Total Cerebral Blood Flow in the Monkey
Measured by Hydrogen Clearance

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Abstract:
Simple hydrogen-sensitive polarographical electrodes of thin platinum wire were inserted into the torcular Herophili of Rhesus monkeys. Hydrogen was administered by inhalation for ten minutes, after which the hydrogen clearance was recorded from torcular blood. At a Paco of 32 mm Hg (SD ± 2.3), flow in the fast flow compartment was 102 ml/100 gm per minute (SD ± 19.1), and flow in the slow flow compartment was 28 ml/100 gm per minute (SD ± 5.8). Mean total cerebral blood flow was 52 ml/100 gm per minute (SD ± 10.5). Coefficient of variation was less than 10%.

Our experience suggests that one may reliably measure average total cerebral blood flow in the experimental setting by following the clearance of hydrogen from torcular blood. The method is relatively simple, inexpensive and radiation-free. It can be easily combined with the standard hydrogen clearance technique for measuring local tissue blood flow, thereby permitting the simultaneous recording of both local and total brain blood flow.

Additional Key Words
inert gas clearance hydrogen polarography platinum electrodes hydrogen inhalation

Introduction
Since the detailed report of Aukland and co-workers in 1964, the hydrogen clearance method has been used widely to measure local blood flow in diverse tissues including brain and spinal cord. The method employs hydrogen-sensitive polaro graphical electrodes of fine platinum wire that develop current proportional to the partial pressure of hydrogen in surrounding tissue. When hydrogen administration is stopped and its concentration in arterial blood falls to zero, the clearance rate of hydrogen from the tissue, reflected as a proportionate decline in current from the electrode, is determined by local blood flow. If the clearance curve is monoeponential, blood flow may be calculated by the formula

\[ F = \frac{\lambda \cdot 0.693}{T_{1/2}} \] (1)

where \( F \) equals blood flow in milliliters per gram per minute, \( \lambda \) is the blood: tissue partition coefficient, which for hydrogen is 1, and \( T_{1/2} \) is the half-time in minutes of the hydrogen clearance curve.

Both Aukland et al. and Neely et al. also inserted these simple electrodes into representative draining veins and thereby measured average total blood flow to entire organs such as heart, skeletal muscle and kidney. If it were applicable with equal ease and validity to the brain, the hydrogen clearance method would become even more useful since both local and total cerebral blood flow could be monitored simultaneously. This paper reports our experience with measuring average total cerebral blood flow in the monkey by following the clearance of hydrogen from torcular blood.

Methods
Rhesus monkeys unselected as to sex and weighing 3 to 5 kg were initially anesthetized with phencyclidine HCl and pentobarbital and intubated endotracheally with a cuffed tube. Catheters were placed in the right femoral artery and vein for monitoring of blood pressure, administration of drugs, and sampling of arterial blood for blood gas analysis before and after each flow study. In some animals a polarographical electrode modified for intra-arterial use was passed into the abdominal aorta via the left femoral artery. The animal was then positioned sphinx-like in a stereotactic head-holder. A 19-gauge needle was passed percutaneously into the cisterna magna and connected to a transducer and polygraph to continuously record the intracranial pressure. Ventilation was maintained at a rate of 36 to 42 per minute. The inspired gas was a mixture of nitrous oxide and oxygen in approximately a 3 to 1 ratio; enough carbon dioxide was added to maintain the desired arterial PCO\(_2\). Lactated Ringer's with tubocurarine chloride was infused by

pump intravenously at a constant rate of 5 ml per kilogram per hour to maintain fluid balance and muscular paralysis during the entire experiment. Body temperature was monitored with a rectal thermometer and maintained between 37° and 39°C with a heating pad.

Part of the skull at inion was removed and an electrode was passed through the dura into the torcular Herophilus and anchored in place with methyl methacrylate. The reference electrode was a self-tapping stainless-steel screw passed into the frontal bone. Hydrogen was added to the inspired gas mixture at the endotracheal tube entrance in a concentration that varied from 5 to 25 vol %, but was constant for each flow determination. Hydrogen was usually given for ten minutes, but in some experiments the period of inhalation was varied from 5 to 30 minutes. At the end of a predetermined period, hydrogen flow was stopped abruptly and the recording of its clearance from the torcular blood began. Flows were obtained during states of normocapnia, hypocapnia and hypercapnia.

The electrode was made of Teflon insulated platinum wire (0.25 mm diameter). At the active end, 3 mm of wire was stripped of insulation and a glass bead was fused to the tip. The electrode was then cleaned in concentrated sulfuric and nitric acid and cathodized in a 5% platinum chloride solution for two seconds at a current density of 5 milliamps per square millimeter to produce a light gray coating of platinum black at the electrode surface.

