

## The Continuous Measurement of Carbon-14 Activity in Circulating Blood by Liquid Scintillation Counting

B. F. J. Page

Department of Metabolism and Pharmacokinetics,  
Huntingdon Research Centre, Huntingdon, England

During the course of work at the Huntingdon Research Centre on the pharmacological aspects of cigarette smoking, a comparison was required between blood levels of nicotine attained by the intravenous route with those achieved via the mouth or the lungs, as it is obtained by smokers. It was felt that this comparison could best be achieved by the administration of Carbon-14 labelled nicotine and subsequent measurement of Carbon-14 levels in blood by liquid scintillation counting. Initial work showed that it would be desirable to be able to monitor the Carbon-14 levels in blood continuously in order to follow rapid fluctuations in blood level of Carbon-14 nicotine during the initial stages of absorption. It was appreciated that this technique would not differentiate between nicotine and other Carbon-14 labelled species resulting from metabolism, but for a comparative study of nicotine absorption this should not matter.

Flow cell devices for the continuous determination of low-energy  $\beta$ -emitting radionuclides in chromatographic column effluents<sup>1, 2</sup> and a flow cell device for determination of high-energy  $\beta$ - and  $\gamma$ -emitting radionuclides in flowing blood,<sup>3</sup> using flow cells fitted to liquid scintillation counters, have been reported. None of these systems was found to be suitable for the determination of low-energy  $\beta$ -emitting radionuclides in flowing blood, and consequently a flow cell for this purpose was constructed from KL211 polystyrene plastic scintillator (Koch-Light Ltd., Colnbrook, England). The U-shaped flow channel was formed in the plastic scintillator so that as large a contact area as possible existed between the blood and the scintillator for events to occur while still presenting the smallest possible area of blood in the field of view of the photomultiplier tubes. As can be seen in Fig. 1, this was achieved by making the flow channel of flat rectangular cross-section (1 in x 0.025 in). The cell (volume 1 ml) was mounted in an I. C. N. -Tracerlab 'Coruflow' SCE542 manual liquid scintillation counter, fitted with dual photomultiplier tubes linked by anti-coincidence circuitry to an I. C. N. -Tracerlab C.M.F.101 dual ratemeter/integrator. Two 'Coruflow' instruments were normally used, one to monitor venous blood and the other to monitor arterial blood, and each instrument

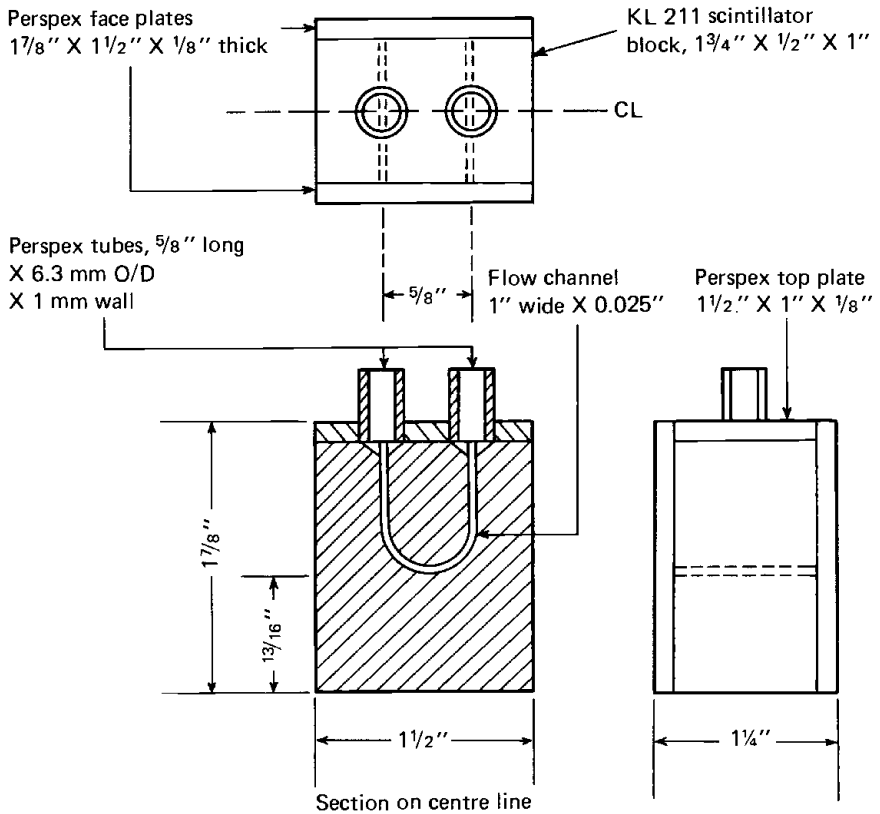


Fig. 1. Plastic scintillator flow cell, manufactured by the Scintillator Division of Koch-Light Ltd., Colnbrook, Buckinghamshire, England.

was connected to one channel of the ratemeter/integrator. The signals from the dual ratemeter/integrator were displayed on a Honeywell 'Electronic 194' dual-pen recorder.

