The Hydrogen Clearance Method in Assessment of Blood Flow in Cortex, White Matter and Deep Nuclei of Baboons

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Abstract: The technique of hydrogen clearance by an inhalation method is discussed. The electronic instrumentation necessary to secure stability and reproducibility from the recordings is described. Clearance rates in gray matter of about 80 ml/100 gm per minute in the cortex and putamen have been obtained, and of about 20 ml/100 gm per minute in white matter. Clearance curves have invariably been monoexponential in character in white matter, and in over half the cases in the putamen. In the remainder of the putamen curves and in 60% of the cortical clearance curves, the curves could be resolved into only two exponentials. Using bicompartamental analysis, the fast-clearing components of biexponential curves in both cortex and deep nuclei gave the same figures as clearance curves of an entirely monoexponential character from these two tissues. The importance of recirculation time, concentration of hydrogen inhalation, and verification of the tissue placement by subsequent dissection are discussed. The capacity of the method to detect sudden changes in flow during clearance is described.

Introduction

The development of the Kety-Schmidt method in 1948 began an increasingly intensive study of the cerebral circulation both in the clinic and in the experimental laboratory. Ingvar and Lassen's development of Kety and Schmidt's principle has resulted in the development of increasingly precise methods for the measurement of regional blood flow by the external recording of clearance of radioactive tracers, usually ^133^Xenon. In 1964, however, Aukland and his colleagues pointed out the possibilities of local tissue blood flow recording in very small areas of brain by the measurement of clearance of the inert gas hydrogen, which had been allowed to saturate the tissue following inhalation, and their method has been used by other authors, either with inhalation alone or by a combination of inhalation and injection or injection alone. The theoretical principles of the hydrogen clearance technique have been fully described by Aukland, and the principles of analysis differ in no substantial way from the techniques of analysis applied to radioactive isotope methods; the half time of the clearance curve obtained is readily translated into flow in milliliters per 100 gm per minute by standard formulae.

In many ways, the extreme smallness of electrodes usable for hydrogen clearance renders it a particularly attractive method for the determination of blood flow in multiple areas of an experimental animal brain, where the electrodes may be placed in deep nuclei by stereotactic techniques, and where tissue invasion is of little consequence provided the electrodes are small. We have used the technique of hydrogen clearance in baboons to determine local blood flow in cortex, white matter and putamen, to compare the response of these three areas during autoregulation to raised intracranial pressure or to diminished perfusion pressure from hemorrhage, to
compare the response of these tissues to increased CO₂ tension, and to analyze the effects of middle cerebral artery occlusion on the superficial and deep gray flow in the middle cerebral territory. The present paper describes the technique in general, and the accompanying paper describes the detection of CO₂ responses and autoregulation in gray and white matter by the method described. Further results in vascular occlusion and on the effects of focally raised intracranial pressure will be presented subsequently.

Methods
Twelve baboons (Papio nubius or Papio cynocephalus) of either sex in the weight range 8 to 20 kg were used. The animals were sedated with phencyclidine (Sernylan) anesthesia induced with a sleep dose of thiopentone (2 to 4 mg per kilogram) administered intravenously, and anesthesia thereafter was maintained with intravenous α-chloralose (50 mg per kilogram). The animals were paralyzed with gallamine triethiodide (1 mg per kilogram) administered intravenously, repeated as necessary, and ventilation was maintained by a Starling pump at a volume adjusted to maintain arterial P CO₂ between 35 and 45 mm Hg. Where the arterial P CO₂ was to be raised, this was done by the addition of CO₂-rich mixtures to the input of the pump. Where hyperventilation was to be induced, this was done by increasing the stroke volume of the pump. End-tidal CO₂ was continuously monitored from the trachea by a Beckman infrared gas analyzer (Model LB1). Systemic arterial pressure was monitored by a P23 BB venous gauge connected to the superior vena cava by a catheter introduced through a femoral vein. In a number of experiments, the central arterial concentration of hydrogen was recorded by a platinum electrode passed up from one femoral artery to the arch of the aorta. Intracranial pressure was measured by an extradural strain gauge introduced into the extradural space by a burr hole in the right postfrontal zone. In a number of experiments, multiple strain gauge insertions were used in the extradural space, together with the establishment of an extradural space-occupying lesion by balloon. These, together with the physiological results of autoregulation to raised pressure and diminished systemic blood pressure, will be reported separately.

