with these tasks were defined during normocapnia using the functional CBF data from all subjects (obtained with a fixed volume of distribution of water of 0.95) as described previously (Lammertsma *et al.* 1990); the anatomical variation between subjects was reduced by convolving the data with a Gaussian filter (full-width half-maximum 20 mm). Any physiological variation resulting from differences in gCBF was corrected for by treating global counts as a confounding variable using an analysis of covariance (ANCOVA; Friston *et al.* 1990). Adjusted condition means and variances were

compared using linear contrasts and the resultant three dimensional maps of  $t$  statistical values (corrected for multiple non-independent comparisons) for the  $P < 0.05$  level of significance were then displayed (Friston *et al.* 1991). The visual activation task produced large rCBF increases bilaterally in the regions around and just superior to the calcarine fissure (see Results), and ROIs were defined on the three planes with the highest values. On each of these planes the voxel with the highest  $t$  value was defined as the centre of a circular region with a volume of thirty-two voxels (i.e. each region approximately 0.5 ml). These regions were then applied to anatomically normalized unsmoothed images which had not been subjected to an ANCOVA correction for global flow. The mean voxel value was then determined for these regions and this was used to determine the rCBF within the visual ROI for each subject in each of the six experimental scans.

#### Statistical analysis

To test the constancy of breathing at different levels of  $P_{ET,CO_2}$  and with visual neural activation, mean values (over each scan) of inspired minute ventilation ( $\dot{V}_I$ ),  $\dot{V}_T$ ,  $f_R$  and  $P_{ET,CO_2}$  were compared between conditions using a two-factor analysis of variance (BMDP) with  $CO_2$  level (low:normal:high) and activation state (rest:activation) as factors. Differences in rCBF between grey and white matter and the dependency of any such differences on the level of gCBF were examined using a two-factor analysis of variance with  $CO_2$  level (low:normal:high) and anatomical site (grey:white) as factors. Differences in gCBF and in rCBF in response to visual neural activation and the dependence of any differences on the level of gCBF were examined using a two-factor analysis of variance with  $CO_2$  level (low:normal:high) and activation state (rest:activation) as factors; for all comparisons  $P < 0.05$  in a two-tailed test was taken as indicating a statistically significant difference. The least significant difference of Fisher (1935) was calculated from the analysis of variance to show the smallest difference between any two means which is statistically significant.

### RESULTS

#### Subjects' comments

The subjects confirmed that during the scans they had remained awake and had kept their eyes open or closed as requested. All subjects were confident that they had been able to maintain a fairly uniform pattern of breathing during the tasks although they all commented that this had been more difficult in the hypercapnic condition due to an uncomfortable feeling of needing to breathe more. Subjects also reported feeling hot and flushed during hypercapnia; by contrast most felt cool or even cold during hypocapnia. There were no reports of any other symptoms during hypocapnia and there was no evidence of tetany. Subjects did not report any difference in the attention required to execute the breathing task either at different  $CO_2$  levels or during neural activation runs.

#### Pattern of breathing

Individual values for breathing pattern and end-tidal  $P_{CO_2}$  ( $P_{ET,CO_2}$ ), at the three levels of  $CO_2$  and during the rest and activation states are shown in Fig. 1. The results of the analysis of variance indicated the expected statistically significant differences in mean  $P_{ET,CO_2}$  levels between hypocapnia (21.0 mmHg), normocapnia (40.0 mmHg) and hypercapnia (54.6 mmHg). Mean  $\dot{V}_I$  was significantly greater at the high  $CO_2$  level (mean = 34.9 l min<sup>-1</sup>) compared with the low (29.8 l min<sup>-1</sup>) and normal (29.7 l min<sup>-1</sup>) levels although there were no significant differences for mean  $f_R$  (low, 16.9; normal, 16.9; high, 18.5 breaths min<sup>-1</sup>) or  $\dot{V}_T$  (low, 1.76; normal, 1.74; high, 1.84 l). Comparison between rest and activation states over the range of  $CO_2$  levels showed that mean  $f_R$  was significantly greater with activation (18.2 min<sup>-1</sup>) compared with rest (16.7 min<sup>-1</sup>); there were no significant differences for  $P_{ET,CO_2}$ ,  $\dot{V}_I$  or  $\dot{V}_T$ .



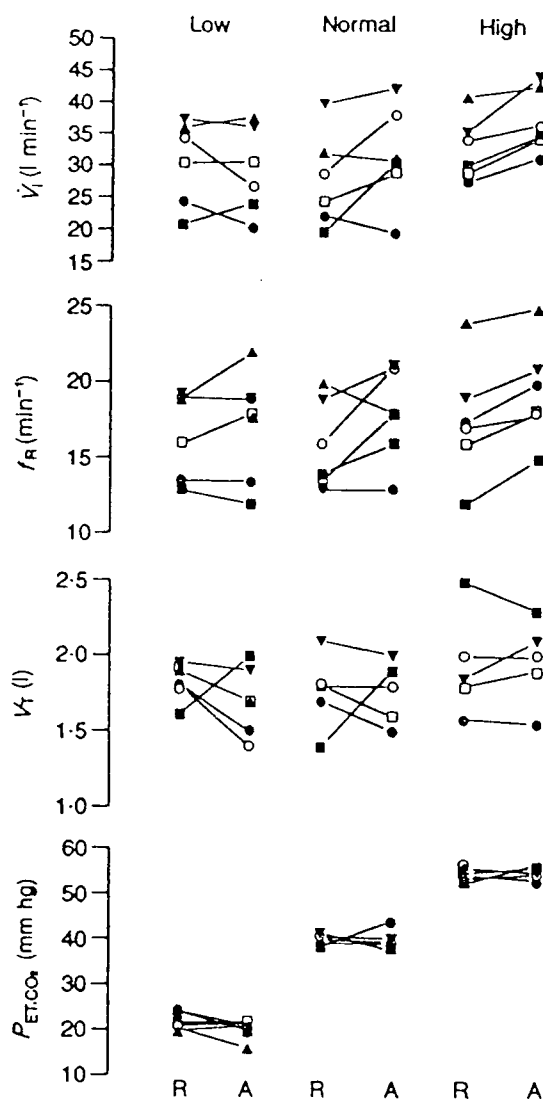


Fig. 1. Average values (over 1 min of  $H_2^{18}O$  infusion) of inspired minute ventilation  $\dot{V}_I$ , respiratory frequency ( $f_R$ ), tidal volume ( $V_T$ ) and end-tidal  $P_{CO_2}$  ( $P_{ET,CO_2}$ ) for each of 6 subjects (●, subject 1; ▼, subject 2; ■, subject 3; ▲, subject 4; ○, subject 5; □, subject 6) in each of the experimental conditions. Low, normal and high represent the three  $P_{CO_2}$  levels at which the scans were performed during resting (R) and visual activation (A) conditions. Note the close matching of ventilatory variables across the experimental conditions and between rest and activation states despite clear differences in  $P_{ET,CO_2}$ .

#### *Blood pressure and arterial blood gas levels*

In five subjects, tested at a different time, the average blood pressures during the steady-state periods of  $P_{CO_2}$  were: 120/75 during hypocapnia, 122/78 during normocapnia and 143/86 mmHg during hypercapnia. Analysis of variance showed that the mean blood pressure ( $\pm$  s.d.) was significantly higher ( $P = 0.018$ ) during hypercapnia ( $105 \pm 10$ ) compared with hypocapnia ( $90 \pm 8$ ) or normocapnia ( $92 \pm 8$  mmHg). In general, there was close agreement in individuals between



arterial  $P_{\text{CO}_2}$  measured at the completion of a scan and end-tidal  $P_{\text{CO}_2}$  averaged over the period of scanning ( $\pm 2$  mmHg); however in a few instances there were discrepancies between the two measurements ( $\pm 5$  mmHg). In view of this,  $P_{\text{ET,CO}_2}$  measurements were utilised in subsequent analyses since they relate better to the period of scanning and do not depend on a single measurement. The average arterial  $P_{\text{O}_2}$  measurements (rest and activation runs combined) were:  $128 \pm 11$  during hypocapnia;  $118 \pm 16$  during normocapnia and  $129 \pm 15$  mmHg during hypercapnia. Analysis of variance showed that there were no statistically significant differences between these levels ( $P = 0.158$ ).

#### *Effect of $P_{\text{CO}_2}$ on global cerebral blood flow*

The values for  $P_{\text{ET,CO}_2}$  and corresponding measurements of gCBF at rest with eyes closed are given for each subject in Table 1. The mean gCBF during normocapnia was  $37.2 \pm 3.9$ ; during hypocapnia it was  $24.2 \pm 4.6$ ; and during hypercapnia it was  $66.8 \pm 7.6$  ml min<sup>-1</sup> dl<sup>-1</sup>. The average ratio (from individual values) of  $\Delta\text{gCBF}:\Delta P_{\text{CO}_2}$  for the low to normal  $P_{\text{CO}_2}$  change was  $0.72 \pm 0.15$  (range 0.44–0.85); and for the normal to high  $P_{\text{CO}_2}$  change it was  $2.00 \pm 0.32$  (range 1.40–2.26) ml min<sup>-1</sup> dl<sup>-1</sup> mmHg<sup>-1</sup>.

#### *Effect of $P_{\text{CO}_2}$ on blood flow in grey and white matter*

For each individual, the measurement of gCBF obtained in each of the three scans (i.e. low, normal and high  $P_{\text{CO}_2}$ ) in the rest (eyes closed) condition was paired with the rCBF measurement for the grey matter region of interest (ROI) and separately with the rCBF for the white matter ROI. These data have been plotted for each of the six subjects in Fig. 2. The mean values with normocapnia for grey and white matter were  $56.8 \pm 10.1$  and  $20.3 \pm 3.4$  ml min<sup>-1</sup> dl<sup>-1</sup> respectively. In each subject, when related to  $P_{\text{ET,CO}_2}$  (Fig. 2A), the increase in rCBF for grey matter is greater than for white matter, particularly in the normal to high  $P_{\text{CO}_2}$  range. Thus in the low to normal  $P_{\text{CO}_2}$  range, the mean changes in blood flow for grey and white matter are  $0.99 \pm 0.60$  and  $0.39 \pm 0.14$  ml min<sup>-1</sup> dl<sup>-1</sup> mmHg<sup>-1</sup> respectively; these changes are significantly different (paired *t* test;  $P = 0.01$ ). For the normal to high  $P_{\text{CO}_2}$  range, the mean change in blood flow for grey matter is  $2.97 \pm 1.07$  and for white matter this ratio is  $1.10 \pm 0.34$  ml min<sup>-1</sup> dl<sup>-1</sup> mmHg<sup>-1</sup>; these ratios are significantly different (paired *t* test;  $P = 0.001$ ). Figure 2B shows the same grey and white matter rCBF values for each subject, but in this case plotted against the prevailing gCBF value at each of the  $P_{\text{CO}_2}$  levels. For each subject there is a nearly linear increase in rCBF as gCBF rises, with intercepts close to zero. The mean ratio over the entire range of gCBF (i.e.  $\Delta\text{rCBF}/\Delta\text{gCBF}$ ) is  $1.53 \pm 0.24$  for grey and  $0.52 \pm 0.18$  for white matter.

Analysis of variance on the pooled data confirms the obvious effect on rCBF for both grey and white matter ROIs when the gCBF is increased by  $P_{\text{CO}_2}$  ( $P < 0.0001$ ); furthermore the rCBF within grey matter was significantly different from that within white matter over the range of  $P_{\text{CO}_2}$  ( $P < 0.0001$ ). The presence of a significant interaction term ( $P < 0.0001$ ) between the  $P_{\text{CO}_2}$  factor and the ROI factor confirms that the pattern of increase in rCBF within grey matter was significantly different from that within white matter as gCBF increased.

*Effect of visual activation on rCBF*

Opening the eyes activated the primary visual cortex bilaterally (see Methods); the three dimensional map of the  $t$  statistical values at normocapnia showed significance at the  $P < 0.05$  level. The rCBF associated with visual activation was determined from the mean voxel values in the visual ROIs (see Methods). These

TABLE 1. Individual and mean values for end-tidal  $P_{\text{CO}_2}$  (in mmHg), regional cerebral blood flow (rCBF; in  $\text{ml min}^{-1} \text{dl}^{-1}$ ) in a visual region of interest and global cerebral blood flow (gCBF; in  $\text{ml min}^{-1} \text{dl}^{-1}$ ).

| Subject number |                   | Condition             |      |                          |      |                        |      |
|----------------|-------------------|-----------------------|------|--------------------------|------|------------------------|------|
|                |                   | Low $P_{\text{CO}_2}$ |      | Normal $P_{\text{CO}_2}$ |      | High $P_{\text{CO}_2}$ |      |
|                |                   | Eyes closed           | Open | Eyes closed              | Open | Eyes closed            | Open |
| 1              | $P_{\text{CO}_2}$ | 24                    | 21   | 39                       | 44   | 55                     | 53   |
|                | gCBF              | 29                    | 28   | 39                       | 48   | 72                     | 68   |
|                | rCBF              | 32                    | 37   | 41                       | 67   | 84                     | 79   |
| 2              | $P_{\text{CO}_2}$ | 22                    | 21   | 39                       | 40   | 55                     | 54   |
|                | gCBF              | 23                    | 23   | 37                       | 39   | 68                     | 72   |
|                | rCBF              | 27                    | 26   | 37                       | 40   | 76                     | 74   |
| 3              | $P_{\text{CO}_2}$ | 24                    | 20   | 41                       | 38   | 53                     | 56   |
|                | gCBF              | 18                    | 19   | 31                       | 32   | 58                     | 57   |
|                | rCBF              | 38*                   | 26   | 32                       | 38   | 62                     | 70   |
| 4              | $P_{\text{CO}_2}$ | 20                    | 16   | 40                       | 40   | 56                     | 55   |
|                | gCBF              | 26                    | 26   | 43                       | 45   | 75                     | 74   |
|                | rCBF              | 27                    | 34   | 48                       | 57   | 78                     | 87   |
| 5              | $P_{\text{CO}_2}$ | 20                    | 21   | 41                       | 39   | 56                     | 54   |
|                | gCBF              | 20                    | 21   | 36                       | 31   | 57                     | 60   |
|                | rCBF              | 27                    | 33   | 41                       | 42   | 66                     | 73   |
| 6              | $P_{\text{CO}_2}$ | 21                    | 22   | 39                       | 40   | 54                     | 54   |
|                | gCBF              | 29                    | 29   | 37                       | 38   | 71                     | 76   |
|                | rCBF              | 37                    | 40   | 44                       | 57   | 80                     | 89   |
| Mean           | $P_{\text{CO}_2}$ | 21.8                  | 20.2 | 39.8                     | 40.3 | 54.8                   | 54.4 |
|                | gCBF              | 24.2                  | 24.3 | 37.2                     | 38.3 | 66.8                   | 67.8 |
|                | rCBF              | 31.4                  | 32.7 | 40.5                     | 50.1 | 74.2                   | 78.7 |

Values are given for each of the three  $P_{\text{CO}_2}$  conditions (low, normal and high) and for the rest (eyes closed) and activation (eyes open) states. \* indicates a presumed invalid measurement due to undefined error (see text).

values are given for each condition for each subject, together with the gCBF and  $P_{\text{ET,CO}_2}$  in Table 1; the mean values for  $P_{\text{ET,CO}_2}$ , gCBF and rCBF are also given. Examination of the data shows one point (subject 3, low  $P_{\text{CO}_2}$ , eyes closed) with a high rCBF which reduces markedly in response to a visual activation; it was thought likely that this point was a result of undefined experimental error. Figure 3 presents the mean data of the group of five subjects (i.e. excluding subject 3) of the effect of visual activation on rCBF at the different gCBFs resulting from changes in  $P_{\text{CO}_2}$ . Analysis of variance on the data from these five subjects shows an overall (i.e. across the range of  $P_{\text{CO}_2}$  studied) significant effect of visual activation

