SUMMARY  We have developed a quantitative autoradiographic technique for the simultaneous measurement of local cerebral blood flow (LCBF) and local cerebral glucose utilization (LCGU) using a combination of ¹⁴C-long-lived and ¹⁸F-short-lived radionuclides as labels. To obtain the LCGU image, a 50-fold greater radioactivity of ¹⁸F than of ¹⁴C was administered and the first exposure was done for 2 hours. Three days later, when most of the ¹⁸F had decayed, a second exposure was done for 5 to 6 days to obtain the LCBF image. ²⁻¹⁸F standards were prepared in each experiment. The technique provides, for the first time, the local glucose flow ratio (LGFR). LGFR, obtained by dividing the LCGU by the LCBF image, was expressed as percent µmol/ml (multiplied by 100). Measurement of these three values in the same animal is expected to prove useful in the investigation of the pathophysiology of the brain. The advantages of this method are that cross contamination is less than 4%, chemical or physical manipulation of the slices is unnecessary, and final results can be obtained within a week.

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Use of Short-Lived ¹⁸F and Long-Lived ¹⁴C in Double Tracer Autoradiography for Simultaneous Measurement of LCBF and LCGU

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TECHNIQUES have been developed for quantitative autoradiographic measurement of local cerebral blood flow (LCBF) using ¹⁴C-iodoantipyrine, and of local cerebral glucose utilization (LCGU) using ¹⁴C-2-deoxy-D-glucose. However, the ordinary single tracer autoradiographic technique has a major disadvantage: it is not possible to measure LCBF and LCGU in the same animal with this technique. This introduces animal-to-animal variability into the final relationship of LCBF and LCGU, especially during pathological states. Since pathophysiology of the implanted brain tumor is complex, heterogeneous local blood flow and local glucose utilization in tumor-bearing brain should be measured in the same brain section. We have accordingly developed a double tracer autoradiographic technique that enables us to measure both values in the same animal and in the same brain section.

Although a number of double tracer autoradiographic techniques have already been proposed, they all require chemical or physical manipulation of the slices. Our technique avoids these steps.

Since this work was completed, an abstract on the use of ¹⁸F-2FDG in double-tracer autoradiographic experiments appeared in the literature.

Methods and Materials

Theoretical Considerations

The theoretic basis of this experiment is the differentation of the energy and half-life of the two radionuclides ¹⁴C and ¹⁸F. Iodoantipyrine and 2-deoxy-2-fluoro-D-glucose (¹⁸F-2-FDG) labelled with ¹⁸F(t½ = 110 min, E avg = 240 KeV, average range in water = 0.62 mm) and ¹⁴C(t½ = 5730 yr, E avg = 45 KeV, average range in water = 0.03 mm) were used. ¹⁸F was the label for the LCGU measurement, and ¹⁴C was the label in the methyl group in position ² of iodoantipyrine for the LCBF measurement.

At exposure time the radioactivity of ¹⁸F in the brain slices was 50 times that of the ¹⁴C. After cutting 20 µm-thick brain sections at −22°C, the first exposure was done for 2 hours to obtain an ¹⁸F image. Three days later (39 half-lives of ¹⁸F) the second exposure, lasting for 5–6 days, was made to obtain the ¹⁴C image. The autoradiographic image from the second exposure was entirely due to the ¹⁴C. In our experiments there was no photographic density from ¹⁸F when the ¹⁴C-exposure was done 3 days after the ¹⁸F-exposure. This finding was confirmed by exposing x-ray film to the ¹⁸F standards used during the first exposure.

Reivich et al12 estimated the lumped constant for FDG in the rat as being 17.9% lower than that determined for DG. The values of LCGU measured simultaneously in the same rats using ¹⁸F-FDG and ¹⁴C-DG agreed with the results of Reivich et al.11 In our experiments the value of 0.397 for the lumped constant, as estimated by Reivich et al, was used when calculating LCGU. Since the rate constants do not greatly influence the final results when Sokoloff’s operational equation is used,12,13 we used the rate constants for DG obtained by Sokoloff et al.2

Preparation of Standards

Tissue concentration of ¹⁴C was measured relative to the commercially available ¹⁴C-methyl methacrylate standards.