Hydrogen gas (British Oxygen) was given directly into the endotrachal tube by a lightly pressurized balloon system, adjusted to constant flow rates so that the hydrogen concentration of the inhaled gases was about 7%. As detailed below, variation in inhaled gas concentration from one clearance to another was unimportant, but we maintained the concentration constant for each individual clearance.

Hydrogen clearance was monitored from cortex, deep white matter and deep nucleus (putamen) by epoxy insulated platinum wire electrodes with a bare conical tip of 1 mm in length and 0.3 to 0.4 mm in diameter. Electrodes were introduced stereotactically after fixation of the animal’s head in a stereotactic apparatus (Narashigi, Model S.N. 3). Stereotactic placements were calculated from the atlases of Riche et al. and Davis and Huffmann. Fixation of the electrodes and of the extradural transducer was by means of acrylic resin.

Recording and Display Circuits
The system used to amplify and display the currents from the platinum wire hydrogen electrodes is shown in figure 1. Up to eight electrodes could be monitored simultaneously by using eight separate amplifiers and a four-channel linear-deflection pen recorder (Rikadenki B-4021), by manually switching between one set of four amplifier outputs and the other.

Each amplifier had its own baseline setting control and offset balance control. The balance control was adjusted for no hydrogen in the tissue until the pen was at baseline for any setting of the gain control. This adjustment permitted the operator to adjust the sensitivity at subsequent peaks of saturation to obtain maximum pen travel during desaturation, thus ensuring maximum accuracy of measurement of the trace, and to commence a new saturation phase, if desired, at any time during the tail of desaturation without having to waste time waiting for the pen to return to baseline.

The chopper-stabilized operational amplifiers used in this system (Analog Devices Ltd, 233J) are specified to have a very low drift rate, less than ±2 pA per degree C in the bias current, so that the contribution toward total drift from this source is negligible compared to that arising from the electrode system itself. In relation to drift, ordinary chloride-silver wire reference electrodes were found to produce too variable a reference voltage, and a sintered Ag/AgCl electrode (Tektronix, Inc, ECG leg electrode) was found to be much better. This was implanted subcutaneously in the animal’s back. After placement of the platinum and reference electrodes and applying the polarizing voltage, it was necessary to allow the electrode system to stabilize in the tissue for about half an hour before clearance measurements with a good baseline could be made.

Most of the electrode noise was eliminated by restricting the amplifier band width to about DC to 0.1 Hz.

Cisternal pressure was monitored in all experiments by an intracisternal needle connected to a strain gauge (Statham Model P23 V or P23 BB).

At the end of each experiment, the position of the electrodes was confirmed, either by briefly diathermizing the electrode or by applying a spot of methylene blue dye to the puncture wound on the cortex after the removal of the calvarium. The brain then was removed, fixed in formalin, and sectioned within a few days of the experiment.

Results
Blood flow determinations (196) under normal conditions in various portions of the baboon brain have been made by hydrogen clearance in 12 experiments. The details of these are given in table 1. Sixty-four determinations in cortex, 56 in putamen and 76 in white matter are analyzed, and it is apparent that a high proportion of these clearance curves had a single exponential character. We have
found that a monoexponential clearance was invariably present in white matter, where the electrodes were shown by autopsy study to be entirely within the deep white matter of the centrum semiovale. In deep nuclei, in 56 curves, we found 31 (55%) to have a monoexponential clearance, the remainder being biexponential in character. Of 64 cortical placements, we found 25 (39%) to be entirely monoexponential, the remaining 39 (61%) being biexponential in character. Where the electrode lay in close proximity to white matter or partially within the white matter, then clearance was invariably biexponential.