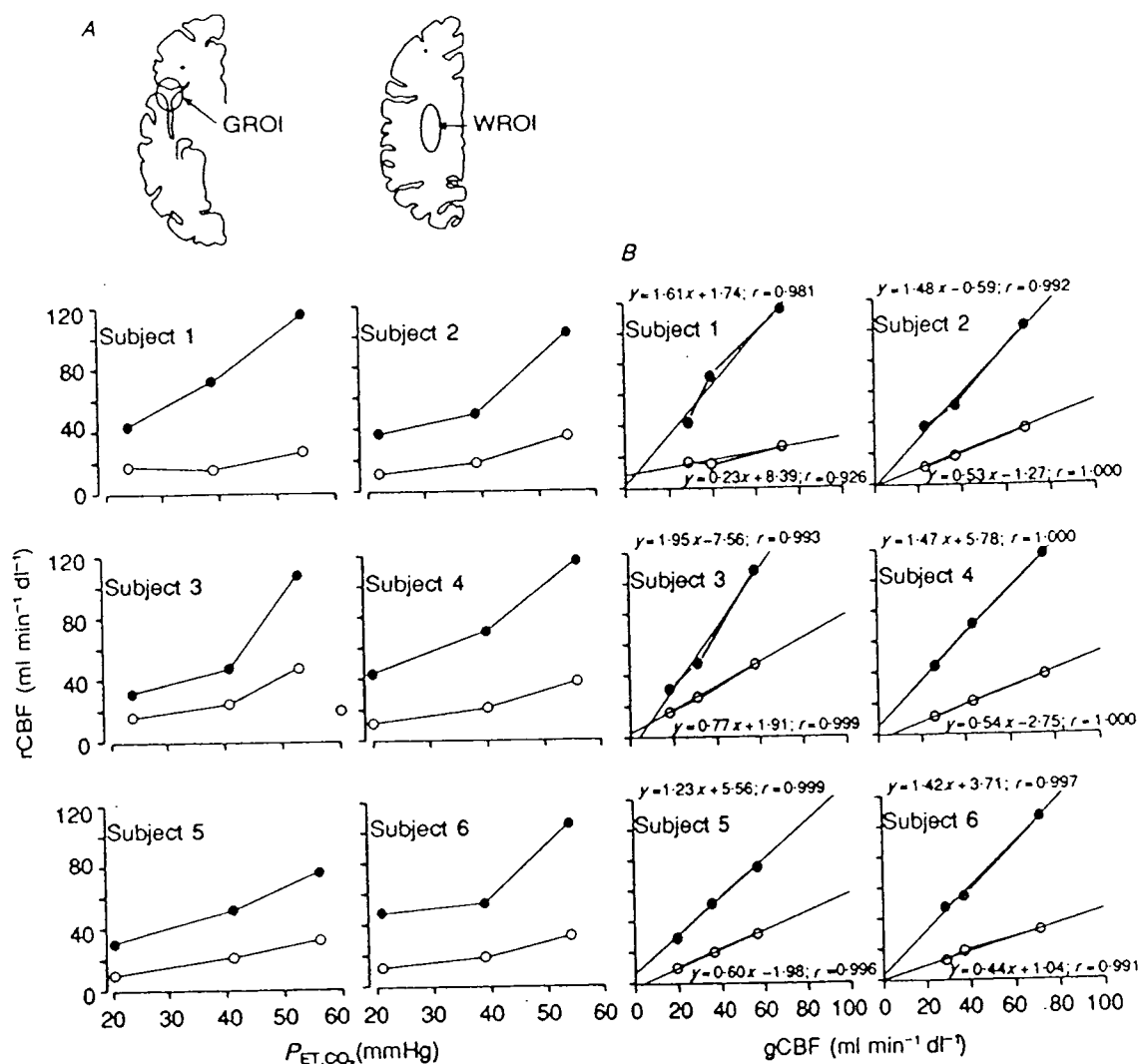


Fig. 2. Values of regional cerebral blood flow (rCBF) in standard cerebral regions comprising predominantly grey matter (●) and white matter (○) for each of six subjects in the absence of visual activation (eyes closed). The inset shows diagrammatically the areas chosen for the grey matter region of interest (GROI; the insula and its operculae from the frontal and temporal lobes) and the white matter region of interest (WROI; the centrum semiovale region). The same rCBF data are plotted separately against the end-tidal  $P_{CO_2}$  ( $P_{ET,CO_2}$ ; Fig. 2A) and global cerebral blood flow (gCBF; Fig. 2B). With respect to  $P_{ET,CO_2}$ , the changes in rCBF are greater for grey matter than for white matter particularly with hypercapnia. With respect to gCBF, rCBF in each subject increased linearly for both grey and white matter; for each subject, regression equations have intercepts close to the origin but slopes are invariably greater for grey matter.  $y$  is rCBF,  $x$  is gCBF and  $r$  is the correlation coefficient.

( $P = 0.028$ ) on rCBF; the same activation produces a minor, non-significant ( $P = 0.083$ ) increase in gCBF. There was no significant interaction term between the  $P_{CO_2}$  factor and the activation factor ( $P = 0.340$ ) on rCBF implying that the effect of activation on rCBF was not dependent on the  $P_{CO_2}$  level and hence on gCBF. Similarly, there was no significant interaction term between the  $P_{CO_2}$  factor and the activation factor ( $P = 0.787$ ) on gCBF.

Analysis of variance on the data from all six subjects (i.e. including subject 3) still shows an overall significant increase in rCBF of visual activation ( $P = 0.031$ ) and a non-significant interaction term between the  $P_{\text{CO}_2}$  factor and the activation factor ( $P = 0.233$ ) on rCBF. The only apparent effect of including the data from subject 3 was to minimize the difference in rCBF due to visual activation at a low  $P_{\text{CO}_2}$  (Table 1, cf. Fig. 3).

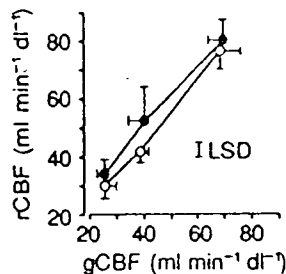


Fig. 3. Mean values ( $\pm$  S.D. bars) of regional cerebral blood flow (rCBF) in the visual cortical ROI with eyes closed (O) and eyes open (●) plotted against global cerebral blood flow (gCBF) resulting from the change in  $P_{\text{CO}_2}$  in 5 of the 6 subjects (see text). Fisher's (1935) least significant difference (LSD) is presented geometrically to indicate the difference between rCBF values necessary to achieve statistical significance at  $P < 0.05$  (see text). At each  $P_{\text{CO}_2}$  level, visual activation (opening eyes) is associated with greater increases in rCBF compared with the change in gCBF.

#### DISCUSSION

The most crucial finding in the present study is the demonstration that neural activation of the visual cortex causes an increase in regional cerebral blood flow (rCBF) which is independent of the prevailing level of global cerebral blood flow (gCBF). It has considerable practical relevance for the normalization of rCBF measurements to take account of variations in gCBF which occur during neural activation studies. A further significant aspect of the present study is that we have been able to use PET to confirm and extend previous observations in man (using 'inert gas' techniques) on the effect of increased arterial  $P_{\text{CO}_2}$  on CBF and its relative distribution to representative areas of grey and white matter.

We have documented a mean range in gCBF from 24–67 ml min⁻¹ dl⁻¹ as end-tidal  $P_{\text{CO}_2}$  was experimentally adjusted between 22 and 55 mmHg. By achieving this at a relatively constant ventilation for each  $P_{\text{CO}_2}$  level, we were able to control for any possible confounding influence that the act of breathing itself might have on gCBF. The average level of gCBF recorded in the present study under resting normocapnic conditions was 37 ml min⁻¹ dl⁻¹. This is somewhat lower than the range of 45–55 ml min⁻¹ dl⁻¹ identified by Lassen (1985) as the 'Gold Standard' in a critical review of the literature. The fact that we obtained a value for gCBF below this range, strongly suggests a systematic error probably arising in the present study from the choice of the middle third of the brain (see Methods) to obtain data for subsequent normalization by a geometrical estimate of *total* intracranial volume; this would include the ventricles and cisternae even though radioactive 'counts' relate essentially to the vascularized regions.

In the present study, we have confirmed previous observations in man that  $P_{\text{CO}_2}$  alterations cause substantially greater changes in CBF above the normocapnic level

than in the hypocapnic range (see Fig. 2). Between 40 and 55 mmHg  $P_{\text{CO}_2}$ , the mean  $\Delta\text{gCBF}/\Delta P_{\text{CO}_2}$  in the present study was  $2.0 \text{ ml min}^{-1} \text{ dl}^{-1} \text{ mmHg}^{-1}$ ; between 40 and 21 mmHg  $P_{\text{CO}_2}$  the mean  $\Delta\text{gCBF}/\Delta P_{\text{CO}_2}$  was  $0.7 \text{ ml min}^{-1} \text{ dl}^{-1} \text{ mmHg}^{-1}$ ; these values are similar to those derived using inert gas techniques (Kety & Schmidt, 1948a; Novack, Shenkin, Bortin, Guloboff & Soffe, 1953). Thus the present findings, taken together with the previous observations cited, lend no support to the view that it is permissible to calculate the slope of the best fitting straight line through gCBF data in the range of  $P_{\text{CO}_2}$  between 20–60 mmHg (Reivich, 1964), or to use such a slope to normalize values of gCBF to a  $P_{\text{CO}_2}$  of 40 mmHg as suggested by Purves (1972).

The average rise in mean arterial blood pressure with hypercapnia in the present study of 13 mmHg for an average elevation of  $P_{\text{CO}_2}$  of 15 mmHg compares well with previous observations (Kety & Schmidt, 1948b; Novak *et al.* 1953). During normocapnia, gCBF in man is independent of arterial blood pressure (Lassen, 1959). However, with cerebrovascular dilatation consequent upon the rise in  $P_{\text{CO}_2}$ , such autoregulation may well be impaired and if this is so, the small elevations in blood pressure documented with hypercapnia may contribute to the increasing  $\Delta\text{CBF}/\Delta P_{\text{CO}_2}$  in the hypercapnic compared to the hypocapnic range.

Since CBF is insensitive to changes in arterial  $P_{\text{O}_2}$  above 60–80 mmHg (Lambertsen, 1961; Borgstrom, Johannsson & Siesjo, 1975) it is unlikely that the small range of mean arterial  $P_{\text{O}_2}$  noted between the different conditions of this study (i.e. 118–129 mmHg) could have had any significant influence on the CBF levels recorded.

The mean values for grey and white matter rCBF found with normocapnia were similar to those reported by Lammertsma *et al.* (1990) using a similar technique. The present study indicates a disproportionate increase in rCBF in grey compared with white matter as  $P_{\text{CO}_2}$  rises above the normocapnic range suggesting that the cerebrovascular resistance falls much more in grey than in white matter under these conditions. The reason for this phenomenon is unclear but it has been reported previously in anaesthetized animals (Hansen, Sultz, Freygang & Sokoloff, 1957; James, Millar & Purves, 1969).

The nature of the relationship between focal CBF changes associated with activation and levels of global CBF is unclear. Since many activation protocols induce only relatively small changes in rCBF compared to the absolute value of gCBF, interpretation of such studies requires that changes in gCBF be carefully considered. Clearly, if there is a non-specific change in gCBF, it is important to be sure that any rCBF change in a ROI is not a mere reflection of the altered gCBF. Changes in gCBF could occur in a single subject over time, and also between individual subjects, as well as resulting from factors, such as exercise, anxiety or changes in acid–base status (as in the present study). In such cases, one would need to normalize for changes in gCBF in order that small changes in rCBF due to activation could be more readily identified. On the other hand, it is possible that the activation task itself may significantly alter global CBF directly through large focal changes; normalization for differences in gCBF in this case might be expected to downgrade or eliminate the effect in which one was interested (Chadwick & Whelan, 1991).

A number of methods have been proposed to take account of global, region-independent, inter- and intra-subject variation during regional neural activation

studies (Horowitz *et al.* 1984; Metter *et al.* 1984; Moeller *et al.* 1987). Fox *et al.* (1988) have proposed a normalization method for gCBF variation which simply involves dividing rCBF by the estimated average gCBF; this proportional model necessarily assumes a relationship between rCBF and gCBF which is both linear and passes through the origin. Friston *et al.* (1990) have compared this proportional model with an additive model in which neural activation in a ROI would result in a similar increase in rCBF over a range of gCBF and have concluded, using a physiological activation protocol (verbal fluency task) that their additive model fits the observed data better than the proportional model. Their conclusion was arrived at through the use of an analysis of covariance which treats gCBF as a confounding 'nuisance' covariate which combines with the 'effect of interest' (rCBF changes solely due to neural activation) to produce variance in what is measured (rCBF changes in the ROI). However, their conclusion may be limited by the relatively narrow variations in gCBF which is likely to have accompanied their activation task.

The present study permits a more direct analysis of the inter-relationships between global and regional blood flow changes encountered during PET studies. By inducing changes in gCBF over a wide physiological range it was possible to examine the effects on rCBF, firstly, in representative areas of grey and white matter in the absence of activation and secondly, in response to a well-defined visual activation task. In this way, the appropriateness of normalization, based on clear-cut changes in gCBF could be judged. Our study confirms that across a wide range of gCBF, the relationship between gCBF and rCBF without activation is well described by a simple linear model for the defined areas of grey and white matter (see Fig. 2B) and also in the visual cortex (Fig. 3). Moreover, this study highlights the fact that the ratio of  $\Delta rCBF/\Delta gCBF$  is much higher for grey matter and clearly demonstrates the need for normalization of even small non-specific changes in gCBF during activation studies. With respect to changes in gCBF in response to visual activation, the present study indicates that at a normal arterial  $P_{CO_2}$ , there is a 23 % increase in rCBF in a visual cortical ROI in the face of only a 3 % non-significant increase in gCBF (Table 1; Fig. 3). Hence, with this activation, any normalization for changes in gCBF (which may have been induced by the activation) would not have greatly limited the ability to identify an increase in rCBF in response to neuronal activation. A different activation, producing much smaller increases in rCBF with a similar increase in gCBF would not have been so easily detected. Since in practice, it is impossible to know to what extent any changes in gCBF reflect non-specific as opposed to activation-related mechanisms, the present results point to the fact that normalization for changes in gCBF is indeed necessary.

The present study demonstrates that the increase in rCBF in the visual cortex, which accompanies opening of the eyes, shows no systematic dependence on the level of gCBF over a wide physiological range. Thus, although the increase in rCBF in response to visual activation was apparently greatest at a normal arterial  $P_{CO_2}$ , there were significant increases in rCBF associated with the same visual activation at both low and high levels of  $P_{CO_2}$  and hence gCBF (Fig. 3). One might hypothesize that the activation-related increase in rCBF at low  $P_{CO_2}$  is reduced because of the intense prevailing vasoconstriction, while at a high  $P_{CO_2}$  there is such an excess of

blood flow that the degree of vasodilatation present may be approaching a maximum. In the range of  $P_{\text{CO}_2}$  studied, and allowing for the relatively small data set, both the presentation of the results shown in Fig. 3 and the non-significant interaction term ( $P = 0.340$ ) between  $P_{\text{CO}_2}$  (gCBF) factor and the activation (rCBF) factor does not provide any basis for rejecting the hypothesis that the plots of rCBF against gCBF with eyes closed or opened are parallel to each other. Hence there is no support for a proportional relationship between rCBF increases with activation and the prevailing level of gCBF. The data support an approximation to an additive model as described by Friston *et al.* (1990) but it must be acknowledged that our limited data set do not allow us to reject the proportional hypothesis.

The results from the present study have a practical significance to those workers measuring rCBF to investigate activation of regional cerebral areas under conditions where global cerebral blood flow could change in a systematic way. Any change in gCBF would necessarily be associated with non-specific changes in rCBF in a region of interest. However, the fact that a more specific neural activation was still detectable over such a wide range of gCBF implies that normalization, on the basis of an 'additive model', should not limit the ability to study focal changes in the face of even substantial changes in total blood flow. If our results with respect to visual activation are representative of other cortical areas, then studies on areas in the brain activated by  $\text{CO}_2$  inhalation (e.g. any cerebral areas associated with respiratory control) should be possible.

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# Detection of Thirty-Second Cognitive Activations in Single Subjects with Positron Emission Tomography: A New Low-Dose $H_2^{15}O$ Regional Cerebral Blood Flow Three-Dimensional Imaging Technique

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**Summary:** Positron emission tomography regional CBF (rCBF) studies of cognitive processes have traditionally required 30–60 mCi of  $H_2^{15}O$  per scan and intersubject averaging to achieve statistical significance. However, intersubject anatomical, functional, and disease variability can make such an approach problematic. A new method that produces significant results in single subjects is presented. It is based upon high-sensitivity three-dimensional imaging and a “slow” bolus administration of <15 mCi of  $H_2^{15}O$  per scan. The method is validated in four normal volunteers using control and auditory-language activation tasks with four scans per condition and statistical parametric mapping analysis. It is demonstrated that the rCBF distribution associated with the

cognitive state is detected during the arrival of radiotracer in the brain. This occurs over 30 s and constitutes a critical temporal window during which stimulation should be performed. A 90-s acquisition time is found to produce results of greater significance than a 60-s acquisition time. The implications of the results and the functional neuroanatomical findings are discussed. This method is suitable for the study of individual functional neuroanatomy in many neuropsychological, pharmacologic, and symptom states in normal subjects and in patients with psychiatric and neurologic disorders. **Key Words:** Functional brain imaging— $^{15}O$ -labeled water—Positron emission tomography—Regional cerebral blood flow—Single-subject analysis—Slow bolus technique.

Functional brain imaging of regional CBF (rCBF) with positron emission tomography (PET) provides an in vivo index of localized synaptic activity associated with cognitive and behavioral states in humans (Raichle, 1987).  $^{15}O$ -labeled water, despite some limitation in diffusion (Eichling et al., 1974), is currently the preferred CBF tracer because of its short half-life (2.05 min), ease of production and use, and low toxicity (Lammertsma and Mazoyer,

1990). Qualitative subtraction studies utilizing 30–60 mCi of  $H_2^{15}O$  per scan and a two-dimensional scanning technique (with interplane septa) have provided valuable information concerning functional neuroanatomy (Posner et al., 1988; Zeki et al., 1991). However, limitations on acceptable doses of administered radioactivity and limitations of scanner sensitivity have necessitated the use of multiple subjects to obtain results of statistical significance. While intersubject averaging may increase the generalizability of results, it introduces additional problems associated with intersubject anatomical, functional, and disease variability (Friston and Frackowiak, 1991; Raichle et al., 1991; Watson et al., 1993). It is therefore desirable to be able to perform within-subject analyses of activation studies. The possibility of single-subject stud-

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**Abbreviations used:** BA, Brodmann's area; 3-D, three-dimensional; NEC, noise equivalent count rate; PET, positron emission tomography; rCBF, regional CBF; SMA, supplementary motor area; SPM, statistical parametric map.

ies also raises the issue of examining transient or randomly occurring brain or mental states that constitute signs or symptoms in individual patients. An ideal single-subject technique should therefore have a well-defined temporal window and permit signal averaging to allow the study of events not under direct experimental control.

