Use of Hydrogen for Measurement of Regional Cerebral Blood Flow

Problem of Intercompartmental Diffusion

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SUMMARY The extreme diffusibility of hydrogen, compared with xenon or krypton, may create serious artifacts when it is used to measure local blood flow with a tissue electrode. The errors are greatest when hydrogen is given by intra-arterial slug injection, and when the electrode is within 2 mm of another tissue compartment, CSF, or air. These all appear to be a consequence of intercompartmental diffusion which can occur at rates of the same order of magnitude as clearance from the tissue by blood flow. No matter how small the electrode, the ultimate spatial resolution of the method appears to be about 2 mm unless quantitative account is taken of diffusion. An important precaution in use of the method is to obtain homogeneous tissue saturation by prolonged inhalation administration.

HYDROGEN IS FREELY diffusible between blood and brain and is metabolically inert, making it a useful indicator for blood flow measurements based on the Fick principle. Since it is relatively easily detected polarographically it would appear to be ideal for measurement of blood flow in very discrete regions. Since the essential measurement is of a clearance rate, there is no need for quantitative calibration of electrode sensitivity, and the only demand for stabili-
Methods

Animal Preparation

Our studies were carried out mainly in adult rabbits under sodium pentobarbital anesthesia, with tracheostomy, mechanical ventilation, and paralysis with gallamine triethiodide (Flaxedil). Arterial blood pressure and expired \( CO_2 \) were monitored. Reference will also be made to observations in several cats with regional cerebral ischemia and infarction made by trans-orbital surgical ligation of one middle cerebral artery and in gerbils with bilateral carotid artery ligation. Sodium pentobarbital was the anesthesia. In cats, the monitoring procedures were the same as in the rabbits, with the addition of EEG recorded from the hydrogen electrodes. In the gerbils, EEG was monitored but blood pressure and expired \( CO_2 \) were not.

Electrodes

Electrodes were 75 micron diameter 90% platinum — 10% iridium wires, electropolished to a 5 micron diameter tip, insulated in glass with a bare tip length of about 200 microns, and platinized by the method described elsewhere to increase the catalytic surface area. Polarization was at +200 mV with a subcutaneous Ag AgCl reference using a simple voltage divider circuit described elsewhere. In most instances a single remote reference sufficed for two or more hydrogen electrodes. However, when there was an order of magnitude difference in hydrogen concentration between two electrodes a separate reference for each electrode was necessary. Examples of such differences were 1) if one was in an ischemic region, 2) if one electrode was much more sensitive than the other, 3) if there were a rapid change at the more sensitive electrode or at that with the greater hydrogen concentration. These might cause an opposite, artifactual, sometimes negative deflection at the other electrode. Amplification and recording were done on a Grass Model 7 D.C. recorder. One electrode was placed in cerebral cortex, another in subcortical white matter in the same hemisphere, about 1 mm beneath the cortex and at least as far from basal ganglia. Apart from placement of one electrode in cortex and one in white matter there was no systematic relationship between their locations. In order to exclude hydrogen diffusion into the atmosphere the area of implantation was covered either with mineral oil, or with dental acrylic which also served to anchor the electrode rigidly to the skull. If acrylic was not used, the electrode was locked in place in an electrode holder anchored to the animal's head holder. Electrode location was subsequently confirmed histologically.

Hydrogen Administration

Hydrogen was administered either by inhalation or injection. During inhalation, various concentrations up to 80% were given for variable periods, from one breath to 15 minutes by which time the tissue hydrogen concentration had reached a stable level. The highest concentrations were needed for the shortest intervals. When hydrogen was given by inhalation, arterial and expired air hydrogen partial pressures were monitored with intra-arterial and tracheostomy tube membrane-covered electrodes.

