Simultaneous Measurement of Blood Flow and Glucose Metabolism
By Autoradiographic Techniques

G. Mies, M.D., I. Niebuhr, and K.-A. Hossmann, M.D.

SUMMARY A double tracer autoradiographic technique using 125I-iodo-antipyrine and 14C-deoxyglucose is presented for the simultaneous measurement of blood flow and cerebral glucose utilization in the same animal. 125I is a gamma emitting isotope with a half life of 8.06 days and can be detected with adequate resolution on standard autoradiographic films. Autoradiograms are made before and after decay of 125I; the time interval between the 2 exposures and the concentration of the 2 tracers is adjusted to avoid significant cross-contamination. In this way, 2 film exposures are obtained which can be processed quantitatively like single tracer autoradiograms.

The validity of the method for the investigation of local coupling of flow and metabolism was tested under various physiological and pathophysiological conditions. Coupling was tight in barbiturate-anesthetized healthy animals, but not under halothane anesthesia where uncoupling occurred in various subcortical structures. Focal seizures induced by topical application of penicillin on the cortical surface led to a coupled increase of metabolism and flow in thalamic relay nuclei but not at the site of penicillin administration where increased glucose utilization was not accompanied by similar increase in blood flow. Both coupled and uncoupled increases in local glucose utilization were observed in spreading depression and in circumscribed areas of experimental brain tumors.

The results obtained demonstrate that double tracer autoradiography allows the very precise local assessment of cerebral blood flow and glucose utilization, and, therefore, is particularly suited to the study of regional coupling processes under various experimental conditions.

METHODS

1. Theoretical Considerations

The present technique is based on the autoradiographic differentiation between 2 radioactive tracers with different half-life and different autoradiographic sensitivities (fig. 1). 125I (half-life 8.06 days) and 14C (half-life 5760 years) are used, the blackening of the photographic film by the gamma-emitting isotope 125I being at least 10 times greater than that by the beta-emitter 14C. When the sections are exposed immediately after the injection of the 2 tracers, the film is blackened almost exclusively by 125I with negligible contamination by 14C. After several weeks, the radio-
activity emitted by $^{131}$I has decayed, and a film exposed to the same brain section is now almost exclusively blackened by $^{14}$C. In order to compensate for the lower sensitivity of the film to $^{14}$C, the second exposure has to be several times longer than the first. $^{14}$C is used to label deoxyglucose for measurement of the metabolic rate of glucose, and $^{131}$I is used for labeling iodo-antipyrine for the measurement of cerebral blood flow.

2. Preparation of Calibrated Autoradiography Standards

a) $^{131}$I standards: $0.2$–$1.1$ μCi of $^{131}$I-iodo-antipyrine were mixed with $0.2$ g gelatine and $0.8$ ml distilled water and heated to $60°$ C until the gelatine dissolved. Aliquots of the suspension were weighed into a counting vial in order to assay $^{131}$I-activity/g suspension medium and placed in a gamma counter (Biogamma II, Beckman Instruments, Fullerton, CA). The rest was frozen at $-70°$ C in methylbutane and mounted on an object holder. Twenty μm thick sections were cut at $-20°$ C with a cryostat (Slee, Mainz, FRG), mounted on coverslips and dried on a hot plate at $60°$ C for $5$ minutes.

To test the accuracy of gelatine standards, brain tissue standards were also prepared. In $3$ rats, different amounts of $^{131}$I-iodo-antipyrine ($30$ to $90$ μCi) were injected intravenously, brains were dissected after one hour, and radioactivity was determined in half of the brain using a beta counter (LS 7000 Beckman Instruments, Fullerton, CA). The other half of the brain was frozen and sections of $20$ μm were exposed for $1$ week for quantitative autoradiography. Calibration curves were obtained by plotting extinction of the autoradiogram against $^{14}$C-radioactivity of the aliquot sample.

b) $^{14}$C standards: Commercially available $^{14}$C methyl methacrylate standards were used for the autoradiographic determination of $^{14}$C-radioactivity. The standards were calibrated against brain tissue analogous to that described for $^{131}$I. In $3$ rats different amounts of $^{14}$C-iodo-antipyrine ($30$ to $70$ μCi) were injected intravenously, brains were dissected after one hour, and radioactivity was determined in half of the brain using a beta counter (LS 7000 Beckman Instruments, Fullerton, CA). The other half of the brain was frozen, and sections of $20$ μm were exposed for $1$ week for quantitative autoradiography. Calibration curves were obtained by plotting extinction of the autoradiogram against $^{14}$C-radioactivity of the aliquot sample.

