Effects observed in the experimental animals with these compounds.

References


Measurement of Regional Blood Flow Using Hydrogen Gas Generated by Electrolysis

KEIJI KOSHU, M.D., KAZUYO KAMIYAMA, M.D., NOBUO OKA, M.D., SHUNRO ENDO, M.D., AKIRA TAKAKU, M.D., AND TATEO SAITO, T.D.*

SUMMARY Electrocchemically generated hydrogen gas was used to measure local blood flow by Stosseck et al. The data obtained by their method, however, did not correlate well with those obtained by hydrogen inhalation.

We have modified the equation proposed by Stosseck, prolonging the stimulus duration in order to increase the amount of hydrogen generated. In dog white matter the resulting clearance curves were formed to be monoexponential both in the living animal as well as after circulatory arrest when all the clearance is by diffusion away from the electrode. The values calculated by our equation correlated well with those obtained by hydrogen inhalation.

From Department of Neurosurgery, Toyama Medical and Pharmaceutical University and Biomedical Science Co. Ltd.*
Address for correspondence: Dr. Keiji Koshu, Department of Neurosurgery, Toyama Medical and Pharmaceutical University, 2630, Sugitani, Toyama, 930-01, Japan.
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ELECTROCHEMICALLY GENERATED H\textsubscript{2} was utilized for the measurement of cerebral blood flow (CBF) by Stosseck et al.\textsuperscript{1} The values obtained using their method differed from those obtained by hydrogen inhalation and were thought to reflect the blood flow in a smaller region of tissue, which Stosseck et al. called "microflow." Their values were greater in general than those found in the H\textsubscript{2} inhalation method and variations in the values obtained were frequent indicating that there would be still problems remaining in its practical application.

We have attempted to measure regional CBF (rCBF) in the dog brain following generation of H\textsubscript{2} gas by electrolysis as Stosseck et al. did, and found that if much more H\textsubscript{2} gas was produced, the changes in H\textsubscript{2} concentration were approximately monoexponential for some minutes both when the dogs were alive and dead.

A simple equation to calculate rCBF has been developed, modified from that of Stosseck equation. This
requires only half the clearance time of generated H$_2$ both in life and during circulation arrest. We have found a high correlation between the data obtained by our method and those by inhalation method.

**Methods**

**Electrode**

Electrodes were made by securing a teflon coated platinum iridium wire (electrode A, 130 $\mu$m$\phi$) to a platinum wire (electrode B, 300 $\mu$m$\phi$), and coating the entire bipolar electrode with acryl-urethane, except for the exposed tips (fig. 1).

**Method of Evaluation**

Stosseck et al. introduced the following equation in their report.

$$\ln \frac{P_{(t)}}{P_{o(t)}} = -\frac{f}{\lambda} t + d(t)$$  \hspace{1cm} (1)

where $\lambda$ = partition coefficient; $P_{o(t)}$ = hydrogen pressure without blood flow; $P(t)$ = hydrogen pressure with blood flow, $t$ = time $d(t)$ = deviation function (assumed constant) $f$ = blood flow.

Considering equation (1), it is pointed out that both $P_{o(t)}$ and $P(t)$ are exponential function. Therefore we expressed them as follows:

$$P(t) = A \cdot e^{-\alpha t}$$  \hspace{1cm} (2)

$$P_{o(t)} = B \cdot e^{-\beta t}$$  \hspace{1cm} (3)

where A, B, $\alpha$ and $\beta$ are constants.

From equations (2) & (3),

$$\frac{P_{o(t)}}{P(t)} = \frac{B}{A} \cdot e^{(\alpha - \beta)t}$$  \hspace{1cm} (4)

$$\frac{d}{dt} \ln \frac{P_{o(t)}}{P(t)} = \alpha - \beta$$  \hspace{1cm} (5)

Meanwhile from equation (1), we get following equation.

$$\frac{d}{dt} [\ln \frac{P_{o(t)}}{P(t)}] = -\frac{f}{\lambda}$$  \hspace{1cm} (7)

Therefore, $f = -\lambda (\alpha - \beta)$  \hspace{1cm} (8)

But since $\alpha$ and $\beta$ is expressed as follows

$$\alpha = -\frac{\ln 2}{T_A}$$

$$\beta = -\frac{\ln 2}{T_B}$$

where $T_A$ and $T_B$ means the half-period of $P(t)$ & $P_{o(t)}$ respectively, and $\lambda$ is assumed as 1, we can get the following equation.

$$f = \ln 2 \left( \frac{1}{T_A} - \frac{1}{T_B} \right)$$

$$= 69.3 \left( \frac{1}{T_A} - \frac{1}{T_B} \right) \text{(ml/100gr/min)}$$  \hspace{1cm} (9)

This shows that if the half periods of the changes in hydrogen concentration on the graphs are obtained from both living animals and those in cardiac arrest, then the rCBF can be calculated easily.