Recent technical and methodologic developments in PET have made it possible to scan with inter-plane septa retracted, enabling three-dimensional (3-D) acquisitions of all possible lines of response within and across planes (Townsend et al., 1989). This results in greater detection of true events and permits scanning at lower administered activities where random coincidence rates and dead time are less problematic. A fully 3-D reconstruction algorithm takes maximal advantage of the increased signal, providing increased sensitivity without a significant decrement in resolution (Townsend et al., 1991).  $H_2^{15}O$  can therefore be given in lower doses, producing significant gains (of four- to fivefold) in noise equivalent count rates (NECs; a signal-to-noise measure) and allowing a greater number of scans to be performed on each subject (Bailey et al., 1991a). The combination of these factors results in increased statistical power.

The aim of this study was to determine whether the increased sensitivity of a 3-D technique would enable the detection of statistically significant changes in rCBF associated with a cognitive activation task in single subjects. To validate the technique and to characterize its temporal window, a single-subject activation paradigm was designed with several considerations in mind.  $H_2^{15}O$  was administered as a "slow" bolus to avoid some of the timing and statistical errors (Dhawan et al., 1986; Iida et al., 1986; Lammertsma et al., 1989) and increased dead time associated with the rapid "autoradiographic" bolus method (Herscovitch et al., 1983; Raichle et al., 1983) while minimizing the decay, CBF nonlinearity (Iida et al., 1991), and task habituation problems associated with the longer stimulation and scanning times of a ramp infusion method (Colebatch et al., 1991). The slow bolus resulted in the delivery of tracer to the brain over a period of 30 s. It was reasoned that this input phase would constitute a critical period during which the rCBF distribution would be detected. Therefore, in the activation condition, 30 s of auditory stimulation was timed to coincide with this 30-s interval. Stimulation before and after the rising interval was also performed to define the boundaries of the temporal window during which brain activity was being measured.  $H_2^{15}O$  was delivered serially in low doses to allow signal averaging within conditions

for a single subject. A range of doses, from 5 to 21 mCi per scan, was used to determine the lowest practical dose that provides sufficient NECs for the detection of a focal rCBF activation in a single subject and to determine the relationship between NECs and administered radioactivity with this technique. Double-frame data acquisition (60 s + 30 s) was used to allow for a comparison of different acquisition times. Acquisition time is known to have significant effects on counting statistics, linearity to flow, intravascular signal, and errors due to dispersion, time mismatch, tissue heterogeneity, and partition coefficient (Koeppel et al., 1987; Kanno et al., 1991). Sixty-second and ninety-second acquisition times were chosen based upon the trade-off between flow information and error sensitivity (Kanno et al., 1987) to determine the parameters of an optimal clinical functional brain-imaging paradigm.

## METHODS

### Subjects

The subjects were four normal male volunteers aged 26–30 years who gave informed consent. All were right-handed and had no history of psychiatric or neurologic illness.

### $H_2^{15}O$ administration

Five to 21 mCi of  $H_2^{15}O$  in 3 ml of normal saline was loaded into intravenous tubing over 20 s and flushed into the subject over 20 s at a rate of 10 ml/min by an automatic pump. After a delay of 35 s, a rise in counts was detected in the head, which peaked 30–40 s later (depending on individual circulation time). The interval between successive  $H_2^{15}O$  administrations was 10 min. The procedure for administering radioactivity was covered under an approval by the local ethical committee and the Administration of Radioactive Substances Advisory Committee (U.K.).

### NEC determination

The global NEC, a measure of signal-to-noise, was calculated for the background- and dead time-corrected image data of subjects 1, 2, and 4 using the following equation:

$$NEC = \frac{[T(1 - SF)]^2}{T + 2fR}$$

where  $T$  is unscattered + scattered true counts,  $R$  is random counts,  $SF$  is scatter fraction ( $=0.31$ , based upon phantom line sources, assuming an average head diameter of 18 cm) (Spinks et al., 1992), and  $f$  is fraction of field of view subtended by the head ( $=18 \text{ cm}/50 \text{ cm} = 0.36$ ) (Strother et al., 1990).

### Control and activation tasks

All four subjects were scanned in two conditions, and in addition, two of the subjects were scanned in a third condition. Four scans were obtained in each condition. Each session therefore consisted of 8 or 12 scans accord-

ingly. The order of the conditions was balanced to avoid systematic time and order effects. In all conditions the subjects were in a supine position with their heads in customized head molds. An intravenous line was placed in the right antecubital vein. Their eyes were closed, earphones were inserted, and a button-pressing device was held in their right hand. The button was linked to a pulse generator that produced a signal (logged every second by a Sun computer) that could be superimposed upon the simultaneously recorded total head counts. The room was dark with only low-level background noise. The experimental paradigm is illustrated in Fig. 1 and the conditions were as follows:

Condition 1: control (all four subjects). The subjects were instructed to relax and to press the button with their right thumb if, and for as long as, they heard sentences spoken through the earphones. No sentences were presented in this condition. This task represents auditory attention and preparatory set. Condition 2: auditory sentence presentation during the rising phase of the head curve (all four subjects). The instructions were the same as for condition 1, but this time prerecorded single words or short sentences (mean length 3.7 words, range 1–12 words) of random content were presented binaurally with normal prosody at an average rate of 2.1 words/s. The auditory stimulation was 30 s in duration, coinciding with the rising phase of the head curve. This task represents auditory sentence monitoring and sustained button pressing. Condition 3a: auditory sentence presentation before the rising phase of the head curve (one subject). The instructions were the same as for condition 1 and the stimuli were the same as for condition 2. However, in this con-

dition the 30 s of auditory stimulation preceded, and finished 2 s before, the rising phase of the head curve. Condition 3b: auditory sentence presentation after the rising phase of the head curve (one subject). Again, the instructions were the same as for condition 1 and the stimuli were the same as for condition 2. However, in this condition, the 30 s of auditory stimulation immediately followed the peak of the head curve.

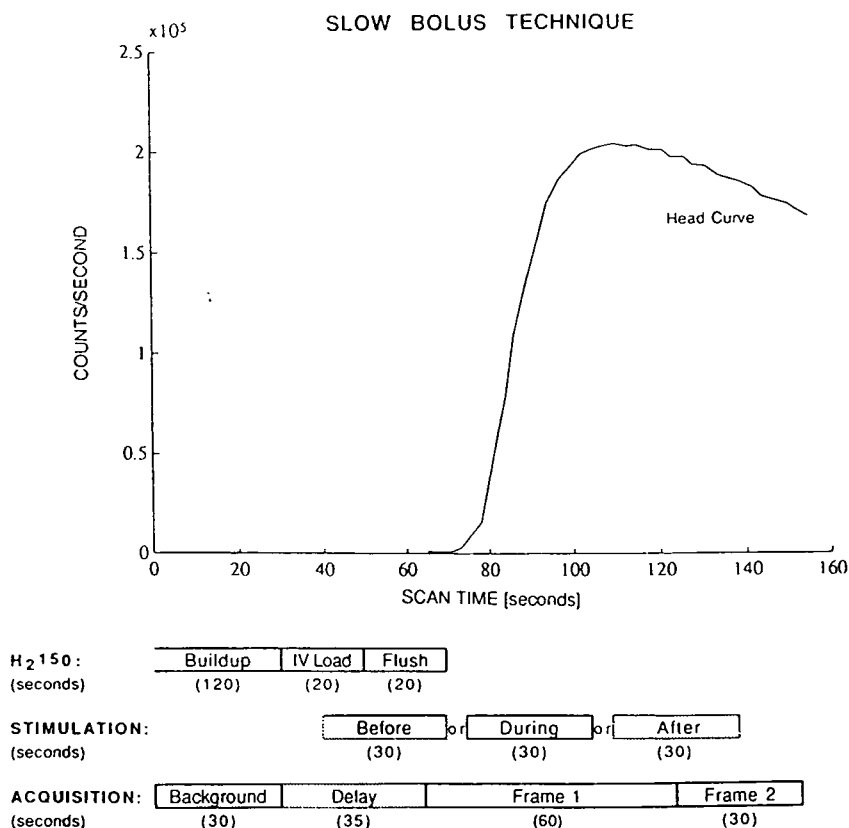
### Data acquisition and image reconstruction

Scans were obtained with a PET scanner (953B; Siemens-CTI, Knoxville, TN, U.S.A.) with the interplane septa retracted (Spinks et al., 1992). This scanner utilizes block technology detectors and contains 16 individual rings, each 6.5 mm thick axially, which results in a total of 31 measured planes (direct and cross). In 3-D mode, the scanner is able to acquire 256 oblique sinograms, one between any pair of opposing detectors. This is unlike the normal two-dimensional mode with septa, which limits sinograms to small (typically fewer than three) ring differences and results in projections orthogonal to the axial plane of the scanner (Bailey et al., 1991a).

A 20-min transmission scan using rotating rods of  $^{68}\text{Ge}/^{68}\text{Ga}$  was performed for attenuation correction. A 30-s frame for background activity correction was obtained before each administration of  $\text{H}_2^{15}\text{O}$ . After a 35-s delay, data were acquired in two consecutive frames of 60 and 30 s, beginning 0–5 s before the rising phase of the head curve. Data from the two frames were summed to generate a 90-s image that could then be compared with the single-frame data of the 60-s image.

The images were reconstructed into 31 slices with a 3-D

**FIG. 1.** The slow bolus technique. The timing of  $\text{H}_2^{15}\text{O}$  administration, cognitive stimulation, and data acquisition are shown with respect to each other and with respect to the head curve (counts per second detected over time). On the x-axis, time = 0 corresponds to the initiation of the scanning (acquisition) protocol, which includes a background frame, delay interval, and two data collection frames. The  $\text{H}_2^{15}\text{O}$  administration protocol begins 90 s earlier with the buildup of radiotracer that is subsequently loaded into the intravenous line and flushed into the patient. In this study, auditory stimulation was performed before, during, or after the rising phase of the head curve.



algorithm (Townsend et al., 1991) in which back-projection was performed at nonorthogonal angles to the axis of the scanner. A Hanning filter with a cutoff frequency of 0.5 cycle/s was used at this stage, giving a transaxial resolution of 8.5 mm full width at half-maximum. The reconstructed images contained  $128 \times 128$  pixels, each measuring  $2.006 \times 2.006$  mm.

### Image analysis

Image analysis was performed on a SPARC 2 Workstation (Sun Microsystems, Europe, Surrey) using statistical parametric mapping (SPM; SPM Software, MRC Cyclotron Unit, London, U.K.) and an interactive image display software package (Analyze; Biodynamic Research Unit, Mayo Clinic). Calculations and image matrix manipulation were performed in PRO MATLAB (Mathworks, New York).

The 31 original contiguous 3.5-mm scan slices were interpolated to 43 planes to render the voxels approximately cubic. All images were aligned on a voxel-by-voxel basis using a 3-D automated algorithm (AIR Software) (Woods et al., 1992). The intercommissural (anterior commissural-posterior commissural) line was identified according to a previously described method (Friston et al., 1989) and the volume transformed into the standard stereotaxic space utilized in the atlas of Talairach and Tournoux (1988). The stereotactically normalized images contained 26 planes of  $2 \times 2 \times 4$ -mm voxels corresponding to the horizontal sections in the atlas. Each image was smoothed with a Gaussian filter 10 mm wide to increase the signal-to-noise ratio and to accommodate normal variability in functional and gyral anatomy for group analysis.

Differences in global activity were removed following an analysis of covariance (Friston et al., 1990) with global counts as covariate and control or activation condition as treatment. This analysis was performed for every pixel and generated a mean pixel value with associated error variance for each condition. Differences in the adjusted condition means were assessed for single subjects and for the group as a whole using the *t* statistic. For each comparison between control and activation conditions, foci of

significant increases and decreases of activity were identified and images of the pixel *t* values were created (Friston et al., 1991). The omnibus significance of these SPMs was assessed by comparing the expected and observed number of pixels above a significance threshold of  $p < 0.01$  or  $p < 0.001$  using the  $\chi^2$ -statistic. The SPM  $\{t\}$  values were also transformed to the unit Gaussian distribution using a probability integral transform so that changes could be reported as Z scores. The SPMs were displayed as volume images of the highest *t* values in three orthogonal projections. To indicate the change in cerebral activity between conditions, the rCBF equivalents were calculated for brain locations of special interest identified as significance maxima on the SPMs and then normalized to 50 ml/dl/min (Mintun et al., 1989). The adjusted rCBF represents a Gaussian-weighted condition mean rCBF over a 12-mm full width at half-maximum sphere centered on a local statistical maximum.

## RESULTS

### $H_2^{15}O$ doses and NECs

Peak head counts ranged from 114,000 to 250,000 counts/s, with 60–81% true events and a 7–14% dead time. Highly significant results ( $p < 0.001$ , omnibus) were obtained in all subjects, including subject 4, who received 5–10 mCi of  $H_2^{15}O$  per scan. Global NEC values rose with increasing dose. NECs per millicurie were plotted against administered dose of radioactivity in the three subjects (1, 2, and 4) for whom data were available (Fig. 2). A linear relationship with a negative slope was observed. Lower doses provided more NECs per millicurie, with a two- to threefold gain at 5 mCi compared with 20 mCi. Over the range of doses, the 90-s acquisition time provided an average gain of 1.6-fold in NECs per millicurie compared with the 60-s acquisition time.

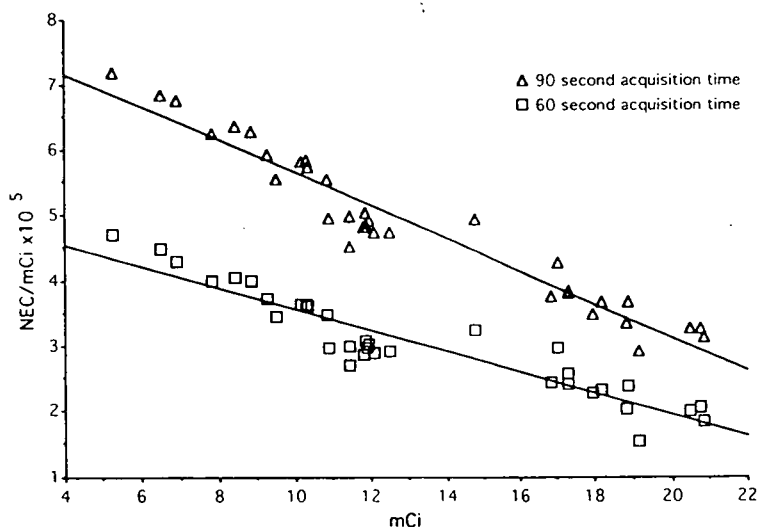


FIG. 2. Noise equivalent count rates (NECs) per millicurie for the range of administered  $H_2^{15}O$  doses in subjects 1, 2, and 4. Lower doses and the 90-s acquisition time produce greater gains in signal-to-noise ratio per millicurie. Linear regression performed on the 90-s acquisition time data reveals the relationship  $y = -0.25x + 8.17$ ;  $R = 0.97$ ,  $p = 0.0001$ . Linear regression performed on the 60-s acquisition time data reveals the relationship  $y = -0.16x + 5.19$ ;  $R = 0.93$ ,  $p = 0.0001$ .

### Significance levels

When the control and activation conditions were contrasted, profiles of significant relative increases and decreases in cerebral activity were obtained. The main comparison was between condition 2 (stimulation during the rising phase of the head curve) and condition 1 (control). In single subjects with four scans in each condition, the profiles of increases (at a threshold of  $p < 0.001$ ) and decreases (at a threshold of  $p < 0.01$ ) demonstrated brain regions comparable with those obtained when intersubject averaging (at a threshold of  $p < 0.001$ ) was performed (Fig. 3). The Z scores for the increases were generally of larger magnitude than those for the decreases (see Tables 2-5). Changes in adjusted rCBF values as small as 5% were detected at a level of  $p < 0.001$ .

### Timing of stimulation

Stimulation before and after the rising phase of the head curve (conditions 3a and 3b) did not result in significant profiles of increased or decreased activity when contrasted with control condition 1 (Table 1).

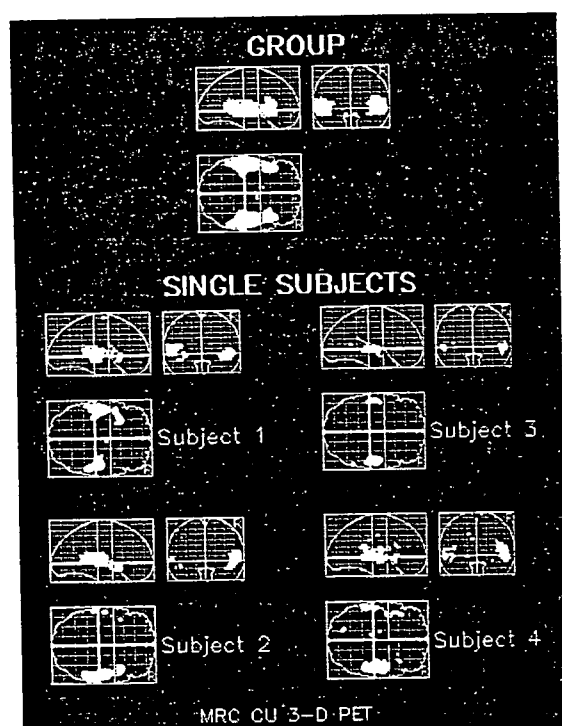


FIG. 3. SPM (t) profiles ( $p < 0.001$ , omnibus) of increased activity for condition 2 (auditory sentence monitoring during the rising phase of the head curve) versus control condition 1 (auditory attention/preparatory set) with a 90-s acquisition time. Sagittal, coronal, and transverse volumetric projections are shown. R, right. The single-subject profiles are comparable with the profile obtained with group analysis.

