High-Speed Automated Discrete Blood Sampling for Positron Emission Tomography

Michael M. Graham and Barbara L. Lewellen

Division of Nuclear Medicine, Department of Radiology, University of Washington, Seattle, Washington

A computer controlled blood sampling system was designed specifically for rapid blood sampling for quantitative PET studies and uses solenoids that pinch silastic tubing, a roller pump and an inexpensive fraction collector. The controlling computer is an Apple II plus. The maximum sampling rate is one sample per 2 sec. Typical sample size is 0.90 ± 0.02 g s.d. The loss of blood per sample is 2.6 ml. Tubing dead space is 1.2 ml. The response to a step change in activity between samples is 91% of the expected activity during high-speed sampling and 99% in the slower sampling mode. The major advantage of this device over flow-through detectors is that the blood is available for further processing to measure plasma or metabolite activities. This device has become a useful tool for quantitative PET studies, resulting in reliable sampling, lower radiation dose to personnel and fewer personnel necessary to conduct a study.

J Nucl Med 1993; 34:1357–1360

Quantitative analysis of positron emission tomographic (PET) studies generally requires blood or plasma time-activity curves as well as tissue activity derived from the PET images. The overall accuracy of calculated metabolic rates or parameter estimates depends on the accuracy of the blood counts as much as on the accuracy of the PET data. Although many PET groups have relied on multiple timed samples taken by syringe, there are a number of reasons to develop an automated blood sampling system. The automatic system is likely to be more reproducible than hand sampling in that identical volumes will be drawn at precise predetermined times. The radiation dose to the blood sampling technician can be significant. At some sites, it is so high that the technicians have to be rotated through the position to keep individual radiation exposures within reasonable limits. High-speed hand sampling requires an intensive team effort with three to five people involved in the process. An automated sampler replaces these people, making the process less confusing and less expensive.

Some groups have approached this problem by using a flow-through detector with continuous withdrawal of blood (*1,2*). This scheme works well for high-speed sampling situations such as first-pass studies of blood flow with ^{15}O water but it is not as suitable for studies taking longer times, such as ^{11}C or ^{18}F studies that often last for 1 hr or more. Although it can be used for such studies, the blood flow through the detector must be maintained at relatively high rates, resulting in significant blood loss or the flow rate can be reduced with resultant loss of temporal resolution.

We have chosen to develop a discrete blood sampler that takes multiple samples into test tubes to minimize blood loss, maintains maximal temporal resolution and provides blood samples which can be processed prior to counting. The processing includes simple steps such as centrifugation to obtain plasma samples, or more complex separations with high-performance liquid chromatography (HPLC) to separate the primary tracer from metabolites.

METHODS AND MATERIALS

Description of the Sampler

The design of the sampler was based on experience gained in the design and use of two previous samplers for blood sampling from small animals (*3,4*). Solenoid pinch valves with silastic tubing are used to control the flow of blood, heparinized saline and air. The arrangement of the tubing, valves and roller pump is shown in Figure 1. The blood samples are delivered into test tubes and then moved with an inexpensive fraction collector (Bio-Rad Model 2110, Richmond, CA). The solenoids, roller pump and fraction collector are all controlled with an Apple II computer containing an ADALAB interface card including a relay card. The Apple II computer has been programmed in Pascal to sequence the opening of solenoids and activation of the pump and fraction collector to define the appropriate series of events to obtain the blood samples at predetermined times. The relay closure sequence can be changed fairly easily by writing a small text file that is read by the Pascal program. The text file can be edited easily to create a new sampling sequence without having to recompile the main program.

All tubing that comes in contact with blood is replaced with new sterile tubing for each study. The ability to replace all the tubing with sterilized new tubing for each study is an essential aspect to eliminate the risk of transmitting any infections to patients. The replaceable tubing includes the tubing to the pressurized, heparinized saline, the patient, the sample site, the vacuum waste bottle and through the roller pump. The waste bottle is replaced each time with a new 250-ml evacuated empty bottle with 5 ml of isopropanol added to prevent foaming. It is connected to

Received Nov. 30, 1992; revision accepted Apr. 1, 1993.
For correspondence and reprints contact: Michael M. Graham, PhD, MD, Division of Nuclear Medicine RC-70, University of Washington, Seattle, WA 98195.

