The use of inert gases was introduced for measuring cerebral blood flow by Kety and Schmidt in 1945¹ and has since found wide application to the brain and other regions. Nitrous oxide has been used for measuring cerebral, myocardial, and renal blood flow,²-⁴ while labelled krypton has been used for the same organs by other investigators.⁵-⁷ The method requires simultaneous measurement of the gas concentration in arterial and regional venous blood throughout saturation or desaturation with the inert gas. Assuming equilibrium distribution of gas between tissue and venous blood at the beginning and end of the sampling period, the blood flow per unit of tissue is readily calculated according to the Fick principle.

The present report deals with a more direct method of measuring local blood flow by continuous recording of tissue hydrogen concentration by means of a platinum electrode inserted into the tissue. By simultaneous recording from two or more electrodes in different parts of an organ it is thus possible to get information about the magnitude and distribution of blood flow within the organ. Furthermore, the possibility of continuous recording of venous concentration with a catheter electrode simplifies the usual method and also allows better characterization of the flow pattern.

The first part of the paper deals with the method used for hydrogen measurement, in vitro tests, and determination of hydrogen solubility in tissue. Then follows an experimental validation of the method for flow measurement in myocardium, renal cortex and skeletal muscle.

Part I. Hydrogen Determination

A. PRINCIPLE

The current generated by oxidation of molecular hydrogen to hydrogen ions at the surface of a platinum electrode was measured. This current is shown to be proportional to the hydrogen partial pressure, which in turn is proportional to hydrogen activity. The circuit consists of a calomel half cell and a platinum electrode. With a high resistance in the external circuit, the platinum electrode potential can be measured relative to the calomel electrode potential. In the absence of interfering substances the potential of the platinum electrode relative to the standard hydrogen electrode potential is given by the Nernst equation:

\[ E = \frac{0.059}{2} \log \left( \frac{a_{H^+}}{a_{H_2}} \right) \]

where \(a_{H^+}\) is the hydrogen ion activity in
mol/liter and $a_H$ the hydrogen partial pressure in atm. The constant applies to a temperature of 25°C. At very low hydrogen partial pressure, however, the measured voltage will be determined by other oxidation-reduction systems invariably present in biological fluids, and will therefore deviate from the value predicted from the above equation. Thus the problem of establishing a baseline and also the inconvenience of a logarithmic scale makes a potentiometric method unsuitable for the present purpose. Hyman showed recently, however, that the current produced in the same circuit with a low external resistance is proportional to hydrogen concentration. No explanation was given for the mechanism involved. His observation has been confirmed, and the following hypothesis is advanced to explain the linear relationship between hydrogen concentration and current. With a low external resistance the platinum electrode becomes polarized, attaining a voltage determined by the calomel electrode, and the current produced depends on the amount of hydrogen available for oxidation at the platinum surface. Although this system differs from usual polarographic methods in having no voltage selector, the polarographic mechanism seems clear from the following observations described in more detail below:

1. With a low external resistance the platinum electrode voltage is equal to the calomel electrode voltage and is essentially independent of hydrogen concentration.

2. The current generated by the electrode is proportional to hydrogen concentration and is not increased by further lowering of the external resistance.

3. The current produced by an electrode at a given hydrogen concentration is increased by stirring the solution.

4. Closing the circuit in the presence of hydrogen gives a high initial current which then gradually declines to a stable level.

5. Current-voltage curves suggest a diffusion current plateau in the region of the standard calomel voltage.

According to this hypothesis the current is limited by the amount of hydrogen brought to the electrode surface by diffusion and convection. The potential at which the oxidation takes place is equal to the standard calomel electrode potential, i.e., $+0.24$V relative to the standard hydrogen electrode. The effect of disturbing substances such as oxygen and hydrogen ions would be expected to be small at this voltage: Applicable oxygen reduction does not occur until a potential $0.2$ to $0.3$V more negative is reached, and hydrogen gas evolution from hydrogen ions is also slow until even more negative potentials are reached.

B. DETAILED METHODS, MEASURING CIRCUIT

Platinum Tissue Electrodes

Tissue electrodes were made from 0.8 mm thick and 10 mm to 30 mm long pieces of platinum wire. The tip was tapered by filing and the other end soldered to a thin nylon-insulated copper wire (no. 30 HCN Nyclad, Belden Mfg. and Co.) (fig. 1). The electrode and the junction to the wire were then insulated by 3 to 5 paintings with lacquer (Damarda Lacquer, grade L. 49369, Bake-
measuring circuit used consists of a standard calomel electrode connected by an agar bridge to the test solution where \( H_2 \) tension is to be measured, the ammeter and platinum electrode (fig. 2). A Keithley Instruments Microvolt-Ammeter, 150A was used in the range having an input resistance of 333 ohms. The ammeter output signal was recorded on a Honeywell-Brown Electronic Recorder with an interposed variable resistance by which the gain of the recorder could be chosen. A three-way switch in the measuring circuit made it possible to read three electrodes alternately, while the current of the remaining two electrodes was shunted through a resistance equal to that of the measuring circuit. As pointed out above, a low resistance circuit is to be preferred for theoretical reasons. It was found, however, that with an external resistance of less than \( 10^5 \) ohm the system became extremely sensitive to electrical noise produced by electrical motors, infusion pumps, and even to walking around in the room. A resistance of \( 10^6 \) ohm was found to give a stable reading from electrodes in the test chamber and in the renal cortex, while a resistance of \( 10^7 \) ohm had to be used for recording in the working myocardium. It is obvious that a current of the order of \( 10^{-7} \) amp, which was usually obtained in the test chamber with a hydrogen concentration of 3% to 5%, will cause a considerable shift in the platinum voltage in a negative direction when a resistance of \( 10^6 \) ohm is used, and thereby increase the rate of oxygen reduction. (The effect on oxidation of hydrogen will be discussed below.) Although the current obtained in tissues with comparable hydrogen concentration was usually less than \( 10^{-7} \) amp, with correspondingly less voltage change, it is nevertheless clear that a lower series resistance should be preferred if a stable reading can be obtained.

C. IN VITRO TESTS OF THE SYSTEM

A closed lucite chamber provided with a magnetic stirrer was used for testing the electrodes (fig. 2). The electrode was mount-
ed with the bare tip projecting into the chamber and the circuit completed with a polyethylene tube KC1 agar bridge (3% agar in saturated KC1) from the chamber to the calomel electrode. Unless otherwise stated, the experiments were performed with the chamber completely filled with air-saturated solutions and stirred continuously.

**Calibration**

With a fresh plated electrode in stirred 0.3 molar phosphate buffer, pH 7.4, a positive current (electrons flowing towards the platinum electrode in the external circuit) of the order of $10^{-7}$ amp was obtained. This positive current diminished rapidly during the first minutes and then gradually attained a constant value in the order of $10^{-8}$ amp. Injection of known amounts of hydrogen-saturated saline produced a rapid change to a less positive or to a negative current, giving a new stable reading in the course of 5 to 10 seconds. Since the time needed for complete mixing of the chamber solution is probably the time limiting factor, the electrode response time could not be tested with this system. Calibration curves obtained by subsequent additions of hydrogen gave a good linear relationship to hydrogen concentrations in the range of 0 to 6% saturation, when an external resistance of $10^5$ ohms was used (fig. 3). With a resistance of $10^9$ ohm, a lower current was obtained at higher $H_2$ concentrations than with a resistance of $10^5$ ohm, the deviation from linearity depending on the sensitivity of the electrode, i.e., the current produced, in accordance with the hypothesis presented above. In the course of hours, the current obtained in a phosphate buffer with a given hydrogen concentration would usually decrease gradually. In heparinized plasma or 5% human albumin in saline, the current remained more stable.

