H₂ Clearance Measurement of Blood Flow: A Review of Technique and Polarographic Principles

Wise Young, Ph.D., M.D.

SUMMARY H₂ clearance is a powerful method for monitoring blood flow. Simple and inexpensive to implement, the method allows multiple in situ determinations of blood flow from any tissue in which a small electrode can be implanted. There is, however, evidence to suggest that H₂ clearance is neither as accurate nor as local a measure of blood flow as generally supposed. Both in theory and practice, it probably cannot accurately determine blood flow rates greater than 100 ml/100 gm/min or localize blood flow to tissue volumes of less than 5 ml. Moreover, its experimental application is complicated by many technical problems hitherto largely ignored by workers in the field. Some of these problems arise from the limitations of the steady state polarographic technique used to measure tissue H₂ concentrations. Other problems stem from the failure to consider possible sources of error in H₂ clearance monitoring; these include interference with the H₂ signal by spurious electrode and tissue currents, and contributions from tissue ascorbate and O₂. Nevertheless, with the appropriate safeguards and qualifications, H₂ clearance is a valid and important approach to measuring blood flow.

The appropriate technology for monitoring tissue H₂, however, had to be developed. In 1956, Misrahy and Clark⁹ recorded the potentials generated by spontaneous oxidation of inhaled H₂ gas with platinum electrodes implanted in animal cerebral cortices. They found that under conditions generally known to increase blood flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials. Conversely, decreasing flow with barbiturate drugs resulted in decreasing flow, i.e., CO₂ inhalation, their electrodes recorded larger positive potentials.

In 1961, Hyman¹¹ introduced a quantitative polarographic technique of measuring tissue H₂ concentration. Instead of recording the potentials generated by spontaneous H₂ oxidation, he monitored the current generated by a platinum electrode, finding that it correlated with H₂ concentration. Auckland et al.² in 1964 modified this polarographic technique by polarizing the electrode to +250 mV, the voltage of a calomel reference electrode. With in vitro studies, they concluded that the technique did linearly estimate tissue H₂ concentration and that interference by substances, such as O₂, H⁺, and ascorbate, was acceptably small. Then they recorded the exponential

From the Departments of Neurosurgery and Physiology and Biophysics, New York University Medical Center, 550 First Avenue, New York, NY 10016.
Reprints: Dr. Young, Department of Neurosurgery, NYU Medical Center, 550 First Ave., New York, NY 10016

desaturation rates of $\text{H}_2$ from animal kidneys and hearts, using both respiratory and arterial routes of $\text{H}_2$ administration. The blood flow rates were estimated from $\text{H}_2$ clearance and found to correlate well with those obtained by venous outflow collection, except for the fastest flow components.

The applicability of the $\text{H}_2$ clearance method to brain blood flow monitoring was quickly established. In 1964 to 1968, Fieschi et al. \textsuperscript{12-16} and Gotoh et al. \textsuperscript{17, 18} and others \textsuperscript{19-25} applied $\text{H}_2$ clearance to the cerebral cortex and obtained reasonable flow values. In 1969, Fieschi et al. \textsuperscript{19} directly compared $\text{H}_2$ clearance with the $^{14}\text{C}$ antipyrene autoradiographic technique, measuring blood flow in cat subcortical nuclei and white matter; good correlation was obtained between these 2 methods. More recently, Lamorgese et al. \textsuperscript{20} and Rowan et al. \textsuperscript{21} reported excellent correspondence between blood flow values obtained by $\text{H}_2$ clearance and 2 other monitoring approaches, the radioactive microsphere injection and the $^{133}\text{xenon}$ gas clearance techniques.

Advantages of $\text{H}_2$ Clearance

$\text{H}_2$ clearance possesses distinct advantages over other blood flow monitoring techniques. First, $\text{H}_2$ clearance can be determined in any tissue where a small platinum electrode can be inserted. Second, multiple flow determinations can be obtained from the same tissue site over long periods of time, unlike autoradiographic or microsphere techniques. Third, blood flow can be estimated from the clearance rate of $\text{H}_2$, independently of the absolute amplitude of the $\text{H}_2$ signal. The only requirement is that the electrode response remains linear and constant over the time course of the clearance. Fourth, the $\text{H}_2$ clearance method is among the most inexpensive blood flow monitoring techniques available. The electronic circuitry is simple and the electrodes are easily manufactured. Hydrogen gas is readily available and requires no elaborate facilities to handle.