For the expired air electrode, a Clark type of configuration was necessary with both reference and hydrogen sensitive anode behind the membrane in a drop of electrolyte. An external reference sufficed for the intravascular electrode. Hydrogen was also given by intracarotid injection, either hydrogen-saturated normal saline or hydrogen-saturated heparinized blood. The latter was achieved by slowly drawing blood into a heparinized glass syringe via a 10 cm length silastic catheter in the common carotid artery. The catheter was within a 5 mm diameter tube of Kel-F, filled with 100% hydrogen. This method provided approximately a ten-fold greater delivery of hydrogen to the brain, than with hydrogen saturated saline. Adequate hydrogen clearance curves were obtained with injection volumes of 0.1–0.2 cc in contrast to 1–2 cc needed with hydrogen saturated saline.

In all rabbit experiments hydrogen was given by injection, by brief (0.5–3 minute) inhalation, and prolonged (> 15 minute) inhalation to equilibrium, in order to permit comparison of the methods of administration.

In a few experiments for special purposes, hydrogen was also applied locally by electrochemical generation, according to the procedure developed by Stosseck, et al. A 100 micron diameter platinized platinum electrode with a separate remote platinum reference was placed at a distance of 100–500 microns from the recording electrode. Utilizing a circuit which generated a constant current, a one second duration pulse of hydrogen was generated by electrolysis of water adjacent to the recording electrode. For a quantitative estimate of blood flow, evaluation of clearance by both diffusion and flow are required. In these experiments the main interest was in the qualitative detection of hydrogen as an index of its diffusion properties.

Cisternal Pressure Changes During Intracarotid Injection

When hydrogen was given by injection, cisternal CSF pressure was monitored via a 20 gauge needle in the cisterna magna, connected to a strain gauge. In about 30% of injections of volumes greater than 0.5 cc injected in one second, there was a transient rise in cisternal pressure, sometimes as high as 30 mm Hg above the resting level. Its duration was usually less than 1 second. In about 5% of injections, following the pressure peak there was a gradual, exponential decrease of the cisternal CSF pressure, reaching the pre-injection level in 3 to 5 minutes. This was taken to be resolution of injection pressure-induced edema. All measurements associated with even a transient CSF pressure rise were excluded from further consideration. When the small volumes of hydrogen saturated blood were injected as described above, there was never a CSF pressure change.

Re-circulation of Hydrogen after Inhalation

When hydrogen was given for long or short periods, arterial and expired air clearance were relatively rapid. Ninety percent clearance was regularly obtained at the intra-arterial electrode within 45 seconds. When expired air was sampled at the tracheal cannula, 90% clearance was noted within 30 seconds. It was relatively difficult to maintain a continuously effective intra-arterial electrode, probably due to blood clot formation on its tip.
Results

Bizarre distortions of the hydrogen saturation and clearance curves were observed. These distortions appeared to be a function of the duration of indicator application, being most frequent after intracarotid injection and ultra short (<0.5 minutes) inhalation. They are less frequent after brief (0.5–3 minutes) inhalations, and occur not at all after prolonged periods of inhalation, usually more than 15 minutes, when saturation equilibrium is reached.

Hydrogen Curves — The Saturation Phase

Following about 25% of intracarotid hydrogen injections, and a nearly equal proportion of very brief (less than 30 seconds) inhalations, gross differences in the rate of tissue saturation at the grey and white matter electrodes were noted (fig. 1). At the grey matter electrode there was a virtual step rise followed by an apparently exponential clearance, but at the white matter electrode the saturation was considerably slower. Although the saturation phase began simultaneously with the grey matter electrode it often required 30 to 60 seconds or more to reach its peak which then coincided approximately with the half clearance time of the cortical electrode. In some cases these two modes of input were seen at a single electrode, one superimposed upon the other (fig. 2), even though the electrode was subsequently proved to be entirely in cerebral cortex.