Table 2 describes the levels of blood flow obtained by the t½ clearance analysis in normal circumstances in the baboon. In cortex, the overall mean flow has been 82.8 ml/100 gm tissue per minute (SD ± 18) in 64 clearance curves. The 25 clearance curves in cortex of monoexponential character gave a mean value of 83.3 ml/100 gm per minute (SD ± 21.2), and the fast component of the biexponential curves gave a fast clearance in 39 curves of 82.5 ml/100 gm per minute (SD ± 15.9). In the putamen, the overall mean of 56 clearance curves was 83.1 ml/100 gm per minute (SD ± 18.6). The 31 monoexponential curves gave a mean flow of 86.1 ml/100 gm per minute (SD ± 22.0), and the 25 biexponential clearance curves a fast component of 79.3 ml/100 gm per minute (SD ± 12.7). The 76 clearance curves from white matter were only monoexponential in character and gave a mean white matter flow of 19.4 ml/100 gm per minute (SD ± 4.5). The results were tested using Student's t test for possible differences between monoexponential and biexponential figures. Thus, the difference between monoexponential clearance in

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**TABLE 1**

<table>
<thead>
<tr>
<th>Analysis of Hydrogen Clearance Curves in 12 Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of measurements</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Cortex</td>
</tr>
<tr>
<td>Putamen</td>
</tr>
<tr>
<td>White matter</td>
</tr>
</tbody>
</table>

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**Figure 1**

The circuit as presently used for hydrogen clearance. It should be noted that some of the records displayed in the paper were made before the present arrangement of feedback capacitors was adopted, and therefore have a higher noise level than subsequent records.
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TABLE 2

Perfusion Rates in Various Tissues in the Baboon Under Normal Circumstances

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of clearance curves</th>
<th>Mean (ml/100 gm per minute)</th>
<th>Standard deviation</th>
<th>p values for mean differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>Overall</td>
<td>64</td>
<td>82.8</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>Monoexponential</td>
<td>25</td>
<td>83.3</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td>Biexponential</td>
<td>39</td>
<td>82.5</td>
<td>15.9</td>
</tr>
<tr>
<td>Putamen</td>
<td>Overall</td>
<td>56</td>
<td>83.1</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>Monoexponential</td>
<td>31</td>
<td>86.1</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>Biexponential</td>
<td>25</td>
<td>79.3</td>
<td>12.7</td>
</tr>
<tr>
<td>White matter</td>
<td>Overall (monoexponential only)</td>
<td>76</td>
<td>19.4</td>
<td>4.5</td>
</tr>
</tbody>
</table>

the cortex and the fast component of the biexponential curves was not significant (p > 0.05) and, similarly, there was no significant difference between monoexponential curves obtained from the putamen and the fast component of the biexponential curves (p > 0.05). Comparing the cortical perfusion rates with those of the putamen, there was no significant difference between the figures obtained from cortical gray matter (overall mean) and those from the putamen (overall mean) (p > 0.05). Comparing the monoexponential curves from cortex and putamen, once again there was no significant difference (p > 0.05), and comparing the biexponential clearance curves (fast components only), there was no significant difference between the perfusion rates obtained between cortex and putamen (p > 0.05).

CALCULATION OF THE "SLOW FLOW" PORTION OF GRAY MATTER CLEARANCE

The slow component of 39 biexponential clearance curves in the cortex gave a value for local flow of 23.5 ml/100 gm per minute (±5.62). In putamen electrodes, the value for 25 placements was 24.5 ml/100 gm per minute (±5.22). These are not significantly different (p > 0.05). We therefore compared the slow components of a group of 37 gray matter placements yielding biexponential clearance with 37 white matter clearances obtained in each instance during the same clearance curve, using Student's t test for paired samples. The slow gray flows gave a mean value of 24.1 ml/100 gm per minute (SD ± 5.39), and white flows gave a mean of 18.8 ml/100 gm per minute (±3.99). These values are significantly different (p < 0.001).

EFFECTS OF VARYING LENGTHS OF INHALATION TIME OF HYDROGEN AND OF VARYING HYDROGEN CONCENTRATION ON THE BLOOD FLOW DETERMINATION

We found that the hydrogen concentration in tissue rises gradually over a period of some minutes to reach a stable plateau, the time taken to reach this plateau depending on the rate of blood flow through the tissue. Thus, at high \( P_{CO_2} \), the time for the plateau to be reached is less than at low levels of \( P_{CO_2} \) when the tissue blood flow is lower, other things being equal. We therefore analyzed the effect of varying inhalation times of hydrogen on the results obtained by the analysis of the clearance curve. Table 3 shows the data from an experiment in which the \( P_{CO_2} \) was kept constant as far as possible over a period of 40 minutes, while flow was assessed repeatedly, inhalation times between one and ten minutes being used. From these data it is apparent that with close agreement of mean systemic blood pressure, perfusion pressure, and intracranial pressure, and the \( P_{CO_2} \) remaining on the whole constant.