Not unexpectedly, the $\text{H}_2$ clearance method has been adopted by many laboratories to study a wide range of blood flow phenomena. Symon et al. \textsuperscript{25-30} have utilized $\text{H}_2$ clearance to study the effects of middle cerebral artery occlusions in baboons. Kobrine et al. \textsuperscript{31-36} and, more recently, Senter et al. \textsuperscript{37-39} applied the method to investigate blood flow autoregulation and traumatic ischemia in the spinal cord. Lubbers et al. \textsuperscript{40-42} examined the relationship of tissue oxygenation and blood flow, taking advantage of the fact that platinum electrodes can be used to detect tissue $\text{O}_2$ concentration as well as $\text{H}_2$. Some \textsuperscript{43-50} have used $\text{H}_2$ clearance alongside ion-sensitive microelectrodes to determine the influence of tissue pH and $\text{K}^+$ concentration on blood flow. $\text{H}_2$ clearance has been used routinely in many laboratories to monitor blood flow in ischemic tissues. \textsuperscript{51-70}

Limitations of the $\text{H}_2$ Clearance Method

As experience accumulated in the use of $\text{H}_2$ clearance, several criticisms of the method have been voiced. First, some workers have objected to the implantation of electrodes within brain tissues, on the grounds that it may cause local injury and consequent alteration of blood flow. \textsuperscript{14, 16, 71, 72} Second, the $\text{H}_2$ clearance curves are often polyexponential, \textsuperscript{14-17, 22-28, 33-38} raising serious questions concerning the use of a single exponential clearance rate for approximating blood flow. Third, the clearance method assumes that arterial concentration of the tracer falls to zero. There is some evidence to suggest that this may not be true in the first minute of $\text{H}_2$ clearance, especially in the smaller arteries and arterioles. \textsuperscript{65, 69, 74} Fourth, platinum electrodes are sensitive to $\text{O}_2$ changes. Although Aukland et al. \textsuperscript{1} have shown that, under normal circumstances, this effect is negligible, substantial error may be introduced in blood flow studies of severely ischemic tissues.

Partially in response to these criticisms, certain standard practices in $\text{H}_2$ clearance have evolved. In order to minimize tissue injury from the electrode implantation, most workers in the field have tended to use smaller and smaller electrodes. Aukland et al. \textsuperscript{1} originally used 2 mm diameter electrodes, but, in recent years, electrodes of 200-250 micra, \textsuperscript{20, 30, 35, 39, 40, 50, 61, 63, 65, 66} 75 micra, \textsuperscript{52-56} and even 1 micron \textsuperscript{46, 51, 52, 57} diameter tips have been favored. Many investigators \textsuperscript{12, 14, 20, 41-48} dealt with polyexponential curves by assuming that independent tissue compartments contribute to the clearance and by treating the clearance curve as a sum of several exponential components. Some researchers avoid the entire issue of polyexponential clearance by scrupulously recording blood flow only from homogeneous tissue such as white matter. \textsuperscript{59} To circumvent artifacts due to recirculated arterial $\text{H}_2$, many investigators simply discard the first 1-2 minutes of the clearance data. Others go so far as to use only 2-3 minute segments of the clearance curve. \textsuperscript{25-30} To reduce contributions from $\text{O}_2$ changes in ischemic tissue, some workers \textsuperscript{25, 33, 37, 41} polarize the electrodes to +650 mV instead of the +250 mV used by Aukland et al. \textsuperscript{1} and others. \textsuperscript{51, 65, 73} At +650 mV polarization, the sensitivity of the electrode to $\text{O}_2$ is less compared to +250 mV.

In general, these empirically derived practices have improved the reproducibility of $\text{H}_2$ clearance data from different laboratories. In a wide variety of animals, including mice, \textsuperscript{51} rats, \textsuperscript{48, 70} cats, \textsuperscript{12, 16} rabbits, \textsuperscript{49-50} and monkeys \textsuperscript{27-30, 61, 67, 68}, and humans, \textsuperscript{17, 18} gray matter blood flows estimated by $\text{H}_2$ clearance typically range from 40-100 ml/100 gm/min. White matter flows cluster around 15-20 ml/100 gm/min. Severe functional deficits usually accompany flow values of less than 5 ml/100 gm/min. In certain laboratories, reproducibility of $\text{H}_2$ clearance flow values is better than 15% \textsuperscript{37, 60, 61, 67, 74} Although data consistency partially vindicates some of these practices, the important question of how accurately $\text{H}_2$ clearance reflects blood flow remains largely unanswered.