¹⁸F standards were prepared in each experiment. From 10 to 100 µCi/g of ¹⁸F-compounds were mixed with one gram homogenized rat brain before the study.

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The radioactivity of $^{18}$F decayed to $\frac{1}{2}$ or $\frac{1}{3}$ until the exposure time. The compounds used to prepare the $^{18}$F-standard were obtained from $^{18}$F-labelled 2-FDG or from $^{18}$F-labelled 2-fluoro-D-mannopyranoside obtained during $^{18}$F-FDG synthesis, or were formed by reacting F with 0.5 N NaOH. The standard was frozen by immersing it in liquid freon-12. Half of it was sliced in a cryostat into 20 μm-thick sections which were then exposed in an x-ray cassette along with brain tissue slices. The other half was assayed for $^{18}$F radioactivity in a gamma scintillation counter to obtain the concentration in μCi/g. Calibration curves were obtained by plotting the extinction of the autoradiogram against $^{18}$F-radioactivity (fig. 1).

Preparation of Animals

Female Wistar rats weighing 200–220 g were fasted, except for water, for 14–16 hours before the experiment. At least 2 hours before the study, the femoral artery and vein were catheterized under light halothane anesthesia. The rat was immobilized in a loose-fitting plaster cast. Arterial blood pH, pCO$_2$, and PO$_2$ were checked by a micro blood gas analyser, and blood pressure was measured just before the CBF measurement. Rectal temperature was maintained with a heating pad at approximately 37°C.

Measurement of LCGU

The method for measuring cerebral glucose utilization using $^{18}$F-FDG is based on the $^{14}$C-DG autoradiographic technique described by Sokoloff et al. $^{2}$ $^{18}$F-FDG was prepared by a method we have described elsewhere. $^{16}$ $^{18}$F-F$_2$ is produced by irradiating 0.5% F with $^{20}$Ne(d, α) $^{18}$F nuclear reaction. $^{17}$ After irradiation $^{18}$F-F$_2$, is bubbled through a solution of sodium acetate in glacial acetic acid at room temperature. Triacetlylglycerol is added, the acid extracted with CH$_2$Cl$_2$, methylene chloride evaporated, and the residue hydrolyzed with 2N HCl. After evaporation under reduced pressure, $^{18}$F-labelled 2-FDG is dissolved in 1 ml of phosphate buffer in saline solution (pH = 7) and sterilized by filtration through 0.2 μm Millipore. Three to four mCi of $^{18}$F-FDG (specific activity 374.8 mCi/mmole) at the end of synthesis, or between 250 and 350 mCi/mmole at the time of administration) in 1 ml of normal saline buffer was injected intravenously as a bolus. Following tracer injection, the arterial blood samples were withdrawn at increasing time intervals up to 44 min. (Animal blood loss could be reduced by using a technique we recently described.)$^{15}$ At 45 min after injection the rat was decapitated. The blood samples were centrifuged and 20 μl of the plasma was used to measure $^{18}$F concentration. The remainder of the plasma sample was used to determine the glucose concentration. LCGU was calculated according to the equation of Sokoloff et al.$^{2}$

Measurement of LCBF

Local cerebral blood flow was measured in the same animal using the iodoantipyrine technique. $^{1}$ Thirty μCi of $^{14}$C-IAP was injected intravenously for 1 minute, 44 min after $^{18}$F-FDG administration. A constant infusion was used and arterial blood samples were withdrawn at 5- to 8-second intervals after injection. The animal was decapitated 1 minute after the start of the $^{14}$C-IAP study and the brain was quickly removed and frozen at −30°C in liquid freon-12.

The autoradiographic tissue preparation was then done in the usual manner, $^{2}$ which requires about 60 minutes from decapitation to exposure. The tissue concentration of $^{14}$C-IAP was determined by the quantitative autoradiographic technique.$^{3,18}$ The concentration of $^{14}$C-IAP in the arterial blood was determined by measuring the radioactivity of arterial samples with a liquid scintillation counter 3 days later, when the $^{18}$F had decayed. LCBF was calculated using the operational equation described by Sakurada et al.$^{1}$ A tissue-blood partition coefficient for iodoantipyrine of 0.8 was used.