3. Measurement of Cerebral Blood Flow

Local cerebral blood flow was measured using the autoradiographic iodo-antipyrine technique described by Sakurada et al. $^{1}$ Seventy μCi $^{131}$I-iodo-antipyrine (specific activity 1.0–4.9 mCi/mg; radionuclidic and radiochemical purity $99\%$; NEN Chemicals, Dreieichenhain, FRG) in $1$ ml Ringer solution was infused intravenously for one minute and arterial blood samples were withdrawn at $10$ second intervals after injection. The animal was sacrificed immediately thereafter by systemic administration of potassium chloride. The brain was quickly removed, frozen at $-70°$ C in methylbutane, $20$ μm sections were dried and then immediately placed together with freshly prepared $^{131}$I- standards on photographic film for $24$ hours. $^{14}$C-standards were also exposed to evaluate cross contamination by $^{14}$C-deoxyglucose (see below). The tissue concentration of $^{131}$I-iodo-antipyrine was determined by quantitative autoradiography, and the blood content of $^{131}$I-iodo-antipyrine by measuring radioactivity in blood samples in a gamma counter during exposure period. Blood flow was calculated according to Reivich et al. $^{2}$ using a tissue-blood partition coefficient for iodo-antipyrine of $0.8$. $^{3}$

4. Measurement of Cerebral Glucose Utilization

Local glucose utilization was measured in the same animals prior to determination of cerebral blood flow (see above). Thirty min before the end of the experiments, $30$ μCi $^{14}$C-deoxyglucose (specific activity
were cannulated with polyethylene catheters, and the arterial blood samples were withdrawn at increasing time intervals from 10 sec to 10 min following tracer injection. After flow measurement the brain was frozen and cut in a cryostat. Eight weeks after the experiment, i.e. after 131I-iodo-antipyrine had decayed to about 3% of the original activity, the sections were placed together with 14C-standards for one week on photographic film. 131I-standards used for the previous flow measurement were also exposed for the determination of cross contamination. Plasma samples were measured in a scintillation counter for assaying 14C-radioactivity and plasma glucose content was determined with a glucose analyzer (Beckman Instruments, Fullerton, CA).

Local glucose metabolism was calculated according to Sokoloff et al. The following rate constants were used: \( k_1 = 0.189, k_2 = 0.245, k_3 = 0.052 \) for grey matter and \( k_1 = 0.079, k_2 = 0.133, k_3 = 0.02 \) for white matter of the rat brain, respectively. The lumped constant for local glucose metabolism was 0.483.

5. Animal Preparation

 Autoradiographic double tracer measurements were performed in 33 BD IX rats of both sexes weighing 150–300 g. The rats were anesthetized either by an intraperitoneal injection of pentobarbital (50 mg/kg) or with 0.8% halothane. The femoral arteries and veins were cannulated with polyethylene catheters, and the animals subsequently tracheotomized, immobilized with curare (Curarin-Asta) and artificially ventilated with 30% oxygen. Body temperature, blood gases and arterial pH were maintained within normal limits. Arterial blood pressure was monitored using a pressure transducer. The heads of the animals were fixed in a stereotaxic frame, and the EEG was recorded with bipolar silver-ball electrodes brought to the cortical surface. Penicillin was replenished every 10 min until the end of the experiment. Seizure was demonstrated by EEG spike activity.

 a) The local 14C-deoxyglucose content was determined by quantitative autoradiography after complete decay of 131I-iodo-antipyrine.

 b) Spreading depression (8 rats): a small burrhole (diameter 1 mm) was made over the right hemisphere and the exposed dura was covered with cotton wool moistened with warm Ringer solution until the start of the experiment. Two microcalomel electrodes were positioned over the left and right side of the frontal brain to record the DC-potential. Spreading depression was evoked by placing a filter paper soaked with 4 molar potassium chloride on the exposed dura, and replenishing the solution every 10 min until the end of the experiment.