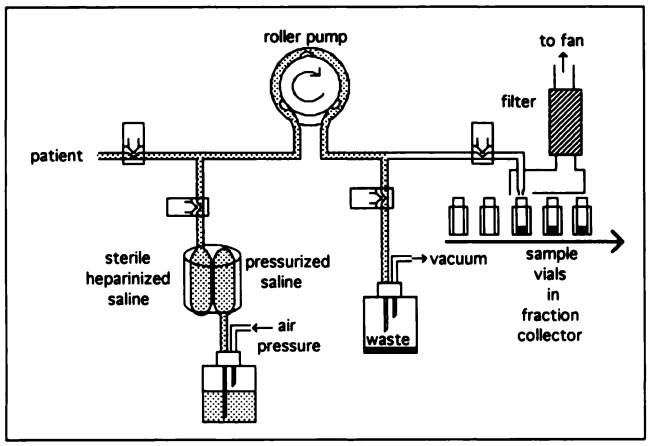

FIGURE 1. Diagram of the tubing, solenoid valves and roller pump in the automatic blood sampler. The dots indicate the portion of the tubing filled with saline at the beginning of a sampling sequence. The filter and fan assembly are for clearing aerosolized blood droplets from the air near the sampling tip.

the tubing with a sterile hypodermic needle and to a second 500-ml waste bottle acting as a trap. The second bottle is connected to a wall vacuum through a 0.22-μ filter to act as a final liquid trap. The saline is 0.9% sterile saline in a 500-ml plastic bag with 2500 U of heparin added to the contents. The bag is placed in a plastic holder along with a second bag connected to a source of pressurized water. This provides a source of sterile heparinized saline. The water for the second bag is pressurized from wall air with a pressure-reducing valve and is monitored with a pressure gauge. A vacuum gauge is also connected to the waste bottle.

As part of a sampler for human blood, it is important to protect personnel working in the vicinity of the sampler from aerosolized blood droplets. This is accomplished with a small fan that withdraws air through a replaceable filter from the region immediately around the sample site where the blood is squirted into heparinized test tubes.

Sampling Sequences

In the slow speed mode (15 sec to 20 min between samples), a roller pump initially withdraws blood at a constant rate into a waste bottle to clear the tubing of diluted blood, and then into the test tube. The test tube is placed in a fraction collector that advances after a sample is obtained. Between samples, the tubing is flushed with saline and saline is back-flushed into the patient's artery. The segment of tubing leading to the sample test tube is also flushed into a second test tube and the residual saline is cleared by opening the sample and vacuum solenoids. This clears the distal tubing contents into the waste bottle. The timing sequence is shown in greater detail in Figure 2.

In the high speed mode (maximum rate of 1 sample per 2 sec) blood is initially shunted to the waste bottle to clear the system dead space and then to the sample vial. Between samples, the distal tubing going to the sample test tube is cleared by suction into the waste bottle. There is no attempt to flush the system with saline because of the time constraints. In essence, this sequence pumps the dead space contents into the test tube and refills the dead space with fresh blood at each time point. The timing sequence is shown in greater detail in Figure 3.

Evaluation of Sampler Characteristics

As part of the evaluation of the sampler, the following characteristics were measured.

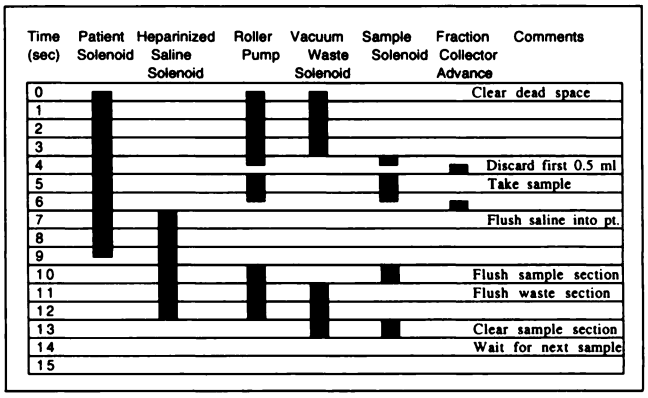

FIGURE 2. Timing sequence for the blood sampler when operating in the slow mode. With this sequence, samples can be obtained at 15-sec intervals. Between samples, heparinized saline is flushed into the patient and the tubing is flushed with saline. The last part of the tubing, leading to the test tubes, is also cleared of blood by suction into the vacuum waste bottle and by flushing clear saline into a test tube. Thus, every other test tube contains a valid blood sample.

Reproducibility of Blood Sample Size. This was measured by preweighing and postweighing a series of test tubes used in a sampling sequence.