Glucose-flow Ratio

$^{18}$F and $^{14}$C images were analyzed quantitatively by both manual densitometry and image analyzer.$^{19}$ The image analyzer system is essentially a computerized scanning micro-densitometer. The optical density of each spot on the autoradiogram is stored in a computer, converted to the actual values for LCGU and LCBF, and displayed on a color monitor. The local glucose flow ratio (LGFR), was calculated by the following equation: \[ \text{LGFR} = \frac{\text{LCGU}}{\text{LCBF}} \times 100 \text{(% μmol/ml)} \]. This formula can be used only for double tracer autoradiograms, that is, when both values are measured in the same animal and in the same slice.

Results

Physiological Variables

Table 1 shows the mean arterial blood pressure, blood gases, and pH recorded just before measurement.

![Figure 1. $^{18}$F and $^{14}$C standard curves in the first exposure (for 2 hours). The relationship between tissue concentration of $^{18}$F and optical density is linear over the concentrations ranging from 5.8 to 42.7 μCi/g. Whole structures were located in the shielded range.](image-url)
of the CBF, and the plasma glucose concentration measured at the start of the experiment. The hematocrit immediately before injection of $^{13}$C-IAP was about 4% lower than at the time of injection of $^{18}$F-FDG.

Validity of the $^{18}$F and $^{14}$C Double Tracer Method

We administered 3 to 4 mCi of $^{18}$F-FDG (1.5 mCi at exposure time) and 30 mCi of $^{14}$C-IAP. The tissue concentration of the radioisotope ranges between 5 and 45 $\mu$Ci/g for $^{18}$F, and between 100 and 900 nCi/g for $^{14}$C. Our preliminary experiment revealed that if the $^{18}$F concentration in tissue approximated the levels mentioned above, a 2-hour exposure was optimal for obtaining the $^{18}$F image.

Figure 1 shows the relationship between optical density and tissue concentration of $^{18}$F and $^{14}$C from various standard sources in the first exposure, which lasted 2 hours. In this figure the scale of the concentration was determined by the ratio of the administered activity of the two tracers (1.5 mCi at exposure time of $^{18}$F, and 30 mCi at exposure time of $^{14}$C). A linear relationship exists between photographic extinction and radioactivity for the $^{18}$F standards over a concentration range from 5.0 to 42.5 $\mu$Ci/g. (Sokoloff et al reported similar findings). These standards covered concentration of the isotope in all structures examined in this experiment. Our single-tracer autoradiographic experiment revealed that tissue concentration of $^{14}$C-DG and $^{14}$C-IAP is close in the normal physiological state, that is, when LCBF and LCGU are coupled. Since coupling is also expected in our double tracer $^{18}$F-FDG experiment, the cross contamination of $^{14}$C in the first exposure would be less than 2%.

In the second exposure, done 3 days later, $^{18}$F standards prepared and used in the first exposure along with $^{14}$C standards and brain slices. By this time $^{18}$F radioactivity decayed completely to $\frac{1}{2}$ of the initial radioactivity, and no extinction produced by $^{18}$F standards was observed in the second exposure.

Simultaneous Measurement of LCGU and LCBF

The results of the simultaneous double tracer measurement of LCGU and LCBF in normal awake rats compared favourably with those obtained by the single tracer autoradiographic technique as described in the literature (table 2). The difference between results obtained by the single tracer and double tracer techniques is less than 10%, and is statistically insignifi-
obtained from the same animal and the same slice. In other double tracer techniques, cross contamination must be subtracted or removed by a physical process and introduction of additional error in the final results cannot be avoided. In the other methods similar to that outlined in this paper, before a $^3$H-2-DG image can be obtained $^{14}$C-4-IAP must be washed away in one of the slices, the contribution to the density from $^{123}$I must be mathematically subtracted, or the beta radiation from $^{14}$C stopped by a thin sheet of plastic or one tracer ($^{14}$C-IAP) extracted from slices, before both functional variables are calculated. These steps reduce the likelihood of an accurate reading, since even slight contamination by the blood flow tracer in the glucose image could prove serious in certain pathological states.