The circuit was designed by Willis and has been described previously. It is a modification of earlier designs, and provides a constant electrode polarization potential of 0.65 volt for a wide range of electrode currents. The output is stable and generally free of artifacts. The unit has eight separate circuits powered by a dual 15-volt power supply, permitting one to record simultaneously from up to eight electrodes. The output is connected to a standard laboratory polygraph for recording of the clearance curves. An on-line digital converter simplified the analysis of data.

**DATA ANALYSIS**

The first 40 seconds of each clearance curve were not used. The remainder was transferred to semilog graph paper. The biexponential clearance curves from the torcular were analyzed by standard curve-stripping techniques. Flows in the fast and slow flow compartments were calculated from the slope of each monoexponential clearance curve using equation (1). To determine average total flow, we assume that the hydrogen concentration achieved at the end of the saturation period is equal to the sum of the Y-axis intercepts of the fast and slow clearance curves at zero time. The straight line fast and slow clearance curves are then extrapolated through the unplotted initial 40 seconds of each curve to obtain the zero time Y-axis intercepts for each compartment. This gives the "weight" of each compartment and allows one to calculate the average total flow

\[
\text{Total flow} = I_1 + I_s
\]

where \( I_1 \) and \( I_s \) are the zero time Y-axis intercepts of the fast and slow compartments, respectively, and \( F_1 \) and \( F_s \) are the flows in the fast and slow compartments.

For animals with both torcular and aortic electrodes, the total blood flow also can be calculated using the familiar formula of Kety and Schmidt, as described by Gotoh et al.

\[
\text{Cerebral blood flow (ml/100 gm per minute) =} \frac{\text{100Vo}}{\int \text{d}(v-a) \text{dt}} (3)
\]

in which the quantity \( \int (v-a) \text{d}t \) is obtained by direct measurement of the area between the aortic and torcular hydrogen clearance curves, and \( \text{Vo} \) is the venous hydrogen tension at time zero.

**Results**

**TORCULAR ELECTRODES**

Torcular clearance curves were always biexponential and usually smooth (figs. 1, 2 and 3). The baseline remained stable for entire experiments, which often were more than ten hours long. Compartmental analysis (equation 2) of 47 flow determinations from 13 experiments yielded the following: at a mean \( \text{Paco}_2 \) of 32 mm Hg (SD ± 2.3), mean flow was 102 ml/100 gm per minute (SD ± 19.1) for the fast flow compartment and 28 ml/100 gm per minute (SD ± 5.8) for the slow flow compartment. Mean total cerebral blood flow was 52 ml/100 gm per minute (SD ± 10.5). The coefficient of variation (standard deviation/mean × 100) for repeated total flow determinations, performed while all parameters known to affect cerebral blood flow were kept as constant as possible, was less than 10%. Increasing the period of inhalation from 10 to 30 minutes had little consistent effect on results (fig. 3). Increasing and decreasing the \( \text{Paco}_2 \) resulted in the anticipated changes in blood flow (figs. 1 and 4).

Ten clearance curves from two animals with both aortic and torcular electrodes were analyzed by the height/area formula (equation 3) as well as compartmentally (equation 2). The height/area formula gave 57.4 ml/100 gm per minute (SD ± 8.7), and compartmental analysis gave 66.7 ml/100 gm per minute (SD ± 7.2). The difference is not significant (paired t test; \( P > 0.05 \)).

![FIGURE 1](http://stroke.ahajournals.org/)

A semilog plot of three successive hydrogen clearance curves from torcular blood in one experiment. Curves are biexponential and respond as expected to changes of \( \text{Paco}_2 \).
A polygraph tracing during a blood flow determination. Hydrogen comprised 25 vol % of inspired gas mixture. During saturation and desaturation there are no significant changes in blood pressure, pulse rate or intracranial pressure.

AORTIC ELECTRODE

Half-time for the decline in hydrogen concentration in the aorta was usually less than ten seconds. In all instances, the hydrogen concentration in aortic blood fell to well below 10% of the original concentration within 40 seconds. Curves were smooth and free of artifacts (fig. 5).

EFFECT OF HYDROGEN INHALATION ON VITAL SIGNS AND BLOOD GASES

Concentrations of hydrogen of up to 25 vol % of inspired gas had little effect on blood pressure, heart rate or intracranial pressure (fig. 2). Before hydrogen inhalation was begun the Pao2 generally ranged between 140 and 150 mm Hg and PcO2 between 31 and 33 mm Hg. During the hydrogen inhalation, the Pao2 would fall approximately 15 mm Hg and PcO2 1 or 2 mm Hg. The blood gases returned to their previous levels shortly after the hydrogen was discontinued.