 Successful maintenance of spreading depression was determined by suppression of EEG activity and the repetitive negative shifts of the DC-potential, as monitored on a polygraph.

 c) Focal penicillin seizures (5 rats): The parietal cortex of the right hemisphere was exposed as described above. Additionally, the dura was split under an operating microscope. Penicillin seizures were initiated by application of 20 μl sodium penicillin G on the cortical surface. Penicillin was replenished every 10 min until the end of the experiment. Seizure was demonstrated by EEG spike activity.

 d) Experimental brain tumors (9 rats): Intracerebral tumors were produced by stereotactical implantation of 2000 suspended cells of a rat glioma clone (RGI 2.2, courtesy Dr. H.-D. Mennel) into the caudate-putamen of the right hemisphere. Animals were kept on a standard diet until the first neurological symptoms appeared (usually after 2 to 3 weeks), and then investigated as described above.

### Results

1. Validation of the Method

 The autoradiographic differentiation between 14C- and 131I-radioactivity was tested by exposing appropriate standards of increasing radioactivity. The first exposure was performed immediately after preparation of the standards for a duration of 24 hours, and the second exposure 8 weeks later for a duration of 7 days (fig. 2). Both tracers exhibited a linear relationship between photographic extinction and radioactivity over a concentration range between 0.2 and 1.4 μCi/g. Extinction by 131I was about 10 times greater than that of 14C because of the greater sensitivity of the film to gamma radiation. For practical purposes, contamination of 131I by 14C was considered to be negligible when the latter contributed to less than 10% of the total extinction since the resolution of quantitative autoradiography is of this order of magnitude (compare fig. 2 for the exposure of standards). As indicated in figure 3, this condition was fulfilled when radioactivity of 131I in the brain tissue was about 1.5 times higher than that of 14C. Only under conditions of ischemic stimulation of anaerobic glucose utilization may significant contamination occur because in the affected region 131I-iodo-antipyrine is decreased and 14C-deoxyglucose increased. In this case, correction of 131I extinction by 14C-activity may be necessary to obtain the correct value.

 After an interval of 8 weeks, brain sections were again exposed for quantitative autoradiographic assessment of 14C. By this time 131I had decayed to less than 3% of the initial activity and was no longer visible on the autoradiogram although the exposure time was increased to one week. Contamination of 14C by 131I, in consequence, was virtually absent.

 According to the data given in figure 2, the following simple test was made in each experiment to determine the adequate autoradiographic differentiation between the 2 isotopes:

 a) The local 14C-deoxyglucose content was determined by quantitative autoradiography after complete decay of 131I-iodo-antipyrine.

 b) The local 14C-deoxyglucose content being known, contamination of 131I-activity by 14C was determined using the 14C-standards as references. Contamination...
Double tracer autoradiography

was considered to be negligible when exposure by $^{14}$C amounted to less than 10% of the total extinction; when more than 10%, the value was subtracted from total extinction.

2. Animal Experiments

a) Control Experiments: In the barbiturate-anesthetized animals, the autoradiographic representation

![Figure 2. Exposure of $^{131}$I- and $^{14}$C-standard immediately after preparation and 8 weeks later. Note the complete decay of $^{131}$I-standards during this interval. Duration of first exposure was 24 hours and of second exposure 1 week.](image)

of local flow and glucose utilization of the same tissue section was almost identical (fig. 4A). In agreement with earlier reports, a homogeneous distribution of flow and metabolism was observed, the activity in the grey matter being about 1.5 times higher than that in the white matter. In the table regional cerebral blood flow and cerebral glucose utilization found in 15 different structures are summarized. The average cortical flow rate was 77.5 ± 1.9 ml/100 g/min, and the average cortical glucose utilization 67.2 ± 2.1 μmol/100 g/min.