$\text{H}_2$ Polarography

The one unspoken assumption shared by all users of $\text{H}_2$ clearance blood flow monitoring is that the polaro-
graphic technique accurately reflects H₂ concentration in tissues. As the following discussion shows, this assumption must be carefully validated in the experimental setting.

Electrode Equations

H₂ polarography is based on the principle that the oxidation reaction, $\text{H}_2 \rightarrow 2\text{H}^+ + 2e^-$, generates electrons.⁷ If provided an acceptor surface, such as platinum, the reaction will donate electrons to the electrode, causing a current flow. The acceptance of the electrons by the electrode is optimized when it is polarized to a positive voltage. When a positively polarized platinum electrode is inserted into a solution containing H₂, the H₂ molecules closest to the electrode surface oxidize to form H⁺ ions. As the H₂ adjacent to the electrode surface becomes depleted, a concentration gradient is established between the bulk solution and the immediate electrode vicinity. This concentration gradient causes a migration of H₂ molecules, controlled by the diffusion coefficient of H₂ in the solution. When the H₂ depletion is sufficiently rapid, the electrode is diffusion limited and is described by Fick’s law⁷⁷-⁷⁸

$$J = D \left( \frac{dC}{dx} \right)$$

where J is the one dimension flow in moles, dC is the concentration gradient over distance x from the electrode. The term J, the flow of H₂ towards the electrode, can be converted into a current term, using Faraday's Law:

$$i_e = nFD \left( \frac{dC}{dx} \right)$$

where $i_e$ is the current density per unit area of electrode surface, n is the number of charges transferred by each H₂ molecule, F is Faraday’s number. This equation is greatly simplified if we assume that the concentration gradient of the diffusion layer is linear:

$$i_e = nFD \left( \frac{C_\text{e} - C_\text{o}}{L} \right)$$

where $C_\text{e}$ and $C_\text{o}$ are the H₂ concentrations in the bulk solution and at the electrode surface respectively; L is the thickness of the diffusion layer. If the electrode oxidation of H₂ is sufficiently extensive such that H₂ at the electrode surface approaches zero, the equation becomes:

$$i_e = nFD \left( \frac{C_\text{e}}{L} \right).$$

Although these equations assume 100% correlation between the electrode reaction and electrical output, this is not usually the case in practice.⁷⁹ No electrode is totally efficient in its energy conversion. A fraction of the current engendered from a given reaction will be dissipated as heat or bypass the electronic measuring circuit as a result of current leakage. Multiple chemical reactions can occur with an electrode inserted into a complex medium, such as brain. These reactions may interact with each other, resulting in cancellation of current. Finally, electrical work not only in the recording electrode but in the reference electrode must be considered, since current flow occurs at both electrodes to complete the circuit. Thus, an inefficient reference electrode will absorb some of the current generated by the recording electrode.

These equations tell us that current is a linear function of bulk H₂ concentration. They, however, do not yield much insight into the role of diffusion parameters, the effect of electrode polarization, and the selectivity of the electrode for different chemical reactions. Since these are crucial factors in the experimental application of H₂ monitoring, they will be discussed below.

Diffusion Error

Most investigators assume that diffusion parameters remain stable during H₂ monitoring. However, there are 2 situations where this assumption may not hold. First, ischemic brain tissues often undergo a fluctuating edema state.⁶⁰,⁶¹ Second, critics of the H₂ clearance method point out that introduction of an electrode into brain tissue may result in a devitalized zone of cells which may serve as a diffusion barrier.⁷⁹,⁸⁰

Auckland⁴ examined the effect of diffusion barriers on H₂ clearance, using a theoretical treatment originally derived for membrane covered electrodes (fig. 1). He calculated that for a membrane of 0.2 mm, the electrode response deviates from H₂ concentration only during the first second of clearance. With a membrane 0.4 mm thick, the electrode response does not approach the H₂ concentration until 16 seconds later. With membranes of 0.6 mm or greater, as much as a minute is required. This calculation suggests that the electrode response should reflect H₂ concentration during most of the clearance curve, despite a substantial diffusion barrier. Especially at low blood flow rates, even thicker diffusion barriers may be tolerated without introducing significant distortion of the H₂ clearance curve. In fact, because a membrane has a defined thickness and diffusion coefficient, it is not subject to the vicissitudes of tissue change. Thus, deliberate use of an artificial membrane⁶² may yield more consistent clearance curves.