Loss of Blood per Sample. This was measured by starting with a clean waste bottle and running the sampler to obtain five samples from a human with ^{11}C carbon monoxide-labeled blood. The decay-corrected count per minute (CPM) per ml of blood was determined (CPM_{blood}), all of the sampled and waste blood was pooled, the volume was measured (V_{total}) and the decay-corrected CPM per ml was determined (CPM_{total}). The total blood volume lost per sample was then calculated from:

$$(V_{total} \cdot CPM_{total})/(CP\ M_{blood} \cdot 5).$$

Dead Space in the Tubing. With a 20-gauge intravenous catheter attached to patient tubing (without a patient), the sampler tubing was filled with saline containing ^{99m}Tc up to the end going into the test tubes. The CPM per ml of the ^{99m}Tc solution was determined. The intravenous catheter was then placed in a beaker containing nonradioactive saline, and the patient and sample solenoids and roller pump were activated. After approximately 20 ml had been pumped through the system, the sampler was turned off. The volume of the pumped saline was determined by weighing

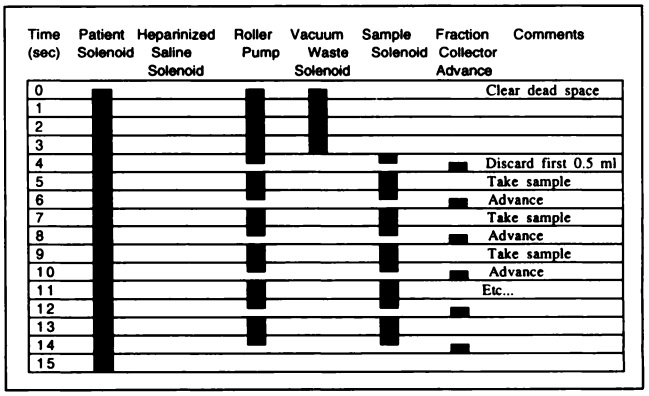

FIGURE 3. Timing sequence for the blood sampler when operating in the fast mode. With this sequence, samples can be obtained at 2-sec intervals.

the test tube, the CPM per ml of the contents was determined and the dead space of the sampling tubing was calculated. The procedure was repeated five times.

Time Delay from Patient to Arrival at Test Tube. The time delay was calculated by dividing the dead space by the measured flow rate of the roller pump.

Dilution of Blood Activity During Sampling. Starting with a clear sampler, blood was sampled from a patient whose blood was labeled with ^{11}C-carbon monoxide. The CPM of the samples was determined by pipetting 0.5 ml from each sample. The correct CPM per ml was determined from blood withdrawn by hand after predrawing 10 ml to clear the tubing of any diluted blood. This measurement is equivalent to the response to a step change in activity since the initial activity is zero.

RESULTS

Blood Sampler Findings

Reproducibility of Blood Sample Size. Running in the rapid sampling mode, sample size was 0.83 g ± 0.02 s.d. Coefficient of variation = 2.4% (n = 20). In the slower sampling mode, sample size was 0.90 ± 0.02 g s.d. Coefficient of variation = 2.2% (n = 20).

Loss of Blood per Sample. In the rapid sampling mode, loss of blood per sample, including the sample itself, was 2.3 ml. In the slower sampling mode the loss was 2.6 ml.

Dead Space in the Tubing. The dead space from the end of the catheter to the point where the blood enters the test tubes is 1.2 ml.

Time Delay from Patient to Arrival at Test Tube. Since the roller pump flow is 4 ml per second, the delay from patient to sampling site is 0.3 sec.

Dilution of Blood Activity During Sampling. In the rapid sampling mode, 91% of the expected activity was present in the first sample with 99% in the next sample. In the slower sampling mode, 99% of the expected activity was in the first sample.

Figure 4 shows eight examples of ^{18}F-fluorodeoxyglucose plasma time-activity curves obtained with this sampler. The fitted curves were obtained with a three-compartment model of the kinetics of an intravascular tracer (5). The coefficients of variation for the fits ranged from 1.8% to 11.9% (mean = 4.9%).