In halothane-anesthetized animals, a distinct local dissociation between flow and metabolism was

![Figure 3. Relationship between tissue concentration ratio of $^{131}$I-iodo-antipyrine and $^{14}$C-deoxyglucose (abscissa) and contamination of $^{131}$I-autoradiogram by $^{14}$C-activity (ordinate, expressed as percent extinction). Measurements were performed in various regions under different experimental conditions (closed circles: regions with coupling of flow and metabolism; open circles: increased glucose metabolism without coupled increase of flow). Note that contamination is less than 10% when tissue concentration ratio is higher than 1.5.](image)

TABLE  Regional Cerebral Blood Flow and Cerebral Glucose Utilization of Barbiturate Anesthetized Rats Measured by Double Tracer Autoradiographic Technique. Values are Means ± SE, n = 6.

<table>
<thead>
<tr>
<th>Structures</th>
<th>Cerebral Blood Flow ml/100g/min</th>
<th>Cerebral Glucose Utilization μmol/100g/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cerebral cortex</td>
<td>77.5 ± 1.9</td>
<td>67.2 ± 2.1</td>
</tr>
<tr>
<td>Lateral geniculate body</td>
<td>74.2 ± 2.6</td>
<td>53.5 ± 4.0</td>
</tr>
<tr>
<td>Medial geniculate body</td>
<td>68.1 ± 3.3</td>
<td>62.5 ± 2.0</td>
</tr>
<tr>
<td>Thalamus: lateral nucleus</td>
<td>73.1 ± 2.7</td>
<td>57.2 ± 3.2</td>
</tr>
<tr>
<td>Thalamus: ventral nucleus</td>
<td>69.7 ± 3.8</td>
<td>52.9 ± 2.8</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>55.7 ± 1.9</td>
<td>38.0 ± 3.3</td>
</tr>
<tr>
<td>Caudate-putamen</td>
<td>80.8 ± 3.9</td>
<td>64.7 ± 3.3</td>
</tr>
<tr>
<td>Hippocampus: gyrus dentatus</td>
<td>63.2 ± 2.7</td>
<td>44.7 ± 2.5</td>
</tr>
<tr>
<td>Accumbens nucleus</td>
<td>74.8 ± 5.4</td>
<td>64.8 ± 5.6</td>
</tr>
<tr>
<td>Mamillary body</td>
<td>65.5 ± 3.0</td>
<td>52.0 ± 3.4</td>
</tr>
<tr>
<td>Amygdala</td>
<td>67.7 ± 6.5</td>
<td>49.1 ± 4.0</td>
</tr>
<tr>
<td>Septal nucleus</td>
<td>72.8 ± 5.0</td>
<td>47.3 ± 4.8</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>64.1 ± 8.8</td>
<td>38.2 ± 2.2</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>47.2 ± 1.9</td>
<td>40.4 ± 1.3</td>
</tr>
<tr>
<td>Cerebellar cortex</td>
<td>82.4 ± 5.2</td>
<td>48.0 ± 5.3</td>
</tr>
</tbody>
</table>
FIGURE 4. Double tracer autoradiographic measurement of blood flow with $^{11}$I-iodo-antipyrine and glucose consumption with $^{14}$C-deoxyglucose in animals anesthetized with barbiturate (A) and halothane (B). Note the uncoupled increase of metabolism in the hippocampus under halothane anesthesia.

apparent. In the stratum pyramidale of the dorsal hippocampus, and in the nucleus parafascicularis of the midbrain, glucose utilization was more than 2 times higher than that in adjacent nuclei, whereas blood flow was only slightly increased (fig. 4B). The average cortical flow and glucose utilization was $110.9 \pm 2.7$ ml/100 g/min and $77.1 \pm 1.9$ μmol/100 g/min, respectively.