Electrode Polarization

The polarization voltage of the electrode determines in part the intensity of chemical reaction at the electrode surface.⁷⁸ In the case of H₂ oxidation, the more positive the polarization, the greater the likelihood of electron transfer. This holds true until the supply of H₂
FIGURE 1. Contribution of diffusion error to electrode response time. The theoretical electrode response, calculated by Aukland,1 for a H₂ diffusion coefficient $D = 3 \times 10^{-5}$ cm$^2$ sec$^{-1}$, $K$ refers to the exponential clearance rate of H₂ in the bulk tissue surrounding the electrode. L is the thickness of the diffusion barrier between the electrode and the bulk tissue. The vertical dashed line at time zero indicates the start of H₂ desaturation from the tissue. The slanted dashed lines represent the H₂ desaturation rate and the solid lines represent the electrode response. The electrode responses for 3 thicknesses of the diffusion barrier were calculated. With increasing diffusion thickness, the electrode response deviates from the desaturation rate.

FIGURE 2. Steady state current-voltage plot of polarographic response. Shiny platinum electrodes are polarized to different voltages, shown on abscissa, with respect to an Ag-AgCl reference electrode. The steady state current generated by each polarization level is represented on the ordinate.

A = Deaerated versus H₂-saturated saline. The deaerated saline was obtained by vacuum boiling; the electrode response to this solution is shown in solid circles. The H₂ saturated solution was made by bubbling deaerated saline solution with 100% H₂ for 5 minutes; the electrode response is shown in open squares. Note current plateau region for the H₂ saline between the polarization levels +0.25 to +0.75 V, signifying diffusion limitation of electrode response.

B = Effect of oxide film on polarographic electrode response to H₂ in saline. The current-voltage plot for H₂ saturated saline is indicated with closed squares. The electrode was then briefly immersed into a solution of concentrated hydrogen peroxide (H₂O₂). The current voltage plot of the H₂O₂ treated electrode (represented with open circles) changes considerably. Note the decreased slope of the response and the absence of a plateau.

C = Effect of 10⁴ ohm series resistance on the polarographic electrode response. A 10⁴ ohm series resistance is placed between the polarographic electrode and the current-measuring circuit. Note the similarity of this electrode response with series resistance to the response from the electrode treated with H₂O₂.
mV, the current response reaches a plateau, signifying diffusion limitation of the reaction rate. At this point, the H₂ concentration at the electrode approaches zero and the electrode reaction rate is controlled by diffusion factors primarily. The increase in current at +800 mV results from electrolysis of H₂O.

Sometimes the electrode cannot convert H₂ rapidly enough to achieve a zero H₂ concentration at its surface, no matter what polarization is used. For example, if an electrode has insufficient active sites, i.e., due to poisoning of the electrode, the current-voltage plot does not have a diffusion-limited current plateau at higher polarization voltages. Figure 2B shows the plot of a platinum electrode which has been exposed briefly to concentrated hydrogen peroxide which deposits a film of non-reacting oxide on the electrode surface. The current response increases without a plateau region. The situation can be simulated by placing a current-limiting resistor in the electrode circuit. In figure 2C, the current-voltage plot is shown of an electrode with a 10 megohm resistor placed in the input. No diffusion limited current plateau occurs. Note that in both cases, the amplitude of the current is low.

Empirically, it can be shown that the non-diffusion limited electrode response does increase with H₂ concentration in a linear fashion. For example, in figure 3, the response of a shiny platinum electrode is plotted against H₂ concentration for different polarization voltages. Below +200 mV polarization, outside of the current plateau region, the current-concentration relationship remains reasonably linear. The disadvantage of a non-diffusion limited system is that the current responses become dependent on electrode parameters, such as capacitance, speed, polarization level, and linearity. However, it is less dependent on diffusion factors in the solution. Thus, a non-diffusion limited approach finds its greatest use when the investigator's confidence in the electrode exceeds faith in the constancy of diffusion factors in the experimental preparation.

The polarization voltage also has an effect on electrode response time. When polarization voltage is increased, reaction intensity does also. Consequently, a steeper concentration gradient will result, taking more time to reach a steady state (fig. 4). Thus, the greater the polarization voltage used, the longer the time required by the electrode to equilibrate. But most important of all, the polarization voltage determines, in part, what chemical substances are oxidized or reduced by the electrode.