TABLE 3

Left Anterior Cortical Electrode

<table>
<thead>
<tr>
<th>N° Inhalation Time (min)</th>
<th>rCBF ml/100 gm per minute</th>
<th>Arterial ( P_{CO_2} ) (mm Hg)</th>
<th>ICP (mm Hg)</th>
<th>Perfusion pressure (mm Hg)</th>
<th>MABP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>72</td>
<td>39</td>
<td>11</td>
<td>137</td>
<td>148</td>
</tr>
<tr>
<td>1</td>
<td>77</td>
<td>39</td>
<td>12</td>
<td>146</td>
<td>158</td>
</tr>
<tr>
<td>2</td>
<td>80.6</td>
<td>39</td>
<td>10</td>
<td>152</td>
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<td>10</td>
<td>69</td>
<td>33</td>
<td>12</td>
<td>153</td>
<td>165</td>
</tr>
</tbody>
</table>

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at 39, flow was repeatable within a fairly narrow band, with an inhalation time of from one to seven minutes. The variation in blood flow of approximately 10% (the shorter inhalation times giving the higher values) may represent no more than the experimental error of the method. Figure 2 shows an example from another experiment in which flows were obtained under the same physiological conditions with an inhalation time of 2.3 minutes, as compared with an inhalation time of 8.3 minutes. Two putamen electrodes and one cortical electrode gave a slightly lower flow with the inhalation time of 8.3 minutes, but once again the figures are a reasonable approximation.

The effect of variation of hydrogen concentration has been assessed by altering the amount of hydrogen in constant volume administration by a Starling pump. Changing the inhaled hydrogen concentration from 7% to 12% has not been found to influence the flow rates obtained, and figure 3 demonstrates curves obtained under these two circumstances. Changing the hydrogen concentration from 7% to 12% was associated with frontal cortical flows of 72.7 ml/100 gm per minute and 73.8 ml/100 gm per minute, both to a five-minute saturation. Parietal cortex gave flows of 80.6 and 83.4 ml/100 gm per minute under the same circumstances. The curves of analysis are shown in the figure.

The effects of varying time of hydrogen inhalation on clearance curves obtained. Two hydrogen clearances following an 8.3-minute and a 2.3-minute inhalation of hydrogen are shown. The lower part of the figure shows the actual hydrogen concentrations recorded from left putamen (a), right putamen (b), and right frontal cortex (c). The upper part of the figure shows the semilogarithmic plots of hydrogen concentrations extracted from the primary curves. The first 40 seconds of the curves are not included, as discussed under figure 4.
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THE QUESTION OF ARTERIAL RECIRCULATION
The hydrogen method has been regarded by some workers as virtually devoid of problems arising from arterial recirculation. The blood-gas partition coefficient for hydrogen is low (0.018) so that hydrogen rapidly disappears from the lungs at the end of inhalation and the arterial concentration is negligible during clearance. However, other workers, notably Meyer and his colleagues, have stated that “arterial desaturation takes many minutes.” We therefore measured the central arterial concentration of hydrogen with a polarographical electrode introduced into the aortic arch from the femoral artery. The shape of the curve for the central arterial concentration of hydrogen proves interesting. This reaches a high level soon after the commencement of the inhalation and then falls slowly but steadily over the period of five or six minutes’ inhalation by some 6% of the initial peak value. The explanation for this is probably abstraction of hydrogen by tissue, and it also has been found in relation to the end-tidal concentration of continuously inhaled Xenon in a closed system by Veall and his co-workers (personal communication). Immediately following the cessation of hydrogen inhalation, the arterial concentration fell dramatically. It reached half peak value in 28 seconds, and 10% peak value in 100 seconds (fig. 4). Calculation of tissue clearance from various fractions of the curve showed that the initial 40 seconds of the clearance curve gave a somewhat slower blood flow than the remainder of the curve, and analysis of these fractions gave similar results. Therefore, it appears that the arterial concentration falling rapidly over the first 40 seconds in fact does blur the clearance figures from this part of the curve but, provided this period of time is discounted, arterial recirculation is demonstrably no longer a problem. It is our view, therefore, that no special precautions are necessary to exclude arterial recirculation, provided the first 40 seconds of the curve are discounted.

