Comparison Between Hydrogen Clearance and Microsphere Technique for rCBF Measurement

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SUMMARY Regional cerebral blood flow was measured repeatedly in anesthetized and immobilized cats under various experimental conditions by recording the clearance of inhaled hydrogen with inserted platinum electrodes and by recording the distribution of 15 μ microspheres labeled with 3 different radioisotopes. The values from both methods in normal cortical tissue were comparable (75.7 ± 23.5 ml/100 g min for H₂-clearance; 67.6 ± 26.2 ml/100 g min for microsphere technique), but were below those recorded in awake cats. With both methods the values could be reliably reproduced (correlation coefficient between measurements: 0.903 for H₂-clearance, 0.754 for microsphere technique). During ischemia induced by temporary occlusion of the middle cerebral artery the microsphere technique usually yielded higher flow values than the H₂-clearance, and did not indicate severe ischemia in 6 out of 20 instances. After restoration of flow, hyperperfusion was observed by the microsphere technique in 2 cases only while H₂-clearance indicated hyperemia in 6 instances. This limited comparability between the 2 methods was also expressed in a low correlation coefficient (0.486) calculated from 139 flow values obtained simultaneously with both methods.

The discrepancy between the methods under pathological conditions might be due mainly to the different recording volumes: while Pt-electrodes record H₂-clearance from a few mm³ or less, tissue samples of 300–700 mg were necessary for the microsphere technique and inhomogeneities of flow may thereby escape detection. The technique for measuring cerebral blood flow in an experimental setup should be selected according to the requirements of the study and according to the limitations of the various methods.

Methods

Preparation of Experimental Model

Results obtained in experiments carried out on 26 normal cats (weighing 1.5–3.0 kg) were used. Anesthesia was initiated in the animals with pentobarbital sodium, 30 mg/kg injected intraperitoneally. Tracheotomy was performed and catheters were placed into the femoral vein, through one femoral artery to the abdominal aorta (for continuous monitoring of blood pressure and withdrawal of blood samples) and through the other femoral artery into the left ventricle (for injection of microspheres). The cats were immobilized with Flaxedil (15 mg/kg initially, thereafter as needed), and mechanically ventilated with a gas mixture containing 25% oxygen, sufficient carbon dioxide to maintain Paco₂ at 27 to 33 mm Hg (0% to 5%), hydrogen gas as needed for flow measurements (intermittently between 5 and 10%) and nitrous oxide. Sixty-seven to 73% nitrous oxide was considered adequately analgesic and sedative in cats. The cat's head was placed in a stereotaxic apparatus and small areas of the Sylvian, middle ectosylvian, or lateral gyrus, which were sufficiently protected by spinal fluid, were exposed through drill holes. In some instances large craniotomies were performed and the underlying dura was resected; in these cats the exposed surfaces of the brain were protected by a pool of warm mineral oil. In 15 cats the left middle cerebral artery (MCA) was exposed transorbitally and freed from its arachnoidal investiture. A polished hook prepared from a No 18 cannula (O.D: 0.0505) was manipulated...
around the MCA, and the optic foramen was sealed around the cannula with tissue glue and a coagulative sponge. The orbit was then filled with epoxy cement. Astylet could be advanced through the cannula and the MCA thus occluded for variable periods of time. This permitted flow measurements before, during and after MCA occlusion.