**Electrode Selectivity**

Theoretically, a polarographic electrode will respond to any reacting chemical species which will exchange electrons with it. However, electron transfer is limited by the potential energy barrier that binds the electron(s) to the reacting molecule or electrode. If this potential energy barrier is matched by the polarization voltage, the likelihood of electron transfer increases. For convenience, we will refer to the optimal polarization voltage of a given reaction as

![Figure 3](http://stroke.ahajournals.org/)

**Figure 3.** Electrode response versus H₂ concentration at different polarization voltages. A saturated H₂ solution was then sequentially diluted with deaerated saline to obtain the % saturated solutions, indicated on the abscissa. The steady state current generated by a shiny platinum electrode at +0.1V, +0.3V, +0.5V, +0.7V, +0.9V polarization are shown. Note the decreased but linear slope of current vs % H₂ saturation with smaller polarization voltages.

![Figure 4](http://stroke.ahajournals.org/)

**Figure 4.** Equilibration time for electrode response for different polarization voltages. The time required for the electrode to reach a steady state current response increases with higher polarization voltages. These data were obtained from an H₂ saturated solution. At the time indicated by an arrow, a step polarization of 0.1V, 0.3V, 0.5V, 0.7V, and 0.9V was applied. At less than 0.5V polarization voltages, the current response typically reaches steady state in less than one minute.
the redox potential. Note that it is not a single threshold whereby electron transfer takes place. Rather, depending on the electrode surface and ability to act as a catalyst, the reaction may take place over a range of voltages. A standard practice is to quote the potential at which the reaction attains 50 percent of its maximal rate, the so-called half-wave potential.

A polarized electrode will oxidize or reduce chemical substances that have redox potentials similar to the polarization voltage. Thus, an electrode polarized to +200 mV will oxidize not only H₂ but ascorbic acid, catecholamines, hemoglobin, succinic acid, to name but a few of the many readily oxidizable substances in the brain. In addition, the same electrode may reduce some substances, i.e., O₂. With sufficient reactant concentration, even reactions with greatly dissimilar redox potentials may interfere significantly with each other. For H₂ monitoring in brain tissues, at least one substance may contribute current to an electrode polarized to +500 mV: ascorbic acid.

Ascorbic acid has a half-wave potential of approximately +300 mV, only +80 mV from the H₂ half-wave potential. On that basis alone, ascorbic acid would be expected to contaminate H₂ currents recorded polarographically. Aukland et al. examined the role of ascorbic acid in H₂ monitoring. They found that the current contributed by 0.1 mM ascorbic acid corresponded to 0.25% of H₂ saturation current, recorded with a +250 mV polarized electrode. The current voltage curve for ascorbic acid, an example of which is shown in figure 5A, shows a plateau region at +500 mV and greater. At +500 mV, the ascorbic acid current is much greater than at +250 mV. They concluded that, for physiological concentrations, the role of ascorbic acid contamination is negligible. They, however, did not realize that brain ascorbic acid levels are 6–8 times higher than in most other tissues of the body, on the order of 1.0 to 3.0 mM (fig. 5B). Aukland et al. also had not anticipated that workers in the field would use +600 mV polarization, where sensitivity to ascorbic acid is maximized.

**Polarographic Instrumentation**

The polarographic technique, because it measures current resulting from an imposed voltage, is conceptually an electrode impedance measuring system. For example, in an electrode polarized to +600 mV, 0.6 microamperes of current implies an electrode resistance of 1 megohm. In theory, the electrical circuitry required to measure the current is simple but practical implementation is harder to achieve. The main problem rests with the small amplitudes of current involved. With the present trend towards the use of smaller and smaller electrodes, often with impedances of 10 megohms or more in H₂ saturated solutions, instrumentation that can resolve nanoamperes or even picoamperes is necessary. Great pains must be taken to ensure that the circuit measures all the current generated by the polarographic electrode. Because such small currents are involved, any spurious leakage of current from the polarographic electrode to the circuit will yield large signal loss.

Presently, 2 types of circuit are being used in H₂ clearance experimentation. The most common circuit places a resistance in series with the electrode and a high impedance amplifier is used to measure the voltage drop across the resistance. As pointed out above, the series resistance must be substantially smaller than the electrode resistance or else the former
becomes current limiting, simulating a poisoned electrode. With the recent advent of cheap high-gain operational amplifiers with current leakage specifications on the order of femtoamperes (10⁻¹⁴ amperes), it has become possible to build current measuring circuits, shown in figure 6. In both cases, a feedback loop is connected between one input and output of the amplifier. The output of the amplifier will be proportional to the current needed to cancel the voltage difference between the 2 inputs, feeding electrons as necessary to maintain either the polarographic electrode or the reference electrode at a given voltage. Both circuits can be used together (fig. 7).