THE POSSIBILITY OF MEASUREMENT OF INCREASE IN FLOW DURING CLEARANCE CURVES
The remarkable reproducibility of this method over many hours has enabled us to detect at any rate sudden and marked changes of blood flow in the course of a clearance curve. This has been so since, as demonstrated in figure 4, the analysis of the clearance has given the same flow levels over various

FIGURE 3
The effect of varying hydrogen concentration on clearance curves. The primary curves of hydrogen concentration in tissue following a four-minute inhalation of hydrogen at a concentration of 7% and then of 12% are shown. Flows from parietal and frontal cortex approximate closely in the two circumstances. The recording obtained from an aortic arch hydrogen electrode indicates the rapid decline in arterial hydrogen concentration following the termination of inhalation.

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The effect of arterial recirculation on various portions of the clearance curve. A four-minute inhalation of hydrogen is shown with a recording of hydrogen concentration from the aortic arch, a cortical electrode and an electrode in white matter. Analysis of the cortical clearance is shown in the semilogarithmic plot under the heading a. Biexponential analysis gives a slow component (line 1), and a fast component for the portion of the curve from beta to gamma shown in line 2 with a flow value of 77 ml/100 gm per minute. The fast component of the curve from alpha to beta (line 2a) gives a somewhat slower flow value of 49.5 ml/100 gm per minute. Thus the first 40 seconds of the curve lie entirely separate from the main fast portion. Above b appears the analysis of the white matter clearance curve—a flow value of 19.8 ml/100 gm per minute.

Discussion

The acquisition of detailed knowledge of the circulation in various parts of the brain remains of paramount importance from the general physiological point of view and for the continued development of treatment in cerebrovascular disease. It has become clear in recent years that the necessary focal accuracy upon which scientific analysis depends is difficult to achieve with the techniques of radioisotope elution where the present limits of collimation, even with the tiny crystals such as have been used by portions of the curve, excluding the first 40 seconds. Therefore, it is no longer necessary to consider flow as an integral of a ten-minute desaturation period, and rapid changes in flow are detectable. This is demonstrated in figure 5, which shows the effect of severely raised intracranial pressure on hydrogen clearance. As saturation was achieved, the animal's intracranial pressure was raised to very high levels, and desaturation virtually arrested. With the sudden release of intracranial pressure from cessation of cisternal infusion and restoration of normal intracranial pressure, a vast increase in flow (the phenomenon of reactive hyperemia) took place and clearance immediately occurred at a high flow rate. The inflexion in the tissue hydrogen concentration is quite clear.

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INTRACRANIAL PRESSURE
(EXTRADURAL TRANSDUCER)

SYSTEMIC BLOOD PRESSURE

WHITE MATTER $F=28.6 \text{ml/100g/min}$

CORTEX $F=173.0 \text{ml/100g/min}$

AORTIC ARCH

**FIGURE 5**

To show the effect of sudden change in intracranial pressure on hydrogen clearance from cortex and white matter. During a four-minute hydrogen inhalation, intracranial pressure was increased by cisternal infusion of Ringer's-lactate, and by the time of saturation had reached very high levels (as demonstrated in the portion of the intracranial pressure record at the top of the figure). Tissue clearance in the cortex and white matter was virtually arrested. Sudden release of the intracranial pressure resulted in a rapid fall in intracranial pressure and in systemic blood pressure and a rapid increase in blood flow shown by a massive increase in clearance of the hydrogen from tissue. Cortical blood flow during this phase of hyperemia was 173 ml/100 gm per minute, and white matter flow 28.6 ml/100 gm per minute. The recording from the central hydrogen electrode in the aortic arch also is shown.

Yamamoto, Feindel and his associates,\(^15\) make point recording even in the cortex difficult, and, because of the size of the probes, render point recording in the deep nuclei impossible. It is clear that even highly collimated external detectors cannot resolve superficial from deep gray flow in the relatively small brain of a baboon. For this reason, a technique which provides highly focal recording of cerebral blood flow will continue to be of interest in the experimental laboratory where the noninvasiveness of technique is not of such primary importance as in the clinic. The necessity for implantation of electrodes in the brain, however, does raise the problem of tissue damage, and Aukland\(^16\) has correctly pointed out that a small zone of tissue damage must surround every implanted electrode. He has analyzed further the delay in clearance curves resulting from a diffusion layer of varying thickness between the actual clearing tissue and the electrode. From this it is clear that while the clearance curve may be somewhat delayed if there is a wide area of tissue damage around the electrode, the slope of the clearance curve will attain in the end the same value as that obtained by an electrode in the immediate proximity of the tissue being cleared.