The "wash away" technique might have another drawback as well: part of the $^3$H-2-DG-phosphate or free deoxyglucose present in extracellular space may be lost during the washing. This is especially important when LCGU is uncoupled from LCBF. A common form of uncoupling is decreased blood flow and steady or increased glucose utilization. In the method described in this paper, when this type of uncoupling is encountered $^{14}$C contamination in the $^{18}$F image is slight, and the combination of the radio-labelled tracers in pathological conditions works in our favor. Reversed uncoupling (decreased glucose utilization and steady or increased blood flow) also occurs. In this case, a 50% decrease in LCGU with flow within the normal range would reduce the tissue concentration of $^{18}$F to roughly 50%, and according to the standard calibration curve (fig. 1), the contamination of $^{14}$C in the $^{18}$F image would still be less than 4%. When either coupling or uncoupling is involved, the method described here produces less cross-contamination than do the other two techniques in which this type of pathology was mentioned. Although both physiological factors are measured in the same animal, they are not measured quite simultaneously because the FDG method involves a weighted average throughout 45 min.

The present study provides the first local glucose flow ratio image. This ratio should be calculated only on slices that are anatomically identical and in which cross contamination between two isotopes can be avoided. LGFR is not critical in the normal brain, which can afford to extract a surplus of glucose but not of oxygen. Since there is little metabolic reserve of oxygen in brain tissue, the regional blood flow can be taken as an indicator for the oxygen supply. In other words, the oxygen supply is correlated with the blood flow. The oxygen supply would accordingly determine the metabolic ratio between aerobic and anaerobic glycolysis. Consequently, the glucose utilization-blood flow ratio could be used as an indicator of aerobic metabolism. If there is a discrepancy between energy demand and oxygen supply, anaerobic glycolysis will occur to compensate for the shortage of oxygen and in this pathological state an increase in GFR will be observed. The GFR obtained in our work ($0.76 \pm 0.06$) compares well with the same ratio calculated from the standard calibration curve (fig. 1).
published data on LCBF\(^\text{1}\) and LCGU.\(^\text{2}\) The GFR is thus an important indicator for assessing cerebral energy metabolism in acute focal ischemic conditions and experimental brain tumors. It should be mentioned that absorption in the tissue slices of beta radiation from \(^{14}\text{C}\) might be higher than that from \(^{18}\text{F}\). This absorption would result in lower optical density which would give an artificially high LGFR in the white matter (table 3).

Since \(^{18}\text{F}\) has a relatively low energy and is a pure positron emitting radionuclide, the resolution of different brain structures in \(^{18}\text{F}\)-autoradiograms is almost as good as that in \(^{14}\text{C}\) images. Because the half-life of \(^{18}\text{F}\) is only 110 min, it is necessary to have a source of supply nearby. This should not prove to be a major problem, however, since \(^{18}\text{F}\) can be produced in any commercial accelerator or nuclear reactor. Most medical research centres now have access to this type of equipment.

The major disadvantage of the method used in our experiment is that the rate and lumped constants for FDG in rats are not known; however, this is not as serious as it might appear. Using 0.397 for the lumped constant, as estimated by Reivich et al.,\(^\text{12}\) and the DG rate constants resulted in excellent agreement in the final values for the glucose utilization calculated for both tracers. Since the model is not highly sensitive to the values of rate constants used, substitution of DG rate constants for FDG rate constants is justified.\(^\text{13,15}\) These results support the validity of the calculation for lumped and rate constants, at least under normal conditions.

Another disadvantage of our method is the need to prepare standards in each experiment. This problem could be overcome by calibrating standards made from a long-lived positron or pure beta-emitter. However, in double tracer autoradiography, these troublesome procedures cannot be avoided except through the method described by Furlow et al.\(^\text{9}\)

The advantages of this autoradiographic method definitely outweigh these drawbacks, however. There are many ways to combine not only \(^{18}\text{F}\)-FDG and other \(^{14}\text{C}\)-labelled compounds, but also \(^{18}\text{F}\)-labelled tracers with \(^{14}\text{C}\)-labelled tracers to measure different biochemical variables in the same animal. The technique therefore has great potential for measuring physiological variables in a number of pathological conditions.

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**References**

Use of short-lived 18F and long-lived 14C in double tracer autoradiography for simultaneous measurement of LCBF and LCGU.

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