To complete the circuit, any current generated by the polarographic electrode must also traverse the reference electrode. In order not to influence the polarographic signal, the reference electrode must be of low resistance, pass current reversibly, and not generate any intrinsic current. Most investigators virtually ignore the reference electrode. Some use stainless steel needles inserted into muscle or a brass screw implanted in the skull. Such electrodes suffer from junction potentials and rectification. Furthermore, any alteration of the chemical environment may change the reference electrode and perhaps even yield polarographic-type currents. Since the reference electrode for H₂ monitoring is negatively polarized with respect to the polarographic electrode, it can reduce O₂ and, therefore, may be sensitive to O₂ changes. The ideal reference electrode should have good electrical contact with the preparation but be isolated from the chemical environment of the tissue. One practical approach is to place the reference electrode in a glass pipette filled with concentrated KCl, bridging this solution through agar to the preparation.

### H₂ Clearance

The basic paradigm of H₂ clearance consists of inserting a positively polarized electrode into tissue, administering H₂ either by respiration or intra-arterially, allowing the H₂ to be cleared from arterial blood, and then monitoring the exponential clearance rate of H₂ from the tissue.

#### Clearance Equation

The equations for calculating blood flow from tracer clearance have been extensively reviewed.
A coefficient of determination ($r^2$), for estimating line fit, can be obtained through least square analysis:

$$r^2 = \frac{[n \sum t_i \ln C_i - \sum t_i \sum \ln C_i]^2}{[n \sum t_i^2 - (\sum t_i)^2][n \sum (\ln C_i)^2 - (\sum \ln C_i)^2]}$$

The value $k$ can be calculated, given a set of points representing $(C_i, t_i)$. Figure 8 shows an example of this calculation.

Most experimental $H_2$ clearance curves are not monoeXponential, however. This has been attributed to the well known fact that nervous tissue is a heterogeneous mixture of white and gray matter which have greatly differing blood flows. We can assume that the different compartments of the brain are cleared independently by blood. This makes it possible to consider the $H_2$ clearance curve as a series of summed exponential terms:

$$C_i = C_o (W_i e^{k_1 t} + W_2 e^{k_2 t} + \ldots W_n e^{k_n t})$$

which represents a biexponential curve. If the electrode is inserted into a predominantly gray matter region of the brain, $W_i$ becomes large relative to $W_2$. In this case, the initial slope of the exponential clearance curve approximates $k_1$. Conversely, if the electrode is inserted into a predominantly white matter region of the brain, $W_i$ is small relative to $W_2$; therefore, $k_2$ can be approximated from the exponential slope in the latter part of the clearance curve. If $W_i$ and $W_2$ are known from histological examination of the tissue surrounding the $H_2$ electrode, it is possible to calculate mean blood flow from the slopes and intercepts of the two exponentials:

$$r_{CBF} = \frac{I_1 k_1 + I_2 k_2}{I_1 + I_2}$$

where $I$ refers to the intercepts and $k$ refers to the rates for compartments 1 and 2.

There are 4 troubling assumptions implicit in the above approach to calculating blood flow from the polarographic signal. The first is that $H_2$ achieves rapid equilibration between vascular and tissue compartments. The second is that the arterial concentrations of white and gray matter are known from histological examination of the tissue surrounding the $H_2$ electrode. It is possible to calculate mean blood flow from the slopes and intercepts of the two exponentials:

$$r_{CBF} = \frac{I_1 k_1 + I_2 k_2}{I_1 + I_2}$$

where $I$ refers to the intercepts and $k$ refers to the rates for compartments 1 and 2.

Rapid Equilibration of $H_2$

Because of the small size and high lipid solubility of $H_2$, most investigators have tacitly assumed that the
equilibration of $\text{H}_2$ between the blood and tissue is sufficiently rapid and complete to allow blood flow estimation. This assumption is partly supported by data. Aukland et al.\textsuperscript{6, 16} and Meyer et al.\textsuperscript{12} found that intra-arterial injection of a bolus of $\text{H}_2$-saturated solution will result in a rapid rise in $\text{H}_2$ current in tissues. Likewise, Halsey et al.\textsuperscript{64} reported that intracarotid injections of $\text{H}_2$-saturated solutions cause virtual step increases in $\text{H}_2$ current monitored in cerebral gray matter. However, we need to examine this assumption critically from another point of view.