The criticism of blood flow recordings in acute experiments following needle detector insertions in cortex or subcortical structures by Brock et al.\(^17\) in 1967 has seemed unjustified to us on a number of counts. Their experiences were based on the use of a
rather large needle, with an external diameter of over a millimeter, whose sensing element was well back from the tip of the instrument (8 to 9 mm) and was in fact 2.5 mm in length (Appelgren et al. 18). Although 1 mm appears to be a small tip diameter, the insertion of this instrument to such a depth past the tissue it is recording of course must involve considerable disturbance, and indeed this was shown in histological sections of the tissue examined. The circumstances are entirely different in a small hydrogen needle whose detecting point is at its tip and whose tip itself is no more than 0.3 to 0.4 mm in diameter at maximum and tapered to a fine point, the whole length of the recording area being 1 mm. Brock et al. further stated that their clearance curves obtained with the large semiconductor probe were monoexponential in type and cited this as an indication of tissue damage, supposing that the fast components had been lost in the trauma produced by the insertion of the needle. In the current experiments, however, the monoexponential curves obtained from cortex or putamen in fact were identical with the fast components of biexponential curves obtained from the same tissue, and both were reasonably high as far as gray matter values are concerned. They do not suggest that monoexponential clearance was typical of damaged tissue, but rather that point recording of a single homogeneous clearance had been obtained. Further, Fieschi and his colleagues in 1968 8 validated hydrogen electrode clearance had been obtained. Further, Fieschi and his colleagues in 1968 8 validated hydrogen electrode measurements in the cat with autoradiographical determinations of blood flow in the same species, and found that there was no significant difference between the average values obtained by the two methods, hydrogen in fact being systematically, though not significantly, the higher. We would conclude in agreement with Fieschi that the hydrogen electrode is in every way superior to the beta detector, and the accompanying paper, demonstrating the preservation of normal autoregulatory and CO₂ responses, would seem to bear out this conclusion. It has been our experience, therefore, that no progressive or serious tissue damage results from these implantations, except in a small number of cases. In only two of more than 90 electrode placements in our experiments has some lack of reactivity of the electrode (for example to CO₂) or an appreciable delay in its response, as pointed out by Aukland, 10 suggested to us that tissue damage had occurred. In each of these instances it was clear before postmortem dissection that this tissue damage must be present, and in each instance a small hematoma around the electrode was found. It was obvious, however, that the results could not be interpreted in the normal way, and in every other instance it has seemed to us that the constancy of response from electrodes after an initial half-hour stabilization, and the lack of obvious tissue damage on dissection of the brain, renders this an adequate method for the accurate experimental assessment of cerebral circulation. The fact that an electrode can differentiate between gray and white matter when placed within a few millimeters of the other tissue indicates the highly focal nature of the recording, and enables stereotactic placement within at least the major nuclei of the brain. This potential is not shared by radioisotopic methods, and although it may be paralleled by the heat clearance method used for many years by Betz and his associates, 19, 20 this method is difficult to express in quantitative terms.