Discussion

Our results suggest that average total cerebral blood flow can be measured with acceptable accuracy and reliability by adding hydrogen to inspired gas and subsequently following its clearance from torcular blood with simple platinum electrodes. Flow values for fast and slow compartments as well as for total cerebral blood flow approximate those reported by others using different methods in both man and other mammals (table 1).17-22 The coefficient of variation also compares favorably with that of other methods. However, some criticisms and objections to this method can be anticipated.

Objections have been raised to the inhalation method of delivering the inert indicator gas.23 When measured by following the clearance of inhaled 133Xenon from the brain with radiation detectors applied to the scalp, cerebral blood flow determinations are subject to errors introduced by recirculation of the gas and by the slow clearance of the gas from the scalp. The latter objection does not apply here since the electrode is immersed in blood flowing only from the brain. Regarding recirculation after prolonged inhalation, all parameters being equal, hydrogen concentration in arterial blood becomes negligible more rapidly than 133Xe because hydrogen diffuses more rapidly than Xenon and has a lower blood-gas partition coefficient. Moreover, by increasing the ventilatory rate while maintaining a physiological Paco2 and PaO2 with appropriate adjustments of the inspired gas mixture, one may increase the rate of clearance of hydrogen from lungs and arterial blood. In our experiments, the aortic electrode recorded clearance curves with half-times of less than ten seconds (fig. 5). Therefore, 40 seconds after hydrogen administration is stopped, arterial hydrogen concentration falls to less than 10% of its original concentration. In this instance, graphic analysis of the portion of the torcular clearance curve that begins 40 seconds after cessation of hydrogen administration in-

A semilog plot of three successive hydrogen clearance curves from torcular blood in one experiment. Changes in the duration of hydrogen inhalation had little consistent effect on clearance curves.

The effect of changing PaO2 on total cerebral blood flow as measured from torcular hydrogen clearance curves following a ten-minute period of inhalation. Six experiments are represented by different symbols.
A polygraph tracing of hydrogen clearance curves simultaneously recorded from the torcular and aorta after a 20-minute period of hydrogen inhalation. Note the rapid decrease in the hydrogen concentration of aortic blood variably yields straight lines on the semilog plot for both the fast and slow compartments of the clearance curve. This supports the assumption that in these experiments, recirculation introduced an error that was acceptably small. We found additional support for this assumption from the results of comparing flows determined by both the compartmental analysis (equation 2) and the height/area analysis (equation 3). We analyzed ten clearance curves by both methods. The resulting cerebral blood flow values did not differ significantly (paired t test; P > 0.05). In the height/area analysis, the error introduced by recirculation is eliminated by subtracting the area under the aortic clearance curve from the area under the torcular clearance curve. In our compartmental analysis, the error introduced by recirculation is minimized by omitting the initial 40 seconds of the recorded torcular clearance curve when arterial hydrogen concentration remains appreciable. The initial part of the clearance curve is subsequently reconstituted by extrapolation from the later segment of the clearance curve formed while the arterial hydrogen concentration is negligible.

Meyer and associates7-23 contend that when hydrogen is given by inhalation, arterial hydrogen desaturation takes many minutes because of recirculation. They provided no documentation for this assumption. On the other hand, Pasztor et al.9 found that the half-time of the hydrogen clearance curve from the aorta was approximately 28 seconds in baboons given the gas by inhalation. They concluded that hydrogen recirculation is not a problem if the first 40 seconds of the clearance curve are discounted. Neely et al.3 found that the half-times of hydrogen clearance curves recorded from the aorta of dogs given hydrogen by inhalation were 12 seconds during normal respiration and six seconds during hyperventilation. Our results in monkeys, which inhaled the gas for ten minutes or longer, are identical to those reported by Neely et al.3 We have concluded that recirculation prevents one from using inhaled hydrogen and a simple torcular electrode to measure cerebral blood flow only when flow is very fast, i.e., with half-times in the fast flow compartment of less than 0.4 minute. In this case both aortic and torcular electrodes can be used and the data analyzed by the height/area formula.

The method is subject to other potential sources of error. Baseline shifts can be troublesome and the cause often can be traced to a defect in the electrode