Kety\textsuperscript{3} suggested an alternative approach to the derivation of a blood flow equation that does not require the assumption of instantaneous tissue-blood equilibrium of tracer. Using equations originally developed for pulmonary uptake of anesthetic gases, he showed that:

$$C_i = C_0 e^{-kt}, \quad k_i = \frac{m_i F_c}{V_i \lambda_i} \quad \text{XVII}$$

for diffusion dependent desaturation of inert gaseous tracers from tissues. This solution differs from equation VII only in that it includes the term $m_i$; $S$ is the diffusion surface of the capillaries per unit volume of tissue, $F_c$ is the velocity flow of blood for the vascular system, and $D'$ is the diffusion coefficient for the tracer per unit capillary surface area. Thus, $m_i$ is a diffusion and flow dependent term. Increases in the value of $S$ and $D'$ bring the value of $k_i$ close to $F_c$, whereas increases in $F_c$ tend to diminish $k_i$. Therefore, this equation predicts that larger capillary surface area and a greater diffusion coefficient for $\text{H}_2$ will enhance while faster blood flow will diminish the apparent blood flow values estimated by $k_i$. This derivation is based on the assumption that the $\text{H}_2$ molecules require a finite time to cross the capillary surface.

It is difficult to evaluate the significance of $m_i$ term for $\text{H}_2$ clearance because the value of $D'$ for $\text{H}_2$ diffusion across capillary surfaces has not been determined. If we assume that $D'$ approximates the diffusion coefficient of $\text{H}_2$ in dilute solutions, $5 \times 10^{-5} \text{ cm}^2/\text{second}$,\textsuperscript{3, 4} we can estimate $m_i$. The capillary surface for cerebral cortex\textsuperscript{31} is about 190-240 cm$^2$/cm$^3$. Therefore, for $D' = 5 \times 10^{-5} \text{ cm}^2/\text{sec}$, $D'S$ is about 0.51-0.72. For $F_c$ of 1.0 cm/min, $m_i$ calculates to 0.4-0.5. Note that $F_c$ has the dimensions of cm/min. If the vascular volume of the cerebral cortex is 1% of total tissue volume, then an $F_c$ value of 1.0 cm/min approximates 1.0 cc/cc/min. Thus, depending on the value of $D'$, the $m_i$ term may contribute as much as 50% error in $k$ for flows greater than 1 cc/cc/min. At lower flow rates, the error is less.

**Arterial $\text{H}_2$ Concentration During Clearance**

The assumption that arterial $\text{H}_2$ concentration falls close to zero during the clearance measurements has been partially substantiated by experimental data. Fieschi et al.\textsuperscript{16} and others\textsuperscript{40, 50} have recorded $\text{H}_2$ concentrations in the major arteries and verified that indeed 95% of the $\text{H}_2$ is cleared within one min. The $\text{H}_2$
levels in the major arteries, however, may not correspond with those in the smaller arteries and arterioles. Stosseck and Lubbers et al.48, 49 have reported that the clearance of H2 from pial arteries lags considerably behind the major arteries; several minutes after H2 is cleared from the aorta, a large fraction of H2 may still be detectable in the pial arteries of the brain. Consequently, they speculated that pre-capillary arterial exchange of H2 with brain tissue occurs. This phenomenon does not necessarily violate the Fick Principle which merely stipulates that no additional tracer is brought to the tissue. It implies, however, that significant H2 transfer can occur between different brain tissue compartments through the vascular space.4, 59 H2 leaking into the arterioles may influence H2 clearance by capillaries and venules downstream.

It should be emphasized that, although early studies do show very efficient clearing of H2 from the arterial blood by the pulmonary system, very few laboratories routinely and systematically monitor arterial H2 levels. For assessment of very high flow components of 200 ml/100 gm/min or more, the first minute of clearance becomes crucial. In such cases, it may be necessary to monitor arterial H2 or end-tidal respiratory H2 to estimate the tissue-blood H2 gradient. The appropriate corrections can then be applied to the clearance equations.

Localization of Blood Flow

Because the polarographic electrode monitors local H2 concentration, many investigators have claimed that H2 clearance represents blood flow of the small volume of tissue immediately surrounding the electrode.19, 22 This volume of tissue, depending on electrode size, has been quoted to be as low as 1 mm3.19, 22, 23, 24 There is, however, some experimental evidence to suggest that this is not so. H2 generated 2-5 mm from the recording electrode has been found to contribute significantly to the current of the electrode within the 10-20 min clearance time course.48-52, 62-64 Furthermore, H2 clearances obtained from small mammals with thin cortical gray matter layers of less than 2 mm thick seldom show a monoexponential fast gray matter component.29, 62-66, 69, 70, 72, 76 whereas H2 clearances from baboon cortex, which has a 5 mm thick gray layer, sometimes do.26, 26, 26, 68 Similarly, investigators52, 67, 72, 74 applying H2 clearance to spinal cords have been unable to demonstrate flow rates compatible with gray matter flow, even with electrodes inserted into the dorsal gray horns. Also, H2 clearances recorded from circumscribed ischemic regions, i.e., produced by middle cerebral artery occlusion, often show higher flow rates than expected.62, 66 These findings cast serious doubts on the ability of H2 clearance to localize blood flow to tissue volumes of less than 5 mm3.

