

**TOTAL CEREBRAL BLOOD FLOW
MEASURED BY HYDROGEN CLEARANCE**

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ABSTRACT

Total cerebral blood flow was measured by sampling hydrogen clearance from torcular blood using simple, hydrogen-sensitive, platinum electrodes. The clearance curves in rhesus monkeys were usually biexponential with a mean flow in the slow compartment of 28 ml/100 g per min (± 5.8 SD), a mean flow in the fast compartment of 102 ml/100 g per min (± 19.1 SD) and a mean total cerebral blood flow of 52 ml/100 g per min (± 10.5 SD). This method is relatively simple and accurate and constitutes a valuable adjunct to local cerebral blood flow measurements using the same technique.

I. INTRODUCTION

Since the detailed report of Aukland and co-workers¹ in 1964, the hydrogen clearance method has been used widely to measure local blood flow in diverse tissues including brain^{2,3,5,8,13,15,16,19,20,23} and spinal cord.¹⁰ The method employs hydrogen-sensitive polarographic 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 monoexponential, blood flow may be calculated by the formula

$$F = \lambda \frac{.693}{T 1/2} \quad (1)$$

where F equals blood flow in milliliters per gram per minute, λ is the blood-tissue partition coefficient, which for hydrogen is 1,^{1,2} and T 1/2 is the half time in minutes of the hydrogen clearance curve.

Aukland et al.¹ and Neely et al.¹⁵ 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. Applicable, with equal ease and validity, to the brain, the hydrogen clearance method becomes even more useful since both local and total cerebral blood flow can 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.

II. MATERIALS AND METHODS

Rhesus monkeys unselected as to sex and weighing 3-5 kg were initially anesthetized with phencyclidine HCl* and pentobarbital and intubated with a cuffed endotracheal tube. Catheters were placed in the right femoral artery and vein for monitoring blood pressure, administration of drugs, and sampling of arterial blood for blood gas analysis before and after each flow study. In some animals a polarographic electrode modified for vascular use was passed into the abdominal aorta via the left femoral artery. The animal was then placed in a stereotactic headholder in a sphinx-like position. 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 36-42/min. 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 solution with tubocurarine chloride added was given intravenously at 5 ml/kg 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 the inion was removed and an electrode was passed through the dura into the torcular Herophili 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 volumes percent, but was constant for each flow determination. Hydrogen was usually given for 10 minutes, but in some

* Sernylan, Bio-Centric Laboratories, Inc., St. Joseph, Missouri

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 begun. Flows were obtained during states of normo-capnia, hypocapnia and hypercapnia.

The electrode was made of Teflon insulated platinum wire (0.25 mm diameter). At the active end, 3 mm of wire were 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 percent platinum chloride solution for 2 seconds at a current density of 5.0 mA per square millimeter to produce a light grey coating of platinum black on the electrode surface.

The circuit was designed by Willis et al. and has been previously described.²⁴ It is a modification of earlier designs providing 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 simplifies the analysis of data.

Data analysis. The data from 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 the 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

$$\text{Total flow} = \frac{I_f + I_s}{I_f/F_f + I_s/F_s} \quad (2)$$

where I_f and I_s are the zero time Y-axis intercepts of the fast and slow compartments, respectively, and F_f and F_s are the flows in the fast and slow compartments.⁷

III. RESULTS

Torcular electrodes. Torcular clearance curves were always biexponential and usually smooth (Figures 1-3). The base line remained stable for entire experiments, which often were more than 10 hours long. Analysis of 47 flow determinations from 13 experiments yielded the following: at a mean PaCO_2 of 32 mm Hg ($\text{SD} \pm 2.3$), mean flow was 102 ml/100 g per min ($\text{SD} \pm 19.1$) for the fast flow compartment and 28 ml/100 g per min ($\text{SD} \pm 5.8$) for the slow flow compartment. Mean total cerebral blood flow was 52 ml/100 g per min ($\text{SD} \pm 10.5$). The coefficient of variation (standard deviation/mean x 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 percent. Increasing the period of inhalation from 10 to 30 minutes had little consistent effect on results (Figure 3). Increasing and decreasing the PaCO_2 resulted in the anticipated changes in blood flow (Figures 1 and 4).