**Influence of Platinum Electrode Potential on Current**

The assumption made above, that the current is diffusion limited, demands a demonstration of a "diffusion plateau," i.e., a voltage range in which the current produced by a given concentration of hydrogen is largely independent of the voltage. An attempt was therefore made to determine the voltage-current curve for the oxidation of hydrogen at the platinum surface. The test chamber was filled with deoxygenated 0.3 molar phosphate buffer, pH 7.4, containing 0.1% human albumin. Hydrogen-saturated buffer was added to give a concentration of approximately 5% saturation. Varying platinum electrode voltage was provided with a 1.5V dry cell and a potentiometer and measured across the external resistance with an electrometer. It was found that a long time was needed to reach a stable current after each change of voltage. Since the sensitivity of the system might decrease considerably throughout one experiment, no absolute current voltage curve can be given. The general shape of the curve as obtained in several experiments is approximately indicated in figure 4 with maximum variation between different electrodes as indicated by the two curves. The curve strongly suggests a voltage range in which the current is diffusion limited, and furthermore that the standard calomel potential falls within this range. However, any change of potential in negative direction might give a lower current.

The decrease in sensitivity with time suggests that the oxidation of hydrogen may also be limited by some barrier within the electrode itself, possibly a reduced rate of the
platinum catalyzed atomization of hydrogen molecules at the platinum surface due to “poisoning.” Experimentally, the current was found to remain proportional to hydrogen concentration even with a marked loss of sensitivity. However, with this lower current the hydrogen concentration at the electrode surface cannot be assumed to be zero, as is the general assumption made for polarographic processes, and accordingly, the area from which hydrogen is derived (“effective diffusion area”) cannot be calculated.

Desaturation of Chamber Fluid

Due to the slowly changing sensitivity, absolute calibration of the electrodes was not possible. Since the procedure finally adopted for flow measurements consisted of measuring the rate of hydrogen clearance from tissue or blood, a pertinent in vitro test would be to study the desaturation of the chamber fluid. This was accomplished by filling the chamber only 1/2 to 2/3 full with 3% to 8% hydrogen-saturated fluid and letting the hydrogen escape by diffusion. Hydrogen given off to the gas phase was removed by constant suction. According to expectation, this gave a single exponential washout curve, showing that any change in sensitivity during the desaturation period was too small to distort the curve (fig. 5). This procedure also provided an opportunity to compare the washout rate recorded simultaneously with two different electrodes. The desaturation of phosphate buffer, 5% albumin in buffer and plasma with constant stirring was recorded in 26 experiments with 7 different pairs of electrodes. The maximum difference between the half-times obtained with any electrode pair was 6.1% and the mean difference 2.6%. As applied to flow measurements, this difference would include the error dependent on differences between electrodes plus the errors involved in reading the curves, plotting on semilog paper and measuring the half-times.

“Nonspecific Effects”

The experiments reported above were made under standardized conditions. In vivo variations with respect to temperature, pH, pO₂, and ascorbic acid concentration might intro-
duce errors and should therefore be investigated.

Oxygen. By flushing the chamber fluid with nitrogen, it was found that oxygen accounts for more than 80% of the positive current obtained in air-saturated solutions. The change in current resulting from a given change in $pO_2$ (oxygen tension) was found to decrease with increasing $pO_2$. At a $pO_2$ in the range of 20 to 60 mm Hg it was found that the effect of increasing $pO_2$ by 10 mm Hg corresponds to reducing hydrogen tension by 1 to 3 mm Hg (equivalent to 0.15% to 0.40% of full saturation).

Hydrogen Ion Concentration. The effect of changing pH was examined by adding small amounts of hydrochloric acid or sodium hydroxide. In plasma with a hydrogen concentration in the range of 0 to 5% saturation the effect of changing pH by 0.1 unit was found to correspond to a change in hydrogen concentration of less than 0.05% saturation, or a change in hydrogen tension of less than 0.4 mm Hg.

Ascorbic Acid. The effect of ascorbic acid was tested by alternately adding known amounts of hydrogen and ascorbic acid to the test chamber containing phosphate buffer. The negative current resulting from 100 μmol/liter of ascorbic acid was found to correspond to an average of 0.25% hydrogen saturation, with variations between different electrodes from 0.1 to 0.35%. (The normal plasma level in dog and man is less than 100 μmol/liter.) A current voltage curve for ascorbic acid showed a half-wave potential of approximately +0.3V versus the standard calomel electrode, with a diffusion limited plateau from +0.5V versus calomel electrode, where the current was more than 20 times higher than at the voltage of the calomel electrode.

Temperature. The effect of temperature changes was studied by warming or cooling the test chamber from the outside, the temperature being measured in the stirred solution. It was found that the effect of raising the temperature was to increase the current, both the positive current in the absence of hydrogen, and the negative current with hydrogen present, i.e., to increase the sensitivity. In the temperature range of 21°C to 26°C and with a hydrogen concentration of 4% saturation, the sensitivity increased roughly by 2.5% for 1°C.

Effect of Stirring. The rate of stirring had no effect on the positive current generated in air-saturated solutions without hydrogen, indicating that the reduction of oxygen is not limited by diffusion at this voltage. However, in the presence of hydrogen, an increase in stirring rate greatly increased the current, i.e., increased the sensitivity. All in vitro experiments therefore had to be carried out with constant rate of stirring.

Comments. From the results presented above it seems clear that changes in pH, temperature and ascorbic acid concentration in the living organism under most conditions will not seriously affect the reading. Although changes in $pO_2$ do have a considerable effect on the current, it should be pointed out that considerable changes in venous oxygen concentration occur with only small changes in $pO_2$. Furthermore, since the method requires a stable blood flow throughout one measurement, great changes in venous or tissue oxygen concentration are unlikely, and the effect of oxygen would not, therefore, seem to limit the usefulness of the method.

Theoretically, a slightly more positive polarizing voltage, +0.1 – 0.2V with respect to the calomel electrode, might seem preferable. This would decrease the effect of oxygen and also assure a voltage well within the plateau of the hydrogen voltage-current curve (fig. 4). A higher positive voltage (>0.2V versus calomel electrode) would greatly increase the effect of ascorbic acid and should probably be avoided. This has not been tested systematically, and it should be emphasized that the final validation must be based on actual flow measurements.

Part II. Blood Flow Measurements

PRINCIPLE

The following theoretical considerations are largely based on Kety's approach to blood-tissue exchange of inert gases, and a more thorough treatment will be found in his reviews on the subject. According to the Fick principle, the amount of gas accumulat-
ed or given off by a homogeneously perfused tissue \( i \) in the time \( dt \) is:

\[
dQi = (Ca - Cv_i) F dt
\]

where \( F \) is the blood flow (arterial flow = venous flow), \( Ca \) is arterial concentration and \( Cv_i \) the concentration in venous blood from that particular tissue. If \( Ci \) is the concentration in the tissue with volume \( W \), we will have:

\[
W dCi = (Ca - Cv_i) F dt
\]

For highly diffusible and lipid soluble gases, such as hydrogen, it may be assumed that the tissue is in instantaneous diffusion equilibrium with venous blood from that particular tissue throughout the whole saturation and desaturation period, i.e., \( Ci = \lambda Cv_i \), where \( \lambda \) is the tissue/blood partition coefficient for the gas. The validity of this assumption has been discussed theoretically by Kety and has been experimentally supported by Jones. Substituting \( Ci/\lambda \) for \( Cv_i \) in equation 2 yields:

\[
W dCi = F (Ca - Ci/\lambda) dt
\]

In the experiments to be described below, the arterial concentration of the test gas is negligible, whence \( Ca = 0 \). Equation 3 then becomes

\[
\frac{dCi}{Ci} = -\frac{F}{\lambda W} dt
\]

Integration of equation 4 between the limits \( t = 0 \) and \( t = t \) yields

\[
Ci = Ci_0 e^{-kt}
\]

where \( k = F/\lambda W \). In terms of concentration in venous blood, equation 5 becomes

\[
Cv_i = Cv_i e^{-kt}
\]

The value of \( k \) is obtained from the slope of a tissue or venous desaturation curve plotted on a semi-logarithmic scale against time: \( k = \frac{0.693}{T/2} \) where \( T/2 \) is the time in min for \( Ci \) or \( Cv_i \) to be reduced to half of its numerical value. Then \( k \) has the dimension \( \text{cc/min/cc} \) (or \( \text{min}^{-1} \)) and is readily converted to flow per vol of tissue \( (F/W) \) when the tissue/blood partition coefficient is known.