Hydrogen Curves — The Clearance Phase

When the hydrogen curves did not reveal such gross distortions as in figure 2, they were re-plotted on semi-log paper to permit graphical compartmental analysis by the usual procedure of curve stripping. Two hundred eighty-five curves from 17 electrodes in 10 rabbits were examined in this way. Five different patterns were evident:

Mono-exponential Curves

Although 8 electrodes were proved to be totally in white matter and 6 totally in grey matter, only 27 of the clearance curves from 8 electrodes were mono-exponential. Seven of these electrodes were in white matter and one in grey. The flow rates following prolonged inhalation to saturation equilibrium, usually 10 to 15 minutes, were in the range of 20 to 30 cc/100 grams/minute, while following injection or very brief inhalation they were 40 to 60 cc/100 grams/minute. Only one grey matter electrode gave mono-exponential curves, and then not consistently. The flow rates were similar to those of the white matter electrodes.

Bi-exponential Curves

These were recorded more often from grey matter electrodes. Following injection there was always a super-fast compartment with a rate of 300 to 1000 cc/100 grams/minute and a relative weight of 1 to 15%. The slow compartment rate was comparable to that of the mono-exponential white matter curves. The compartmental structure of these curves was essentially the same whether the electrode was entirely in grey matter or at the junction of grey and white matter. Some white matter electrodes yielded curves of this type, though less consistently. When hydrogen was given by a brief inhalation of less than 5 minutes, the fast compartment rate was 150 to 250 cc/100 grams/minute with relative weight 1 to 10%. Following prolonged inhalation to saturation equilibrium, the fast compartment flow was 70 to 100 cc/100 grams/minute with relative weights again 1 to 10%. At about half of the electrodes which yielded bi-exponential curves with injection or brief inhalation, prolonged inhalation to saturation equilibrium converted the clearance curve to mono-exponential form with a flow range of 20 to 30 cc/100 grams/minute.

Tri-exponential Curves

Only one example was seen at a grey matter electrode which otherwise yielded bi-exponential clearance curves. The
rates of the 3 compartments were 500 cc/100 grams/minute, 250 cc/100 grams/minute, and 55 cc/100 grams/minute. The relative weights were 4%, 6%, and 90%.

**Bizarre Curves — Accelerating Slope**

At electrodes usually yielding mono-exponential (slow) curves there was sometimes noted a progressive increase in the rate of clearance with time after injection or curve peak (fig. 3). This comprised 14 of 43 slow clearance curves from white matter electrodes after injection (33%) and 13 of 56 slow clearance curves after brief (0.5 to 5 minutes) inhalation (23%). They were not seen following prolonged inhalation to saturation equilibrium.

**Sigmoid Curves**

Sigmoid curves were a configuration, as seen in figure 4, consisting of a nominal initial fast component, a second slow component, and a final fast component. There were 8 of these following injection, 4 following brief inhalation, but none after prolonged inhalation. We never observed a reverse configuration, i.e., slow-fast-slow, nor more than three phases.

**Hydrogen Saturation and Clearance in Cerebral Infarction**

Six cats were studied 1-6 hours after transorbital ligation of one middle cerebral artery. In these the EEG was isoelectric over the ipsilateral hemisphere. Oxygen electrodes in immediate proximity to the hydrogen electrodes revealed relative tissue $P_O_2$ less than 10% of their pre-occlusion levels. Hydrogen saturated saline was injected in the ipsilateral common carotid artery. There was no initial appearance of hydrogen at the electrode in the ischemic region though normal appearing clearance curves were noted at electrodes in the opposite hemisphere. However, beginning 1 to 10 minutes after injection, hydrogen current began to rise at the electrode in the ischemic region. It continued to increase over a period which varied from 10 to 30 minutes and was then followed by a gradual decline, ultimately clearing after 30 to 60 minutes. In order to exclude the effect of pressure of the intra-arterial injection, hydrogen was also given by brief inhalation (30 to 60 seconds). The same course of extremely slow saturation and desaturation occurred, always beginning several minutes after complete arterial clearance. At subsequent pathologic study the hydrogen electrodes were found to be within the infarction.