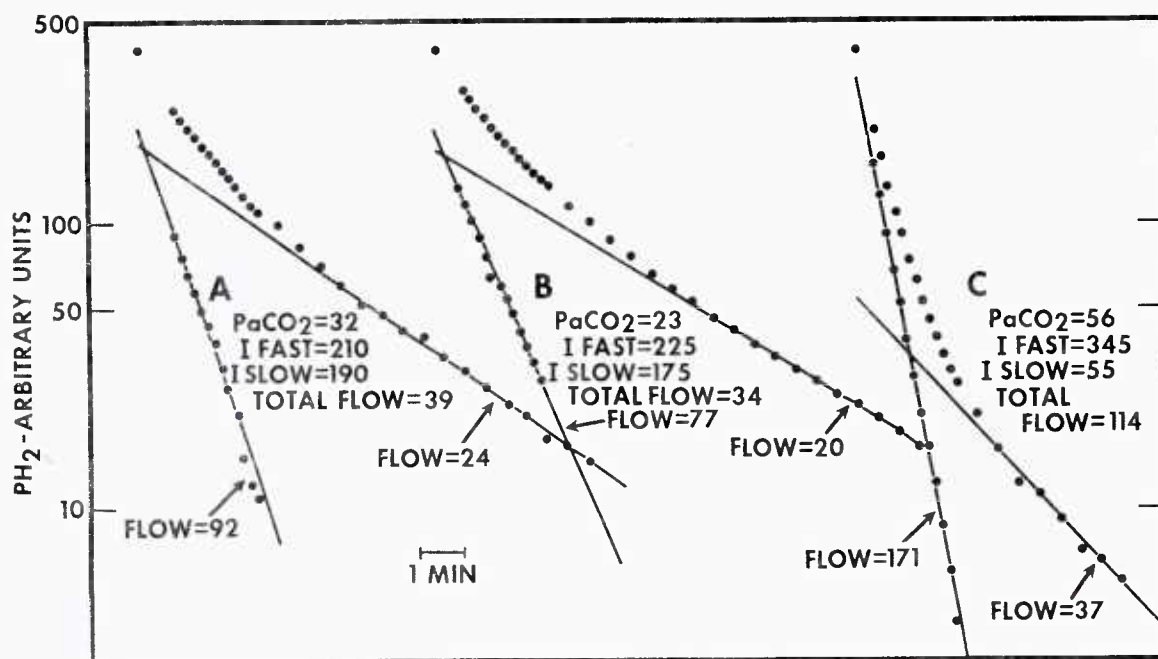


Figure 1. A semilog plot of three successive hydrogen clearance curves from torcular blood in one experiment. Curves are biexponential and respond as expected to changes of PaCO_2 .

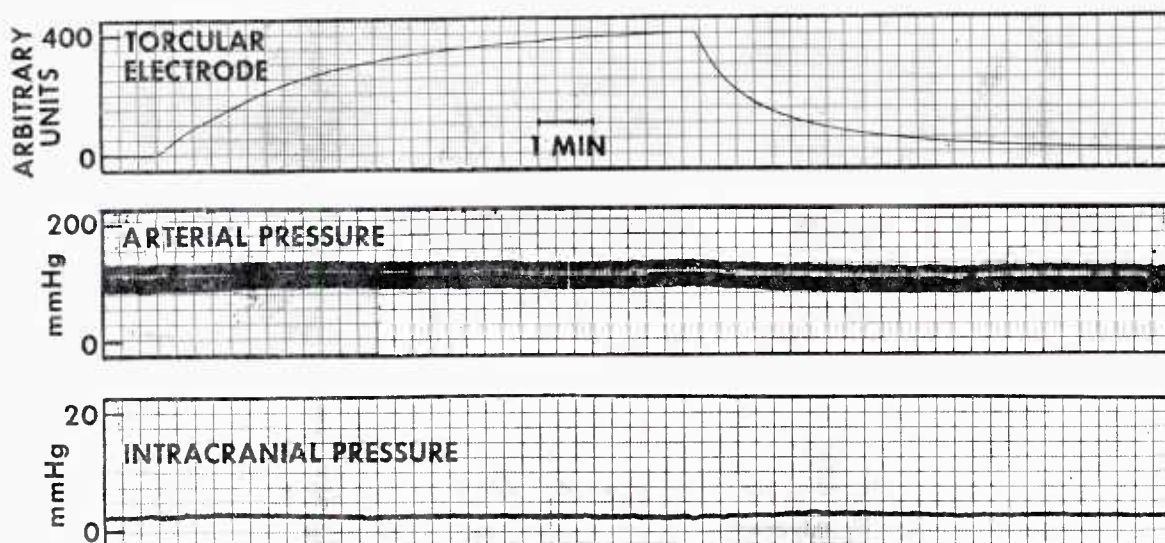


Figure 2. A polygraph tracing during a blood flow determination. Hydrogen comprised 25 volumes percent of inspired gas mixture. During saturation and desaturation there are no significant changes in blood pressure, pulse rate or intracranial pressure.