### Acquisition time

The 90-s acquisition time produced whole-brain significance profiles with higher  $\chi^2$ -values and (with one exception) more significant  $p$  values than did the 60-s acquisition time (Table 1). The exact location of local maxima within a given functional area varied with the acquisition time in a nonsystematic fashion. The 90-s acquisition time provided higher NEC values and generally produced higher Z scores. It also resulted in less variability among the subjects (and between individual subjects and the group) with regard to the percent change in adjusted rCBF for a given functional region (Tables 2-5).

### Group analysis with 90-s acquisition time

With intersubject averaging, the main rCBF increases in activation condition 2 relative to control condition 1 were detected in the superior and middle temporal gyri bilaterally [Brodmann's areas (BAs) 21, 22, 38, 41, 42] (Table 3). These areas constitute auditory primary and association cortices, including Wernicke's area. The overall maximum ( $Z = 10.47$ ) was located in area 22 on the right (Talairach coordinates  $x = 48$ ,  $y = -20$ ,  $z = 0$ ). Increases were also seen in Broca's area and its right-sided homologue (BA 44,45). Other areas with smaller or less significant foci of activation included right supramarginal gyrus area 40, right insula, right supplementary motor area (SMA) 6, right prefrontal area 10, bilateral inferior frontal area 47, and right hypothalamus.

With intersubject averaging, rCBF decreases in activation condition 2 relative to control condition 1 were detected bilaterally in visual primary and association cortices (BA 17, 18, 19), parietal and multimodal association cortices (BA 5, 7, 37, 39, 40), prefrontal association cortices (BA 9,10), anterior (BA 24, 32) and posterior (BA 23, 29, 31) cingulate gyrus, premotor areas (BA 6, 8), and cerebellum (Table 5).

### Single-subject analysis with 90-s acquisition time

In subject 1, rCBF increases in activation condition 2 relative to control condition 1 were detected bilaterally in auditory primary and association cortices, insula, and SMA. Increases in Broca's area and left inferior frontal area 47 were also observed. The overall maximum ( $Z = 6.86$ ) was located in area 22 on the right ( $x = 44$ ,  $y = -20$ ,  $z = 0$ ) (Table 3). rCBF decreases were detected bilaterally in visual primary and association cortices, parietal and multimodal association cortices, prefrontal association cortices, anterior and posterior cingulate gyrus, premotor areas, and cerebellum (Table 5). Very small isolated areas of less significant decrease were detected in the left thalamus, parahippocam-

TABLE 1. Whole-brain significance profiles (activation versus control): stimulation before, during, and after rising phase of head curve

| Subject no.        | Acquisition time (s) | SPM significance threshold | SPM increases |          | SPM decreases |          |
|--------------------|----------------------|----------------------------|---------------|----------|---------------|----------|
|                    |                      |                            | $\chi^2$      | p(HO)    | $\chi^2$      | p(HO)    |
| Stimulation during |                      |                            |               |          |               |          |
| 1                  | 60                   | p < 0.01                   | 621           | <0.00001 | 58            | <0.00005 |
|                    |                      | p < 0.001                  | 491           | <0.00001 | 3.4           | 0.00396  |
|                    | 90                   | p < 0.01                   | 1,205         | <0.00001 | 946           | <0.00001 |
|                    |                      | p < 0.001                  | 935           | <0.00001 | 81            | <0.00001 |
| 2                  | 60                   | p < 0.01                   | 1,207         | <0.00001 | 389           | <0.00001 |
|                    |                      | p < 0.001                  | 892           | <0.00001 | 13            | <0.00001 |
|                    | 90                   | p < 0.01                   | 1,155         | <0.00001 | 763           | <0.00001 |
|                    |                      | p < 0.001                  | 917           | <0.00001 | 6.1           | <0.00001 |
| 3                  | 60                   | p < 0.01                   | 57            | 0.00006  | 247           | <0.00001 |
|                    |                      | p < 0.001                  | 66            | <0.00001 | 8.9           | <0.00001 |
|                    | 90                   | p < 0.01                   | 216           | <0.00001 | 701           | <0.00001 |
|                    |                      | p < 0.001                  | 147           | <0.00001 | 50            | <0.00001 |
| 4                  | 60                   | p < 0.01                   | 1,481         | <0.00001 | 0             | 1        |
|                    |                      | p < 0.001                  | 540           | <0.00001 | 0             | 1        |
|                    | 90                   | p < 0.01                   | 2,143         | <0.00001 | 367           | <0.00001 |
|                    |                      | p < 0.001                  | 983           | 0        | 5.6           | 0.00030  |
| Group              | 60                   | p < 0.001                  | 3,545         | 0        | 4,189         | 0        |
|                    | 90                   | p < 0.001                  | 4,234         | 0        | 8,619         | 0        |
| Stimulation before |                      |                            |               |          |               |          |
| 3                  | 60                   | p < 0.01                   | 0             | 1        | 0             | 1        |
|                    |                      | p < 0.001                  | 0             | 1        | 0             | 1        |
|                    | 90                   | p < 0.01                   | 0             | 1        | 0             | 1        |
|                    |                      | p < 0.001                  | 0             | 1        | 0             | 1        |
| Stimulation after  |                      |                            |               |          |               |          |
| 4                  | 60                   | p < 0.01                   | 0             | 1        | 0             | 1        |
|                    |                      | p < 0.001                  | 0             | 1        | 0             | 1        |
|                    | 90                   | p < 0.01                   | 0             | 1        | 0             | 1        |
|                    |                      | p < 0.001                  | 0             | 1        | 0             | 1        |

The  $\chi^2$ -statistic is a measure of the significance of the observed number of pixels (versus the expected number of pixels under the null hypothesis) that exceeded the threshold indicated. The p(HO) values indicate the probability that the observed profile may have occurred by chance. Stimulation during the rising phase of the head curve resulted in highly significant profiles, while stimulation before or after the rising phase did not.

pal gyrus, and motor cortex and in association cortices bilaterally.

In subject 2, rCBF increases were detected bilaterally in auditory primary and association cortices and insula. Increases in the right-sided homologue of Broca's area and left inferior frontal area 47 were also observed. The overall maximum ( $Z = 8.26$ ) was located in area 22 on the right ( $x = 52$ ,  $y = -18$ ,  $z = 8$ ) (Table 3). rCBF decreases were detected bilaterally in visual primary and association cortices, parietal and multimodal association cortices, prefrontal association cortices, anterior and posterior cingulate gyrus, premotor areas, and cerebellum (Table 5). Very small isolated areas of less significant decrease were detected in the right thalamus and in sensorimotor cortices bilaterally.

In subject 3, rCBF increases were detected bilaterally in auditory primary and association cortices. Increases in the left insula were also observed. The overall maximum ( $Z = 5.49$ ) was located in area 21/22 on the right ( $x = 52$ ,  $y = -12$ ,  $z = -4$ ) (Table 3). rCBF decreases were detected bilaterally in visual primary and association cortices, prefrontal

tal association cortices, anterior and posterior cingulate gyrus, premotor areas, and cerebellum (Table 5). Very small isolated areas of less significant decrease were detected in the right caudate and in sensorimotor and association cortices bilaterally.

In subject 4, rCBF increases were detected bilaterally in auditory primary and association cortices, insula, supramarginal gyrus, and SMA. Increases in Broca's area and its right-sided homologue and in left inferior frontal area 47 were also observed. Very small isolated areas of activation were detected in the anterior and posterior cingulate gyrus and in area 18. The overall maximum ( $Z = 7.15$ ) was located in area 22 on the right ( $x = 50$ ,  $y = -10$ ,  $z = -4$ ) (Table 3). rCBF decreases were detected bilaterally in visual primary and association cortices, prefrontal association cortices, posterior cingulate gyrus, and premotor areas. Decreases in the left anterior cingulate gyrus and right cerebellum were also observed (Table 5). Very small isolated areas of less significant decrease were detected in the right parahippocampal gyrus and in association cortices bilaterally.

TABLE 2. Increases in brain activity (activation versus control), 60-s acquisition time: single-subject and group analyses ( $p < 0.001$ )

| Regions of activation |   | Maxima and BAs | Subject 1                                  | Subject 2                           | Subject 3                        | Subject 4                            | Group                            |
|-----------------------|---|----------------|--------------------------------------------|-------------------------------------|----------------------------------|--------------------------------------|----------------------------------|
| Temporoparietal       | R | Z score        | 5.25                                       | 8.51                                | 5.45                             | 6.64                                 | 8.48                             |
|                       |   | (x, y, z)      | (46, -24, 0)                               | (52, -22, 8)                        | (48, -6, 0)                      | (50, -8, 0)                          | (44, -14, 4)                     |
|                       |   | % rCBF change  | 13.5                                       | 17.6                                | 11.3                             | 16.7                                 | 10.1                             |
|                       |   | BAs            | 21, 22 <sup>a</sup> , 42, insula           | 21, 22, 38, 40, 41, 42 <sup>a</sup> | 21, 22 <sup>a</sup> , 38, 41, 42 | 21, 22 <sup>a</sup> , 40, 42, insula | 21, 22 <sup>a</sup> , 38, 41, 42 |
|                       | L | Z score        | 5.71                                       | 3.84                                | 3.80                             | 4.75                                 | 7.00                             |
|                       |   | (x, y, z)      | (-52, -26, 0)                              | (-48, 0, -12)                       | (-52, -8, -8)                    | (-50, -38, 0)                        | (-52, -16, 4)                    |
|                       |   | % rCBF change  | 11.8                                       | 13.3                                | 11.3                             | 10.5                                 | 12.2                             |
|                       |   | BAs            | 21 <sup>a</sup> , 22 <sup>a</sup> , 40, 42 | 21 <sup>a</sup> , 22, 38, 42        | 21 <sup>a</sup> , 22             | 21 <sup>a</sup> , 22, 38, insula     | 21, 22 <sup>a</sup> , 38, 42     |
| Inferior frontal      | R | Z score        | c                                          | 4.31                                | c                                | 3.32                                 | 4.07                             |
|                       |   | (x, y, z)      |                                            | (38, 24, 4)                         |                                  | (34, 16, 16)                         | (42, 26, 8)                      |
|                       |   | % rCBF change  |                                            | 7.3                                 |                                  | 7.3                                  | 5.8                              |
|                       |   | BAs            |                                            | 45 <sup>a</sup>                     |                                  | 44 <sup>a</sup> , 45 <sup>a</sup>    | 6, 45 <sup>a</sup> , 47          |
|                       | L | Z score        | 3.85                                       | 5.12                                | b                                | b                                    | 5.38                             |
|                       |   | (x, y, z)      | (-46, -6, 12)                              | (-44, 28, -4)                       |                                  |                                      | (-44, 16, -8)                    |
|                       |   | % rCBF change  | 9.8                                        | 16.2                                |                                  |                                      | 8.7                              |
|                       |   | BAs            | 6 <sup>a</sup>                             | 47 <sup>a</sup>                     |                                  |                                      | 6, 45, 47 <sup>a</sup>           |

Values are the Z score, Talairach atlas coordinates, and percent change in adjusted regional CBF (rCBF) for regional maxima. The coordinates are in mm; x is the lateral distance from the midline (positive = right), y is the anterior-posterior distance from the anterior commissure, and z is the height above the intercommissural line. R, right; L, left.

<sup>a</sup> Brodmann's area (BA) in which the local maximum Z score occurred.

<sup>b</sup> Region present at an SPM{t} threshold of  $p < 0.01$ .

<sup>c</sup> Region not present at an SPM{t} threshold of  $p < 0.01$ .

## DISCUSSION

### The single-subject paradigm

Single-subject analysis avoids the confounding effects of anatomical, functional, and disease variability in normal subjects and in patients with psychiatric or neurologic disease. This permits a more

rigorous correlation of specific brain states with particular cognitive, perceptual, affective, and behavioral states. In addition, the effect of individual task performance, strategy and laterality can be more easily assessed. Within-subject PET-magnetic resonance coregistration, which provides a direct mapping of function onto structure, can

TABLE 3. Increases in brain activity (activation versus control), 90-s acquisition time: single-subject and group analyses ( $p < 0.001$ )

| Regions of activation |   | Maxima and BAs | Subject 1                            | Subject 2                         | Subject 3                         | Subject 4                                | Group                                          |
|-----------------------|---|----------------|--------------------------------------|-----------------------------------|-----------------------------------|------------------------------------------|------------------------------------------------|
| Temporoparietal       | R | Z score        | 6.86                                 | 8.26                              | 5.49                              | 7.15                                     | 10.47                                          |
|                       |   | (x, y, z)      | (44, -20, 0)                         | (52, -18, 8)                      | (52, -12, -4)                     | (50, -10, -4)                            | (48, -20, 0)                                   |
|                       |   | % rCBF change  | 11.4                                 | 13.8                              | 13.8                              | 11.6                                     | 10.7                                           |
|                       |   | BAs            | 21, 22 <sup>a</sup> , insula         | 21, 22 <sup>a</sup> , 38, 41, 42  | 21 <sup>a</sup> , 22 <sup>a</sup> | 21, 22 <sup>a</sup> , 40, 42, insula     | 21, 22 <sup>a</sup> , 38, 40, 41, 42           |
|                       | L | Z score        | 6.80                                 | 3.65                              | 3.61                              | 4.50                                     | 8.67                                           |
|                       |   | (x, y, z)      | (-54, -22, 4)                        | (-58, -28, 4)                     | (-50, -10, 0)                     | (-56, -42, 0)                            | (-52, -22, 0)                                  |
|                       |   | % rCBF change  | 10.2                                 | 7.9                               | 12.7                              | 7.3                                      | 9.4                                            |
|                       |   | BAs            | 21, 22 <sup>a</sup> , 41, 42, insula | 21, 22 <sup>a</sup> , 38          | 22 <sup>a</sup>                   | 21 <sup>a</sup> , 22, 38, 40, 42, insula | 21 <sup>a</sup> , 22 <sup>a</sup> , 38, 41, 42 |
| Inferior frontal      | R | Z score        | <sup>b</sup>                         | 3.13                              | <sup>b</sup>                      | 3.23                                     | 4.91                                           |
|                       |   | (x, y, z)      |                                      | (40, 20, 8)                       |                                   | (36, 20, 16)                             | (44, 24, 12)                                   |
|                       |   | % rCBF change  |                                      | 8.4                               |                                   | 5.1                                      | 5.9                                            |
|                       |   | BAs            |                                      | 44 <sup>a</sup> , 45 <sup>a</sup> |                                   | 45 <sup>a</sup>                          | 44, 45 <sup>a</sup> , 47                       |
|                       | L | Z score        | 4.80                                 | 3.44                              | <sup>b</sup>                      | 3.47                                     | 6.55                                           |
|                       |   | (x, y, z)      | (-48, 10, 8)                         | (-42, 12, -8)                     |                                   | (-42, 24, 0)                             | (-46, 16, -8)                                  |
|                       |   | % rCBF change  | 5.2                                  | 9.7                               |                                   | 6.9                                      | 9.0                                            |
|                       |   | BAs            | 44 <sup>a</sup> , 47                 | 47 <sup>a</sup>                   |                                   | 6, 45, 47 <sup>a</sup>                   | 44, 45, 47 <sup>a</sup>                        |

Values are the Z score, Talairach atlas coordinates, and percent change in adjusted regional CBF (rCBF) for regional maxima. The coordinates are in mm; x is the lateral distance from the midline (positive = right), y is the anterior-posterior distance from the anterior commissure, and z is the height above the intercommissural line. R, right; L, left.

<sup>a</sup> Brodmann's area (BA) in which the local maximum Z score occurred.

<sup>b</sup> Region present at an SPM{t} threshold of  $p < 0.01$ .