Application to Hydrogen Gas Measured with the Platinum Electrode

The high diffusion coefficient of hydrogen and the low water/gas partition coefficient (rapid removal by the lungs) make this gas highly suitable for flow measurements. By means of the platinum electrode it is possible to follow arterial and venous hydrogen concentration (when a representative vein is accessible), and the flow may be calculated according to the formula used for the nitrous oxide method. It would also seem possible to calculate the flow from arterial and tissue concentration, according to equation 4. It should be kept in mind, however, that the electrode current at a given time is determined by the availability of hydrogen molecules for oxidation at the electrode surface (or more precisely by the hydrogen tension gradient at the electrode surface), and not by the absolute concentration in the solution or tissue. This means that a change in hydrogen tension in the bulk tissue is reflected as a change in the tension gradient at the electrode surface with a time lag. The delay will depend on the thickness of the “diffusion layer,” which again depends on 1) the thickness of a noncirculated area of blood, tissue fluid or fibrin surrounding the electrode, representing a true diffusion area and, 2) the blood flow, which tends to dissipate any tension gradients produced by the hydrogen consumption at the electrode. Since a delay of less than one second was observed in the most highly perfused tissue investigated (renal cortex), the first factor would seem to be of minor importance. For tissues with low blood flow, such as skeletal muscle, it can be calculated from the observed current that the hydrogen consumption is sufficient to create a hydrogen tension gradient (presumably zero or very low tension at the surface) extending for several hundred microns into circulated tissue, thus explaining the marked delay observed in this organ. (Due to the high intra-vascular stirring effect the lag will probably be negligible in arteries and veins.) When a steady state is reached, however, the concentration “seen” by the electrode will fall at the same rate as the true tissue concentration, and equation 7 may be used for flow calculation. A considerable delay also precludes the application of the “initial slope method” used by Ingvar and Lassen to calculate the average flow in a heterogeneously perfused tissue.
Also in venous desaturation curves, where no lag was observed, equation 7 seems preferable to the calculation by the integrated area, since it obviates correction for the artery to vein transit time.

**Partition Coefficient**

In order to calculate blood flow from tissue saturation or desaturation curves it is necessary to know the solubility of hydrogen in the tissue involved relative to the solubility in blood, i.e., the tissue/blood partition coefficient. Even if electrode calibration in tissue and blood should be possible, this could not answer the question, since the electrode response is determined by hydrogen tension and not by hydrogen content. Accordingly, hydrogen has to be transferred from tissue to blood, or water, before being measured. The following method was adopted: A known weight of tissue slices (2 g to 4 g) and approximately 5 cc 0.3 M phosphate buffer pH 7.4 was equilibrated with 100% hydrogen in a 25-cc tared syringe for two hours under constant shaking. The gas phase was renewed several times to remove nitrogen and oxygen given off. As much as possible of the buffer solution was then squeezed out, leaving the tissue and 0.5 cc to 1 cc fluid, assessed by weighing the whole, syringe. Five to 10 cc of phosphate buffer (without hydrogen) was then added, great care being taken to avoid air bubbles. The syringe was weighed again to give the exact amount of buffer added, and the content then mixed on a rocker for at least two hours. In order to remove tissue slices from the syringe outlet the syringe was centrifuged at low speed for a few minutes. The hydrogen concentration of the supernatant was then tested by injecting known amounts into the test chamber alternately with 100% hydrogen saturated buffer for calibration. To avoid any effect on electrode characteristics by the sudden appearance of the protein-containing solution, the chamber was filled with plasma or albumin solution. Hydrogen concentration of the tissue extract obtained in this way could then be used to calculate the tissue/buffer partition coefficient. Values obtained for kidney tissue in four experiments were as follows (each value based on the average of two to four determinations of tissue extract concentration with two different electrodes): 0.98, 0.94, 0.90 (cortex) and 0.92 (medulla), averaging 0.94.

The solubility in blood compared to buffer was determined by alternately injecting known volumes of completely saturated buffer and blood into the test chamber containing blood or plasma. Four determinations gave the following values for the blood/buffer partition coefficient: 0.93, 0.96, 0.99, 1.00, with an average of 0.97. Plasma tested against whole blood showed no difference. It is concluded that the kidney tissue/blood partition coefficient cannot be distinguished from unity with the present method. Measurements in other organs have thus far not been performed. From the data available for other gases in different tissues, however, it seems likely that the hydrogen tissue/blood partition coefficient is close to unity in most tissue, including brain.

**MYOCARDIAL BLOOD FLOW**

The homogeneous anatomical structure of the myocardium and also reports indicating a uniform distribution of myocardial blood flow suggest this tissue as suitable for validating the method. This was accomplished, in eight experiments on perfused dog heart, by comparing direct flow measurements with myocardial blood flow estimated from simultaneous hydrogen desaturation curves obtained from either coronary venous blood or from the myocardium.

**Methods**

Dogs were anesthetized with pentobarbital sodium iv, 30 mg/kg. A disk oxygenator primed with fresh heparinized dog blood and thermostatically maintained at 37°C served the double purpose of oxygenation and preventing recirculation of hydrogen. A roller pump delivered blood from the oxygenator to the ascending aorta via a catheter through the left subclavian artery. The descending aorta and the right brachiocephalic

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8 Procedure described above gives the partition coefficient as hydrogen solubility in g tissue/cc blood, with the dimension cc/g. The blood flow calculated using this partition coefficient will therefore have the dimension cc/min/g.
artery were tied off. Perfusion pressure was continuously recorded by a strain gauge transducer and a Sanborn recorder. Myocardial venous blood was drained by gravity into a venous reservoir by a catheter introduced through the azygos vein and the right atrium into the right ventricle. The superior and inferior venae cavae were ligated. A catheter was also introduced into the left ventricle to drain blood flow from the Thebesian veins; this flow was usually less than 5% of venous outflow through the coronary sinus. Preparations with higher left ventricular flow, probably indicating aortic insufficiency, were excluded. Coronary venous outflow was measured with a graduated cylinder for two-minute periods before and after each hydrogen desaturation run. Results were generally constant within a few percent, and the average of the two values was used for comparison with flow calculated from desaturation curves.

One platinum electrode was mounted in the venous cannula as close to the heart as possible, while one or two electrodes were inserted into the myocardium of the left or right ventricle. The calomel reference electrode was connected to the dog by means of a polyethylene tube-KCl agar bridge introduced subcutaneously through an incision in the groin. To assure a reliable connection the subcutaneous pocket was filled with saline. The electrode current was measured with the system described above, in most experiments using a series resistance of 10\(^5\) ohm and in a few desaturation curves a resistance of 10\(^6\) ohm.