This apparent “reservoir” of tissue hydrogen was sometimes responsive to systemic arterial blood pressure changes, but the response was variable. Sometimes an increase in blood pressure would increase the hydrogen current though the current did not immediately decrease in turn when the blood pressure decreased. At another time, an increase in blood pressure would cause a decrease in tissue hydrogen concentration, the decrease then persisting when the blood pressure declined. On 8 of 10 occasions, when the tissue hydrogen concentration was rising in the saturation phase, an increase in blood pressure augmented the rate of hydrogen saturation, while in 7 of 11 occasions, when the tissue hydrogen concentration was clearing, the blood pressure rise accelerated the clearance (fig. 5).

**Diffusion of Locally Generated Hydrogen**

In a gerbil, hydrogen was generated locally by electrolysis of water at an electrode in the left occipital pole. Clearance
of the locally generated hydrogen was documented by a hydrogen electrode placed within about 200 microns. Another hydrogen electrode was placed in the right frontal pole, about 1 cm distant from the generating electrode. No hydrogen was detected initially at the remote electrode during successive pulse generations. The animal was then sacrificed and the hydrogen generation pulses were continued. The hydrogen pulses were 1 second in duration, at a frequency of 1 per minute. The generation current was about $10^{-4}$ Amp. The hydrogen electrode adjacent to the generating electrode continued to show clearance of the hydrogen away from the generating region by diffusion but with a progressive buildup of baseline hydrogen content. After about 30 minutes hydrogen became detectable at the remote electrode, about 1 cm from the generating electrode.

**Discussion**

The only possible interpretation of the cat focal infarction and the postmortem gerbil experiments is that hydrogen can diffuse considerable distances through non-perfused tissue. If this is so, we suggest that diffusion of hydrogen may occur through perfused tissue, independent of the perfusion, providing an additional mechanism of saturation and clearance. How this might account for the bizarre curve configurations reported here is as follows:

**Saturation Phase After Injection**

Two simultaneous patterns of saturation after hydrogen injection are illustrated in figure 1 where one electrode was in grey matter and one in white matter. In that figure, the saturation time recorded from the white matter electrode is of the same order of magnitude as the half clearance time of the grey matter (several seconds). We offer the suggestion that this super-fast component of grey matter clearance is due to diffusion of hydrogen from grey to white matter, and, conversely, that a portion of the white matter saturation originates from grey matter by diffusion, in addition to direct delivery of hydrogen by blood.

**The Clearance Phase**

Of the two bizarre forms of clearance curves, the most common was characterized by a progressively accelerating slope (fig. 3). Although this could be due to non-steady state, i.e., increasing flow, review of the experimental protocols revealed no reason to believe that the flow changed. There was no change in blood pressure, $P_{CO_2}$ or EEG. Moreover, such curves were less frequent after brief inhalation, than with injection, and did not occur at all with prolonged inhalation to saturation equilibrium. The more likely interpretation, we think, is that the apparent slow clearance at the beginning of the curve was due to continuing input of indicator into the tissue in early portions of the curve, arriving, we believe, by indicator diffusion from adjacent faster-loading compartments. The delayed input cannot be attributed to recirculation since monitoring with an intra-arterial electrode confirms that hydrogen clearance is always complete in the first pass through the lungs after injection. Conversely, the late accelerated component of the curve may be partly due to diffusion of some of the indicator into more rapidly clearing grey matter.

The occasional finding of sigmoid shaped curves (fig. 4) may be explained by the combination of a small super-fast clearing compartment and a slowly saturating one, a less gross form of the curve in figure 2, which was made evident only by semi-log reploting of the curve.

Slower white matter clearance with prolonged inhalation is probably due to greater saturation of the slowest perfused and non-perfused compartments in the region of the electrode so that they come to be represented more dominantly in the curve at its peak. These compartments would include damaged tissue around the electrode and CSF, as well as result from the microcirculatory heterogeneity of the grey and white matter. Each comprises many "mini compartments" with different perfusion rates, sometimes very slow or zero. If our estimate is correct (see below) that the electrode is recording $H_2$ concentration in a radius up to 2 mm, then the interfering contribution of damaged tissue to the slowest flow compartments around a small electrode would be minimal, though it might be substantial with a large electrode.