The analysis of the clearance curves obtained by the hydrogen inhalation method has proved of some interest. Fieschi and his colleagues 13 found that the characteristic clearance of hydrogen in gray matter could be expressed by a biexponential function. This has not been our invariable experience. All electrode positions having been carefully checked, we can be certain that monoexponential clearance is invariably present in the white matter. The greater proportion of cortical electrode placements (61%) could be resolved into a biexponential function, but a high proportion (39%) were entirely monoexponential. It is somewhat difficult to obtain accurate intracortical placement of a hydrogen electrode in the baboon since the cortex is quite thin, and although we have customarily placed the electrode obliquely in an endeavor to retain it entirely within the cortex, a certain number of placements have left the electrode tip close to white matter. The biexponential curves reported from the cortex in this paper, however, were those in which the electrode was certainly within the cortex, although perhaps one portion of it might have been fairly close to white matter. It is still not possible to be certain that the biexponential character refers to a mixture of clearances in the small region of cortex, some laminae of which were no doubt perfused more rapidly than others, or whether contamination from nearby white matter may have played some role. It is interesting to note that a very much higher proportion (55%) of deep nuclear hydrogen clearance curves were monoexponential in character, resolution into two exponentials being found only in the remaining 45%. As Fieschi 13 pointed out in the cat, however, the deep nuclei of animals are not entirely homogeneous in anatomical character. Thus, the putamen of the baboon contains clear lamellations and the biexponential character of clearance might well be due to the admixture of tissues clearing at different rates within the gray matter itself. We know from the work of Sokoloff and his associates 21, 22 with autoradiographical techniques that different perfusion rates can be demonstrated clearly within areas of the cortex and deep gray
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matter, and there is no reason why a sufficiently focal electrode should not record a mixture of different clearing tissues even within one nucleus. The hydrogen electrode, however, renders it possible to record a monoexponential curve even from gray matter in the cortex or putamen, and we feel that this probably represents placement of the small electrode within a single homogeneously perfused area of the gray matter. The hydrogen method in our hands has given remarkably stable values for the perfusion of gray matter, in both the cortex and deep nuclei. An overall mean of 82.8 (±18.0) ml/100 gm per minute in 64 curves of the cortex and of 83.1 (±18.6) ml/100 gm per minute in 56 curves from the putamen would seem to indicate that there is no significant difference in the perfusion rate of gray matter measured overall in these two sites. Resolution of monoexponential and biexponential curves has shown that the monoexponential curve expresses the fast component of a bicompartamental analysis, and we would feel, therefore, that the biexponential curve contains an admixture of slower clearing tissue which may with justification be abstracted from true gray or nuclear flow. It is of interest that the second component of such an analysis is significantly more rapid than the monoexponential clearance obtained in our white matter clearance curves. This suggests that the slow component of nuclear or cortical flow, while it may contain some admixture of non-nuclear clearance, is on the whole composed of more slowly clearing elements of the gray matter itself than from an admixture of intercalated white matter.

The occurrence of a reasonably high number of even cortical gray matter curves which could be expressed by a monoexponential function indicates a further possibility of the hydrogen clearance method which, as far as we know, is not shared by any of the other tissue clearance techniques, that is, the capacity to detect a rapid change in blood flow during the elution of the tracer element. The characteristic of all our hydrogen curves has been that the later part of the curve invariably either fits a monoexponential clearance, as in white matter, in the majority of deep nuclear curves, or in about 40% of cortical gray matter curves, or contains a tail of slower clearing elements. Where the unresolved or natural clearance curve from the tissue shows a sharp increase in clearance at the end of the curve, it therefore is clear that tissue blood flow has increased. This enables the calculation of a rapid increase in tissue blood flow, as for example during hyperemia, to be performed with presaturation of the tissue and is of considerable use, therefore, in the assessment of deep nuclear reactivity during hyperemic phases. Of course, it is impossible to detect reduction of flow with certainty; for example, were hydrogen inhalation and tissue saturation to precede a reduction in flow, the clearance curve would alter in such a way as to render confusion with an unusually slow tail impossible to avoid. However, the capacity to detect sudden change in flow is of considerable utility and could at least suggest even a fall in flow.

The time of inhalation of hydrogen and its concentration has not been found critical in our experiments. Although we have not been able to substantiate the opinion statistically, we have been left with a clear impression that very short inhalation times tend to give flows slightly on the high side, but that provided a period of longer than two minutes is employed, then constant flow rates are obtained. We therefore have used an inhalation period of three minutes routinely in experiments to allow ample saturation time even at low levels of blood flow, as for example during hypocapnia. Under these circumstances, it appears that over serial determinations of flow, the error of the method is less than 10%, other conditions being equal. Also, it is clear that the concentration of hydrogen administered to the animal is not critical. This is certainly true in animal experiments, where the animal is breathing 100% oxygen, so that the addition of even as high a concentration of hydrogen as 12% to the inhalation mixture does not substantially reduce the oxygen saturation of the animal's blood. Thus, for example, P O2 levels between 450 and 600 torr are commonly obtained in the baboon breathing 100% oxygen, and a modest reduction to less than 300 torr may be seen with hydrogen concentrations even up to 12%, quite insignificant as far as the oxygen saturation of the blood is concerned, and completely without risk of incidental tissue hypoxia. However, it is certain that inhalation of high concentrations of hydrogen in inspired air may alter the oxygen saturation of the blood, which of course would involve the risk of hypoxic alteration in cerebral perfusion and therefore should be avoided.