The apparatus was calibrated using both water and whole blood and counting efficiency was found to lie between 1.5 and 3%, depending on the individual cell used. There was some variability between cells, which tended to increase with use. The background count-rate of the cells also increased slowly with use and when this was unacceptable the cells were discarded. Alteration of blood flow-rate through individual cells produced no change in the measured Carbon-14 over the range 0–100 ml min<sup>-1</sup>, as long as there was a constant level of Carbon-14 in the blood passing through the cell. However, when the level of Carbon-14 in the blood passing through the cell was fluctuating, changes in flow produced large differences in the measured Carbon-14 until the Carbon-14 reached a constant level. For this reason, the blood flow through each cell was routinely monitored using a Nycotron blood flowmeter equipped with extracorporeal flow probes. The signals from the blood flowmeters were recorded on a Devices Polygraph recorder.

The baboon was chosen as the experimental animal because it is large enough for the necessary surgical operations and it is a primate. Baboons were anaesthetised with nembutal and given 5000 units of heparin intravenously every 2 h during each experiment, to prevent the blood clotting in the flow cells. The blood was either obtained from the common carotid arteries and internal jugular veins or from the

femoral artery and vein. In each instance the total blood supply was routed through the flow cell system and returned to the blood vessel from which it was taken. It was necessary to pump the venous blood through the cell system, using a peristaltic pump. (The pressure drop through the flow cell system was normally 15–25 mm Hg.) The total blood volume external to the animal through each cell system was of the order of 5–10 ml, depending on the vessels cannulated. The blood was routed to and from the cells through black silicone rubber tubing to prevent the passage of radiation into the cell housing which could give an unacceptable increase in background count-rate. We have used this system to study the absorption of nicotine by the baboon from various sites. Figure 2 shows a typical trace obtained following the

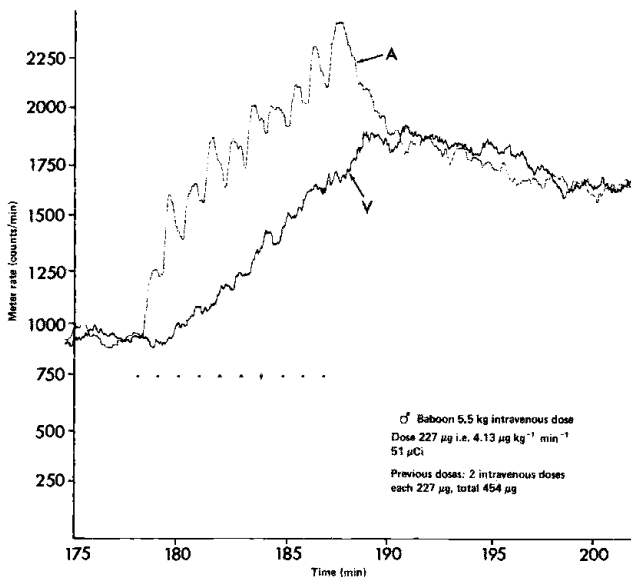


Fig. 2. Arterial (A) and venous (V) concentrations of Carbon-14 (relative units) after repeated i.v. administration of  $^{14}\text{C}$ -labelled nicotine into the radial vein of a baboon. Black triangles represent doses of nicotine. Each dose was 0.1 ml containing 22.7  $\mu\text{g}$  (5.1  $\mu\text{Ci}$ ) nicotine.

intravenous administration of  $^{14}\text{C}$ -nicotine. N-Methyl- $^{14}\text{C}$ -nicotine was obtained from the Radiochemical Centre, Amersham, at a specific activity of 223  $\mu\text{Ci mg}^{-1}$  (free base) and used either as the bitartrate in 0.9% saline solution or buffered to pH 8.5 in sodium bicarbonate buffer. Each dose consisted of 1 ml of solution, administered as 0.1 ml by syringe at one-minute intervals (black triangles on the figure represent each 0.1 ml dose). The trace marked A represents arterial blood Carbon-14 activity and the trace marked V venous blood Carbon-14 activity. In all cases, the ratemeter was used with a time constant of 30 s, in order to give a smoother trace, and blood flows varied between 5 and 40  $\text{ml min}^{-1}$  depending on the individual animal.