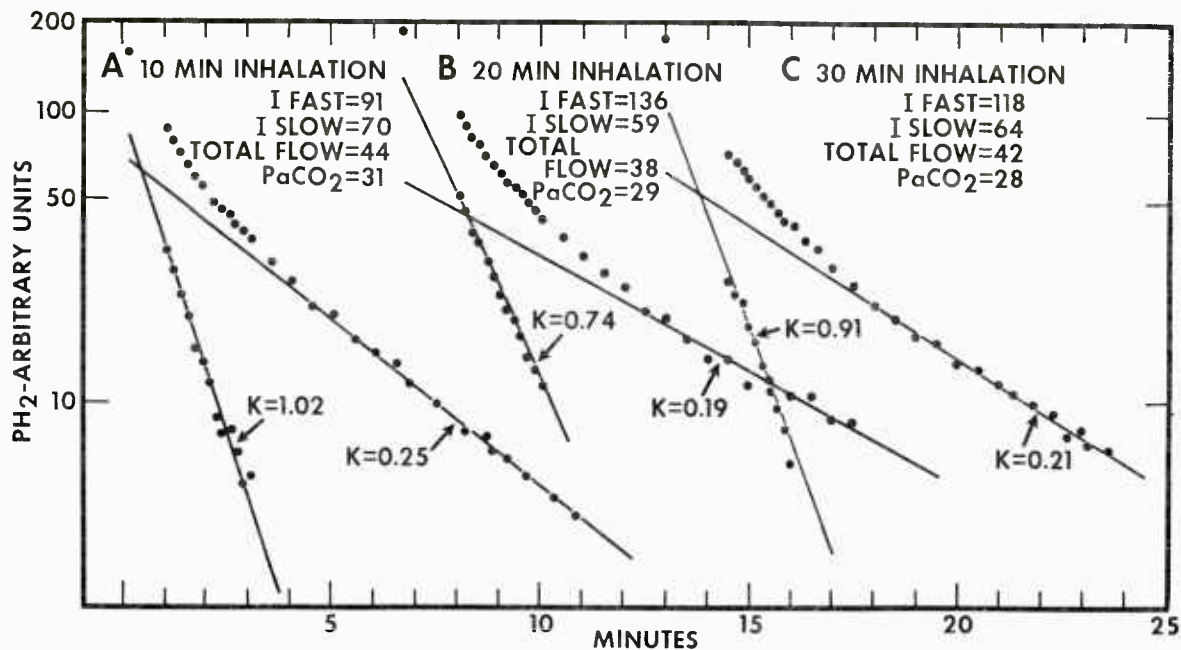


Figure 3. A semilog plot of three successive hydrogen clearance curves from torcular blood in one experiment. Changes in the duration of hydrogen inhalation had little consistent effect on clearance curves.