TABLE 4. Decreases in brain activity (activation versus control), 60-s acquisition time: single-subject and group analyses

| Regions of activation |   | Maxima and BAs | Subject 1<br>( $p < 0.01$ ) | Subject 2<br>( $p < 0.01$ )      | Subject 3<br>( $p < 0.01$ )  | Subject 4<br>( $p < 0.01$ ) | Group<br>( $p < 0.001$ )     |
|-----------------------|---|----------------|-----------------------------|----------------------------------|------------------------------|-----------------------------|------------------------------|
| Visual                | R | Z score        | 2.94                        | 3.00                             | 3.78                         | 3.09                        | 5.44                         |
|                       |   | (x, y, z)      | (24, -58, -8)               | (28, -74, 20)                    | (26, -74, 20)                | (24, -88, 12)               | (24, -84, 12)                |
|                       |   | % rCBF change  | 7.7                         | 11.4                             | 6.7                          | 7.0                         | 6.4                          |
|                       | L | BAs            | 19 <sup>a</sup> , 37        | 18, 19 <sup>a</sup>              | 17, 18 <sup>a</sup> , 19, 37 | 17, 18 <sup>a</sup> , 19    | 18 <sup>a</sup> , 19, 37     |
|                       |   | Z score        | 3.75                        | 3.63                             | 2.99                         | 3.38                        | 4.62                         |
|                       |   | (x, y, z)      | (-40, -46, -16)             | (-20, -70, 24)                   | (-18, -54, -4)               | (-2, -72, 20)               | (-28, -86, 8)                |
| Parietal/association  | R | % rCBF change  | 13.2                        | 10.1                             | 7.0                          | 6.8                         | 6.0                          |
|                       |   | BAs            | 17, 18, 19, 37 <sup>a</sup> | 18 <sup>a</sup> , 19             | 18, 19 <sup>a</sup>          | 17, 18 <sup>a</sup> , 19    | 17, 18, 19 <sup>a</sup> , 37 |
|                       |   | Z score        | 3.12                        | 3.03                             | 2.96                         | 2.51                        | 4.82                         |
|                       | L | (x, y, z)      | (34, -66, 24)               | (32, -68, 24)                    | (10, -58, 40)                | (30, -70, 24)               | (14, -58, 40)                |
|                       |   | % rCBF change  | 10.5                        | 9.5                              | 11.1                         | 6.1                         | 6.2                          |
|                       |   | BAs            | 7, 39 <sup>a</sup> , 40     | 5, 7, 39 <sup>a</sup>            | 7 <sup>a</sup> , 39, 40      | 39 <sup>a</sup>             | 7 <sup>a</sup> , 39, 40      |
| Prefrontal            | R | Z score        | 3.77                        | 2.99                             | 3.67                         | 2.84                        | 4.49                         |
|                       |   | (x, y, z)      | (-4, -52, 40)               | (-4, -38, 44)                    | (-4, -28, 48)                | (-26, -46, 24)              | (-14, -58, 44)               |
|                       |   | % rCBF change  | 8.8                         | 6.7                              | 9.0                          | 10.6                        | 7.4                          |
|                       | L | BAs            | 7 <sup>a</sup> , 40         | 5, 7 <sup>a</sup> , 40           | 5 <sup>a</sup> , 7, 39, 40   | 7, 39 <sup>a</sup>          | 5, 7 <sup>a</sup> , 40       |
|                       |   | Z score        | 3.45                        | 2.64                             | 3.08                         | <sup>b</sup>                | 3.44                         |
|                       |   | (x, y, z)      | (24, 40, -8)                | (14, 46, 12)                     | (34, 40, -4)                 |                             | (22, 54, 12)                 |
| Anterior cingulate    | R | % rCBF change  | 15.0                        | 6.8                              | 7.5                          |                             | 4.8                          |
|                       |   | BAs            | 10 <sup>a</sup>             | 9, 10 <sup>a</sup>               | 9, 10 <sup>a</sup>           |                             | 10 <sup>a</sup>              |
|                       |   | Z score        | 3.16                        | 3.30                             | 2.62                         | 3.28                        | 5.49                         |
|                       | L | (x, y, z)      | (-28, 8, 40)                | (-32, 44, 24)                    | (-32, 48, -8)                | (-24, 50, 20)               | (-32, 54, 16)                |
|                       |   | % rCBF change  | 8.0                         | 5.4                              | 9.3                          | 8.6                         | 7.6                          |
|                       |   | BAs            | 9 <sup>a</sup> , 10         | 9 <sup>a</sup> , 10 <sup>a</sup> | 10 <sup>a</sup>              | 9, 10 <sup>a</sup>          | 10 <sup>a</sup>              |
| Posterior cingulate   | R | Z score        | 2.54                        | 3.00                             | 2.54                         |                             |                              |
|                       |   | (x, y, z)      | (10, 24, -8)                | (20, 38, 8)                      | (6, 30, 24)                  |                             |                              |
|                       |   | % rCBF change  | 15.3                        | 9.3                              | 8.4                          |                             |                              |
|                       | L | BAs            | 24, 32 <sup>a</sup>         | 24 <sup>a</sup>                  | 32 <sup>a</sup>              |                             |                              |
|                       |   | Z score        | 2.87                        | 3.06                             | 2.47                         | 2.54                        |                              |
|                       |   | (x, y, z)      | (-24, 40, 8)                | (-4, 14, 36)                     | (-20, 34, 0)                 | (-2, 42, 4)                 |                              |
| Premotor              | R | % rCBF change  | 9.6                         | 6.9                              | 7.4                          | 9.8                         |                              |
|                       |   | BAs            | 32 <sup>a</sup>             | 24, 32 <sup>a</sup>              | 24 <sup>a</sup>              | 32 <sup>a</sup>             |                              |
|                       |   | Z score        | 2.72                        | 2.68                             | 2.62                         | 2.80                        | 4.50                         |
|                       | L | (x, y, z)      | (10, -62, 8)                | (26, -68, 12)                    | (0, -36, 40)                 | (4, -64, 12)                | (2, -36, 36)                 |
|                       |   | % rCBF change  | 7.3                         | 9.6                              | 6.3                          | 8.3                         | 5.0                          |
|                       |   | BAs            | 31 <sup>a</sup>             | 31 <sup>a</sup>                  | 23, 31 <sup>a</sup>          | 31 <sup>a</sup>             | 31 <sup>a</sup>              |
| Cerebellum            | R | Z score        | 2.92                        | 3.18                             | 2.62                         | 2.63                        | 4.58                         |
|                       |   | (x, y, z)      | (-4, -52, 36)               | (-10, -30, 36)                   | (0, -36, 40)                 | (-16, -68, 12)              | (-14, -60, 12)               |
|                       |   | % rCBF change  | 7.8                         | 7.3                              | 6.3                          | 8.3                         | 5.9                          |
|                       | L | BAs            | 31 <sup>a</sup>             | 23, 31 <sup>a</sup>              | 31 <sup>a</sup>              | 31 <sup>a</sup>             | 29, 31 <sup>a</sup>          |
|                       |   | Z score        | <sup>b</sup>                | 3.75                             | 2.92                         | 2.38                        | 4.29                         |
|                       |   | (x, y, z)      |                             | (2, 12, 44)                      | (18, -18, 48)                | (18, 0, 56)                 | (24, 12, 56)                 |
|                       | R | % rCBF change  |                             | 6.6                              | 11.1                         | 6.5                         | 4.6                          |
|                       |   | BAs            |                             | 6 <sup>a</sup>                   | 6 <sup>a</sup>               | 6 <sup>a</sup>              | 6 <sup>a</sup>               |
|                       |   | Z score        | 2.68                        | 2.83                             | 4.03                         | 2.79                        | 4.20                         |
|                       | L | (x, y, z)      | (-14, 14, 60)               | (-18, -12, 56)                   | (-26, 30, 44)                | (-32, 20, 40)               | (-22, 0, 52)                 |
|                       |   | % rCBF change  | 5.1                         | 9.2                              | 9.4                          | 4.7                         | 6.2                          |
|                       |   | BAs            | 6 <sup>a</sup> , 8          | 6 <sup>a</sup> , 8               | 6, 8 <sup>a</sup>            | 6, 8 <sup>a</sup>           | 6 <sup>a</sup> , 8           |
|                       | R | Z score        | 2.79                        | 2.57                             | <sup>b</sup>                 | 2.33                        | 3.93                         |
|                       |   | (x, y, z)      | (6, -76, -12)               | (6, -72, -24)                    |                              | (34, -56, -16)              | (36, -60, -16)               |
|                       |   | % rCBF change  | 7.6                         | 11.0                             |                              | 5.3                         | 4.8                          |
|                       | L | Z score        | 2.64                        | 2.62                             | 2.92                         | <sup>b</sup>                | 3.45                         |
|                       |   | (x, y, z)      | (-42, -46, -24)             | (-34, -32, -24)                  | (-26, -46, -16)              |                             | (-42, -44, -20)              |
|                       |   | % rCBF change  | 12.2                        | 10.2                             | 8.0                          |                             | 5.2                          |

Values are the Z score, Talairach atlas coordinates, and percent change in adjusted regional CBF (rCBF) for regional maxima. The coordinates are in mm. x is the lateral distance from the midline (positive = right), y is the anterior-posterior distance from the anterior commissure, and z is the height above the intercommissural line. R, right; L, left.

<sup>a</sup> Brodmann's area (BA) in which the local maximum Z score occurred.

<sup>b</sup> Region not present at an SPM(t) threshold of  $p < 0.01$ .

also be performed. Case studies of the same patient in different symptom and treatment states are possible as well. Group analysis is not precluded. Indeed, a combined within-subject and group analysis is easily performed and can help to clarify the relationship between subject-specific and relatively invariant modes of processing.

The single-subject paradigm described in this article relies fundamentally on 3-D acquisition and re-

construction techniques that provide more statistical information for a given administered dose of radioactivity imaged for a given period of time. Using a scanner with retractable septa (now commercially available), it is therefore possible to use one-half to one-quarter of the conventional dose per scan and perform more scans without increasing the risk to the subject. In fact, with this technique, the lowest doses were found to result in the greatest gain in

TABLE 5. Decreases in brain activity (activation versus control), 90-s acquisition time: single-subject and group analyses

| Regions of activation |   | Maxima and BAs | Subject 1<br>( $p < 0.01$ )          | Subject 2<br>( $p < 0.01$ ) | Subject 3<br>( $p < 0.01$ ) | Subject 4<br>( $p < 0.01$ )           | Group<br>( $p < 0.001$ )     |
|-----------------------|---|----------------|--------------------------------------|-----------------------------|-----------------------------|---------------------------------------|------------------------------|
| Visual                | R | Z score        | 3.85                                 | 3.48                        | 3.67                        | 3.44                                  | 5.45                         |
|                       |   | (x, y, z)      | (22, -80, -4)                        | (10, -76, 12)               | (30, -72, 12)               | (2, -74, 20)                          | (36, -78, 20)                |
|                       |   | % rCBF change  | 8.0                                  | 8.9                         | 6.3                         | 4.9                                   | 6.0                          |
|                       | L | BAs            | 18 <sup>a</sup> , 19, 37             | 17 <sup>a</sup> , 18, 19    | 18, 19 <sup>a</sup> , 37    | 17, 18 <sup>a</sup> , 19              | 17, 18, 19 <sup>a</sup> , 37 |
|                       |   | Z score        | 3.46                                 | 3.60                        | 2.87                        | 3.48                                  | 5.57                         |
|                       |   | (x, y, z)      | (-38, -62, -12)                      | (-24, -72, 20)              | (-34, -78, 16)              | (-40, -72, 4)                         | (-36, -72, 0)                |
| Parietal/association  | R | % rCBF change  | 9.0                                  | 6.4                         | 4.9                         | 8.1                                   | 5.8                          |
|                       |   | BAs            | 18, 19, 37 <sup>a</sup>              | 17, 18 <sup>a</sup> , 19    | 19 <sup>a</sup>             | 18, 19 <sup>a</sup> , 37 <sup>a</sup> | 18, 19 <sup>a</sup> , 37     |
|                       |   | Z score        | 3.55                                 | 3.04                        | 3.60                        | 2.85                                  | 6.07                         |
|                       | L | (x, y, z)      | (30, -54, 28)                        | (0, -16, 44)                | (12, -64, 36)               | (16, -70, 40)                         | (10, -60, 40)                |
|                       |   | % rCBF change  | 6.6                                  | 6.1                         | 8.9                         | 7.5                                   | 6.0                          |
|                       |   | BAs            | 5, 7, 39 <sup>a</sup> , 40           | 5, 7 <sup>a</sup> , 39, 40  | 5, 7 <sup>a</sup> , 40      | 7 <sup>a</sup>                        | 5, 7 <sup>a</sup> , 39, 40   |
| Prefrontal            | R | Z score        | 3.84                                 | 3.04                        | 3.37                        | 2.94                                  | 5.36                         |
|                       |   | (x, y, z)      | (-32, -50, 32)                       | (0, -16, 44)                | (-44, -36, 40)              | (-20, -60, 44)                        | (-26, -54, 40)               |
|                       |   | % rCBF change  | 6.3                                  | 6.1                         | 6.3                         | 8.6                                   | 4.6                          |
|                       | L | BAs            | 7, 39 <sup>a</sup> , 40 <sup>a</sup> | 7 <sup>a</sup>              | 7, 39, 40 <sup>a</sup>      | 7 <sup>a</sup> , 39, 40               | 7, 39, 40 <sup>a</sup>       |
|                       |   | Z score        | 2.72                                 | 2.36                        | 3.77                        | 2.89                                  | 4.13                         |
|                       |   | (x, y, z)      | (26, 16, 32)                         | (24, 18, 32)                | (34, 24, 36)                | (12, 44, -8)                          | (26, 56, 16)                 |
| Anterior cingulate    | R | % rCBF change  | 4.9                                  | 5.8                         | 6.5                         | 8.8                                   | 4.9                          |
|                       |   | BAs            | 9 <sup>a</sup>                       | 9 <sup>a</sup>              | 9 <sup>a</sup> , 10         | 10 <sup>a</sup>                       | 9, 10 <sup>a</sup>           |
|                       |   | Z score        | 3.22                                 | 2.95                        | 2.70                        | 3.51                                  | 5.36                         |
|                       | L | (x, y, z)      | (-22, 56, 12)                        | (-26, 50, 24)               | (-32, 52, 0)                | (-34, 42, 8)                          | (-28, 48, 16)                |
|                       |   | % rCBF change  | 5.5                                  | 6.5                         | 8.2                         | 6.8                                   | 5.4                          |
|                       |   | BAs            | 9, 10 <sup>a</sup>                   | 9, 10 <sup>a</sup>          | 10 <sup>a</sup>             | 9, 10 <sup>a</sup>                    | 9, 10 <sup>a</sup>           |
| Posterior cingulate   | R | Z score        | 2.86                                 | 3.20                        | 2.48                        | <sup>b</sup>                          | 4.47                         |
|                       |   | (x, y, z)      | (16, 18, 40)                         | (20, 40, 12)                | (16, 0, 44)                 |                                       | (16, 18, 40)                 |
|                       |   | % rCBF change  | 6.8                                  | 8.8                         | 5.4                         |                                       | 5.1                          |
|                       | L | BAs            | 32 <sup>a</sup>                      | 24, 32 <sup>a</sup>         | 24 <sup>a</sup>             |                                       | 32 <sup>a</sup>              |
|                       |   | Z score        | 3.30                                 | 3.29                        | 2.91                        | 2.52                                  | 4.50                         |
|                       |   | (x, y, z)      | (-18, 34, 16)                        | (-24, 16, 28)               | (-10, -2, 36)               | (-2, 40, 4)                           | (-20, 14, 40)                |
| Premotor              | R | % rCBF change  | 9.2                                  | 10.6                        | 8.6                         | 8.4                                   | 5.0                          |
|                       |   | BAs            | 24, 32 <sup>a</sup>                  | 24 <sup>a</sup> , 32        | 24 <sup>a</sup> , 32        | 24 <sup>a</sup>                       | 32 <sup>a</sup>              |
|                       |   | Z score        | 3.34                                 | 3.31                        | 3.44                        | 3.19                                  | 5.87                         |
|                       | L | (x, y, z)      | (22, -54, 20)                        | (12, -32, 36)               | (28, -46, 36)               | (10, -62, 16)                         | (4, -38, 40)                 |
|                       |   | % rCBF change  | 6.8                                  | 6.6                         | 11.4                        | 8.9                                   | 5.1                          |
|                       |   | BAs            | 31 <sup>a</sup>                      | 31 <sup>a</sup>             | 23, 31 <sup>a</sup>         | 23, 29, 31 <sup>a</sup>               | 23, 31 <sup>a</sup>          |
| Cerebellum            | R | Z score        | 3.22                                 | 2.67                        | 2.51                        | 2.68                                  | 5.54                         |
|                       |   | (x, y, z)      | (-10, -56, 12)                       | (-4, -40, 40)               | (-18, -16, 40)              | (-16, -62, 16)                        | (-14, -62, 16)               |
|                       |   | % rCBF change  | 7.0                                  | 5.7                         | 8.6                         | 6.3                                   | 5.2                          |
|                       | L | BAs            | 23 <sup>a</sup> , 31 <sup>a</sup>    | 31 <sup>a</sup>             | 31 <sup>a</sup>             | 23, 29, 31 <sup>a</sup>               | 29, 31 <sup>a</sup>          |
|                       |   | Z score        | 3.15                                 | 3.23                        | 3.33                        | 2.37                                  | 4.73                         |
|                       |   | (x, y, z)      | (20, 30, 48)                         | (10, -24, 56)               | (36, 10, 44)                | (24, 30, 44)                          | (22, 16, 48)                 |

Values are the Z score, Talairach atlas coordinates, and percent change in adjusted regional CBF (rCBF) for regional maxima. The coordinates are in mm: x is the lateral distance from the midline (positive = right), y is the anterior-posterior distance from the anterior commissure, and z is the height above the intercommissural line. R, right; L, left.

<sup>a</sup> Brodmann's area (BA) in which the local maximum Z score occurred.

<sup>b</sup> Region not present at an SPM(t) threshold of  $p < 0.01$ .

signal per millicurie, and significant results were obtained in subject 4 with only 5–10 mCi of  $H_2^{15}O$  per scan. A dose range of 10–15 mCi can be recommended as this amount balances the gains achieved at lower doses with the increased counting statistics obtained at higher doses. Even with the low doses used in this study, activations as small as 5% were detected when two conditions were com-

pared using just four scans for each condition. Four scans per condition minimizes the effects of variations in response magnitude and permits signal averaging, while allowing several conditions to be contrasted in a single study session for an individual subject. If more sampling of each condition is desired, five or six scans per condition can be performed.