The doubts were reinforced recently by the experiments of Stosseck and Lubbers et al.48, 49, 49, 52 They introduced a new approach to H2 blood flow monitoring, by generating H2 electrochemically in tissue instead of introducing it through the arterial blood. The time course of the H2 signal was monitored with an electrode situated 300 micra from the H2 generating electrode. Using a complex formula describing the influence of blood flow on the time course of H2 diffusion from one point to another, they calculated blood flow values that they claim are measures of truly local blood flow or "microflow." Although this technique is not very precise, and involves certain questionable assumptions concerning the homogeneity, isotropicity, and constancy of blood flow during the local clearance studies, the blood flow values obtained often differ markedly from ones obtained by the standard H2 clearance method.

Cross flow between compartments further complicates the issue of H2 clearance localization. Recently, Halsey et al.64 examined the flow rates of rapidly loading compartments, using the differential loading technique of intra-arterial H2 injections. They obtained evidence suggesting that rapidly loading compartments can transfer directly to slower loading compartments. When H2 was given by brief inhalation of 1-3 minutes duration, the initial slope of the clearance curve was 150-250 ml/100 gm/min, much higher than hitherto reported. After prolonged inhalation of H2 to complete saturation, the fast compartment slope was reduced to 70-100 ml/100 gm/min. With short injections of H2 saturated solutions intra-arterially, they occasionally encountered ultra-fast slopes suggestive of 300-1000 ml/100 gm/min. Halsey et al.64 attributed these findings to the great diffusability of H2 in brain tissues.

Baseline Shifts

Most investigators assume that the polarographic signal in the absence of H2 remains constant. A common practice is to use the baseline at the end of H2 clearance as indicative of zero H2. This practice may lead to erroneous flow estimates should a baseline change occur during clearance. Figure 9 shows an example of an H2 clearance in which the pre-clearance baseline is different from the post-clearance baseline. Depending on which baseline is assumed, the value of k may be more than 50% in error.

How stable is the resting polarographic current monitored from brain tissues? It is well known that ascorbic acid concentrations fluctuate in brain tissues, especially under conditions of stress.100 For example, during experimental ischemia induced by middle cerebral artery (MCA) occlusion, a significant decrease in tissue ascorbate concentration may occur over a short period of time.63 This fall in ascorbate levels may be reflected in the resting oxidation current monitored from ischemic cortex, as shown in figure 10. The recordings were obtained from the MCA territory of both hemispheres of a cat. The MCA on one side was occluded for an hour. Before occlusion, a resting current of about 200 nanoamperes was present in both hemispheres, recorded with platinum electrodes polarized to +500 mV. Shortly after occlusion, the polarographic current from the ischemic side fell
to 40 nanoamperes. In the contralateral hemisphere, the resting current gradually declined over the 5–6 hours of the experiment. Thus, in this situation, if the baseline change was not carefully monitored, blood flow estimates would be erroneously high.

Another possible cause of baseline shift is tissue impedance increase associated with edema formation.

During cerebral ischemia, tissue impedance may rise from 10^2 to 10^4 ohms. If the electrode impedance is on the same order of magnitude, the tissue impedance change should have relatively little effect. However, should large polarographic electrodes be used, ones that pass more than 50 microamperes, significant error may result.

Acknowledgment

This work was supported in part by grants NINCDS-2P50-NS10164-07A and NINCDS-1R01-NS 15590-01. I wish to thank Dr. Allen Hirschfeld for his help in gathering some of the literature references, Professor Eugene S. Flamm for helpful discussions, and Janet Grana for her excellent typing.

References


47. Stosseck K: Hydrogen exchange through the pial vessel wall and its meaning for the determination of local cerebral blood flow. Pfliigers Arch 320: 111-119, 1970

48. Stosseck K: Quantitative detection of spontaneous changes of...
microflow on corresponding locations of the cerebral cortex. Eur Neurol 6: 264–268, 1972
H2 clearance measurement of blood flow: a review of technique and polarographic principles.
W Young

Stroke. 1980;11:552-564
doi: 10.1161/01.STR.11.5.552

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/11/5/552