b) Spreading Depression: Spreading depression was induced in 8 rats under barbiturate anesthesia by topical application of 4 molar potassium chloride on the exposed parietal cortex, i.e. in the center of the territory supplied by the middle cerebral artery. Measurements were started 15 min after the initiation of spreading depression, i.e. after steady state conditions were present. Glucose utilization increased by more than 66% in the cortex supplied by the middle cerebral artery, but there was a sharp demarcation between this and the brain territory supplied by the anterior cerebral artery in which glucose utilization was not increased (fig. 5A). In the opposite hemisphere and in subcortical structures, glucose utilization was slightly depressed. In comparison to normal control animals, glucose utilization was approximately 15% lower. Cortical blood flow increased in parallel with the increased glucose utilization, but there was not such a sharp demarcation between the affected territory and that of the anterior cerebral artery. In subcortical structures, cerebral blood flow, in contrast to glucose utilization, was not decreased, indicating an uncoupling of flow and metabolism.

b) Penicillin Seizures: In 5 rats under halothane anesthesia, focal seizures were evoked by topical application of penicillin on the exposed cortical surface. EEG recording revealed focal spiking during the whole period of measurement, suggesting that steady state conditions were present during the observation period. As demonstrated in figure 5B, a striking uncoupling of the metabolic rate of glucose and blood flow was present in the area of penicillin application. In this region, the metabolic rate for glucose was increased by more than 100% but blood flow did not change significantly. A coupled increase of both flow and metabolism was found in the lateral geniculate body and in the ventromedial nucleus of the hypothalamus. A dissociation between metabolism and
Blood flow also occurred in the stratum pyramidale of the dorsal hippocampus and in the nucleus parasfascicularis; however, this was not a consequence of penicillin seizures but was due to halothane anesthesia (see above).

**d) Experimental Brain Tumors:** In 9 rats experimental brain tumors were produced by implantation of a rat glioma cell clone deep in the right hemisphere. Within a few weeks a spherical tumor developed which frequently had a necrotic center. In this region, blood flow and glucose utilization were zero (fig. 5C). In the margin of the tumor glucose utilization was greatly enhanced, indicating an increased metabolic activity of the tumor tissue. In some circumscribed regions a dissociation between increased glucose utilization and decreased blood flow was noted which suggests that in these regions anaerobic stimulation of glucose metabolism occurred.
SIMULTANEOUS BF, GLUCOSE METABOLISM BY AUTORADIOGRAPHY

Mies et al.

Discussion

Despite the great interest in coupling mechanisms between brain function, blood flow and metabolism, only 2 short communications have been published in which autoradiographic double tracer techniques have been employed for evaluating local blood flow and local glucose consumption in the same brain section.11,12 The technique is based on the different half lives of two tracers, 131I and 14C. Since the half life of 131I is only 13 hours, the same section exposed twice at an interval of a few days shows 131I and 14C activity on the first, and 14C-activity alone on the second autoradiogram. 131I-activity can then be determined by subtracting the second from the first autoradiogram.

The present method is very similar to this technique with 2 exceptions. 131I was used instead of 14C because this isotope has a longer half life and so can be shipped to the customer by normal transport means. 131I-iodo-antipyrine was further given in a dose so that contamination by 'C-disintegrations. As has been demonstrated by earlier observations which have been reported previously by several authors.13,14 It has not been noted, however, that there is an overlap of those regions in which flow and glucose metabolism are activated. The increase in glucose consumption was strictly confined to the cortex supplied by the middle cerebral artery, i.e., the region of potassium chloride application, but there was also an increase in blood flow in the medial part of the hemisphere which is supplied by the anterior cerebral artery. Coupling of flow and metabolism, in consequence, was less strict than would appear from single tracer autoradiograms from different animals.

The time lag between the administration of 14C-deoxyglucose and 131I-iodo-antipyrine also has to be considered. Most of the deoxyglucose activity in the brain is accumulated during the initial 10 to 15 min after tracer administration, i.e., at an earlier time than the injection of 131I-iodo-antipyrine, which normally is performed 30 min after the 14C-deoxyglucose bolus. Under physiological conditions this is probably not of importance, but an error may be introduced when, under pathological conditions, steady state conditions are not present during this interval.