The hydrogen method has differed in its technique of application in the hands of various authors. Thus, Fieschi, one of the first workers to use the method, used a period of 10 to 15 minutes' inhalation of hydrogen, followed by a two-minute period of infusion of saline equilibrated with hydrogen into a lingual artery in the cat. Meyer and his associates, on the other hand, have had considerable experience in the use of hydrogen, particularly in intravascular flow recording, and have used a bolus injection of hydrogen-saturated saline into the lingual artery in the monkey. Our experimental design was concerned to compare tissues in the two hemispheres, and in areas of the same hemisphere in carotid and vertebrobasilar distribution, and we did not feel, therefore, that a bolus injection into the carotid circulation could be
used. The use of a centrally injected (aortic) bolus is a possibility, but the theoretical considerations of the propriety of use of techniques of analysis in a bolus which is already somewhat dispersed by the time it reaches the hemisphere (as discussed by Hütten et al.) led us to concentrate on the use of the inhalation technique. The main problem with this technique was clearly the complication introduced by recirculation of hydrogen. With hydrogen, however, the very small molecular size and peculiarly favorable partition coefficient between blood and lung air results in an extremely rapid disappearance of the tracer from the blood. Our results have clearly shown that an artifact introduced by recirculation problems is evident only within the first 40 seconds of the clearance curve and that if this period of time is removed from the analysis, a uniform rate of blood flow is detectable thereafter. Where very high flow rates are superimposed on a period of low clearance, as for example in the development of hyperemia following a period of slow flow, arterial recirculation, of course, is no problem since the arterial concentration has fallen to zero, often many minutes before the production of the fast flow.

We devoted considerable attention to the problems of electronic stability which are peculiar to the method, namely, those of recording very low signal currents (of the order of 10^{-8} amps) with a bandwidth extending down to DC and with stringent requirements on low drift in the electrode-amplifier system. We found that one common cause of unstable recording from the hydrogen electrodes was the use of an unsatisfactory reference electrode. Thus, a standard silver/silver chloride electrode gave a somewhat varying half-cell potential, and we would suspect this to be characteristic of the majority of bimetallic reference electrodes. The use of a sintered silver/silver chloride electrode, however, overcame this, and our system now will maintain a constant baseline over the course of an entire experiment, occupying five or six hours. It should be noted that, although the external polarizing voltage applied to the electrode is 400 mV (with the platinum positive), the total polarization applied to the hydrogen electrode is, in fact, the sum of this external voltage and the half-cell potential of the silver/silver chloride electrode. In our case, this amounts to a total of about 700 mV (platinum positive). Using this external voltage and polarity, we have found the hydrogen electrodes entirely insensitive to changes in concentration of oxygen either in vivo or in vitro, P_O2 being varied from 600 mm Hg to 100 mm Hg by removal of oxygen from the gas mixture. The construction of the platinum electrodes has been found to require meticulous inspection of the insulation; we have used microscopic inspection, and also saline immersion of the entire system before use, to check insulation.

The hydrogen clearance method in our hands appears to be an excellent technique for the measurement of regional blood flow in small areas of the brain. Although it is an invasive technique, the electrodes are small, and tissue damage seems to be of acceptable minimal proportions. It is likely that the areas from which the hydrogen electrodes record, although small, are themselves beyond the immediate area of tissue damage, and therefore reflect perfusion through relatively normal tissue. In this respect, the high rates of clearance obtained by the hydrogen electrode give evidence of good perfusion through the tissue, and the maintenance of CO2 reactivity and of autoregulatory characteristics, as presented in the following paper, show entirely normal cerebrovascular responses in the region of these small needles.

References

HYDROGEN CLEARANCE METHOD

The Hydrogen Clearance Method in Assessment of Blood Flow in Cortex, White Matter and Deep Nuclei of Baboons

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