### Temporal window

The 3-D slow bolus technique, as described, has a temporal window of 30 s. This is long enough to provide adequate sampling while brief enough to minimize patient distraction and task habituation. To our knowledge, this is the shortest discrete activation to be demonstrated with PET to date, and the first time tracer arrival time has been used to define the temporal window in a clinical activation paradigm. The results for conditions 3a and 3b demonstrated that stimulation before and after the rise of the head curve did not contribute significantly to the resultant image, regardless of acquisition time. This underscores the tight temporal coupling between rCBF and localized neuronal activity as well as the primacy of the radioactivity input phase. Such information can be of help in the design of stimulation studies and in the analysis of images of transient or fluctuating conditions. For routine studies 45 s of stimulation can be recommended, as this duration allows the subject to become engaged in the task a few seconds before the head curve rises and ensures that stimulation occurs throughout the rising phase in subjects with slightly different circulation times. The shorter stimulation time of 30 s may be used if the head curve is monitored during the scan and the delivery of stimulation is timed precisely to correspond to the rising phase. In addition, the contribution to an image of a brief, variable sign or symptom may be determined using a correlational analysis relating the timing and duration of the measured event to the timing and duration of the rising phase.

### Acquisition time

The similarity in the SPM profiles obtained with 60- and 90-s acquisition times is consistent with the demonstration that the image is predominantly determined during the 30-s critical phase. There was, however, some variation in the location of significance maxima within a given region of activation. The data for the two acquisition times indicate that the 90-s acquisition time provided better counting statistics with a greater signal-to-noise ratio and resulted in higher Z scores. The 90-s acquisition time also resulted in less variability among adjusted rCBF change measurements. These findings are in keeping with the observation of improved signal-to-noise ratios with 90- to 120-s acquisition times for the autoradiographic method (Kanno et al., 1981). They also suggest that radiotracer diffusion and washout do not pose a significant problem over this time scale. Therefore, a single 90-s frame is recommended for use in this paradigm.

### Single-subject versus group analysis

In the main comparison between condition 2 (stimulation during the 30-s rising phase of the head curve) and condition 1 (control), the single-subject analysis for rCBF increases produced highly significant results comparable with those obtained with the group analysis ( $p < 0.001$ , omnibus). This level of significance has been shown to protect against false-positives (Bailey et al., 1991b). Results may also be viewed at the  $p < 0.01$  level to identify statistical trends or areas of slightly lesser, but nonetheless genuine, activation.

The rCBF decreases were more widespread and variable and tended to be of lesser significance. With single-subject analysis, areas of maximal decrease were significant at the  $p < 0.001$  level; however, a  $p < 0.01$  threshold produced a profile that was more like that of the group analysis (which was performed at the  $p < 0.001$  level). While normalization for global activity may necessarily result in small, widely distributed decreases, statistically significant negative peaks are thought to be of import, particularly when more complex behavioral states are compared (Fox, in Chadwick and Whelan, unpublished observation, 1991). Such decreases may reflect inhibition due to system connectivity (Frich et al., 1991), selective attention with depression of modalities not being attended to (H. Jenkins et al., personal communication), or relative increases in activity during a nonrest control task.

While there was good concordance overall between the SPM profiles for the single-subject and group analyses, not every area observed in the group SPM was observed in each of the single-subject SPMs, and not every area observed in each single-subject SPM was observed in the group SPM. In other words, the single-subject profiles were similar, but were not all identical. For example, subject 2 had a greater degree of lateralization in superior temporal response, and subject 3 had less inferior frontal activity (present at the  $p < 0.01$  level), associated with the activation task. This may be due to individual functional-anatomical variability in organization or strategy regarding language functions (Mazziotta et al., 1982; Ojemann, 1983). To the extent that this is the case, any discordance may be informative and may provide further evidence for the value of single-subject studies. Alternatively, the degree of intersubject variability may not always be so great (Fox and Pardo, 1991), and discordance may reflect a lack of specificity in the tasks employed or a lack of sensitivity of the technique. In either event, it should be noted that, in most cases, areas of discordance were those with lower Z scores that were present on only one plane.

### Areas of cerebral activation

The main areas of increased activity associated with the activation task (representing auditory sentence monitoring and sustained button pressing) constitute a bilateral auditory language network. Bilateral superior and middle temporal (BA 21, 22, 38, 41, 42) and even left inferior frontal (BA 45, 47) activations have been observed when subjects simply listen to words (Nishizawa et al., 1982; Petersen et al., 1988; Frith et al., 1991; Zatorre et al., 1992). When lexical, phonological, pitch, semantic, or (silent or vocal) articulatory processes are involved, unilateral or bilateral insula, parietotemporal (including BA 40), and inferior frontal (BA 44, 45, 47) areas may be seen as well (Roland et al., 1981; Mazziotta et al., 1982; Frith et al., 1991; Wise et al., 1991; Zatorre et al., 1992; Démonet et al., 1992). These areas, which were observed in this study, function as components of neurocognitive networks (Mesulam, 1990) and may be activated automatically with the presentation of meaningful stimuli (McClelland and Rumelhart, 1981).

Right-sided superior temporal maxima, as observed in this study, have been found in many of the studies of verbal listening tasks cited above. This may reflect a lateralized difference in resting and activation rCBF values and their variability, a lateralized attentional component, or, with stimuli of random content such as employed in this study, a lateralized affective component (Heilman et al., 1975). The minimal SMA activity detected and lack of primary motor region activity reflect the fact that there was essentially no finger movement or volitional planning during the activation task; the button remained depressed in response to an external stimulus throughout the entire rising phase of the head curve.

The areas of decreased activity associated with the activation task/increased activity associated with the control task (representing auditory attention and preparatory set) include visual, prefrontal, multimodal association, premotor, cerebellar, and cingulate regions. If these findings are due to regionally specific phenomena, one reasonable interpretation is that visual and high-order association cortices are inhibited or less active during the auditory sentence monitoring/button-pressing task, while the parietal, cingulate, and SMA areas that constitute the posterior and anterior attentional systems (Posner and Rothbart, 1991) are more active during the auditory attention/preparatory set control task. It is unclear whether the cerebellar signal is the result of cerebellar involvement in motor preparatory set or the result of an inaccuracy in the mapping of inferior occipital signal at the bottom of

the scan onto the Talairach atlas. This raises two relevant points. First, SPM analysis does not require a priori determination of regions of interest. It is therefore more likely that new findings will occur, which can be tested and explored in subsequent studies. Second, the Talairach atlas represents one person's postmortem brain. The resultant problems associated with atlas mapping can be avoided by using single-subject techniques, such as this one, that permit intrasubject PET-magnetic resonance coregistration.

### CONCLUSION

We have described a PET paradigm utilizing high-sensitivity 3-D scanning with low doses of  $H_2^{15}O$  that allows the detection of small, statistically significant changes in rCBF associated with cognitive/behavioral states in single subjects. It consists of four scans per condition with each scan characterized by a slow bolus of 10–15 mCi of  $H_2^{15}O$ , a 30-s critical period (corresponding to the arrival of radiotracer in the brain) during which the rCBF distribution is detected, 30–45 s of cognitive stimulation, and a 90-s acquisition time. This method was investigated and validated in normal volunteers using control and activation tasks and SPM analysis. It is a potentially valuable tool for the study of functional neuroanatomy in various neuropsychological, pharmacologic, and disease states. In addition, its well-defined temporal window provides a means of recording the maximum response associated with an experimental task and may allow the imaging of transient or randomly occurring mental/brain states that are not under direct experimental control.

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"THE HAMMERSMITH PHILOSOPHY FOR PET CHEMISTRY AUTOMATION"

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We have only automated production of robust chemical syntheses for routine use on a weekly scheduled basis;  $^{18}\text{F}$ FDG,  $^{11}\text{C}$ -methylations &  $\text{H}_2^{15}\text{O}$ . We use programmable logic controllers (PLC's) to control temp, time and flow. "Set up" and "run" programmes are assembled in "ladder logic" and stored on an "EPROM". During "set up" clean dry reaction vessels, product reception vials & reagents are installed. HPLC product peak is "cut" by remote control valve operation. For  $^{11}\text{C}$  methylations an automated product formulation system is integrated with the HPLC "cut peak" and delivers the product via a membrane filter to the delivery vial. A PLC controlled  $^{18}\text{F}$ FDG unit, incorporating a closed glassy carbon reactor in a one pot system for fluorination and hydrolytic deprotection has been built. Enriched  $\text{H}_2^{18}\text{O}$  is recovered and the product is purified by HPLC.  $\text{H}_2^{15}\text{O}$  is infused from a unit housed under the PET scanner couch. It is supplied with  $^{15}\text{O}_2$ , 5%  $\text{H}_2$  & saline for infusion. Solution & gas are separated by a sterile, pyrogen free water permeable membrane. Infusions are controlled from the scanner console where infused doses are displayed using an "on board" radiation detector. These systems are operated by a small team of trained technicians within a Good Manufacturing Practice framework. Independent Quality Assurance is provided, to qualify for a "specials" Radiopharmaceutical Manufacturing Licence.

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## THE HAMMERSMITH PHILOSOPHY FOR PET CHEMISTRY AUTOMATION

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### INTRODUCTION

Clinical scientific research using PET requires access to a wide variety of radio-pharmaceuticals. We have chosen only to automate the production of those accessible via robust chemical syntheses. Three types of system are in routine use on a weekly scheduled basis. They are for [ $^{18}\text{F}$ ]-2 fluoro-2 deoxy-D-glucose([ $^{18}\text{F}$ ]FDG) production, [ $^{11}\text{C}$ ]-methylations and  $\text{H}_2^{15}\text{O}$  infusions. As most PET Radiopharmaceutical syntheses generally require only control of valves, temperature, time and flow, we have chosen to use industrial programmable logic controllers (PLC's) with sensing and feedback of temperature and multiport valve position where essential. "Set up" and "run" programmes are assembled in "ladder logic" and stored on an erasable programmable read only memory (EPROM). During the "set up" procedure clean dry reaction vessels, SEP- PAC<sup>®</sup>s, precursors, reagents, filters and product reception vials are installed. During the "run" sequence the processing proceeds automatically the operator intervenes only to "cut" the preparative HPLC product peak by remote control valve operation. For [ $^{11}\text{C}$ ] methylations an automated product formulation system is integrated with the HPLC "cut peak" and delivers the product via a membrane filter to the delivery vial. A PLC controlled [ $^{18}\text{F}$ ]FDG unit, incorporating a closed glassy carbon reactor in a one pot system for fluorination and hydrolytic deprotection has been built. Enriched  $\text{H}_2^{18}\text{O}$  is recovered and the product is terminally purified by HPLC. Bedside infusions of  $\text{H}_2^{15}\text{O}$  are carried out using a unit which is housed under the PET scanner couch which is supplied with [ $^{15}\text{O}$ ] oxygen in 1%  $\text{O}_2/\text{N}_2$ , 5%  $\text{H}_2/\text{N}_2$  and saline for infusion. The gas and solution phases are permanently separated by a water permeable membrane which is maintained sterile and pyrogen free. Infusions are controlled from the scanner control room and infused doses are displayed there using an on board radiation detector. These systems are in routine use operated by a small team of trained technicians within a Good Manufacturing Practice (GMP)



framework. Quality Assurance (QA) is provided by an independent person in order to qualify for our "specials Radiopharmaceuticals" Manufacturing Licence.

## AUTOMATION OF [ $^{18}\text{F}$ ]FDG PRODUCTION

The nucleophilic fluorination of methyl 4,6-O-benzylidene-3-O-methyl-2-O-trifluoromethanesulfonyl- $\beta$ -D-mannopyranoside using Kryptofix® - 222(K-222) and [ $^{18}\text{F}$ ] fluoride (Hamacher et al 1986) has become the synthetic route of choice for the preparation of [ $^{18}\text{F}$ ]FDG. Having reviewed the available commercial devices on offer at the time we chose to assemble our own system based on a single glassy carbon reaction vessel (see Figure 1). The processing sequence is controlled by a Toshiba EX-40+ programmable controller. This is used to reproduce the various timed steps in the [ $^{18}\text{F}$ ]FDG synthesis reliably without any intervention from the operator. Thus, once the system is set up, [ $^{18}\text{F}$ ]FDG can be produced automatically. The system consists of two parts: the reaction and primary purification system, and the HPLC final purification system. The reaction and the HPLC systems are designed to be easily removed from the hot-cell should either need to be repaired or replaced. Most of the liquid transfers on the system are achieved by helium overpressure. In the final stages, however syringe drives are used in order to achieve greater control over the transfer rates.

### [ $^{18}\text{F}$ ]Fluoride Production.

[ $^{18}\text{F}$ ]fluoride is produced by the proton irradiation of 80 % oxygen-18 enriched water via the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reaction. The target is closed with a 2 Bar imposed overpressure, any spray or condensed steam produced during the irradiation is recondensed and pumped back into the target chamber. In addition any radiolytic gases ( $\text{H}_2$  and  $\text{O}_2$ ) are recombined using a single pellet of palladium on alumina catalyst to avoid excessive pressure rises. After irradiation, the solution containing the [ $^{18}\text{F}$ ]fluoride is transferred using the 2 Bar overpressure via a 40 m of teflon tube (i.d. 0.87 mm) to the [ $^{18}\text{F}$ ]FDG synthesis system. The recirculation pump and the target fill syringe drive and fill/empty valves are remotely controlled by a PLC.

### [ $^{18}\text{F}$ ]Fluoride Recovery.

Due to the expense of enriched water, it is desirable to recover it for reuse. This is achieved by trapping the [ $^{18}\text{F}$ ]fluoride on an anion exchange resin column (3mm x 20mm, BioRad AG 1-X8 carbonate form)

and allowing the enriched water to pass into a collection vial. The water can then be distilled and reused. The [ $^{18}\text{F}$ ]fluoride is then eluted using potassium carbonate (1ml 4.6 mg/ml) which is transferred into the reaction vessel.

#### **[ $^{18}\text{F}$ ]Fluorination of the Precursor.**

Before the [ $^{18}\text{F}$ ]fluoride can be incorporated into the triflate precursor, the dry complex  $[\text{K-222}][^{18}\text{F}]$  must be formed by adding a solution of K-222 in acetonitrile to the reaction vessel. The reaction mixture is then dried in a single stage evaporation. The main reaction vessel is made of glassy carbon which is both chemically inert and has good thermal conductivity. The evaporation and drying is achieved by heating the reaction vessel whilst blowing a stream of helium onto the liquid surface. The vapours are removed using a vacuum pump. The flow rates are set to provide a positive pressure in the reaction vessel to stop the liquid bumping during evaporation. If this were to occur, then [ $^{18}\text{F}$ ]fluoride would be lost to the vacuum pump and the vessel walls! When dry, the precursor in acetonitrile is added to the reaction vessel and [ $^{18}\text{F}$ ]fluoride is incorporated by nucleophilic substitution of the triflate. The reaction vessel can be heated at one of two temperatures by a CAL 9000 controller using one or two band heaters as desired. It can be cooled with a compressed air jet. Two heaters are used for drying and one is used for refluxing. The heating, cooling and liquid transfer processes are all controlled by the PLC program (see Table 1 for program sequence).

#### **Hydrolysis.**

Before hydrolysis, the protected  $^{18}\text{F}$ FDG is transferred onto a C-18 SEP-PAC<sup>®</sup> which is then washed with Hydrochloric acid (0.1M) to remove K2.2.2. The product is then eluted into the reaction vessel with ethanol which is subsequently removed by evaporation. The Hydrochloric acid (1ml.2M) is then added and heated. Again the sequence of events is controlled by the PLC.

#### **Purification.**

Before HPLC purification the solution is transferred through a C-18 SEP-PAC<sup>®</sup> and a resin column 8mmx40mm (BioRad AG 11 A8) to remove organic by-products and to neutralise the acid. A syringe drive with pulse control is used to provide an accurate flow rate of 0.5 ml/min to ensure effective neutralisation. If this is not achieved then the product will be acidic and damage to the HPLC column will result. The

product is then drawn up using the second syringe drive and transferred to the loop of the HPLC injector valve. A BioRad HPX-87P column is eluted with water at 0.5ml/min. The purified product "peak" is "cut" by manual remote control of valve 20 while observing the radioactivity trace.

### Performance of the System

The labelling efficiency corrected to start of synthesis and before HPLC is 50%. Losses incurred during HPLC result in an overall efficiency of 30%.

Figure 1 Scheme of PLC-controlled apparatus for the preparation of  $^{18}\text{F}$ FDG.