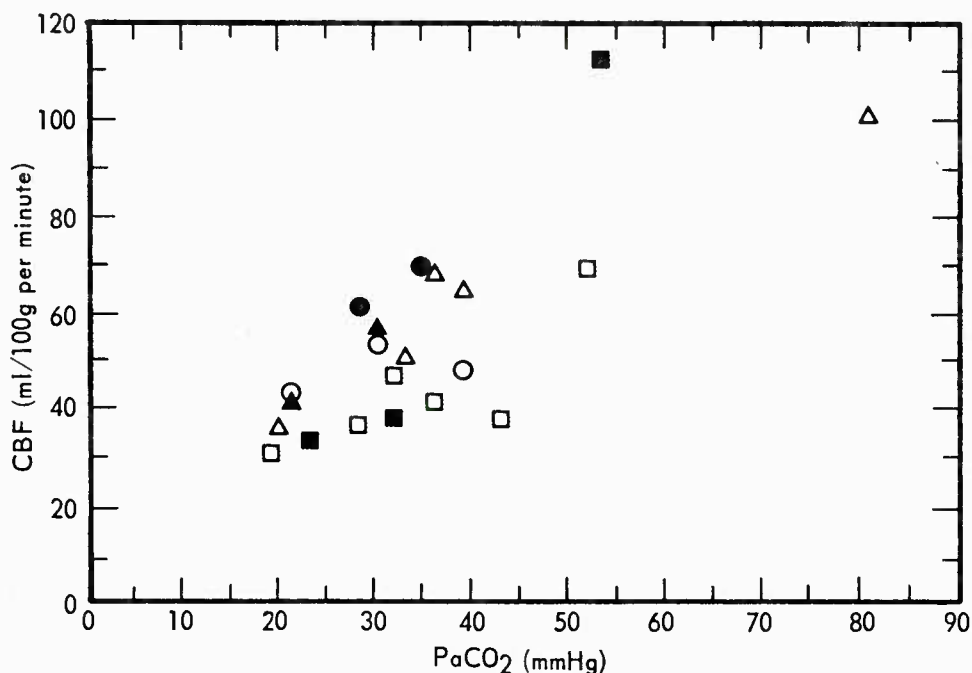


Figure 4. The effect of changing PaCO_2 on total cerebral blood flow as measured from torcular hydrogen clearance curves following a 10-minute period of inhalation. Six experiments are represented by different symbols.

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

Effect of hydrogen inhalation on vital signs and blood gases. Concentrations of hydrogen of up to 25 volumes percent of inspired gas had little effect on blood pressure, heart rate or intracranial pressure (Figure 2). Before hydrogen inhalation was begun

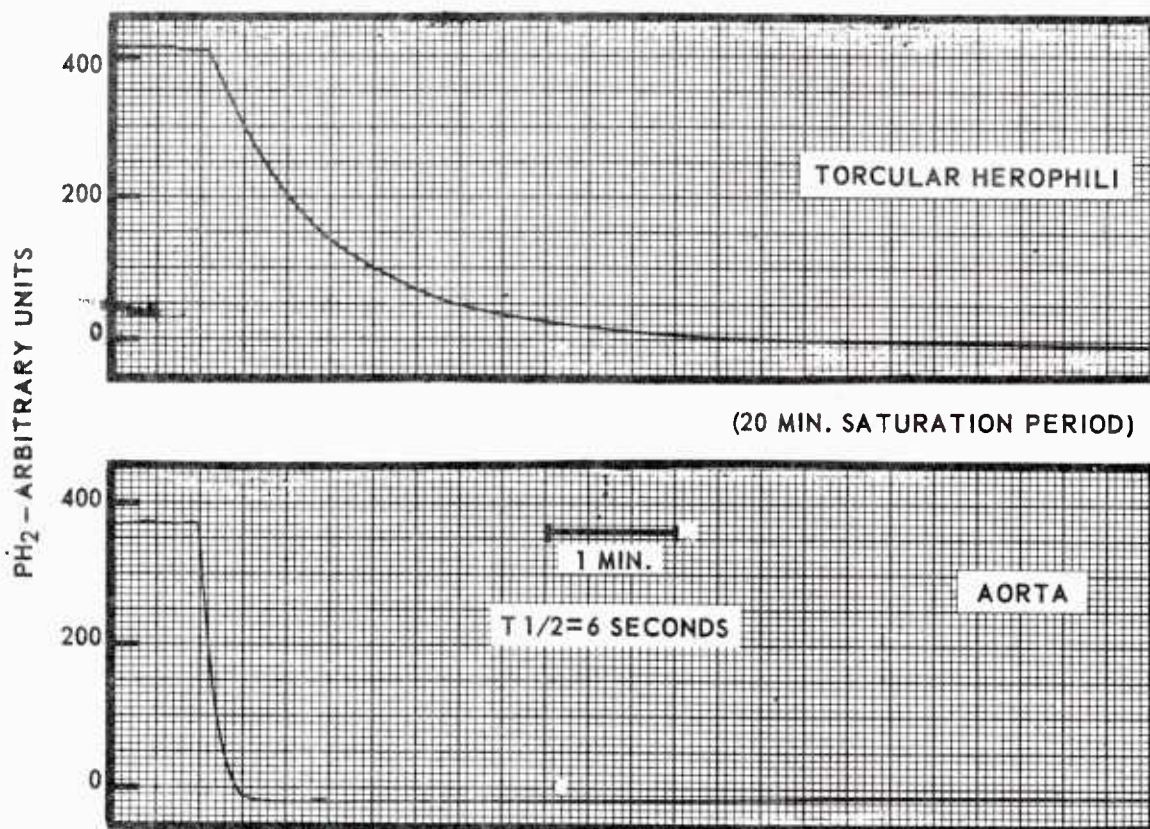


Figure 5. A polygraph tracing of hydrogen clearance curves simultaneously recorded from the torcular and aorta after a 20-minute period of hydrogen inhalation. Note the rapid decrease in the hydrogen concentration of aortic blood.