In the traces shown, the flow did not vary during the experimental period indicated. Figure 2 clearly shows an individual peak as each dose is injected, whereas these have been effectively damped out in the venous side. The blood was obtained for this experiment from the common carotid arteries and internal jugular veins, so the difference between the venous and arterial Carbon-14 levels could represent to some extent uptake by the brain.

Figure 3 shows the trace obtained following administration of  $^{14}\text{C}$ -nicotine into the lungs *via* the trachea. As can be seen, this is essentially similar to that obtained by the i. v. route, though the arterial peaks are almost absent, and up-

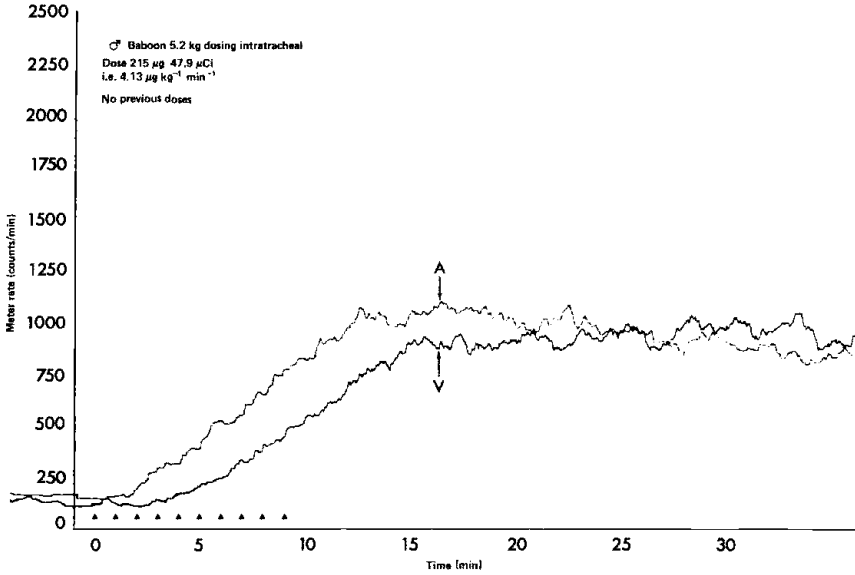


Fig. 3. Arterial (A) and venous (V) concentrations of  $^{14}\text{C}$  (relative units) after repeated administration of  $^{14}\text{C}$ -nicotine into the trachea of a baboon. Black triangles represent doses of nicotine. Each dose was 0.1 ml containing 21.5  $\mu\text{g}$  (4.8  $\mu\text{Ci}$ ) nicotine.

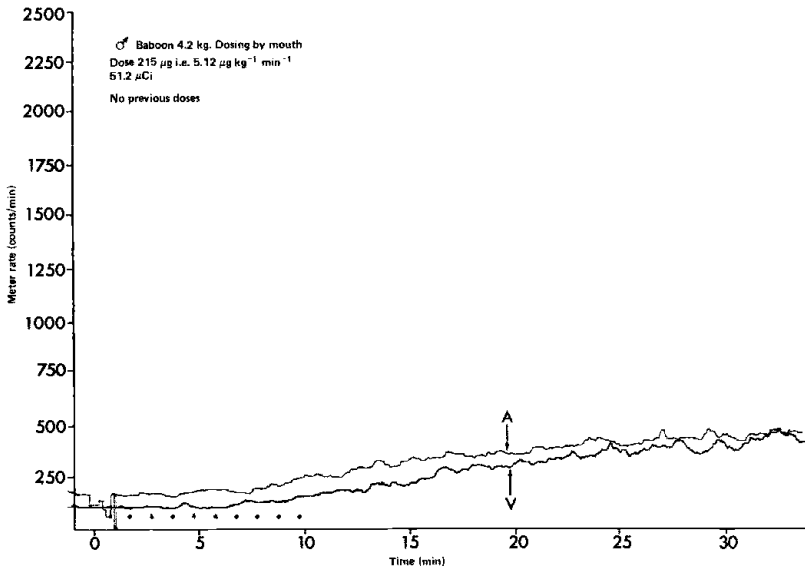


Fig. 4. Arterial (A) and venous (V) concentrations of  $^{14}\text{C}$  (relative units) after repeated administration of  $^{14}\text{C}$ -nicotine into the mouth of a baboon. Black triangles represent doses of nicotine. Each dose was 0.1 ml containing 21.5  $\mu\text{g}$  (4.8  $\mu\text{Ci}$ ) nicotine.