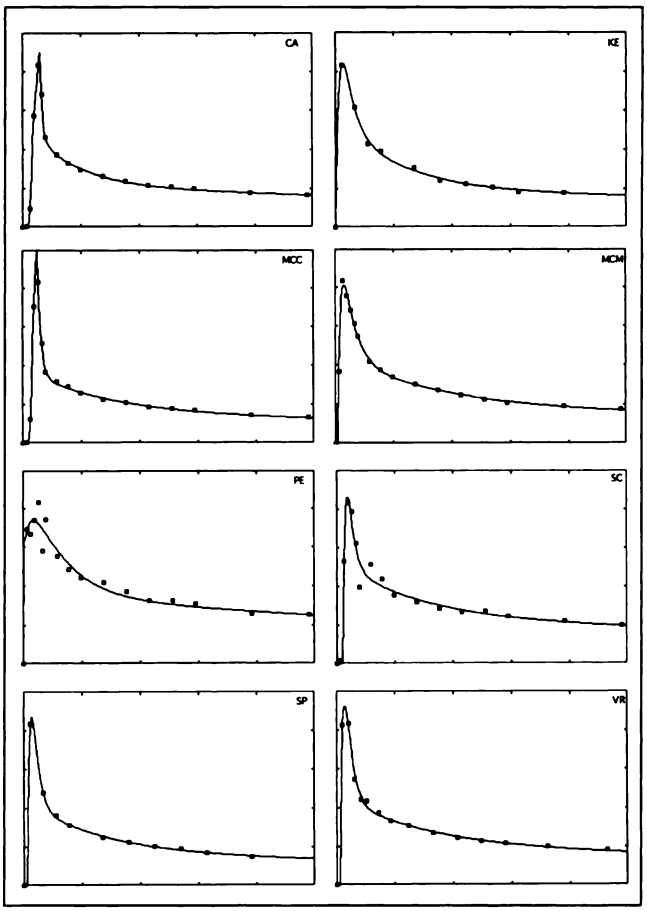

FIGURE 4. Typical ^{18}F-fluorodeoxyglucose plasma time-activity curves obtained with the automated blood sampler using the slow sequence. The points shown are for the first 25 min of the study. The fitted curves are obtained with a three-compartment model of the kinetics of intravascular tracers.

DISCUSSION

There are two major approaches to automated blood sampling for PET studies: flow-through detector systems and discrete sampling. Several groups are using flow-through detector systems, particularly for high temporal resolution of arterial blood following bolus injection of the radiotracer. The temporal resolution is essentially equal to the volume of blood viewed by the detector divided by the flow. The advantages of this approach are that data is acquired rapidly and blood handling is minimized. Disadvantages are that it is less useful for long studies because of limitations on the amount of blood that can be withdrawn and counting is limited to whole blood only.

Discrete sampling has the disadvantages of having lower temporal resolution, requiring more handling of blood and being more mechanically complex. However, one important gain is achieved with discrete sampling; direct access to the blood samples. This means it is possible to manipulate the blood prior to counting. Examples of such manipulation include centrifugation to count plasma, acidification to drive off carbon dioxide and HPLC to determine metabolites.

Automated sampling is most important when high-speed sampling is needed and manual sampling with syringes is not feasible. Although it is not absolutely necessary when the interval between samples is longer (>20 sec), it is still very useful. This is because it takes the samples in a reproducible manner with minimum blood loss and at a preprogrammed time. Inevitably, when manual sampling is used, the operator occasionally becomes distracted and samples are not taken at the correct times. Finally, radiation exposure to research personnel is significantly reduced and fewer personnel are needed to conduct a study.

Although there are several programmable fraction collectors available, none are designed for direct arterial blood sampling and they do not have the capability of back-flushing heparinized saline into the arterial line in between samples.

The automated blood sampler described here has evolved significantly from the first version (*6*). The major changes that took place were the replacement of an old liquid scintillation belt used for test tube movement with a compact, inexpensive fraction collector and redesigning the placement of the solenoids and pump to absolutely minimize tubing dead space. The characteristics of the sampler (described in the Results section) are very acceptable. The blood sample sizes are reasonably reproducible, but this is not particularly critical since the samples are almost always pipetted prior to counting. The loss of blood per sample is acceptable. This amount means that in a typical sampling sequence of 25 samples, the total blood loss is only 65 ml. This is so low largely due to the small dead space in the tubing which is achieved by careful design and the use of small-bore tubing. The 0.3-sec time delay from artery to test tube is so short that it rarely needs to be corrected. In the rapid sampling mode when most of the contents of the tubing are pumped into the test tube while the tubing is filling for the next sample, there is some blurring of one sample into the next. If arterial activity is changing very rapidly, then this will require some correction. In most situations, including bolus injection, the difference from one sample to the next is relatively small so the correction factor is less than 2%. Overall, we found these characteristics very encouraging, confirming our opinion that this was an accurate method to obtain blood samples. This current design has now been in operation for over 3 yr and has proven to be a reliable and essential tool in conducting PET studies at the University of Washington.

ACKNOWLEDGMENT

This work was supported by NIH grant CA42045.

REFERENCES

1. Eriksson L, Kanno I. Blood sampling devices and measurements. *Med Prog Technol* 1991;17:249–257.
2. Muzic, Jr. RF, Nelson AD, Muswick GJ, Voelker WH, Miraldi F. A device for continuous monitoring of arterial positron concentration using a gamma detector [Abstract]. *J Nucl Med* 1988;29:833.
3. Graham MM. A semiautomatic blood sampler for the rat. *J Appl Phys* 1971;30:772–773.
4. Graham MM, Abbott GL. High-speed, bubble-segmented blood sampler for the rat. *J Appl Physiol* 1984;57:1284–1287.
5. Graham MM. Parameter optimization programs for positron emission tomography [Abstract]. *J Nucl Med* 1992;33:1069.
6. Graham MM, Lewellen BL. High speed automated discrete blood sampling for PET [Abstract]. *J Nucl Med* 1988;29:879.