the PO_2 generally ranged between 140 and 150 mm Hg and the PCO_2 between 31 and 33 mm Hg. During hydrogen inhalation, the PO_2 would fall approximately 15 mm Hg and the PCO_2 , 1 or 2 mm Hg. The blood gases returned to their previous levels shortly after the hydrogen was discontinued.

IV. DISCUSSION

Our results suggest that average total cerebral blood flow can be measured with acceptable accuracy and reliability by following the hydrogen clearance in torcular blood with simple platinum electrodes. Flow values for fast and slow compartments as well as for total cerebral blood flow are close to those reported by others using different methods in both man and other mammals (Table I). 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.¹⁴ When measured by following the clearance of inhaled ^{133}Xe 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 that flows only from the brain. As regards recirculation after prolonged inhalation, all parameters being equal, hydrogen concentration in arterial blood becomes negligible more rapidly than ^{133}Xe because hydrogen diffuses more rapidly than xenon and has a lower blood-gas partition coefficient. Moreover, by increasing the ventilatory rate while maintaining a physiologic $PaCO_2$ and PaO_2 with appropriate adjustments of the inspired gas mixture, one may increase the rate of

Table I. Cerebral Blood Flow Values Reported for Man and Other Mammals. Superscripts After Authors' Names Refer to References in this Paper.

Author	Species	Method	PaCO ₂ (mm Hg)	Cerebral blood flow*		
				Fast	Slow	Total
Kety and Schmidt ⁹	rhesus and spider monkey	N ₂ O inhalation	?			22 - 66
Kety and Schmidt ⁹	man	N ₂ O	?			41 - 78
Lassen et al. ¹¹	man	intra-arterial ⁸⁵ Kr	?			60 ± 13
Reivich ¹⁸	rhesus monkey	jugular flowmeter	41			49
Harper ⁶	man	N ₂ O inhalation	40			50 - 55
Gotoh et al. ⁴	man	H ₂ inhalation	?			37 - 78
Høedt-Rasmussen et al. ⁷	man	arterial ¹³³ Xe	39			50 ± 5
McHenry et al. ¹²	man	arterial ¹³³ Xe	35 - 42	77 ± 6	22 ± 1	52 ± 3
Shinohara et al. ²¹	man	arterial H ₂	?	118 ± 19	26 ± 5	43 ± 4
	rhesus	H ₂ inhalation	?			45 ± 6
Petruk et al. ¹⁷	rhesus	arterial ¹³³ Xe	35 - 45			49 ± 13
Present series	rhesus	H ₂ inhalation	33 ± 2	102 ± 19.1	28 ± 5.8	52 ± 10.5

* ml/100 g per minute ± standard deviation

clearance of hydrogen from lungs and arterial blood. In our experiments, the aortic electrode recorded hydrogen clearance curves with half times of less than 10 seconds (Figure 5).

Therefore, 40 seconds after hydrogen administration is stopped, arterial hydrogen concentration falls to less than 10 percent 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 invariably yields straight lines

on the semilog plot for both the fast and slow compartments of the clearance curve. This further supports the assumption that in these experiments, recirculation introduces an error that is acceptably small. Yet Meyer and associates^{13, 14} have stated that after inhalation of hydrogen, its recirculation results in arterial desaturation that takes many minutes, although they provided no documentation for this assumption. Aortic half times of about 28 seconds were reported by Pasztor et al.¹⁶ in baboons given hydrogen by inhalation, and they concluded that hydrogen recirculation is not a problem if, after hydrogen inhalation is stopped, the first 40 seconds of the clearance curve are discounted. The desaturation curves from the aorta that we obtained after prolonged inhalation of hydrogen are almost identical to those of Neely et al.¹⁵ who reported half time values of 12 seconds with normal respiration and 6 seconds with hyperventilation. We have concluded that recirculation becomes a problem only when cerebral blood flow is very fast, with half times in the fast flow compartment of less than 0.4 minute. In this instance one might employ an aortic electrode to obtain the difference between arterial and venous hydrogen concentrations at each point along the clearance curve. Flow can then be determined by measuring the area between the arterial and venous clearance curves, as is done with the nitrous oxide method.⁹

The hydrogen clearance method is subject to other potential sources of error. Base-line shifts can be troublesome and the cause often can be traced to a defect in the electrode insulation. Changes in arterial PO_2 and PCO_2 , brought about by the addition of hydrogen to the inspired gas mixture, can affect both base-line 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 PO_2 and PCO_2 .