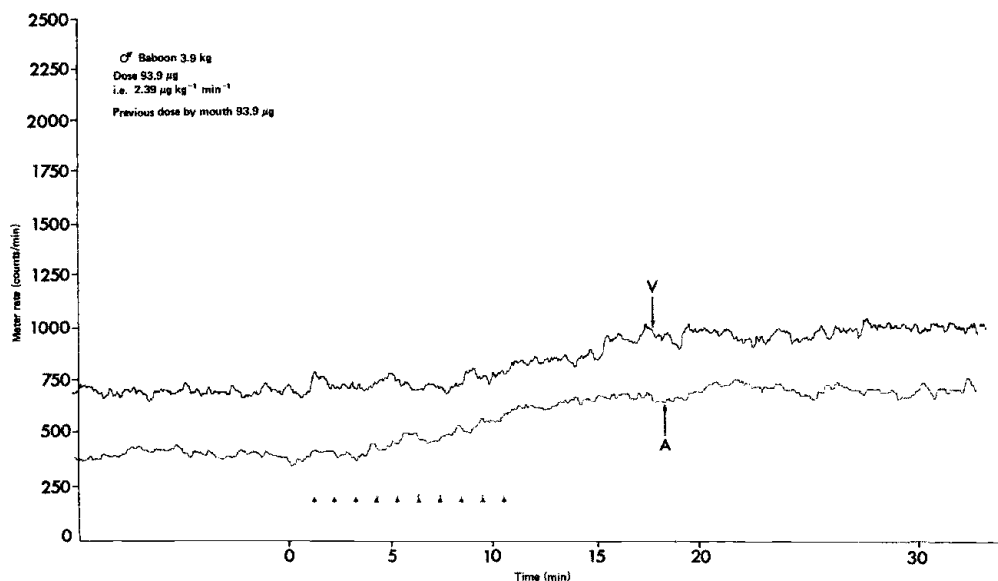


Fig. 5. Arterial (A) and venous (V) concentrations of  $^{14}\text{C}$  (relative units) after repeated administration of  $^{14}\text{C}$ -nicotine into the nose of a baboon. Black triangles represent doses of nicotine. Each dose was 0.1 ml containing 9.4  $\mu\text{g}$  (2.1  $\mu\text{Ci}$ ) nicotine.

take is delayed. Figure 4 shows the trace obtained following the sublingual administration of  $^{14}\text{C}$  nicotine in sodium bicarbonate buffer. Here the trachea and oesophagus were tied off and blocked with vaseline and bone wax to prevent absorption taking place anywhere but in the mouth. The cells were set up to monitor blood from the femoral artery and femoral vein. Figure 4 clearly shows the absorption is much delayed and very much slower by this route. Figure 5 shows the trace obtained following the administration of the dose into the nose. As can be seen, this route demonstrates an absorption pattern somewhere between that obtained by the i.v./intratracheal routes and the mouth. These experiments are illustrative of the uses to which the technique described can be put. We are at present developing the technique to study not only absorption patterns of drugs, but also uptake of drugs by various organs, and we feel that it should be applicable to a variety of problems.

## REFERENCES

- 1 L. Schutte, *J. Chromatogs.* **72**, 303 (1972).
- 2 E. Loos, *Anal. Biochem.* **47**, 90 (1972).
- 3 J. Roba, R. Roncucci, and G. Lambelin, *Analyt. Lett.* **2**, 325 (1969).

## DISCUSSION

N.G.L. Harding: I could not read the time scales of your traces, so I wonder if you could comment upon the features of flow cell systems, namely blood coagulation, haemolysis and infection, which could be important in long-term recovery experiments or work with human patients.

**B.F.J. Page:** Our work to date has only involved terminal experiments with baboons. Anaesthetised animals have been successfully connected to the flow cell system for up to 8 h in the experiments carried out so far. Peristaltic pumps are used to pump the blood through the flow cell systems and to maintain a constant flow rate, so some haemolysis could be occurring. Blood coagulation has not been found to be a problem as long as the animal is kept adequately heparinised.