Hydrogen Clearance

Regional cerebral blood flow (rCBF) was measured by recording the appearance and clearance of \H_2\ administered by inhalation. Recording electrodes were prepared from 70% platinum — 30% iridium wire 250 \mu\ meter in diameter. The wire was electrolytically sharpened to a tip of about 10 \mu\ meter and isolated with glass, except for a free area of 300–600 \mu\ meter at the tip. The exposed tip was covered with platinum black for improved recording properties. These electrodes had an impedance of 70 to 100 kOhm at 10 Hz.18 Usually, one electrode was inserted into the Sylvian or ectosylvian gyrus (area supplied by the MCA) and one into the lateral gyrus (area supplied by the anterior cerebral artery); an indifferent electrode was placed in the cat's neck muscle. A polarizing voltage of 400 mV, positive to the recording electrode, was applied and compensated via a bridge circuit. The slow potential changes proportional to the concentration of \H_2\ at the electrode tip were DC-amplified with a gain of 100, passed through a low-pass filter with a cut-off frequency of 0.1 Hz and recorded on a polygraph. For rCBF measurement, \H_2\ was added in a concentration of 5–10% to the gas mixture until an adequate deflection of the recording pen was observed on the polygraph. Administration of \H_2\ was then interrupted and its clearance from the brain recorded. To show the monoexponential rate of clearance, clearance curves were often plotted on semilogarithmic paper. The CBF was calculated according to the equation \( f = \lambda / (0.693/T/2) \), in which \( \lambda \) is the brain-blood partition coefficient for the indicator (= 1 for \H_2\), 0.693 the natural logarithm of 2, and \( T/2 \) the time required for the clearance curve to diminish to one-half. With \( f = 41.58/T/2, \) CBF in milliliters per 100 gram per min was obtained.

Microsphere Method

Measurements were made according to procedures described by Marcus et al.11 and Fan et al.,29 with slight modifications. Microspheres 15 ± 3 \mu\ meter in diameter, labeled with \(^{141}\text{Ce}\) (145 keV, 32.5 days half-life), \(^{186}\text{Rh}\) (497 keV, 39.8 days) and \(^{48}\text{Sc}\) (889 and 1120 keV, 84 days), respectively, were utilized (NEN-Trac, supplied by New England Nuclear). A 0.5 ml suspension of 3–6 \times 10^8 microspheres in 10% dextran (with 0.01% Tween 80 to prevent aggregation), having a specific activity of 0.1 mCi/ml, was injected into the left ventricle for each measurement. Prior to injection, the vial containing the suspension was vigorously agitated and placed in an ultrasonic sonicator for 2 min. Then 0.5 ml suspension was aspirated into a syringe and immediately injected over a period of 30 sec. Withdrawal of blood was begun 10 sec prior to injection and continued for 1 min at a constant speed of 3 ml/min (Harvard infusion withdrawal pump). Three injections of microspheres were made at different stages of the experiments. When the experiment was completed, the cat was killed by injecting potassium chloride, an overdose of pentobarbital or air intravenously. The brain was removed and cut into 5 mm frontal sections. The slice through the marks of the electrodes positions was dissected, and cortex and white matter were separated yielding wet tissue samples of 300 to 700 mg. The radioactivities of each tissue sample and of the blood samples (divided into aliquots) and the weights of each tissue sample were entered into a PDP 12 minicomputer (Digital Equipment Corp.). The computer program resolved the activity of each isotope, made corrections for background and scatter from other isotopes, and calculated the radioactivity per g of tissue sample (C_t). The flow rate (f) per unit weight of the tissue sample was calculated as \( f = C_t \cdot V/C_a \), where \( V \) gives the volume (3 ml/min) and \( C_a \) the radioactivity of the reference blood sample.

The frontal slices adjacent to the one used for counting microsphere radioactivity were prepared for microscopic examinations. The number of trapped microspheres was counted and the condition of the tissue (infarcted or not) was judged. In serial histologic sections of 5, 10, 15, and 20 \mu\ meter thickness the trapped microspheres were counted in an area of 5 × 5 mm. In the 15 \mu\ meter sections, trapped microspheres per tissue volume were calculated to be 42666 to 125333 ms/cm^3, permitting estimation of the number of microspheres trapped per injection to be between 14222 and 41778 ms/cm^3. When a single injection was performed after MCA occlusion, 6666 ms/cm^3 were trapped on the occluded and 17066 ms/cm^3 on the patent side. Therefore, the number of microspheres per tissue sample can be expected to be above 2000 in all instances.

Results

Flow in Undamaged Cortical Areas

Pt-electrodes were advanced into the cortex of the lateral or ectosylvian/Sylvian gyrus of the left hemisphere of the cat. Therefore, flow measurements from the areas supplied by the anterior and middle cerebral arteries could be compared as obtained with \H_2\ and microsphere method. The flow values measured in 39 undamaged brain areas were 75.7 ± 23.5 ml/100 ml g/min with the \H_2\ clearance and 67.6 ± 26.2 ml/100 ml g/min with the microsphere technique. The mean difference between the 2 methods was 7.4 ± 35.8 ml/100 g/min.