**DC Current for H$_2$ Generation**

As a preliminary experiment, we recorded H$_2$ concentration after electrochemical generation of H$_2$ gas in a dead dog's brain.

After intravenous administration of pentobarbital anesthesia (35 mg/kg) to an adult mongrel dog (12 kg), right frontal craniotomy was performed and the electrode was implanted into the white matter to a depth of about 1 cm from the brain surface. Two silver chloride disc electrodes were also implanted subcutaneously. Electrode A was used for measurement of H$_2$ concentration and electrode B was for H$_2$ generation. After cardiac arrest by 10% KCL solution i.v., 5--100 $\mu$A DC current was passed between the electrode B (the cathode) and a disc electrode to generate H$_2$ gas.

Simultaneously the changes in polarographic current due to H$_2$ oxidation were recorded at electrode A (fig. 2). The surgical table was one which allows for...
securing the dog and maintaining body temperature (Toyorko, TO-10), so that the animal’s body temperature was kept within a physiological range throughout the experiment. A digital stimulator (WPI, M 1800) was used to administer the DC current; the polarographic current was detected on a PHG 300 (Tokai Irika) and recorded on a recorder TI 102 (Tokai Irika). The voltage reading on the PHG 300 was 600 mV and recording was done 12 times at the same site.

When the peak of $H_2$ concentration is taken as 0, it was found that all 12 recordings fit monoexponential curves up to 10 or 15 minutes. A typical example is shown in figure 3.

We obtained the half period of the approximately exponential curves, and the reciprocal of them were plotted on the ordinate, with the time of stimulation plotted on the abscissa, as shown in figure 4. It is seen that the lower the current used or the shorter the period of stimulation, the larger the reciprocal of the half period. Considering equation (9) we thought that the

\[ \frac{1}{T_{1/2}} = 0.025 \times \text{mA} \]

\[ \frac{1}{T_{1/2}} = 0.05 \times \text{mA} \]

\[ \frac{1}{T_{1/2}} = 0.1 \times \text{mA} \]

**Figure 3.** Time course of hydrogen concentration in the experiment in cardiac arrest (a). Its logarithm showed linear change during the first 14 minutes (b).

**Figure 4.** Graph showing the correlation between the stimulation time and the reciprocal of the half period of the respective curve.
smaller $1/T_1$ would be better. We therefore considered that, using stimulation of 2—6 seconds and 50—100 $\mu$A, the volume of $\text{H}_2$ changing due to diffusion could be obtained at relatively low levels.

**Tests of the Method**

Four adult mongrel dogs weighing about 10 kg each were used for the experiment. Under intravenous administration of pentobarbital (25 mg/kg), endotracheal intubation was done followed by right frontal craniotomy of 2 cm diameter. Electrodes were then placed as in the preliminary experiment. The dogs were immobilized with pancuronium bromide and maintained under controlled respiration (AIKA, R-60). Continuous monitoring of arterial blood pressure was done from the right femoral artery. After it was confirmed that blood pressure and arterial blood gases had become stabilized, a clearance curve was obtained after 5 minutes of inhalation of 10% $\text{H}_2$ gas. About 20 minutes later, the changes of $\text{H}_2$ concentration were recorded following 2 seconds of 100 $\mu$A DC current stimulation. Similar experiments were performed at 4 and 6 seconds of stimulation following the measurement with $\text{H}_2$ inhalation respectively. Finally, cardiac arrest was produced by intravenous administration of KCL solution and, clearance curves were made at 100 $\mu$A DC current ranging between 2, 4 and 6 seconds stimulation each.

The clearance curves obtained using the inhalation method showed large oscillations in 2 of our measurements, but in the other 10 measurements roughly monoexponential curves were seen. In the experiment on the living animals, the electrolytic method produced a monoexponential curve for up to about 7 minutes (fig. 5).

Following cardiac arrest, results similar to those in the preliminary experiment were obtained. That is to say, monoexponential curves were obtained for up to 10—15 minutes in all animals.

Using equation (9), values of $r\text{CBF}$ were obtained by local $\text{H}_2$ generation method, and these values were compared with those obtained using the $\text{H}_2$ inhalation method, as shown in the table.