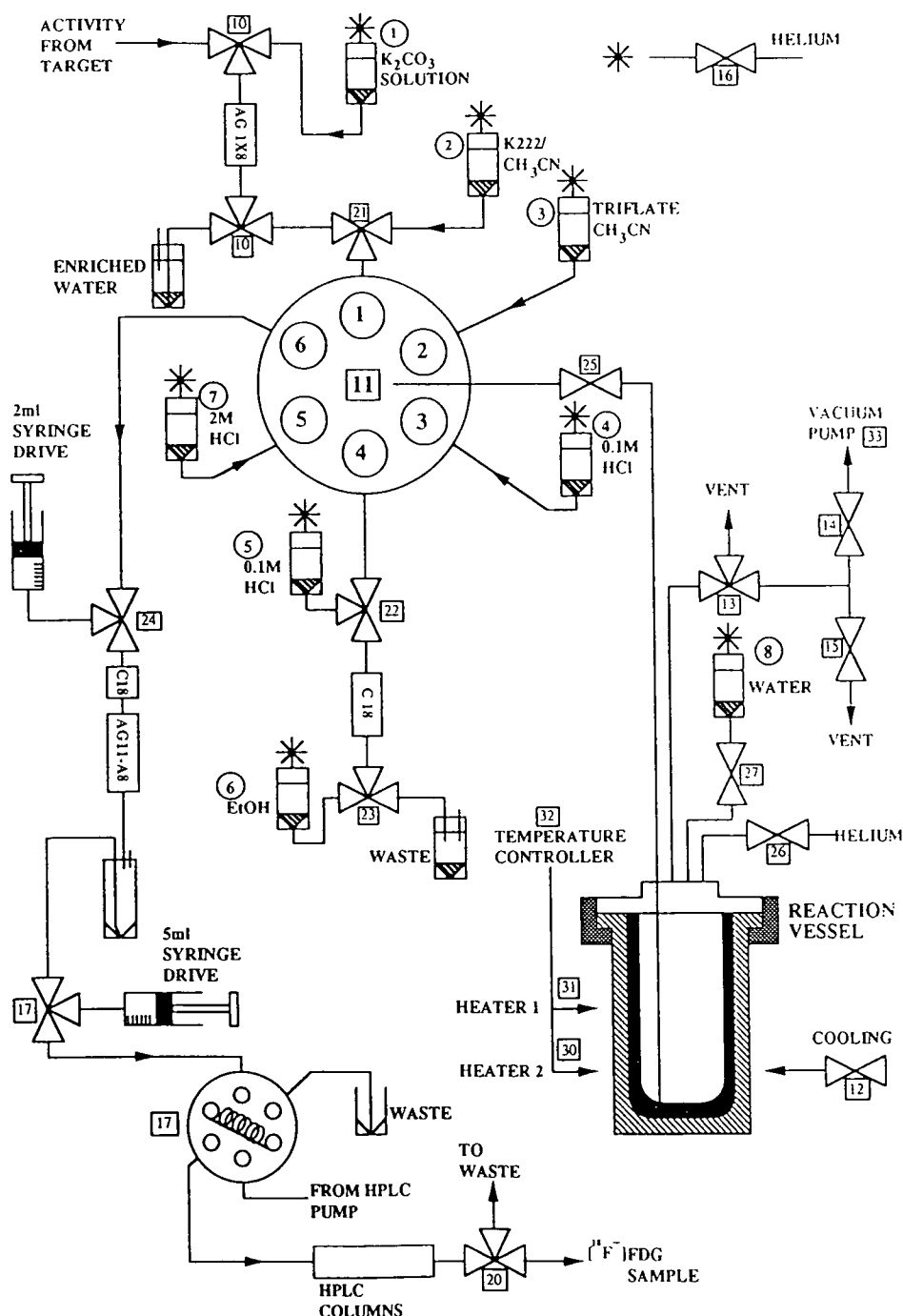


Table 1 Program sequence for the PLC used for the preparation of [ $^{18}\text{F}$ ]FDG

## SETUP MODE

| Step No. | Description                              | Outputs                                                                                                |
|----------|------------------------------------------|--------------------------------------------------------------------------------------------------------|
| 0        | Empty Vial 4                             | 11 (Posn. 4),16,25                                                                                     |
| 1        | Empty Reaction Vessel to Waste           | 11,13,16,25,26                                                                                         |
| 2        | Empty Vial 5                             | 16,22,25                                                                                               |
| 3        | Empty Reaction Vessel to Waste           | 13,16,25,26                                                                                            |
| 4        | Empty Vial 6                             | 16,23,25                                                                                               |
| 5        | Empty Reaction Vessel to Waste           | 13,16,25,26                                                                                            |
| 6        | Empty Vial 7                             | 11 (Posn. 5),16,25                                                                                     |
| 7        | Syringe Out Reaction Vessel to waste (1) | 11 (Posn. 6),25,Syringe drive up 60 sec,24,<br>Syringe drive down 60 sec                               |
| 8        | Syringe Out Reaction Vessel to waste(2)  | Syringe drive up 60 sec,24,Syringe drive<br>down 60 sec                                                |
| 9        | Syringe Out Reaction Vessel to waste(3)  | Syringe drive up 60 sec,24,Syringe drive<br>down 60 sec                                                |
| 10       | Syringe Out Reaction Vessel to waste(4)  | Syringe drive up 60 sec,24,Syringe drive<br>down 60 sec                                                |
| 11       | Empty Vial 8                             | 11 (Posn. 4),16,27                                                                                     |
| 12       | Empty Reaction vessel to Waste           | 13,16,25,26                                                                                            |
| 13       | Empty Vial 1                             | 10,11 (Posn. 1),16,25                                                                                  |
| 14       | Empty Vial 2                             | 16,21,25                                                                                               |
| 15       | Empty Vial 3                             | 11 (Posn. 2),16,25                                                                                     |
| 16       | Empty Reaction Vessel to Waste           | 11 (Posn. 4),13,16,25,26                                                                               |
| 17       | Blow Out Lines                           | 16,23,25,26,27                                                                                         |
| 18       | Step 17+Vacuum                           | 13,14,16, 23,25,26,27,33 Vacuum Pump on                                                                |
| 19       | Step 17+Dry Pot (Heat)                   | 13,14,16,23,25,30 Heater 2 on, 31 Heater on<br>32Temperature Controller on (100°C)<br>33Vacuum Pump on |
| 20       | Cooling+Vent                             | 12,14,15,23,25                                                                                         |

Note: Output 11 is to a 6 port rotary valve with position feedback to the PLC. Outputs 30,31 and 32 are connected via the PLC to allow for set point and temperature feedback

## RUN MODE

| Step No. | Description                                                 | Outputs                                                                                              |
|----------|-------------------------------------------------------------|------------------------------------------------------------------------------------------------------|
| 0        | Elute $^{18}\text{F}$ with $\text{K}_2\text{CO}_3$ (Vial 1) | 10,16,25                                                                                             |
| 1        | Add K222 (Vial 2)                                           | 16,21,25                                                                                             |
| 2        | Dry off solvents                                            | 13,14,16,25,26,30 Heater 1 on,31 Heater 2on,32<br>Temperature controller on(100°C),33 Vacuum Pump on |
| 3        | Cool+Vent reaction vessel                                   | 12,14,15,30 Heater 1 on,32<br>Temperature controller on (85°C)                                       |
| 4        | Add precursor(Vial 3)                                       | 11 (Posn. 2),16,25,30 Heater 1on,<br>32 Temperature controller on(85°C)                              |
| 5        | React precursor                                             | 30 heater 1 on,32 Temperature<br>controller on (85°C)                                                |
| 6        | Add 0.1M HCl (Vial 4)                                       | 11 (Posn. 3),12,16,25                                                                                |
| 7        | Pass through SEP-PAC®                                       | 11 (Posn. 4),13,16,25,26                                                                             |
| 8        | Add 0.1M HCl (Vial 5)                                       | 16,22,25                                                                                             |
| 9        | Pass through SEP-PAC®                                       | 13,16,25,26                                                                                          |
| 10       | Elute SEP-PAC® with EtOH (Vial 6)                           | 16,23,25                                                                                             |
| 11       | Evaporate solvents                                          | 13,14,16,25,26,30 Heater 1on,31 Heater 2 on<br>32Temperature controller on(100°C),33Vacuum Pump on   |
| 12       | Vent reaction vessel                                        | 14,15,30 Heater 1 on,32<br>Temperature controller on (100°C)                                         |
| 13       | Add 2M HCl (Vial 7)                                         | 11 (Posn. 5),16,25,30Heater 1 on,31 Heater 2 on<br>32Temperature controller on(100°C)                |
| 14       | Hydrolyse                                                   | 30 Heater 1 on,31 Heater 2 on,32<br>Temperature controller on(100°C)                                 |
| 15       | Pass solution through columns<br>Add water (Vial 8)         | 11 (Posn. 6),12,16,Syringe drive up 60secs<br>25,Syringe drive down 60secs,24,27                     |
| 16       | Pass solution through columns                               | Syringe drive up 60 secs,25,<br>Syringe drive down 60 secs,24                                        |
| 17       | Pass solution through columns                               | Syringe drive up 60 secs,25,<br>Syringe drive down 60 secs,24                                        |
| 18       | Fill HPLC syringe                                           | HPLC syringe drive up                                                                                |
| 19       | Load HPLC Loop                                              | Syringe drive down (manual control)                                                                  |
| 20       | Inject loop onto column                                     | Manual control                                                                                       |
| 21       | Cut Peak                                                    | 20 (Manual control)                                                                                  |

## AUTOMATION OF $^{11}\text{C}$ -METHYLATIONS

Many  $^{11}\text{C}$ -labelled compounds of great interest to PET clinical science have now been prepared by the methylation of suitable precursors using  $^{11}\text{C}$ iodomethane and the number continues to grow. At Hammersmith Hospital, in order to respond to the varied needs of a busy clinical research programme, a hot-cell has been successfully equipped with a  $^{11}\text{C}$ -methylation facility that can be quickly set up and operated to produce radiopharmaceutical batches for clinical use (see Figure 2). The sequence of operations that is needed to achieve each radiosynthesis is controlled in timed steps by a Programmable Logic Controller (PLC). This incorporates a 'plug-in' module that can be programmed to suit each individual radiosynthesis (Clark and Dowsett, 1992). (see Table 2 for program sequence for [O-methyl- $^{11}\text{C}$ ] Diprenorphine )

### $^{11}\text{C}$ Carbon Dioxide Production.

$^{11}\text{C}$ Carbon dioxide is produced by the proton irradiation of high purity nitrogen gas via the  $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$  reaction. For labelling operations it is advantageous to concentrate the  $^{11}\text{C}$ carbon dioxide into a few millilitres from a volume of several litres of target gas. An automated cryotrap fabricated from a small coil of stainless steel tubing (20 cm  $\times$  1/16" o.d.) cooled in liquid argon is used. The use of liquid argon avoids the problematical co-trapping of significant volumes of nitrogen which occurs if liquid nitrogen is used as the coolant. The valves and cryotrap movement are controlled by the PLC's programme.

### Production of $^{11}\text{C}$ Iodomethane.

The synthesis of  $^{11}\text{C}$ iodomethane using the reaction of  $^{11}\text{C}$ carbon dioxide with lithium aluminium hydride (LAH) and subsequent reaction with hydriodic acid is now widely applied (for a review see Crouzel et al., 1987). The major problem in this radiosynthesis is maintenance of the high specific radioactivity required for  $^{11}\text{C}$ -labelled receptor ligands. This can principally be achieved by the use of high quality LAH in small quantities under carefully controlled carbon dioxide free atmospheres and by working quickly. The heating, cooling and sequencing of the valves for  $^{11}\text{C}$ iodomethane production are controlled by PLC programme.

### **[<sup>11</sup>C]-Methylation of Precursor.**

The conditions under which the precursor is methylated depend critically on the class of compound of interest and the subsequent work-up and purification procedures. An automated reaction vial penetrator is used. This device allows the relevant precursor to be introduced together with the reaction solvent and base in a septum-sealed vial. [<sup>11</sup>C]Iodomethane can then be distilled into the precursor solution via a round-tipped side-hole needle, which can be motor-driven through the septum under programme control. The needles can then be withdrawn and the vial lowered into the heating bath. The <sup>11</sup>C-methylation takes place for a pre-programmed time. The reaction mixture is extracted by re-inserting the needles and transferred to the loop of an HPLC injection valve. Again this sequence of events is controlled by the PLC.

### **HPLC Purification.**

For some compounds, where the base and/or solvent are detrimental to the performance of the HPLC, sample-enrichment can be included in the programme of operations. Here an injection loop (10 mL) is loaded with an aqueous wash of the reaction vial and the contents of this loop are then loaded onto a silica C-18 cartridge (30mmX4mm). This cartridge is washed with water and then back-eluted onto the main silica C-18 column for purification using the chosen eluent. The PLC controls the sequence of operations for the multi-port valves necessary to accomplish these steps. The HPLC eluate that contains the required labelled product is identified by the operator by monitoring radioactivity and UV absorbance, and collected in the flask of a rotary evaporator by manual remote operation of the fraction collect/waste valve

### **Product Formulation.**

Solvent is removed from the product by evaporation under reduced pressure using a micro-rotary evaporator, modified to allow it to be raised or lowered under the control of the PLC. Finally, under programme control, the formulation solvent is introduced into the flask and the resultant radioactive solution transferred via a Millipore® filter into a shielded shipping vial outside the hot-cell.

### Performance of the System.

The system has been used routinely to produce clinical batches of S-[N-methyl- $^{11}\text{C}$ ]nomifensine (Ulin et al., 1989) and [O-methyl- $^{11}\text{C}$ ]raclopride (Ehrin et al., 1987), [N-methyl- $^{11}\text{C}$ ]SCH 23990 (Halldin et al., 1986) and [O-methyl- $^{11}\text{C}$ ]Diprenorphine (Luthra et al 1991). The production of [O-methyl- $^{11}\text{C}$ ]raclopride in particular benefitted from the sample-enrichment facility. The production parameters for these radiosyntheses are listed below.

a. S-[N-methyl- $^{11}\text{C}$ ]Nomifensine.

[ $^{11}\text{C}$ ]Iodomethane is trapped in a solution of desmethyl-S-nomifensine (5 mg) in ethanol (400  $\mu\text{L}$ ). Then the reaction pot is sealed and heated at  $90^\circ\text{C}$  for 5 min. The reaction mixture is purified by HPLC on a  $\mu$ -Porasil<sup>TM</sup> column (30 x 0.8 cm i.d.; Waters Associates Inc.) eluted with chloroform-methanol (90:10 v/v) at a flow rate of 3 mL/min. The product is formulated in isotonic saline which is sterilised by Millipore<sup>®</sup> filtration. A decay-corrected radiochemical yield of 23% from [ $^{11}\text{C}$ ]iodomethane provides 370–555 MBq of S-[N-methyl- $^{11}\text{C}$ ]nomifensine ready for injection with a specific radioactivity of 15000 MBq/ $\mu\text{mol}$ . (product no longer required routinely).

b. [O-methyl- $^{11}\text{C}$ ]Raclopride.

[ $^{11}\text{C}$ ]Iodomethane is trapped in DMSO (400  $\mu\text{L}$ ) containing desmethyl-raclopride (2.5 mg) and sodium hydroxide solution (5M; 10  $\mu\text{L}$ ). The reaction pot is sealed and heated at  $90^\circ\text{C}$  for 5 min. The reaction mixture is purified by sample enrichment followed by reverse phase HPLC using a  $\mu$ -Bondapak<sup>TM</sup> C-18 column (30 x 0.8 cm i.d.; Waters Associates Inc.) eluted with methanol-10mM ammonium dihydrogen phosphate solution (60:40 v/v) at 3 mL/min. The product is formulated in isotonic saline which is sterilised by Millipore<sup>®</sup> filtration. A decay-corrected radiochemical yield of 25% from [ $^{11}\text{C}$ ]iodomethane provides 1019 MBq of [O-methyl- $^{11}\text{C}$ ]raclopride ready for injection with a specific radioactivity of 21100 MBq/ $\mu\text{mol}$ . (average of 10 preparations).

c. [N-methyl- $^{11}\text{C}$ ]SCH 23390.

[ $^{11}\text{C}$ ]Iodomethane is trapped in acetone (400  $\mu\text{L}$ ) containing desmethyl-SCH 23390 (1 mg). The reaction pot is sealed and heated at  $90^\circ\text{C}$  for 10 min. The acetone is then evaporated. The reaction mixture is recovered in 2ml of HPLC eluent and purified by HPLC on  $\mu$ -Porasil<sup>TM</sup> column (30 x 0.8 cm i.d.; Waters Associates Inc.) eluted with chloroform-methanol-aq. ammonia (90:1:0.1 by vol.) at 3 mL/min. The product is formulated in isotonic saline which is sterilised by Millipore<sup>®</sup> filtration. A decay-corrected radiochemical yield of 40% from [ $^{11}\text{C}$ ]iodomethane provides 1400 MBq of [N-methyl- $^{11}\text{C}$ ]SCH 23390 ready for injection with a specific activity of 19600 MBq/ $\mu\text{mol}$ . (average of 10

preparations).

d. [O-methyl- $^{11}\text{C}$ ]Diprenorphine.