Hydrogen saturated saline was infused into the arterial line close to the aorta. Two different types of infusions were used: 1) Continuous infusion with a syringe infusion pump at a rate of approximately 3% of blood flow until a constant current was obtained from the electrodes. In some experiments the infusion was started at a higher rate and then reduced after \(\frac{1}{2}\) or 1 min, in order to shorten the saturation period. 2) Rapid injection by hand of 5 cc to 10 cc hydrogen saturated saline.

In order to avoid myocardial edema most experiments were stopped after approximately one hour of perfusion. Nevertheless, in two instances the heart was obviously swollen at the end of the experiment. The heart was taken out immediately after stopping the perfusion and weighed after emptying the cavities of blood.

**Results**

The current obtained in the absence of hydrogen (residual current) was generally positive and small compared to the negative current resulting from 3% hydrogen saturation. Since this residual current, which has to be subtracted from the total current, might change slowly in the course of an experiment, sufficient time for practically complete desaturation (five or six half times) was allowed in each run. The current obtained with approximately 3% hydrogen saturation was of the order of 10\(^{-8}\) amp and usually well below 10\(^{-7}\) amp, regardless of whether a series resistance of 10\(^6\) or 10\(^5\) ohm was used. Artifacts synchronous with the heart contractions were much more pronounced with the lower resistance and often made it necessary to change to 10\(^5\) ohm. Another source of artifacts was a variable grounding of the animal to the oxygenator via blood flowing down the wall of the venous reservoir. It was found that this could be avoided best by letting the blood run or drip freely through the air from the venous catheter.

Typical desaturation curves from one myocardial and one venous electrode are shown in figure 6, where the net current (current due to hydrogen oxidation) is plotted in arbitrary units on a logarithmic scale against time.

**Figure 6**

Desaturation of myocardium recorded from the wall of the left ventricle (solid dots) and coronary venous blood (circles). Isolated, perfused dog heart. Intravenous injection of hydrogen-saturated saline. Directly measured venous outflow: 82 cc/min/100 g.
time. The linear fall in electrode current on this scale indicates a single exponential decay of myocardial and coronary venous hydrogen concentration. Essentially the same curves were obtained in 27 desaturation runs in eight experiments, with the exception of two consecutive venous curves which will be described below.

No difference was found between curves recorded with a series resistance of 10$^5$ and 10$^6$ ohm respectively. Furthermore, a single exponential desaturation curve was obtained after equilibration with continuous infusion as well as after a rapid injection of hydrogen saturated saline. The time relationship between myocardial and venous desaturation curves was variable. Frequently, the decrease in venous electrode current started ahead of the decrease in myocardial electrode current, the latter often attaining a linear slope more gradually (fig. 6).

Blood flow was calculated from the slope of the curves (equation 7), assuming a tissue/blood partition coefficient of 1.00. The mean ratio between myocardial blood flow obtained from 19 venous desaturation curves in six experiments and the simultaneously measured venous outflow/100 g of tissue was 0.97 with a range of 0.82 to 1.05 and a standard deviation of 0.072. In individual experiments even better correlation was obtained, as shown in figure 7, where different experiments are indicated by different symbols.

Two consecutive desaturation runs were excluded from this summary because of an unusual shape of the curves: In the semilog plot the curves showed an upward convexity and did not attain a slope corresponding to venous outflow until 25% of the initial concentration had been reached. Although a flow corresponding to venous outflow could be obtained (in retrospect) from this late portion of the curves, 20% to 25% lower values were obtained in the routine reading by using an earlier, almost linear portion of the curves. The reason for these abnormal curves is not clear, but seems most likely in some way to be related to the dead space represented by the right heart cavities.

Almost as good agreement was found between left ventricular myocardial blood flow calculated from tissue desaturation curves and directly measured venous outflow (fig. 8). The mean ratio to venous outflow for 17 desaturation curves, representing nine different
electrode sites in six experiments was 0.97 with a standard deviation of 0.088 and a range of 0.84 to 1.15.

Five desaturation curves from the right ventricle in two experiments gave a mean ratio to venous outflow of 0.89 (fig. 8), not significantly different from that obtained in the left ventricle.

In an additional two experiments in which the heart became edematous before the end of the experiment, a good correlation to venous outflow was obtained both for tissue and venous desaturation measurements, although the ratios were as high as 1.3, obviously due to an overestimation of heart weight.

Comments. The fairly good agreement between local blood flow measured in different parts of the myocardium and venous outflow, and also the single exponential washout curve obtained in coronary venous blood indicates an essentially homogeneous myocardial blood flow without appreciable arteriovenous shunting. This is further supported by the uniform appearance of desaturation curves obtained after constant infusion of hydrogen for several minutes and after single rapid injection. In the presence of more than one compartment with different flow, the latter technique will exaggerate a fast component, most easily detected in venous desaturation curves. It should be admitted, however, that the dead space represented by the ascending aorta and the right heart cavities might act to conceal the effect of a small fast component or a shunt. Measurement within the coronary sinus in intact animals with a catheter electrode might therefore provide more precise information on this problem. However, the failure to demonstrate any appreciable arteriovenous shunting is in good agreement with MacLean et al. who found that only 2% to 4% of microspheres with a diameter of 20 \( \mu \) would pass through the coronary circulation. Furthermore, the homogeneous distribution of microspheres arrested in the myocardium agrees well with the present finding of a homogeneous blood flow. The same conclusion was reached by Herd et al. based on external counting of myocardial desaturation of krypton, and is also compatible with uptake curves for tritiated water.

The observed delay in tissue curves conforms with the assumption of a diffusion layer around the electrode. However, the good agreement between flow calculated from tissue curves and coronary venous outflow also shows that the diffusion layer does not affect the slope of the desaturation curve.

When comparing the calculated flow obtained from hydrogen desaturation with venous outflow it should be noted that absolute agreement depends on using the correct weight for calculating venous outflow per 100 g of tissue. Edema, not obvious by inspection, and retention of variable amounts of coronary blood (which should be included in the weight) may certainly have introduced errors. Furthermore, the somewhat wider scattering of the flow readings obtained from tissue electrode curves might result from small local differences in flow and does not necessarily indicate lower accuracy than for flow values obtained from the venous electrode.

Good correlation to venous outflow permits two more important conclusions: The blood/myocardial partition coefficient for hydrogen, which has not been measured directly, must be close to 1.00. (The partition coefficient derived from the mean ratio to venous outflow gives a figure of 1.03. With the uncertainties inherent in this indirect approach, however, it seems more reasonable to take the partition coefficient as 1.00.) Furthermore, the presence of the electrode within the tissue does not seem to interfere with the capillary flow in the area from which hydrogen is delivered to the electrode surface.

RENAL BLOOD FLOW

The availability of other methods for estimating renal blood flow lends this organ to validation of a new method of estimating blood flow. However, the fact that the kidney consists of different tissues with presumably differing blood flows is a disadvantage and will be discussed later.

Methods

Two different kinds of experiments were performed: 1) comparison of the hydrogen method...
with directly measured venous outflow; 2) comparison with PAH clearance.