A significant correlation between the two values was found ($r = 0.9398; p < 0.01$) (fig. 6).

**Discussion**

The hydrogen clearance method is an excellent procedure to measure regional flow. However, in order not to produce changes in arterial $\text{PaCO}_2$, $\text{PaO}_2$ etc., it is necessary to make the animals inhale a constant low concentration of $\text{H}_2$ gas, making this method somewhat inappropriate for experiments in small animals. Moreover a long time is needed to measure blood flow if the region is ischemic, because the time required for saturation of $\text{H}_2$ is long. In this respect, the method reported by Stosseck et al. is advantageous.

Unfortunately, in the local $\text{H}_2$ generation method, $\text{H}_2$ is produced within an extremely small region.
Therefore, diffusion to the surrounding areas cannot be disregarded. This forms a striking contrast to the inhalation method of Aukland et al.

Stosseck et al. made measurements of changes in $H_2$ concentration after electrical stimulation both in living and dead animals. Taking the former change as $P(t)$ and the latter as $P_0(t)$, they showed that $\ln \frac{P(t)}{P_0(t)}$ could be expressed as a linear function and the slope obtained expressed the blood flow $F$.

Unfortunately, in order to obtain $\ln \frac{P(t)}{P_0(t)}$ laborious process was required. First, the actual values of $P(t)$ and $P_0(t)$ for each time were obtained then $\ln \frac{P(t)}{P_0(t)}$ for each time was calculated using a computer and plotted on a graph and the slope was obtained. Through this complicated maneuver they obtained the blood flow.

In actual practice, about 50% of their measurements over a period from 10 to 30 seconds fit the linear function; and even so these values tended to be larger than those obtained with the inhalation method. Stosseck et al. explained that a smaller region was measured and introduced the term "microflow." Stosseck et al. utilized the changes of $H_2$ concentration between 10 to 30 seconds following the peak of the clearance curves. We think that the volume change of $H_2$ due to diffusion is so large during the first minute that the Stosseck's method may contain a large error.

With our modification much more $H_2$ gas is generated, resulting in a monoeponential clearance between 1 and 7 minutes after the peak of $H_2$ concentration. rCBF data calculated by equation (9) correlated well with those of inhalation method.

According to Stosseck et al., no recognizable histological alterations were observed when DC current ranging from 0.4 to 7.5 $\mu$A of 1.9 sec duration were applied to the brain surface. They only observed some increased dye affinity of the cytoplasm.

We used DC current of 100 $\mu$A ranging from 2 to 6 seconds, which is much higher than those used by Stosseck et al. We have yet to investigate the in vivo effects of 2–6 second stimulation of 100 $\mu$A strength used in our experiments, but we have found that no coagulation was visible to the naked eye in egg white using continuous 100 $\mu$A DC current except for the bubble formation of $H_2$ gas. In comparison with the amount of histological damage done by electrode implantation, we believe that this is negligible.

Tissue damage expected by electric current may be mainly due to the effect of heat produced. Production of heat is expressed by Joule's law ($F_{\text{Rt}}$). Thus for example stimulation (100 $\mu$A for 2 sec, or 10 $\mu$A for 20 sec) will produce the same amount of $H_2$ gas, since the amount of $H_2$ gas generated by electric stimulation is proportional to the electric current (Faraday's law). But there would be a ten fold difference in heat production between the two; less with longer generation time. In the former instance it is expressed as $F_{\text{Rt}} = 2 \times 10^4R$ and in the latter as $F_{\text{Rt}} = 2 \times 10^3R$.

The principle advantages in the local $H_2$ generation method are that measurements can be made without the use of $H_2$ gas, and in comparison with the inhalation method, it requires less time.

The disadvantage is that absolute values cannot be determined during the experiment. In order to calculate the absolute values, it is necessary to observe the changes due only to diffusion after cardiac arrest. But since the changes due to diffusion in a given volume of tissue are thought to be constant if the stimulation parameters are held fixed it is sufficient to determine this value beforehand and adjust the values obtained during the experiment accordingly.

In the case where the relative changes in rCBF are being followed, it is thought that our method will be useful for rCBF measurements, particularly in the light of the extreme simplicity of the apparatus and procedure and the fact that compared to other methods a relatively large number of measurements can be made.

**Table: rCBF Calculated by Respective Manners**

<table>
<thead>
<tr>
<th>No.</th>
<th>Inhalation method</th>
<th>Electrolytic method</th>
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(ml/100gr/min)

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