Mass transfer, storage, and utilization of \( O_2 \) in cat cerebral cortex

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A TRANSPORT MECHANISM (facilitated diffusion) for \( O_2 \) in some tissues has been postulated by Wyman (19), and Longmuir (11, 12) suggests an oxygen carrier in some tissues. In addition, Longmuir (9) describes a dependence of \( V_{O_2} \) on \( P_{O_2} \) in rat liver, whereas Gore (5) describes a similar relationship in frog sartorius muscle; and the studies of Chance (2) on mitochondrial respiration indicate a probable \( K_m \) (\( K_m \) being the \( P_{O_2} \) at which metabolism is reduced to one-half the maximum rate) in all tissues. The availability of a micro oxygen electrode (17) offered a unique approach to these problems. In order to determine what kind of transport mechanism exists in cat cerebral cortex, \( P_{O_2} \) profiles were measured in freshly excised tissue. The data were found to be consistent with the classical diffusion equation at \( P_{O_2} \) values in the range of 25 mm Hg and above. Since the data are consistent with the classical diffusion equation they provide the information necessary for calculation of the rate of consumption and diffusion for \( O_2 \) in the tissue.

The solubility coefficient for oxygen cannot be readily measured by ordinary means in a metabolizing tissue and thus, the Krogh diffusion coefficient (diffusion coefficient multiplied by solubility coefficient) is ordinarily reported. To our knowledge, the method to be described here is the only technique for estimating the solubility coefficient as well as determining the rate of \( O_2 \) consumption and diffusion in soft labile tissue.

METHODS AND MATERIALS

For these experiments, 2- to 3-kg cats of mixed breeding and of both sexes were used. Tissue \( P_{O_2} \) was measured with recessed micro \( O_2 \) electrodes (17). These electrodes have long tapering tips of 1-2 \( \mu \) diameter and no tissue distortion was observed through the microscope during penetration. The oxygen electrode current was measured with a Keithley 416 electrometer and electrode depth was monitored via a potentiometer attached to the micrometer used to introduce the electrode into the tissue (see Fig. 1). The solution used for electrode calibration and tissue perfusion was a physiological salt solution (154 mM NaCl, 5.6 mM KCl, 5.0 mM NaHCO\(_3\), and 2.8 mM CaCl\(_2\)) with 100 mg/100 ml glucose added. It was continuously bubbled with either 98% He-2% CO\(_2\), 25% \( O_2 \)-2% CO\(_2\)-73% N\(_2\), 2% \( O_2 \)-2% CO\(_2\)-96% N\(_2\), or 50% \( O_2 \)-2% CO\(_2\)-48% N\(_2\) in bottles maintained at 37 C in a constant-temperature bath. The 2% CO\(_2\) gas mixtures maintained the pH of the bicarbonate-buffered solution at 7.2-7.3. A valve arrangement allowed a rapid change of the solution flowing through the tissue chamber. Electrode calibration was checked near the solution inlet before and after each tissue penetration. All profiles in tissue were taken with 50% \( O_2 \) saturated solution flowing.

The animals were anesthetized with 40 mg/kg of pentobarbital given intraperitoneally. The head of the animal was then shaved and the top of the brain exposed. To expose the brain, a midline incision was made from anterior to posterior in the scalp and the entire top of the skull was removed by cutting around it with a dental drill. Bleeding was controlled with Gelfoam (Upjohn), the dura was carefully removed from the exposed cortex, and the brain was covered with a saline-soaked gauze sponge.

A slice of cortical tissue about 0.5 cm x 1 cm x 1 mm was removed from the cat with a scalpel. The pia was carefully removed from the slice and the tissue was mounted in the chamber as shown in Fig. 2. Oxygenated solution at 37 C was continuously flowed through the chamber. Bleeding was controlled by covering the wound area with Gelfoam and the cat was maintained as a source of additional tissue slices. Under observation with a microscope, the oxygen electrode tip was brought to the top uncut surface
The procedure used to obtain data for estimation of the solubility coefficient consisted of mounting fresh tissue slices as mentioned before. However, the electrode was introduced into the tissue, without recording depth, to a position where the \( P_{O_2} \) was less than 25 mm Hg. The perfusing solution was then switched from 50% \( O_2 \) to 2% \( O_2 \) and the change in \( P_{O_2} \) with time was recorded. As this procedure required very little time, several determinations were made in each tissue slice.

RESULTS AND CALCULATIONS

The data shown in Fig. 4 are first penetration profiles in 13 tissue slices from three cats. In order to determine whether this data can be described by the classical diffusion equation, the equation was rearranged and the data plotted in the following way. The equation,

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - Vo_2
\]

where \( C \) is oxygen concentration, \( D \) is the diffusion coefficient for oxygen, \( x \) is depth into the metabolizing media, \( t \) is time, and \( V_{O_2} \) is oxygen consumption per unit volume of tissue, is found in numerous texts (1, 3) and its derivation and solution will not be discussed here. Under the conditions of this study, the boundary conditions are those of an infinite plane with one-dimensional diffusion such that the \( P_{O_2} \) falls to zero at some depth into the tissue. The critical thickness or depth to zero \( P_{O_2} \) was always less than 150 \( \mu \) and, thus, the width and length (5 mm x 10 mm) are more than 30 times the thickness, making diffusion from the edges negligible. The steady-state solution (see ref 6) is

\[
C = \frac{V_{O_2}}{2D} \sqrt{\frac{2V_{O_2}}{D} C_0 X} \quad (2)
\]

where \( C_0 \) is oxygen concentration at the tissue surface. Inherent in this equation are the assumptions that the tissue is a homogenous medium with a constant rate of \( O_2 \) consumption which is independent of \( P_{O_2} \) for all oxygen
FIG. 3. A: record of a PO2 profile in a tissue slice. Solid line indicates PO2 and dashed line depth of electrode from an initial position above tissue. B: initial portion of a PO2 profile. C: continuation of PO2 profile in B after increasing sensitivity of PO2 channel.