Reproducibility of Flow Values

In 18 instances repeat simultaneous flow measurements with \H_2\ and microsphere methods were performed without alterations of experimental conditions. The time intervals between the 2 measurements varied between one and 3 hours. With the \H_2\
clearance the second value was found to be slightly lower than the first (mean decrease 1.3 ± 7.6 ml/100 g/min); correlation coefficient (0.903) and slope of regression line (0.879) between the first and second value proved the good reproducibility of the method. Flow values obtained with microspheres increased slightly with time (mean increase 3.2 ± 14.7 ml/100 g/min), correlation coefficient (0.754) and slope of regression line (0.877) between the first and second value were not as high as with H₂ clearance.

Flow in Ischemic Brain Regions

In 13 experiments cortical ischemia was produced by occluding the left MCA for 30 to 120 min. The effect of the occlusion could be observed under the microscope as stoppage or diminishment of flow in the pial vessels. In 7 instances flow alteration was also seen at the site of the electrode in the lateral gyrus. In all the experiments a drop in perfusion to at least one third (14.4 ± 11.4 ml/100 g/min) of the resting value (mean 85.3 ± 29.4 ml/100 g/min) was found with the hydrogen technique, and a decreased flow was also recorded in the 7 less perfused lateral gyri (mean 22.1 ± 9.4 ml/100 g/min). By the microsphere technique, a flow decrease in the MCA area was not observed in 5 experiments, and was less evident in the others (mean decrease to 33.2 ± 14.1 ml/100 g/min). Flow decreases in the lateral gyrus were observed in 5 cases (mean 25.8 ± 6.0 ml/100 g/min). After reopening of the MCA, relative hyperperfusion was found in 6 MCA areas by H₂-clearance (mean 180.7 ± 52.5 ml/100 g/min), but only in 2 MCA areas by microsphere technique (96 and 134 ml/100 g/min). Arterial blood pressure and PaCO₂ did not change significantly during and after MCA occlusion.

Correlation Among Flow Values Obtained by Hydrogen Clearance and by Microsphere Technique

The flow values measured by the microsphere technique from cortical tissue supplied by the middle or anterior cerebral artery were compared to the values recorded by hydrogen electrodes placed into the Sylvian/ectosylvian or lateral gyrus, respectively. All pairs of values from undamaged, ischemic or hyperemic tissue were taken for the calculation of product moment correlation coefficients. Over the 139 pairs of flow values a regression line (fig. 1: \( f_{\text{ms}} = 36.0 + 0.404 f_{\text{H₂}} \)) with a correlation coefficient \( r = 0.486 \) was obtained. A further analysis for low and high values was performed, grouping the flow values according to the H₂ recording: while the mean flow of all measurements was identical for both methods (\( f_{\text{H₂}} = 60.9, f_{\text{ms}} = 60.6 \) ml/100 g/min), values in low flow areas were higher with microsphere technique than with H₂-clearance, and values in high flow areas were lower with microsphere technique than with H₂-clearance (table).

Transient Perfusion Changes During Application of Microspheres

To test the influence of the microspheres on cerebral tissue perfusion, H₂-clearance was recorded
TABLE Comparison Between H2 and MS-Flows in Dependency of Flow Values (Grouped According to H2 Measurement)

<table>
<thead>
<tr>
<th>Groups</th>
<th>According to H2-flow</th>
<th>MS-flow</th>
<th>H2-flow</th>
<th>Flow Values (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;20</td>
<td>21-30</td>
<td>31-40</td>
<td>41-50</td>
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<tr>
<td>n</td>
<td>17</td>
<td>11</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>H2-flow (ml/100 g/min)</td>
<td>11.8</td>
<td>27.1</td>
<td>38.2</td>
<td>46.4</td>
</tr>
<tr>
<td>MS-flow (ml/100 g/min)</td>
<td>6.1</td>
<td>3.0</td>
<td>3.1</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Repeatedly and the microspheres injected during clearance of H2. The disturbance of perfusion could be demonstrated by the change in clearance rate (fig. 2), which was especially prominent in tissues with low flow. The influence of microspheres on flow lasted only for a few minutes and subsequent H2-clearance recordings yielded the same rates as before injection of the microspheres. Concomitant changes in blood pressure were not observed, and the decrease in clearance rate had to be due to occlusion of some capillary beds.