**Tissue-Tissue Disequilibria**

As already implied, it is incorrect to infer from the delayed input in some of the white matter curves that instantaneous equilibrium of indicator between blood and tissue does not occur, contrary to the basic requirement for applying the usual compartmental model, e.g. as in the Ingvar-Lassen method with $^{133}$Xenon or $^{85}$Krypton. In the first place, this would be paradoxical since hydrogen is so much more diffusible than krypton or xenon. Our interpretation is that the foregoing phenomena are a consequence of this great diffusibility: the hydrogen diffusion to the electrode often comes from further away than the immediately adjacent capillary. That hydrogen can diffuse a considerable distance in a short time is shown by the experiment with local hydrogen generation in the gerbil postmortem.
Although blood-tissue equilibrium occurs practically instantaneously, initial tissue-tissue disequilibria occur at the same time and secondary equilibration of these take place within the time of the indicator clearance as a consequence of the greater diffusibility of hydrogen. Though tissue-tissue disequilibria are doubtless present after slug injection of larger molecule indicators, e.g., xenon or krypton, their secondary equilibration does not occur since their clearance by blood flow is so much faster than their diffusion from tissue to tissue. The reason that separate compartments are evident with external detection is that the detector is looking at these compartments simultaneously, though the indicator in each is effectively restricted to it. This would be analogous to the situation with a large hydrogen electrode several millimeters in length, in contact with both grey and white matter. The finding of two compartments in our experiments is unique because the electrodes were small enough to be confined within a single tissue most of the time.

The diffusion hypothesis helps to explain the consistent finding of super-fast grey matter clearance (350 to 1000 cc/100 grams/minute). Such values noted here and first described in the previous report from our laboratory, are greatly in excess of grey matter blood flow in other species, and in the rabbit measured with "Krypton. A similar interpretation applies to figure 2 which shows two obvious compartments saturating separately at one cortical electrode — a fast one which saturates immediately and begins to clear while a second slow compartment saturates slowly giving the curve a secondary hump.

An additional argument in favor of the interpretation of diffusion as a clearance factor accounting for the super-fast flow in rabbit grey matter, is its relatively low reactivity to CO2. In our previous report this was an average increase of about 25% with 10% CO2 administration in contrast to an average 55% increase for the slow compartment.

Alternate Hypotheses

We have considered the possibility that these super-fast clearances might be due to proximity of the electrodes to an artery. Though this is imaginable, it seems unlikely: in our previous report, the frequency distribution of clearance rates after intracarotid injection revealed the peak frequency among the super-fast rates. It seems unlikely that the majority of our electrodes were near arteries. Although super-fast clearance at these same electrodes was never seen following prolonged inhalation administration of H2, to achieve intercompartmental equilibrium, these would have been obscured by recirculation.

Since many of the conclusions reached here derived from observations of bizarre configurations of clearance curves, we have carefully reflected for a long time on the possibility that simple mechanical artifacts and experimental errors could account for some of them. These would include non-steady state conditions, trauma to tissue due to acute electrode implantation, movement of the electrode from one tissue compartment to another during a measurement. All of the experimental records have been carefully reviewed with these considerations in mind. Insofar as the steady state of the animal could be defined in terms of arterial blood pressure, respiratory PCO2 and PO2, CSF pressure, and EEG, this condition was maintained in all instances. This would not, of course, exclude a spontaneous change in local blood flow. However, since similar bizarre curves were never seen following prolonged inhalation of hydrogen with similar systemic steady state conditions it seems to us that the blood flow did not change during these measurements.

A further reservation would be some focal change due to the intracarotid injection. We endeavored to minimize this possibility by the CSF pressure monitoring and by keeping the injectate volume small by using H2 saturated blood as described above. Further, we think flow changes are an unlikely explanation for these bizarre curve shapes since the frequency of their occurrence was more a function of the duration of indicator administration, than the method of administration. They were most frequent with intracarotid injections, less with brief inhalation, and did not occur at all with prolonged inhalation. For the same reason, it seems unlikely that electrode movement could account for these curve abnormalities. Monitoring conditions were the same regardless of the mode and duration of H2 administration, and electrode implantation.