### Table 1

<table>
<thead>
<tr>
<th>Author</th>
<th>Species</th>
<th>Method</th>
<th>PaCO₂ mm Hg</th>
<th>Flow fast</th>
<th>Flow slow</th>
<th>Total flow</th>
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<tr>
<td>Kety and Schmidt815</td>
<td>rhesus and</td>
<td>N₂O inhalation</td>
<td>?</td>
<td>22 - 66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lassen et al.17</td>
<td>man</td>
<td>N₂O inhalation</td>
<td>?</td>
<td>41 - 78</td>
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<td></td>
</tr>
<tr>
<td>Reivich18</td>
<td>rhesus</td>
<td>Jugular flowmeter</td>
<td>41</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harper19</td>
<td>man</td>
<td>N₂O inhalation</td>
<td>40</td>
<td>50 - 55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gotoh et al.16</td>
<td>man</td>
<td>H₂ inhalation</td>
<td>?</td>
<td>50 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hjørd Rasmussen et al.20</td>
<td>man</td>
<td>Arterial 133Xe</td>
<td>39</td>
<td>52 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McHenry et al.21</td>
<td>man</td>
<td>Arterial 133Xe</td>
<td>35 - 42</td>
<td>43 ± 4</td>
<td></td>
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<tr>
<td>Shinohara et al.6</td>
<td>rhesus</td>
<td>Arterial H₂</td>
<td>?</td>
<td>45 ± 6</td>
<td></td>
<td></td>
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<tr>
<td>Petruk et al.22</td>
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<td>Arterial 133Xe</td>
<td>35 - 45</td>
<td>49 ± 13</td>
<td></td>
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<tr>
<td>Present series</td>
<td>rhesus</td>
<td>H₂ inhalation</td>
<td>32 ± 2</td>
<td>52 ± 10.5</td>
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</table>

*ml/100 gm per minute ± standard deviation.
insulation. Changes in arterial Po₂ and Pco₂, brought about by the addition of hydrogen to the inspired gas mixture, can affect both baseline and blood flow directly. This source of error is minimized by keeping the hydrogen concentration in the inspired gas as low as possible and by adjusting the inspired Po₂ and Pco₂. Reading of curves from the polygraph tracing, replotting them on semilog paper, and calculating the slopes and half-times by hand are additional sources of error. Analysis of data becomes simpler and more accurate with the use of an on-line digital readout system and a computer programmed to do the curve-stripping and to calculate by linear regression analysis the least-squares best fit of the monoexponential fast and slow clearance curves.

Objection has been raised to the use of simple platinum wire as a vascular electrode. Gotoh et al. contend that platinum wires cannot be used for insertion directly into the blood stream because “they are sensitive to other unknown substances affecting the oxidation-reduction system in the blood besides hydrogen gas.” This is contrary to the experience of Aukland et al. and Neely et al., who placed simple platinum wire electrodes in representative draining veins of skeletal muscle, heart and kidney and obtained calculated flow values that were close to the actual flow values obtained by direct measurement. Our torcular electrodes remained stable and briskly responsive for more than ten hours, also supporting the view that simple wire electrodes reflect accurately the partial pressure of hydrogen in blood when blood gases, acid-base balance, and diverse reducing substances remain stable during a clearance determination.

Recognizing the problem of recirculation with the inhalation method, we initially used the technique of Shinohara et al., which employs saline saturated with hydrogen injected rapidly as a bolus into the internal carotid artery. We found that torcular clearance curves so obtained often had three slopes — an initial very rapid clearance approaching the rate of arterial desaturation; a second slope with half-time of less than one minute, which dominated most of the clearance curve; and a third component representing the slow compartment (fig. 6). We encountered two major difficulties in interpreting the torcular clearance curves obtained by intra-arterial injection of hydrogen in saline: first the peak hydrogen concentration achieved at the onset of desaturation together with the initial very rapid period of desaturation may be a reflection of shunt diffusion that is not representative of effective cerebral blood flow. Such an initial very fast component of a clearance curve followed by two slower ones has also been recorded by Aukland et al. from the renal vein after intra-aortic injection of hydrogen in saline. Second, the clearance curve is dominated by the fast flow compartment, which receives the major proportion of the injected hydrogen. Only a small amount of hydrogen equilibrates with tissue of the slow flow compartment. Consequently, during the latter half of the clearance curve, when the slow flow dominates, the signal-to-noise ratio is low and artifacts may interfere with accurate measurement of the hydrogen concentration in torcular blood. On the other hand, after ten minutes of inhalation even the tissues of the slow flow compartment are usually more than 90% saturated with hydrogen. As a result, the latter half of the clearance curve, which is largely determined by flow through the slow compartment, is smooth and relatively free of artifacts. As pointed out by Aukland et al., the slow flow compartment can also be saturated with prolonged intra-aortic infusion of saline saturated with hydrogen, which also avoids the problem of recirculation. With the modifications noted, however, the inhalation technique of delivering hydrogen appears to be equally valid and has the advantages of avoiding fluid overload, of being simpler, and of providing more hydrogen to the tissue, thereby yielding smoother clearance curves.

**Acknowledgments**

Dr. Martin Reivich reviewed the manuscript and provided helpful criticisms. Mr. Walter Stringfield gave technical assistance.

**References**


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Stroke. 1974;5:512-517
doi: 10.1161/01.STR.5.4.512
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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