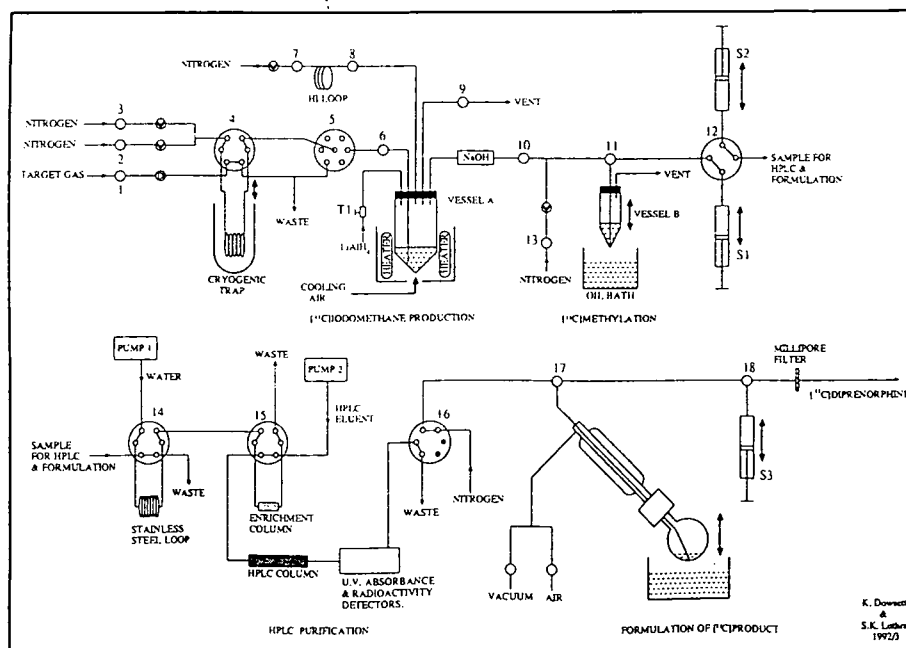
[ $^{11}\text{C}$ ]Iodomethane is trapped in DMSO (300  $\mu\text{L}$ ) containing (3-O -trityl,6-O -desmethyl)diprenorphine (2.0 mg) and sodium hydride (4mg ). The reaction vial is sealed and heated at 95°C for 5 min. The reaction vial is then raised back onto the needles and hydrochloric acid (250  $\mu\text{L}$ , 2M) is slowly added. The reaction vial is then lowered into the oil bath and heated at 95°C for a further 2 mins. to remove the protecting group.

After hydrolysis, the reaction mixture is purified by sample enrichment followed by reverse phase HPLC using a  $\mu\text{-Bondapak}^{\text{TM}}$  C-18 column (30 x 0.8 cm i.d.; Waters Associates Inc.) eluted with acetonitrile-10mM ammonium formate solution (45:55 v/v) at 3 mL/min. After evaporating the HPLC solvent, the product is formulated in isotonic saline which is sterilised by Millipore® filtration. A decay-corrected radiochemical yield of 25% from [ $^{11}\text{C}$ ]iodomethane provides 1051 MBq of [6-O-methyl- $^{11}\text{C}$ ]-Diprenorphine

ready for injection with a specific radioactivity of 28600 MBq/ $\mu\text{mol}$ . (average of 10 preparations).

A second hot-cell has been equipped with two very similar systems to extend the range of routinely available  $^{11}\text{C}$ -labelled compounds within each working day, for example, [N-methyl- $^{11}\text{C}$ ]PK 11195 (Camsonne et al., 1984; Cremer et al., 1992), [N-methyl- $^{11}\text{C}$ ]flumazenil (Mazière et al., 1984) and L-[N-methyl- $^{11}\text{C}$ ]deprenyl (Fowler et al., 1987).

Figure 2. Scheme of PLC-controlled apparatus for labelling radiopharmaceuticals by methylation with [ $^{11}\text{C}$ ]iodomethane.





8A.

Table 2 Program sequence for the PLC used in the preparation of [11C] Diprenorphine.

SETUP MODE.

| Step No. | Description                                                                                                       | Outputs                       |
|----------|-------------------------------------------------------------------------------------------------------------------|-------------------------------|
| 0        | Start                                                                                                             | All off                       |
| 1        | Wash HI loop,remove methylation vial, wash line from HCl syringe to vial, wash line from ammonia syringe to vial. | 7,8,vessel B manual, 10,11,12 |
| 2        | Pump storage eluent to formulation rig,                                                                           | 16                            |
| 3.       | Wash line from formulation syringe to rotary evaporator.                                                          | 17,18                         |
| 4        | Wash out rotary evaporator.                                                                                       | Rotary evap.                  |
| 5        | Load ammonia syringe.                                                                                             | S2 manual                     |
| 6        | Load HCl syringe.                                                                                                 | S1 manual                     |
| 7        | Load formulation syringe.                                                                                         | S3 manual                     |
| 8        | Purge target and apparatus for 3minutes.                                                                          | 1,2,3,5,6,9                   |
| 9        | Pressurise pot and flush HI loop                                                                                  | 2,3,6,7,8                     |
| 10       | Seal pot, and pressure test                                                                                       | All off                       |
| 11       | Slow flush through apparatus                                                                                      | 3,6,9                         |
| 12       | Load vial                                                                                                         | Vessel B manual               |
| 13       | Vial up, nitrogen on.                                                                                             | Vessel B up,13                |
| 14       | Load HI loop                                                                                                      | 3,6,7,8,13                    |

RUN MODE

| Step No. | Description                                                     | Outputs                                                 |
|----------|-----------------------------------------------------------------|---------------------------------------------------------|
| 0        | Change over from setup                                          | All off                                                 |
| 1        | Flush apparatus, load LAH into pot                              | 2,3,6,9,13                                              |
| 2        | Trap down, wait 10secs, empty target 150secs                    | Cryogenic trap down ,1,4,13                             |
| 3        | Trap up, dispense 2 minutes                                     | Cryogenic trap up,3,6,9,13                              |
| 4        | Evaporate THF for 3 minutes                                     | Heater, 2,3,6,9,13                                      |
| 5        | skip ( an unnecessary step was deleted here)                    |                                                         |
| 6        | Cool pot and flush line for 2.5 minutes                         | Cooling air,2,3,6,9,13                                  |
| 7        | Add HI for 2 secs                                               | 7,8,9                                                   |
| 8        | Heat reaction for 2 minutes (optional nitrogen pulse for 0.4 s) | Heater,13 (manual 2,6)                                  |
| 9        | Switch off nitrogen through vial, vent Mel pot for 2 seconds    | Heater,10                                               |
| 10       | Distil Mel for 90 seconds                                       | Heater,2,6,10                                           |
| 11       | Lower vial, wait 5 minutes                                      | Vessel B down                                           |
| 12       | Raise vial, add HCl                                             | Vessel B up,11,S1                                       |
| 13       | Lower vial, wait 2 minutes                                      | Vessel B down                                           |
| 14       | Raise vial, extract reaction mixture into ammonia               | Vessel B up,11,12                                       |
| 15       | Load sample into loop                                           | S2 manual                                               |
| 16       | Transfer to enrichment, 3.5 minutes                             | S2 manual,14                                            |
| 17       | Load onto HPLC, 2 minutes                                       | 15                                                      |
| 18       | HPLC and cut peak into rotary evaporator                        | 15, 16 manual                                           |
| 19       | Vacuum on, wait 30 secs,                                        | Vacuum on, lower flask, rotation on, Rotary evap. down. |
| 20       | Evap up, wait 3s, add saline                                    | Rotation on, 17,18,S3                                   |
| 21       | Draw up saline                                                  | 17,18,S3                                                |
| 22       | Dispense product through millipore                              | S3 manual                                               |

### Components of the $^{18}\text{F}$ FDG and $[^{11}\text{C}]$ Methylation Systems.

Wherever possible commercial components are used with little or no modification. A Toshiba EX40 industrial programmable logic controller, which uses ladder logic was chosen. The programme information is stored on a plug in Electronically Erasable Programmable Read Only Memory (EEPROM) module which can be interchanged readily. The controller contains relays that are suitable for low power (240 V a.c.; 2 A) and low voltage (24 V d.c.; 2 A) operations. Most of the valves and motorised drives are controlled directly by these relay outputs. The 240 V operated devices, such as heaters and pumps, are operated via isolating solid state relays. The two- and three-port Teflon<sup>TM</sup> diaphragm valves for liquid and vapour handling are by Angar Scientific [part nos 3682NC2430 and 368232430 for 24 V and up to 2 bar (30 psi) operation]. They have 1/4" x 28 threads in flat-bottomed ports suitable for use with low pressure chromatography fittings (e.g. 'Altex<sup>TM</sup>', 'Omnifit<sup>TM</sup>'). For gas handling the two- and three-port stainless steel valves are by Skinner [Honeywell Lucifer, part nos B2 RX 127 DC-2 and B14 DK 1075 DC-2 operated at 24 V for pressures up to 12 bar (330 psi) and 5.3 bar (80 psi), respectively]. The multi-port rotary valves are by Rheodyne. The low pressure Teflon<sup>TM</sup>/Kel-F<sup>TM</sup> valves with 1/4" x 28 fittings are 3-port (part no 5301/5300 ) and six-port distribution valves with pneumatic actuators (part no 5011P). The HPLC valves are also by Rheodyne and are pneumatically-actuated stainless steel and Vespel<sup>TM</sup> (Dupont polyimide) or Kel-F<sup>TM</sup> rotary valves (part no 7010P). The rotary evaporator is a UV Micro (Heidolph) and has been modified to be raised and lowered pneumatically. The glassy carbon reaction vessel (70mmx16mm I.D) is made of sigradur® (HTW GmbH,D-8901 Meitingen). The reaction vial for methylation is a Reacti-vial<sup>TM</sup> (Pierce, 1 mL) with a Teflon<sup>TM</sup>-faced silicon septum 3 mm thick retained by a Quickfit<sup>TM</sup> (Corning) SQ 13 cap. The penetration needles are by Hamilton with rounded tips and side holes (part no 100.090.218). The syringe drives and septum penetrator drives are of 'in house' design and manufacture and are 'screw and nut' devices driven by electric motor. The HPLC detectors are by Mini Instruments for radioactivity detection (model 7-10C with a MC10 G-M tube) and by Severn Analytical for UV absorbance detection (model SA 6506).

## AN AUTOMATED SYSTEM FOR THE PREPARATION AND INFUSION OF [15O]WATER AT THE BEDSIDE.

### Introduction

The important blood flow tracer, [15O]water, may be readily prepared by a variety of routes. However, several problems need to be addressed if safe and effective injections or infusions are to be prepared. Of primary concern is that the product should be free from any harmful impurities, both chemical and radioactive, and be sterile and apyrogenic. Due to the short half-life of oxygen-15, quality control of each sample or batch would be impractical. However, most groups carrying out work in this area have evolved safe operating procedures (SOPs) which have been shown to be capable of maintaining the required standards. The measurement and delivery of the prepared doses of [15O]water also requires careful attention both for the administrator and for the recipient. As a typical injected bolus of [15O]water would be 3-3.7 GBq (80-100 mCi) the radiation dose to the administrator is of great concern. Automated injectors and infusers are at advanced stage of development in several laboratories. The device we have developed at Hammersmith is described below.

### Design and Operation of the [15O]Water Infuser.

The 'bedside' [15O]water infuser (nicknamed 'R2D2') recently developed at the MRC Cyclotron Unit (Hammersmith Hospital) (Clark and Tochon Danguy, 1992) is shown schematically in Figure 3. [15O]Water is synthesised in the 50mm thick lead-shielded bedside device by mixing cyclotron-produced [15O]oxygen (in 1% oxygen/ 99% nitrogen) with 5% hydrogen/95% nitrogen over a palladium catalyst in an low voltage(24-0-24Vac.100VA) oven at 200°C. The gaseous output is led to a semi-permeable membrane interface (Visking-Medicell International,U.K.) sandwiched between Perspex blocks (see Figure 4). The other side of the interface is flushed with sterile saline using a pump (IVAC 560) approved for medical application. . [15O]Water diffuses rapidly across the membrane and is taken up into the sterile saline. This is then infused into the subject for study via a shielded G-M tube and sterile Millipore® filter. The fluid handling valves, for control of the infusion parameters, are three-port membrane valves (Angar/Asco, 368232430 24VDC operation). 'On-line' radioactivity detection is based on passing the radioactive saline through a loop of Teflon™ tubing (1/16" o.d.) wound around the G-M tube (Philips ZP-1300). The counts are taken into a scaler-ratemeter (Mini Instruments 6-90) modified with

adjustable pre-scaler to allow direct calibration as 'mCi infused'. This calibration is routinely checked against a standard ionisation chamber. All controls and power supplies are designed and built to comply with electromedical safety standards. Unused product is allowed to decay in a delay vessel consisting of a 250ml volume coil of polypropylene tubing housed within the unit before discharge into a sterile bag external to the lead shield. The device can be operated to give a constant infusion for any predetermined time at a flow rate of 10 mL/min. Alternatively bolus infusions can be prepared and administered. This is achieved by accumulating a "bolus" dose in the membrane exchanger whilst the saline flow is temporarily interrupted. The amount of radioactivity infused is determined by the mode of operation of the PET scanner (CTI-Siemens Neuro-PET) and is typically 560 MBq (15 mCi) for 'septa out' and 3 GBq (80 mCi) for 'septa in'.

### Infuser maintenance.

Pharmaceutical maintenance involves cleaning and sterilising the membrane exchanger prior to assembly and washing the fluid system with isotonic saline solution while the millipore filters are in place. Pyrogen tests (LAL) and sterility tests are carried out to validate the assembly techniques. Routine operation requires replacement of the disposable components, such as the pump tube, saline bag, millipore filters and infusion catheters, followed by pyrogen tests (LAL).

Figure 3 Schematic diagram of the bedside  $H_2^{15}O$  infuser

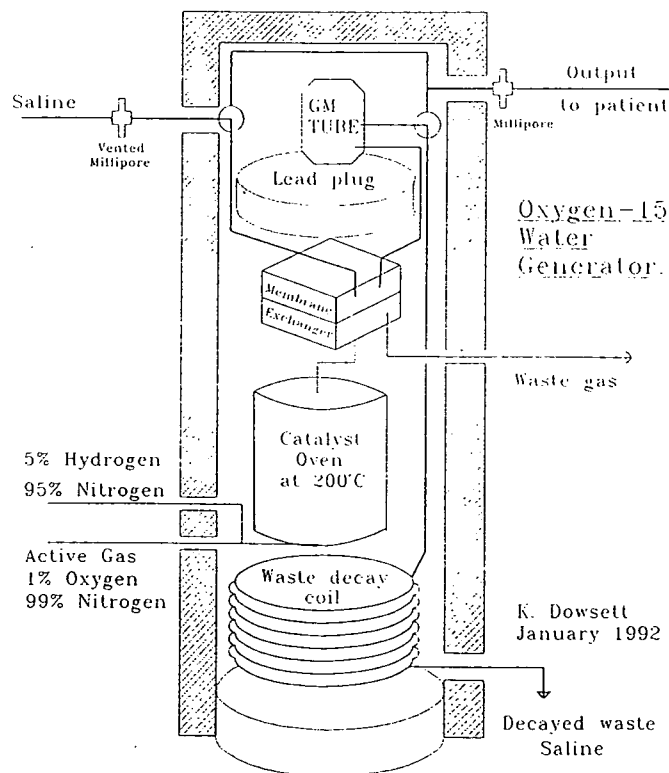
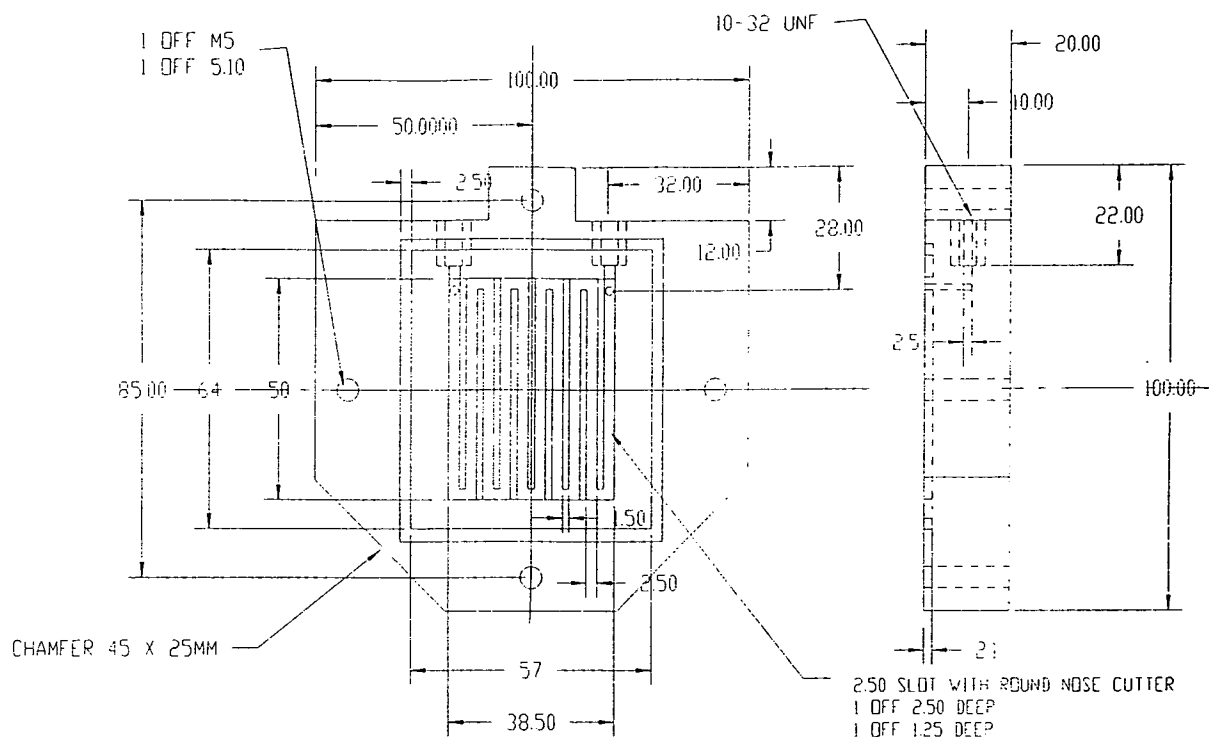


Figure 4 Membrane exchanger design as used in the  $\text{H}_2^{15}\text{O}$  infusor  
(Material acrylic)



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