1) DIRECT MEASUREMENT OF RENAL VENOUS OUTFLOW

Experiments were performed on 12 kg to 25 kg mongrel dogs. Anesthesia was induced with 30 mg/kg of pentobarbital sodium iv, and maintained with subsequent smaller doses. Sufficient and constant ventilation was provided by a positive pressure respirator attached to a tracheal tube. Heparin was given to prevent clotting in the extracorporeal circuit. The left renal vein was exposed through a midabdominal incision and cannulated with a Bardic catheter no. 16. Renal venous blood was drained into a graduated cylinder, and from here pumped back to the femoral vein with a finger pump. Blood flow was estimated directly by measuring venous outflow; 2) comparison with PAH clearance. In some experiments a higher priming concentration was used in order to shorten the equilibration time. No adverse effects on the animal were observed to result from the breathing of hydrogen.

The kidney was excised after completion of the experiments. Electrodes were dissected out and the position of each one recorded. Only curves from electrodes clearly in the renal cortex (and not in the outer medulla) are included. The kidney was then weighed, after having been divided and drained for approximately 10 min, and this final weight was used for calculating venous outflow per 100 cc of cortex per min (see below).

2) RENAL BLOOD FLOW ESTIMATED BY PAH CLEARANCE

In this set of experiments the kidney was left more undisturbed in order to investigate higher flow. Pentobarbital sodium anesthetized dogs were given a priming and sustaining infusion of PAH in saline at a rate providing a constant plasma concentration of 1 mg% to 3 mg%. Urine was collected for 10- to 15-minute periods from the left ureter. Arterial blood samples were drawn approximately at the midpoint of each clearance period for PAH and hematocrit measurement. PAH was determined in plasma and urine by the method of Bratton and Marshall.19

Renal Cortex

The washout of hydrogen was recorded from 17 different electrode sites in the renal cortex in five experiments. To provide essentially instantaneous desaturation of arterial blood, hydrogen was given by infusion of hydrogen-saturated saline into the ascending aorta through a catheter introduced from the right carotid artery. Varying degrees of equilibration were obtained by: a) rapid injection of 2 ml to 5 ml, b) infusion by hand of 5 ml to 15 ml over a period of 15 to 60 sec at a rate adjusted so that the electrode current remained roughly constant, c) hydrogen respiration for 5 to 20 min, followed by intra-aortic infusion maintaining constant electrode current, for approximately 1 min, thus allowing time for hydrogen to be removed from the lungs. Due to the rapid desaturation only one electrode could be recorded at a time with the one channel equipment used. A series resistance of 106 ohm was used in these experiments.

Renal Vein

In five experiments the renal venous desaturation curve was recorded with a catheter electrode introduced into the inferior vena cava via the right jugular vein, and then manually guided into the renal vein through a midline incision. The ovarian (spermatic) vein was tied off. In some experi-
ments the kidney was denervated by stripping the renal artery in order to obtain maximal flows. Heparin was given to prevent clotting on the electrode. Hydrogen was given by infusion or by respiration plus infusion as described above. A series resistance of $10^5$ ohm was used in three of the five experiments. In one experiment an infusion of mannitol was given to provide sufficient urine flow for measuring PAH clearance at considerably reduced renal blood flow. The PAH clearance procedure was as described above.

The kidney was removed after completion of the experiments, divided and drained for approximately 10 min and then weighed.

**Results**

1. **COMPARISON TO DIRECTLY MEASURED VENOUS OUTFLOW**

In the absence of hydrogen a positive current (electron flow towards the platinum electrode) of the order of $10^{-8}$ amp was generally obtained. This "residual current" was found to be influenced by the rate of renal blood flow or factors associated with blood flow ($pO_2$, pH). Considerable changes in blood flow during one desaturation period would therefore complicate the estimation of "net current" ($i_{net}$, current resulting from oxidation of hydrogen), and several desaturation curves had to be discarded for that reason. The net current resulting from 2% to 4% hydrogen saturation was usually less than $10^{-7}$ amp and seemed to be related to the active electrode area and also to the blood flow itself. The current obtained from an intra-arterial electrode was usually higher than that obtained simultaneously from a renal cortical electrode of comparable size.

**Renal Cortex**

Typical desaturation curves obtained simultaneously with two cortical and one intra-arterial electrode are shown in figure 9, where the net current (in arbitrary units) is plotted on a logarithmic scale against time. The linear fall in electrode current from the time when arterial concentration becomes negligible suggests a single exponential decay of tissue hydrogen. Essentially the same desaturation curves were obtained from 17 different electrode positions in eight experiments. However, in a few curves there was obviously a fast initial component, since the flow calculated from the integrated area between arterial and tissue curve (equation 4) for the first 30 to 50 sec came out higher than when a later part of the curve was used. Some curves also suggested the presence of a somewhat slower component, although the evaluation of this is uncertain since reliable readings could not easily be obtained below 10% of the initial concentration under the conditions of these experiments.

A time difference between desaturation curves obtained simultaneously from two different cortical electrodes was frequently found. The difference, which might be as much as 15 sec, did not seem to be related to the slope of the curves. Since this delay would preclude an exact calculation of the integrated area, the flow was calculated from the slope after 40 to 50 sec (equation 7). The
Renal cortical blood flow obtained from one tissue electrode ($F_{H_2}$) compared to directly measured venous outflow ($F_v$). Hydrogen respiration. Mean ratio, $F_{H_2}/F_v = 1.00$, indicated by straight line.

Local cortical blood flow calculated in this way from any particular electrode position showed a fairly constant relationship to venous outflow in the range of 30 to approximately 150 cc/min/100 g throughout experiments of several hours duration (fig. 10). However, different electrode positions frequently gave consistently different flow values (table 1). With venous outflow higher than approximately 150 cc/min/100 g the ratio between cortical flow estimated from tissue electrodes and venous outflow tended to become lower (fig.

![Figure 10](image)

**Figure 10**

Cortical blood flow obtained from one tissue electrode ($F_{H_2}$) compared to directly measured venous outflow ($F_v$). Hydrogen respiration. Mean ratio, $F_{H_2}/F_v = 1.00$, indicated by straight line.

![Figure 11](image)

**Figure 11**

Cortical blood flow obtained from one tissue electrode ($F_{H_2}$) compared to directly measured venous outflow ($F_v$). Hydrogen respiration. Straight line indicates mean ratio, $F_{H_2}/F_v$ for flows < 150 cc/min/100 g = 1.07.

**Table 1**

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Outer 1/3 of cortex</th>
<th>Middle 1/3 of cortex</th>
<th>Inner 1/3 of cortex</th>
</tr>
</thead>
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<tr>
<td>1</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>1.06 (3)</td>
<td>0.96 (3)</td>
<td></td>
</tr>
<tr>
<td>3</td>
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<td></td>
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</tr>
<tr>
<td>4</td>
<td>1.06 (1)</td>
<td>1.22 (1)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.00 (9)</td>
<td>1.31 (6)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
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<td>1.40 (5)</td>
</tr>
<tr>
<td>7</td>
<td>1.08 (4)</td>
<td>0.89 (2)</td>
<td>0.95 (2)</td>
</tr>
<tr>
<td>8</td>
<td>1.09 (5)</td>
<td>0.82 (3)</td>
<td>0.96 (2)</td>
</tr>
<tr>
<td>Mean</td>
<td>1.04</td>
<td>1.08</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Mean ratio for all 17 electrode positions 1.05 (sd ± 0.16).

Range for 70 individual desaturation curves 0.66 to 1.59.
Only periods with venous outflow less than 150 cc/min/100 g are therefore included in table 1, which shows the mean ratios for 17 different electrode positions, including 70 desaturation curves in eight kidneys. No significant difference was found between superficial and deeper parts of the cortex. When an electrode was moved to a new position within the cortex during an experiment, the local cortical flow/venous outflow ratio would usually change, indicating that the considerable scattering of the ratios was not due to factors inherent in the different electrodes.