Discussion

Normal Values

The rCBF values of undamaged brain tissue as recorded in this study by the hydrogen clearance method are lower than those obtained in autoradiographic studies with freely diffusible tracers: Landau et al.1 reported flow values of 1.38 ± 0.12 ml/g/min in the cat's sensory motor cortex, Reivich et al.2 1.09 ± 0.04 ml/g/min, and Sakurada et al.1 1.03 ± 0.04 with 14C-antipyrine and 1.99 ± 0.22 ml/g/min with 125I-iodoantipyrine. However, our values (mean 75.7 ± 23.2 ml/100 g/min) agree well with those obtained by other investigators with hydrogen clearance (79 ± 22 ml/100 g/min,3 82.8 ± 18 ml/100 g/min,4) and the mean value in normal tissue is not significantly higher than that obtained with the microsphere technique (67.6 ± 26.2 ml/100 g/min); the values reported by most authors for the microsphere technique are lower (43 ± 6 and 48 ± 5 ml/100 g/min,56 44.7 ± 3.6 ml/100 g/min57). With both methods a good reproducibility of flow values was observed over long time periods. The flow values as measured with the H2-technique and the microsphere method in this series of experiments, which were below those recorded in awake cats,1,3 may well be influenced by anesthesia, especially pentobarbital. Furthermore, contamination of investigated brain with less perfused tissue could not be ruled out: this contamination may be due to difficulties in dissecting the brain cortex for counting radioactivity. The volume of brain from which electrodes recorded H2-concentration could also be inhomogeneous as indicated by non-monoexponential decay of some clearance curves.34

Comparative Studies with Different Methods

When different methods were used for cerebral blood flow studies in the same experiment, the measured flow values did not agree in all investigations: even the application of differently diffusible tracers for autoradiographic studies influenced the measured flows: Sakurada et al.3 and Ohno et al.38 reported higher flows with 14C-iodoantipyrine than with 14C-antipyrine, and this difference was attributed to the low diffusion coefficient of antipyrine into the

Figure 2. Repeated determinations of H2-clearance with injection of microspheres during the second recording showing the influence of microspheres on microcirculation: after injection of microspheres clearance rate is changed and would now indicate a flow of 93.6 ml/100 g/min. Ten min later (third curve) clearance rate recovered and flow (182.3 ml/100 g/min) is nearly as high as before microsphere injection (203.8 ml/100 g/min, first recording).
brain. Values obtained after short infusion schedules with \( ^3 \)H-nicotine did not differ from those using iodoantipyrine, but longer nicotine infusion yielded lower values due to back diffusion.\(^{28} \) That \( ^4 \)C-antipyrine is not ideally suited for rCBF measurements was demonstrated also in a comparison to flow values obtained with \( ^4 \)C-ethanol, \( ^3 \)H-H\(_2\)O and \( ^133 \)Xe\(^{29} \); the antipyrine method grossly underestimated cortical blood flow in most instances, and tissue sampling techniques using the other isotopes gave values which did not approach those calculated from integrated arterial and tissue activity curves according to the Kety-Schmidt technique. However, the values with the \( ^4 \)C-antipyrine method were not significantly different from those with hydrogen clearance in the caudate nucleus.\(^{30} \) Flow values calculated from \( ^133 \)Xe clearance recorded in the sagittal sinus were in close agreement with those obtained with the microsphere technique.\(^{30} \) In different sets of experiments, flow values measured with microspheres were close to or higher than those obtained with clearance techniques.\(^{30} \) However, all these flow values were low when compared to the cortical values given for awake animals.\(^{1,4} \) Only Horton et al.\(^{32} \) measured flow values with the microsphere technique as high as those obtained with the iodoantipyrine method in most regions of the normal rat's brain, but studies under pathological conditions producing very high or low flow rates were not done. All these comparative studies indicate that measured flow values are dependent on the method used. A comparison of results obtained with different methods must be undertaken with extreme caution.