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 read-out 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.⁴ 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.¹ and Neely et al.,¹⁵ 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 10 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.,²¹ which employs saline saturated with hydrogen injected rapidly as a bolus into the internal carotid artery. 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 1 minute, which

dominated most of the clearance curve; and a third component representing the slow compartment (Figure 6). We encountered two major difficulties in using 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 diffusion²² that is not representative of effective cerebral blood flow. Such an initial very fast component

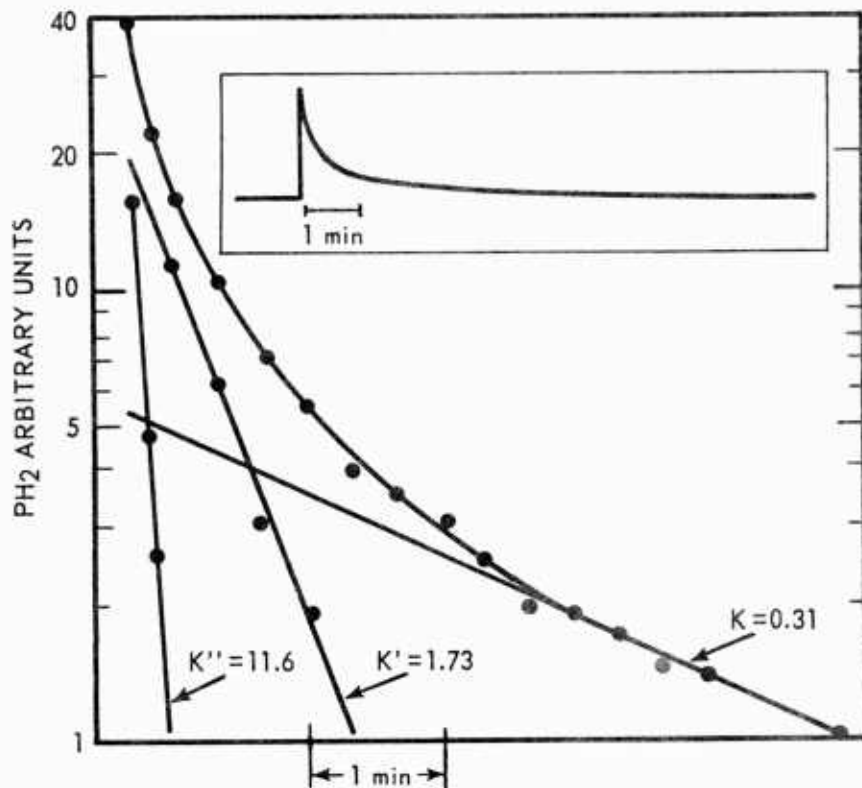


Figure 6. A hydrogen clearance curve from the torcular after a single rapid injection into the internal carotid artery of 3 ml of saline saturated with hydrogen. The polygraph curve (inset) has been replotted on semilog paper and resolved into three components, the most rapid of which (K'') equals the rate of arterial desaturation.

of a clearance curve followed by two slower ones has also been recorded by Aukland et al.¹ 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 10 minutes of inhalation even the tissues of the slow flow compartment are usually more than 90 percent 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.,¹ the slow flow compartment can also be saturated with a 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 being simpler, of providing more hydrogen to the tissue thereby yielding smoother clearance curves, and of avoiding fluid overload.

V. SUMMARY

Our experience suggests that one may reliably measure average total cerebral blood flow in the experimental setting by following the clearance of inhaled hydrogen from torcular blood with simple hydrogen-sensitive polarographic electrodes of thin platinum wire. This procedure is relatively simple, inexpensive, radiation-free,

and augments the usefulness of the hydrogen clearance method of studying cerebral blood flow by permitting the simultaneous recording of both local and total brain blood flow.

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