Renal Vein

Nine venous desaturation curves obtained in two experiments also showed a nearly linear slope from the time when arterial concentration became negligible and down to approximately 10% of the initial concentration. Due to varying flow and changing baseline an exact evaluation of the last part of the desaturation curve was not possible. Calculation of blood flow from the integrated area suggested a fast initial component in these curves. The flow calculated from the slope from 50 sec on, showed good agreement with venous outflow (fig. 12), with average ratios of 1.00 and 0.93 in the two experiments respectively.

Effective renal blood flow in these experiments varied between 260 and 500 cc/min/100 g. Out of 17 electrode sites five gave single exponential desaturation curves with slopes practically independent of the degree of initial equilibration. Flow rates calculated from the slope showed a fairly constant relationship to ERBF, as shown in the example given in figure 13. In all 35 desaturation curves were recorded during 17 clearance periods. The average ratio to ERBF/100 g for the five electrode sites varied between 0.82 and 1.21, with an average of 1.04.

Desaturation curves from the remaining 12 electrode positions were found to be more or less dependent on the degree of equilibration. With a rapid injection a fast initial component was observed, occasionally with a slope considerably exceeding what would be expected from the average flow. With several
minutes' equilibration a slower, multi-exponential curve was obtained. Attempts to calculate the flow from these curves, either by using the integrated area under the curves or by solving in different components, gave flow values lower than expected and a poor correlation to ERBF.

Renal Vein

Desaturation curves obtained from a catheter electrode in the renal vein were generally better technically than those obtained from tissue electrodes. Practically no changes in residual current could be detected in the relatively short time needed for complete desaturation, permitting the curves to be read down to 95% desaturation or lower. Furthermore, no evidence for a delayed response was observed when a resistance of 10 ohm was used.

The ERBF in these experiments was in the range of 250 to 500 cc/min/100 g, usually decreasing spontaneously throughout the experiments. The shape of the desaturation curves was found to be greatly dependent on the time used for saturation, indicating a heterogeneous flow within the kidney. Three curves obtained from one experiment obtained after rapid injection, 50 sec and 11 min equilibration respectively are shown in figure 14. With a rapid injection, a fast initial component with a half-time of less than 2 sec (initial slope having a half-time of ca. 4 sec) was consistently found. The relative magnitude of this fast component is greatly exaggerated, however, as evident from the curve obtained after 50 sec saturation (fig. 14, curve C). With this kind of saturation the fast component was found to account for 5% to 10% of the total amount of hydrogen present in the renal vein at the start of desaturation. It should be emphasized, however, that the present technique does not permit any exact appraisal of the relative size of the fast component.

The second slope, linear or nearly so, down to 90% desaturation or lower, thus representing the greatest part of the flow (presumably cortical flow), was found to be the same after rapid injection as after 30 to 60 sec saturation. (The difference in half-times between curves A and C in figure 14 was ac-
accompanied by a corresponding decrease in ERBF.) With equilibration for 10 to 15 min a multi-exponential desaturation curve was obtained (fig. 14, curve B), obviously by addition, or rather magnification, of a much slower component, presumably representing the renal medulla or part thereof. As shown in the diagram, subtraction of this component gives a curve practically identical to that obtained after 50 sec equilibration. The flow calculated from the second slope, presumably cortical flow, showed a fairly constant relationship to ERBF, as shown in the experiment presented in figure 15, in which mannitol infusion was given in order to provide sufficient urine flow for PAH clearance. Four more experiments, in which no osmotic agents were given, also showed a good correlation to ERBF (fig. 16), although with a lower ratio, the difference probably being due to the different final kidney weight with and without mannitol.

Comments. Several uncertainties are inherent in the use of the renal cortex for evaluation of the method. First of all, the measured venous outflow is the sum of cortical and medullary blood flow, the latter probably constituting a small proportion of the total flow. However, even if the cortical flow itself were known exactly it has to be converted to flow per unit of tissue by dividing by the weight of the cortex, which is not readily assessed. It was found by weighing the kidney clamped in vivo and then again after unclamping and dissecting out the electrodes that the kidney would lose 20% to 30% of its weight, obviously depending on the blood and fluid content at the time of clamping. This weight loss would approximately compensate for the weight of the medulla, and the total flow divided by this weight would therefore be expected to be close to the true cortical flow per unit of tissue. It is obvious, however, that this arbitrary procedure may introduce considerable scattering in the ratios between cortical flow estimated from hydrogen desaturation and directly measured outflow from one kidney to another.

The flow range tested in the experiments with hydrogen respiration and directly measured venous outflow, i.e., 35 to 230 cc/min/100 g, is far below normal cortical flow, indicating an increased renal vascular resistance. It is conceivable that there may be greater local differences in cortical blood flow under these circumstances than under more physiological conditions, and that the considerable variations of flow values obtained from different electrode positions in the same kidney may give a correct picture of the intracortical flow distribution. This is in agreement with the patchy distribution of cortical flow found in rats subjected to hind limb ischemia and in rabbits given epinephrine injections. On the other hand, the random scattering of flows throughout the cortex disagrees with reports of diversion of blood flow from the outer cortex in rabbits after various stimuli and in dog kidneys having been freed from their peritoneal attachments. The poor correlation between cortical flow and renal venous outflow above 150 cc/min/100 g with this technique requires some comments: When hydro-

![Diagram](image-url)
gen respiration is stopped, it takes 40 to 50 sec before arterial blood is desaturated to below 10% of the initial concentration. The decay of tissue concentration will therefore not reflect the flow until the tissue concentration has been reduced to 30% or lower. With any inhomogeneity of flow in the area surrounding the electrode, the remaining part of the desaturation curve will mainly reflect the slower components, thus tending to underestimate the average flow. The general conclusion seems warranted that unless the flow is very homogeneous, the slope calculation cannot be used at flows exceeding 150/cc/min/100 g, when saturation and desaturation is obtained with respiration. It might seem possible to circumvent this problem by calculating the flow from the integrated area between the arterial and tissue desaturation curve. However, the delay observed rather frequently in the tissue curves, probably due to a virtually unstirred diffusion layer surrounding the electrode, might introduce considerable and unpredictable errors in the calculated flow.

Frequent failure of measuring the high normal cortical blood flow even with hydrogen infusion might be explained by the following consideration: The tip of an electrode inserted in a tissue must be surrounded by a non-circulated area of blood, exudate, or devitalized tissue of variable thickness. With slow desaturation (low flow), hydrogen can presumably be brought to the electrode surface over relatively great distances without much distortion of the average tissue desaturation curve. With a rapid desaturation (half-times of the order of 10 sec), however, the delivery of hydrogen to the electrode in amounts proportional to the mean tissue concentration would seem to be more dependent on the circulation in the immediate surroundings of the electrode, and it is conceivable that different portions of the electrode might be provided with widely different concentrations of hydrogen. It is also clear that when the delay caused by the diffusion area becomes large relative to the half-time, the correct slope of the desaturation curve will not be obtained. Thus, one part of the electrode might reach a high degree of desaturation at the time when desaturation of another area has barely started. Although admittedly speculative, this hypothesis would explain the multi-exponential curves, the dependency on the degree of desaturation, and also the tendency to yield too low values when starting desaturation from a completely saturated tissue.

On the other hand, the few electrode positions giving a single exponential decay irrespective of the degree of saturation show that under favorable circumstances, presumably a uniform diffusion area and circulation around the electrode, it is possible to measure flows as high as 500 cc/min/100 g with a tissue electrode.