**Differences Between \( \text{H}_2 \) and Microsphere Technique**

The 2 methods compared in this study are different in many respects: While \( \text{H}_2 \)-clearance is directly recorded and yields immediate results, microspheres are injected at various stages of an experiment and the actual measurements are performed much later after perfusion has been changed repeatedly and the animal has been killed. That such perfusional changes may falsify recorded flow values has been shown in ischemic myocardium.\(^{30,31} \) where microspheres were lost with time elapsed (12-24 hours) after an early post-occlusion particle injection. Similar investigations are not available for focal cerebral ischemia.

The validity of the microsphere technique is related to the number of particles trapped in a tissue sample: Buckberg et al.\(^{35} \) have found that despite some non-randomness in the distribution, errors were usually under 20% as long as each sample had over 400 microspheres. On the other hand, the injection of large numbers of microspheres may influence microcirculation by blocking a relevant number of capillary beds. Such hemodynamic influences could be demonstrated by injecting microspheres during an \( \text{H}_2 \)-clearance. However, with the amount of microspheres injected in these studies such influences were short-lasting and subsequent \( \text{H}_2 \)-clearance yielded the same flow rates as recorded before microsphere injection. The relationship between amount of injected microspheres consistent with no physical changes and number of microspheres per tissue sample necessary for reliable flow determinations limits the size of the tissue sample. Usually tissue samples have to be rather large with respect to micro-circulatory units — in our experiments 300–700 mg.

While the volume of flow determination is accurately defined in the microsphere measurements, the area from which hydrogen is derived, i.e. the effective diffusion area or the tissue volume, cannot be calculated. With constant flow, there is a relationship between size of the electrode tip and the recording volume: for microelectrodes, this volume was estimated to have a diameter of 50 \( \mu \)m\(^{36} \) electrodes with 15 \( \mu \) tips recorded flow from only a few capillaries; with 100 \( \mu \) electrodes the flow through several capillaries was recorded and, therefore, the measurements were more constant.\(^{34} \) Meyer et al.\(^{36} \) assumed a recording volume of 0.5 mm\(^3\) for their 250 \( \mu \) electrodes, while Halsey et al.\(^{34} \) discussed recording volumes of a few cubic millimeters. With local generation of \( \text{H}_2 \) the resolution was better defined and about 1 mm.\(^{34} \) However, these volumes are very small when compared to the tissue samples necessary for microsphere determinations. The volume from which \( \text{H}_2 \) is derived is also dependent on the flow: when slow desaturation (= low flow) \( \text{H}_2 \) can diffuse to the electrode surface over relatively great distances due to its high diffusion coefficient of \( 3.10^{10} \text{ cm}^2/\text{sec} \). With rapid desaturation (\( T/2 \) in the order of 10 sec) the delivery of \( \text{H}_2 \) to the electrode would be more dependent on the circulation in the immediate surrounding tissue, because \( \text{H}_2 \) diffusing from larger distances would be washed away before reaching the electrode. Due to the trauma caused by the electrode, its tip is surrounded by a non-circulated area of blood, exudate and devitalized tissue of variable thickness. The thickness of this diffusion layer does not affect the slope of the desaturation curve, it only causes a delay in start of \( \text{H}_2 \)-clearance after saturation.\(^{37,38} \)

The best agreement between the flow measurements with the 2 methods was found for values between 60 and 90 ml/100 g/min. For values below 60, the flow determined with the microsphere technique was usually higher than that recorded with \( \text{H}_2 \)-clearence; when \( \text{H}_2 \)-clearance was close to 0 and stagnation of the circulation could be observed in the pial vessels, a flow between 10 and 35 ml/100 g/min was still found with the microspheres. With high flows concomitant with a red, hyperperfused cortex after transient circulatory arrest, the microsphere technique usually gave much lower values than the \( \text{H}_2 \)-clearance. In both instances subsequent hemodynamic alterations washing microspheres out of or into the damaged tissue could contribute to these discrepant measurements. A significant shunting of microspheres due to the opening up of arteriovenous anastomoses during post-ischemic hyperemia as observed in the dog\(^{39} \) is not likely, because no difference was found in the filter capacity of the cat's brain for microspheres of 15 and 50 \( \mu \) diameter during post-ischemic recirculation.\(^{40} \) However, microsphere radioactivity could not be
measured in the cerebral venous blood in our experiments, therefore an arteriovenous shunt effect could not be ruled out completely. The grossly different recording volumes could also be responsible for the varying results. As observed with multiple H₂ electrodes, neighboring brain regions may have different flows under certain pathologic conditions, which are averaged out in the large tissue volume used for microsphere determinations.