Finally, a problem which is not related to the double tracer technique itself is the validity of the 14C-deoxyglucose method under pathophysiological conditions. The rate constants described in the original publication1 were determined in the normal brain of awake rats. These constants may be different in diseased brain and the method may not adequately represent the actual cerebral glucose utilization rate.

Even with these methodological limitations in mind, the advantage of the present double tracer technique for studying local coupling mechanism is immediately apparent. In the examples given, various types of local coupling and uncoupling of flow and metabolism were noted, even in the same tissue section. It is evident that these changes would have remained undetected either by conventional arterio-venous sampling techniques because this gives only information about global changes, or by single tracer autoradiograms because this precludes correlation in the same animal.

Another problem is the resolution of 131I-autoradiography. Because of the high energy of this isotope little auto-absorption within the tissue section occurs and thus results in appreciable scatter, particularly when relatively thick sections are used. There are indications, however, that smearing of antipyrine during drying of the sections rather than scatter is the main limiting factor for local resolution. When 14C-iodo-antipyrine and 131I-iodo-antipyrine are injected simultaneously, no appreciable difference in the quality of the autoradiograms is noted, indicating that under practical conditions this problem is of minor importance.

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The various experimental models which have been investigated in this study were mainly used for testing the method, and the results obtained will be described later in more detail. It may be of interest, however, to stress a few observations of more general interest. In animals under halothane anesthesia, a striking increase in metabolic activity was observed in the pyramidal layer of the dorsal hippocampus and in the nucleus parafascicularis of the mesencephalon which was not coupled to an appropriate increase in blood flow. This was in contrast to barbiturate-anesthetized animals, and may be of interest in the interpretation of post-anesthetic side-effects which have been observed using this agent.

The increase in blood flow and the metabolic rate of glucose during spreading depression is in agreement with earlier observations which have been reported previously by several authors.13,14 It has not been noted, however, that there is an overlap of those regions in which flow and glucose metabolism are activated. The increase in glucose consumption was strictly confined to the cortex supplied by the middle cerebral artery, i.e., the region of potassium chloride application, but there was also an increase in blood flow in the medial part of the hemisphere which is supplied by the anterior cerebral artery. Coupling of flow and metabolism, in consequence, was less strict than would appear from single tracer autoradiograms from different animals.

Following intracerebral tumor implantation 2 different forms of local increase in glucose consump-
tion were noted. In some areas glucose consumption was coupled to a parallel increase in blood flow, whereas in others blood flow was decreased. The latter situation can be best explained by a stimulation of anaerobic glycolysis in regions with reduced blood flow. A similar dissociation has also been described by Ginsberg et al.\(^a\) in cats following middle cerebral artery occlusion, indicating that one should be cautious in evaluating an increase in local glucose consumption without knowledge of the associated change in blood flow.

Regional changes in blood flow and glucose consumption during topical penicillin seizures have been reported in 3 communications using rats and monkeys.\(^{11, 12, 18}\) In these reports an increase in both flow and metabolism in the region of penicillin application was noted, but a precise evaluation of coupling was not possible because only one tracer per animal was given. The present study demonstrates that the cortical increase in glucose consumption by far exceeds that of regional blood flow. Only in the thalamic relay nucleus was there a coupled increase in blood flow and metabolism. This finding differs from previous observations regarding a coupled activation of flow and metabolism following generalized bicuculline seizures.\(^a\) It should be considered, however, that the time lag between flow and metabolic investigation may introduce an error, as has been discussed above, and that cortical but not yet subcortical blood flow may have already returned to normal during the time of isotope injection.

Although preliminary, these findings demonstrate that both coupling and uncoupling of blood flow and metabolism are highly regional phenomena which differ considerably in different animals and even in various parts of the same brain. A reliable evaluation, therefore, is only possible by using double tracer techniques, the resolution of which is high enough to demonstrate these regional differences. The present method may be a useful tool for this purpose.

### References

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