Venous desaturation curves obtained with intra-aortic hydrogen infusion (fig. 14) deserve some comments. The very rapid initial rate of desaturation, approaching the curve of arterial desaturation, shows that a fraction of renal venous blood has not been equilibrated with tissue or has been equilibrated with a very small volume of tissue. Three explanations seem possible: 1) direct arteriovenous shunts; 2) short-circuiting of hydrogen in the outer medulla; 3) establishment of a considerable concentration gradient between capillary blood and average tissue.

1. No convincing anatomical demonstration of true arteriovenous shunts in the dog kidney seems to have been presented. This is consonant with the low venous recovery (1.5%) of glass spheres with an average diameter of 19 μ injected into the renal artery in dogs.24 On the other hand, short capillary channels from vas efferens to veins, bypassing the peritubular capillary network, have recently been suggested,25 providing a possible route for hydrogen “shunting.”

2. A short-circuiting of hydrogen between the ascending and descending limbs of the vasa recta in the outer medulla, as proposed for oxygen by Levy and Sauceda,26 seems possible. Taking place within the vascular bundles in this area, it is conceivable that very little tissue saturation would be involved.

3. The third possibility, establishment of a considerable concentration gradient within the tissue, would also be expected to produce a
fast initial slope. Although the average capillary density in the renal cortex is high,\textsuperscript{8} it is clear that separation of the capillaries by the tubules with diameters of 40 \(\mu\) to 80 \(\mu\)\textsuperscript{27} creates diffusion distances exceeding by far that obtained from the average capillary density. According to a formula derived by Kety,\textsuperscript{8} when the gradient is established a single exponential desaturation curve would be expected, the slope of which is reduced by a factor exponentially related to blood flow. The present observation that the flow calculated from the second slope showed no tendency to fall off relative to ERBF with increasing blood flow (figs. 15 and 16) would therefore cast doubt on this explanation. Although the present study does not provide information to decide among the mechanisms discussed above, the physiological implication will be much the same, namely, that a part of the renal blood flow is not effectively presented to the renal parenchyma.

The second slope of the venous desaturation curve is assumed to represent desaturation of the renal cortex, as indicated by the good correlation to ERBF (figs. 15 and 16). An exact evaluation of the absolute values is not possible, however. The ratio of 0.83 to ERBF/100 g drained kidney weight corresponds to a ratio of 1.15 to 1.20 to ERBF/100 g clamped kidney weight, the latter being theoretically more correct if the medullary blood flow/100 g is of the same order of magnitude as cortical flow. Furthermore, due to incomplete extraction of PAH, the ERBF is 10\% to 25\% lower than the total renal blood flow. The difference, the “ineffective flow” might partly be represented by the fast initial component observed here, although it is very unlikely that this fast component could be accounted for by flow to the renal capsule, pelvis, and perirenal fat, as proposed by Smith\textsuperscript{28} to explain the failure of 100\% PAH extraction.

The third component, \(B\), (fig. 14) presumably contains the desaturation of the renal medulla. However, recirculation of hydrogen may contribute considerably to this late portion of the curve, which may not therefore give a true picture of the medullary venous desaturation. Furthermore, due to counter-current exchange of hydrogen in the medulla, a single exponential venous desaturation curve will not be expected for this region, and it seems impossible therefore to estimate medullary flow from the composite renal venous desaturation curve.

**SKELETAL MUSCLE**

**Methods**

In order to test the method in a low flow range, two experiments were performed on dog skeletal muscle, (anesthesia as described for kidney experiments). \(M.\) gracilis was isolated as described by Renkin and Rosell.\textsuperscript{20} All visible nerves were divided. The dog was then heparinized and the muscular vein was cannulated and drained into a graduated cylinder, and from here returned to the dog by means of a Bowman finger pump. Venous outflow was estimated by stopping the pump and measuring the accumulation of blood in the cylinder. The flow range tested was from 4 to 10 cc/min/100 g, based on the weight of the excised muscle. One or two electrodes were inserted into the central part of the muscle, and another electrode was mounted in the venous cannula close to the muscle giving a dead space of less than 1 cc between the muscle and the electrode. A constant ventilation was provided by a respirator and hydrogen gas was given for at least 15 min before starting the desaturation.

**Results**

A venous and a tissue desaturation curve are given as an example in figure 17. Saturation had been obtained by giving hydrogen at a constant, relatively high rate for 4 min and then at an approximately 50\% lower rate for 14 min. (The priming was not sufficient to produce an overshoot in either curve.) The tissue washout curve shows considerable delay relative to the venous curve and then an apparently single exponential decay, giving a flow close to the measured venous outflow. On the other hand, the venous curve shows a pronounced fast component. Assuming complete initial equilibration (see below), graphical analysis as shown in figure 17 indicates that the fast component accounts for 47\% of the blood flow. From the desaturation rates of the two components (0.65 and 0.0825 min\textsuperscript{-1} respectively) it is readily calculated that the blood representing the fast component has been equilibrated with only 10\% of the tissue.
The average flow calculated from these figures is 13.9 cc/min/100 g, i.e., considerably higher than the venous outflow of 9.7 cc/min/100 g. Five additional venous desaturation curves all had the same shape as that shown in figure 17. The calculated average flow showed a fairly constant relationship to venous outflow, although the absolute values were invariably too high.

Twelve tissue desaturation curves were recorded with five different electrode positions. From two electrode positions a fast initial slope was obtained, while the remaining three gave a single exponential decay with more or less delay, as shown in figure 17. Although variations in venous outflow and changing baseline in several runs complicated interpretation of the curves, the flow obtained from the slope (second slope in curves with a fast component) seemed to give a fairly constant relationship to venous outflow in the range of 4 to 10 cc/min/100 g, with individual ratios to venous outflow varying between 0.85 and 1.4.

Comments. Low hydrogen clearance and presence of different clearance rates in the muscle exaggerates errors inherent in the method due to lack of a fixed baseline and absolute calibration. Even a small and slow change in residual current may introduce considerable errors in a slow component, since desaturation has to be carried out for at least one hour.

Fast initial rate of hydrogen desaturation, presumably correctly recorded in the vein, deserves some comments. Half-time for this component, about 1 min, is certainly longer than would be produced by a true arteriovenous shunt. Furthermore, an arteriovenous shunt could not explain the fast component observed in some tissue curves. This conforms with the recovery of only 4.3% of intra-arterially injected 30 μ spheres in dogs30 and 1% in cat muscle,31 indicating that arteriovenous shunt must be rare in skeletal muscle. Two other explanations should be considered: 1) considerable venous-tissue gradient for hydrogen in homogeneously perfused tissue; 2) heterogeneous muscular blood flow.

1) The relatively high capillary density of muscle (Kety8) combined with low flow would seem to preclude any appreciable H₂ gradient. However, if only a small fraction of the total number of capillaries are open in the resting muscle, diffusion distances might possibly be great enough to produce considerable gradients. As discussed for the kidney, this would produce a fast initial desaturation of venous blood, followed by a single exponential washout, the slope of which gives too low an average flow. Although this might explain the venous desaturation curve, appearance of a fast initial component in a tissue curve by this mechanism would require that a tissue electrode would reflect local venous concentration rather than average tissue concentration, which does not seem likely. Furthermore, a similar two component uptake of antipyrine in muscle was found by Renkin,32 who also
presented evidence that the fast component was not diffusion limited. Although the experimental conditions were different from those in the present study, these findings make the hypothesis of a diffusion limitation for hydrogen unlikely.