Relevant Observations by Others

Stosseck was the first to point out the problems resulting from the intercompartmental diffusion of hydrogen, when he noted a nominal "blood flow" clearance rate from intraventricular fluid in the cat of around 20 cc/100 grams/minute. This observation, and the related one of diffusion of the hydrogen indicator from arterial walls resulting in an arterio-venous shunt which bypassed capillaries, led him to develop the local hydrogen method. This enables noting diffusion which actually accounts for more of the clearance than does flow.

Pasztor, et al. described fairly reproducible grey and white matter blood flow compartments in baboons when hydrogen was administered by inhalation. It would appear, therefore, that the flow artifact due to intercompartmental diffusion is not a problem in the larger brain with thicker cortex. Our own experience would suggest that during inhalation administration, intercompartmental equilibrium is largely achieved. In the studies of Pasztor, et al. it is interesting to note that the grey matter clearance was somewhat faster with short inhalation times, perhaps representing a small, unrecognized, diffusion clearance component, or effect of damaged tissue around the electrode.

Kobrine and Doyle used hydrogen inhalation to study spinal cord flow in monkeys. They reported flow rates for dorsal horn grey matter electrodes which would be more appropriate for white matter (17 cc/100 grams/minute). Due to indicator diffusion between grey and white matter, this measured flow was most likely a weighted mean for the whole dorsolateral quadrant of the spinal cord. This is similar to our inability to demonstrate appropriate grey matter clearance rates (70 to 90 cc/100 grams/minute) in rabbit cortex. If the 1 mm thickness of monkey spinal cord dorsal horn grey, and the 2 mm thickness of rabbit cortex — in both of which selective grey matter flow is not demonstrable — and the 5 mm thickness of baboon cortex, in which grey matter flow is separately demonstrable, are taken together a fair estimate of spatial resolution of the
Hematogenous hydrogen method would be about 2 mm.

The diffusion of hydrogen into and out of a sizable infarction (fig. 5) further illustrates its sometimes surprising behavior. The interpretation of the blood pressure effects on the tissue hydrogen "reservoir" is based on its location relative to arterial and venous components of preserved circulation. When most of the hydrogen is on the arterial side, and some of it intravascular, an increase in blood pressure in presence of defective autoregulation would push more hydrogen farther into the previously non-perfused region. When it is mainly on the venous side, a rise in blood pressure increases flow at the margin of the infarct causing an increase in clearance rate.

Precautions in Use of Hydrogen Method

It appears from these experiments that intracarotid slug administration of hydrogen will produce highly unreliable results due, we believe, to pronounced tissue-tissue disequilibria. Prolonged regional perfusion with hydrogen saturated blood might be a possible way to avoid this problem but it would be much more awkward than simple inhalation administration. The only advantage that regional perfusion might provide would be the opportunity to examine the first 45 seconds of clearance, when recirculation would still be present after inhalation. In either case, prolonged administration appears to be the only way to obtain reasonably equal saturation of various tissue compartments. It also avoids distorted curves due to clearance of indicator by diffusion from one compartment to the other, resulting in continuing saturation of one and clearance of another. This problem is especially important in small brains. Judging by the Pasztó et al. experience, this problem is less critical in large brains unless the electrode is at the junction of two different tissue compartments.

Even after achieving saturation equilibrium, however, it should be appreciated that during clearance any inhomogeneity of flow in the tissues will again set up diffusion gradients. The significance of this is that the putative focal flow measurement at the electrode will be influenced by flow at some distance away, perhaps up to 2 mm. Thus, the original promise of the hydrogen method to measure flow in micro-regions seems unlikely to be realized. The present method probably measures flow as the average for a few cubic millimeters. There may be a prospect for more discrete measurement with recognition and adequate mathematical treatment of the diffusion problem. One important approach to this has been the Stosseck-Lübbers local generation method which probably has a resolution of about 1 mm.

References

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