Selection of Methods for rCBF Measurements

The desirable technique must be selected according to the limitation inherent in the various methods for CBF measurement: if accurate measurements of absolute flow values are wanted, only autoradiographic methods with completely freely diffusible tracers fulfill all criteria, but the measurement can only be made once and the method has a minimal time window. The other methods permitting repeated flow determinations usually yield lower absolute values, especially under high flow conditions. When flow is to be determined a few times under stable conditions and hemodynamics do not change greatly during the course of the experiment, the microsphere technique may be applied; it yields reliable results from closed skull preparations, and the tissue sample may also be used for determinations of H₂O content and concentration of electrolytes and metabolites. This method also permits measurement of the flow in various parts of the brain, including deep structures. When short-lived changes in perfusion are to be followed in small tissue volumes, the H₂-clearance method has advantages: it permits an unlimited number of flow determinations over short time intervals and is well-suited to follow diverging temporal perfusion patterns in neighboring cortical regions during and after arterial occlusion; however, the electrodes must be advanced into the tissue, and regions not in the vicinity of the electrode tip are lost for the measurement.

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Lipoprotein Abnormalities in the Pathogenesis of Cerebral Infarction and Transient Ischemic Attack

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SUMMARY HDL- and LDL-cholesterol levels were determined by a heparin-Ca precipitation method in 89 survivors of cerebral infarction (CI) (75 males, 14 females) and 14 patients with transient ischemic attacks (TIA) (8 males, 6 females). The mean values of HDL-cholesterol concentration and HDL:LDL-cholesterol ratio for both sexes of CI patients were significantly lower than those of the healthy controls (37 males, 14 females). These values for CI patients were significantly lower than in patients with various diseases excluding cardiovascular disease, hepatic disease, hyperlipidemia, diabetes mellitus and degenerative disorders of the nervous system (46 males, 43 females). In patients with TIA, these differences were statistically significant only for men. Based on the patient's history, clinical signs and symptoms and the findings of computerized tomography and 4-vessel angiography, male CI patients were divided into 2 sub-groups, CI believed to be in the distribution of a perforating artery and CI in the distribution of a cortical artery; it was found that the HDL-cholesterol level and HDL:LDL-cholesterol ratio were significantly lower in the cortical artery group than in the perforating artery group, suggesting that these lipoprotein abnormalities may play a part in the pathogenesis of CI, particularly of the cortical artery area infarction.

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CONFLICTING REPORTS have been published about the significance of hyperlipidemia in the development of cerebrovascular disease. Some investigators, who have examined survivors of attacks of ischemic cerebrovascular disease, have observed elevated mean concentrations of serum cholesterol and/or triglyceride (TG). 39 while others found no increase in serum lipid levels. 40 In contrast, epidemiological studies conducted in several areas of Japan indicated that the incidence of cerebral infarction was inversely related to the mean level of serum cholesterol; 41, 42 ischemic cerebrovascular disease was found to be associated with a low serum cholesterol level in Japan, where the mean value of serum cholesterol has rarely exceeded 200 mg/dl.

Prospective cohort studies should provide the most reliable information on blood lipids as a risk factor. In the Framingham study, an association of blood lipid with the development of atherothrombotic cerebral infarction under age 60 was statistically significant only for men. Regardless of the associated lipoprotein pattern, the risk of infarction increased in proportion to the serum cholesterol level, but pre-beta lipoprotein levels were unrelated to the risk when associated cholesterol levels were taken into account. 43 Two prospective studies in Japan, one conducted in Hisayama 44 and the other in Akabane and Asahi, 45
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