2) Heterogeneous flow in the sense of separate parallel anatomical compartments with different flows could certainly explain a two exponential curve obtained both from venous and tissue electrodes (the latter happening to be on the boundary between two compartments). Another type of heterogeneous flow, which seems more likely, would be a marked variability in the amount of tissue supplied by different capillaries. Short capillaries with high flow might thus act as "physiological shunts," as discussed by Renkin and Rosell. It should be emphasized that the two compartment solution obtained by graphical analysis may be more or less artificial, although the marked difference between the half-times of the first and later parts of the venous curve would suggest a bimodal distribution of muscular blood channels, rather than one population with a very wide, normal distribution. The present experiments do not give sufficient evidence for any one of these hypotheses. However, the physiological implication is the same, namely that a considerable portion of the muscular blood flow is effectively presented only to a small portion of the tissue. Due to incomplete equilibration at the start of desaturation, the proportion of this "non-nutrient flow" may be somewhat overestimated. The marked delay observed in several tissue curves is caused presumably by hydrogen depletion of a considerable tissue layer around the electrode, as discussed above. It is conceivable that a marked delay might conceal a fast initial component more or less completely.

The results presented above are insufficient for a final validation of the use of the method in skeletal muscle. Fairly constant relationship between venous outflow and flow calculated from tissue desaturation curves suggests that reasonably reliable results might be obtained with improvements in the technique, and certainly measurements in intact muscle would be easier since the extracorporeal circuit used for reference flow measurements in this study tends to interfere with the recording. An obvious advantage of the method is that it could be used in man with only cutaneous anesthesia.

General Discussion and Conclusions
The use of hydrogen desaturation rate as a measurement of local blood flow rests on two assumptions: 1) instantaneous equilibration between tissue and venous blood from that particular tissue; 2) no recirculation of hydrogen.

1. Although theoretical considerations may provide some information on the validity of this assumption, the actual calculations depend on several parameters related to the anatomical structure of the functioning organ which are not readily assessed. The empirical test of comparing the washout rate to independent flow measurements, as reported above, strongly suggests that the assumption is valid within experimental errors, except possibly in the renal cortex with its extremely high flow.

2. The assumption of no recirculation is obviously an approximation, since a small fraction of blood will always pass the lungs without being cleared (generally well below 10%). Due to the low blood/gas partition coefficient for hydrogen, 0.018 compared to 0.051 and 0.44 for krypton and nitrous oxide, respectively, the alveolar hydrogen tension will be kept low and arterial concentration will therefore be reduced nearly proportional to mixed venous blood. When hydrogen has been given systemically, the desaturation of mixed venous blood is determined by the distribution of the cardiac output and on the length of the saturation period. Considering a homogeneously perfused tissue, the effect of recirculation will depend on how fast mixed venous blood is desaturated relative to this particular tissue. Without going into details it may be stated that the effect of recirculation may be kept at a minimum by using a short administration period—for instance, rapid intra-aortic infusion for the most highly perfused tissues, while a...
relatively longer period of administration, covering one half-time or more, should be used for tissues in the intermediate or low flow range. Under these conditions the "slope" calculation, neglecting recirculation seems justifiable, but it should be emphasized that these considerations apply only to a homogeneously perfused tissue. For correct evaluation of a venous desaturation curve from a heterogeneously perfused organ, as for instance the kidney, the administration period has to be long enough to provide complete saturation of the slowest compartment, i.e., the renal medulla. Unfortunately, this also implies that the concentration of mixed venous blood will be much higher than the average tissue concentration except for the very first portion of the desaturation curve. Recirculation through the fast compartment (cortex) might thus contribute considerably to the total venous hydrogen content and considerably influence the late part of the venous desaturation curve, which would otherwise reflect the slow compartment. This problem can be circumvented either by recording arterial desaturation or by local intra-arterial infusion of hydrogen saturated saline. Since the correction for recirculation would involve determination of very low arterial concentrations and greatly increase the experimental error, local infusion seems preferable.

Use of hydrogen breathing for saturation will certainly influence the first part of the desaturation, during which the bulk of hydrogen is removed from lung gases, but this can be avoided by changing to intra-aortic infusion for the last minute of the saturation period. With this combination an essentially square wave arterial curve is obtained with infusion of a minimal volume of fluid.

Lack of a fixed baseline and absolute calibration makes it necessary to carry out the desaturation for five to six half-times for maximum accuracy. In the flow range of the myocardium this means that each determination takes five to ten minutes, during which time the blood flow should remain constant. The method is therefore not suitable for following rapid changes in blood flow. (By assuming a constant baseline, which is usually a good approximation, determinations can be repeated more frequently.) The time necessary also makes the method inconvenient in tissues with very low blood flow.

The main virtue of the method is its applicability to repeated measurements of local blood flow in tissues from which representative venous blood is not available, and the experiments above indicate that this can regularly be accomplished at blood flow less than 150 cc/min/100 g, and under favorable conditions at flows up to 500 cc/min/100 g. The results also imply that the presence of the electrode within the tissue does not seriously influence blood flow in the area from which hydrogen is delivered to the electrode surface. Hydrogen diffusion from arteries to the tissue electrode is a conceivable source of error. With the size of the electrodes used, this would presumably influence only a part of the electrode, producing a very rapid initial desaturation. Since this was not observed in any of the tissues studied, and since a good correlation to venous outflow was generally found, it is concluded that diffusion from arteries is not a major source of error. The flow limitation mentioned above is irrelevant for most tissues, and the method should therefore be applicable to a number of tissues not investigated in the present study. An obvious application is the measurement of blood flow in different structures within the central nervous system. Preliminary experiments on cat brain by C. Fieschi have given flow values for gray and white substance of the order of magnitude expected from other studies (personal communication).

Chronic implantation of tissue electrodes and subsequent flow measurements in unanesthetized animals seems possible from preliminary studies.

Use of hydrogen gas recorded with a venous electrode has the same limitation as methods using other inert gases, namely the problem of finding a representative vein. The advantage would be the continuous recording of gas concentration in situ, avoiding the distortion of fast components which may result from...
sampling via long tubes, thereby allowing a better characterization of the flow distribution. Furthermore, since collection and analysis of multiple blood samples are unnecessary, the method is more simple and more rapid.

Summary

When a platinized platinum electrode is polarized at the potential of the standard calomel electrode, the current generated is proportional to the concentration of dissolved hydrogen gas. The effects of physiological variations in oxygen tension, pH, temperature, and ascorbic acid concentration were found to be negligible. Although absolute calibration in vivo is not possible, the rate of tissue hydrogen saturation or desaturation can be measured by needle-shaped electrodes inserted into the tissue. Arterial and venous concentration can be measured with catheter electrodes. Solubility of hydrogen gas in kidney slices was found to be the same as in blood. With the assumption that tissue is in instantaneous diffusion equilibrium with local venous blood with respect to hydrogen, the local blood flow per volume of tissue can be calculated according to the Fick principle from the rate of tissue desaturation when arterial concentration is lowered to zero.

The method was tested on dogs in myocardium, kidney, and skeletal muscle. Hydrogen was administered by respiration or by intraarterial infusion of hydrogen-saturated saline, giving an arterial concentration of 3% to 5% saturation. Tissue desaturation curves were recorded simultaneously from two to three tissue electrodes. Good agreement with flow measured simultaneously with other methods was obtained in myocardium and renal cortex, while the data on skeletal muscle do not permit any definite conclusion. The main virtue of this method lies in the fact that repeated measurements of local blood flow can be obtained without access to arterial or local venous blood. Reliable measurements of regional blood flow were also obtained from continuously recorded venous desaturation curves, which also provide information on the distribution of blood flow.

Acknowledgment

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References