FIG. 4. Solid curve represents mean PO2 ± SE at each depth as determined from 13 tissue slice profiles; dashed curve is a plot of classical diffusion equation using constants determined from tissue data.

FIG. 5. Plots of transformed PO2 profile data. Curves were determined by taking ln or (P0 − P)/X transform of all PO2 profile data points (each depth in each profile) and calculating a mean and standard error from transformed data at each depth. P0 = 248 mm Hg at X0.

It is rearranged to:

\[
P - \frac{P_0 - P}{X} = \sqrt{\frac{2V_{O_2}P_0}{DS}} - \frac{V_{O_2}X}{2DS}
\]

Thus, if the data are plotted as (P0 − P)/X versus X and if the classical diffusion equation with its assumptions describes the transfer of O2 in the tissue, the plot should be a straight line. While there is considerable variation due to...
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slight temperature changes and inability to determine precisely the tissue surface, the plot of the means of the converted data points in the form \((P_0 - P)/X\) versus \(X\) is consistent with a straight line, as seen in Fig. 5.

Since the variation in the data is considerable, it was decided to see whether another diffusion equation might describe the data equally well. The same assumptions were made with the exception that the \(J_{O_2}\) was assumed to be proportional to the \(P_{O_2}\) or oxygen concentration at any point. This yields the equation:

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial X^2} - Q C = 0
\]

where \(Q\) is oxygen consumption per unit volume of tissue per unit of concentration. The steady-state solution to equation 5 is:

\[
C = C_0 e^{-\sqrt{Q/X}} \quad \text{or} \quad P = P_0 e^{-\sqrt{Q/X}}
\]

Thus, if this equation described oxygen transfer in the tissue, the natural log of the data should plot as a straight-line function of \(X\). Figure 5 shows that it does not and the implication is that the diffusion and utilization of \(O_2\) by cat cerebral cortex is described by the classical diffusion equation, equation 3. However, while it is not evident from Fig. 5, the incremental change in \((P_0 - P)/X\) is 0.527 between 40 and 60 mm Hg, 0.525 between 60 and 80 mm Hg, and 0.485 between 80 and 100 mm Hg. This indicates a probable decrease in \(J_{O_2}\) in the tissue between 14.6 and 1.50 mm Hg. At the increased sensitivity, the electrode was introduced into the tissue without regard to depth from the surface; \(P_{O_2}\) versus depth was then recorded and plotted from 25 mm Hg down to less than 0.5 mm Hg. As can be seen from Fig. 6 this expanded plot of \((P_0 - P)/X\) versus \(X\) is not consistent with a straight line and indicates that a \(K_w\) or critical \(P_{O_2}\) may exist at about 2 mm Hg.

In view of the fact that the data plot as a straight line at high \(P_{O_2}\) is predicted by the rearranged classical diffusion equation (equation 4), \(V_{O_2}\) and \(DS\) can be calculated from the data. In Fig. 4 it can be seen that there is a straight-line gradient for \(O_2\) in the solution for about 40 mm Hg above the tissue, indicating that a boundary-layer equivalent to 40 mm Hg of nonflowing solution exists over the tissue and indicates that the \(\Delta P/\Delta X\) (where \(\Delta P\) is a small incremental change in \(P_{O_2}\) and \(\Delta X\) is a small increment of tissue thickness) in the solution over the tissue, the flux of \(O_2\), is:

\[
M = DS \frac{\Delta P}{\Delta X}
\]

The mean depth to zero \(P_{O_2}\) (\(1.58X_0/13\)) in the 13 tissue slices was 100 ± 4.0 mm Hg and thus, the volume of tissue being supplied with \(O_2\) by the above flux can be calculated and the \(V_{O_2}\) determined. \(V_{O_2} = (\text{flux } \times \text{area})/\text{volume of tissue}\) and the calculated value is 0.0898 ml \(O_2/cm^2\) tissue-min.

Similarly a value for \(DS\) was calculated by noting that the slope of the plot of equation 4 contained only \(V_{O_2}\) and \(DS\), with \(V_{O_2}\) already known. From individual plots of \((P_0 - P)/X\) versus \(X\) for each of the 13 profiles, \(V_{O_2}/2 DS\) was determined graphically. The mean was found to be 2.65 \(X 10^{-2} \pm 0.314 \times 10^{-2} \) mm Hg/\(\mu l\). Substituting \(V_{O_2} = 0.0898 \text{ ml } O_2/cm^2\cdot \text{min} , DS = 0.129 \text{ ml } O_2/cm^2\cdot \text{min} \cdot \text{atm} \) across 1 \(\mu l\), or \(DS = 1.29 \times 10^{-5} \text{ ml } O_2/cm^2\cdot \text{min} \cdot \text{atm} \) across 1 cm of tissue.

The critical depth \(X_0\) used for calculating \(V_{O_2}\) was determined by taking the mean of the critical depths for the 13 individual profiles. The slope of the plot of \((P_0 - P)/X\) used for calculating \(DS\) was determined by taking the mean of the slopes of the 13 individual plots. The mean values were obtained in this way in order to provide meaningful estimates of the standard error of these parameters.

\(V_{O_2}\) and \(DS\) could also be calculated by graphically reading \(X_0\) from Fig. 4 and the slope of \((P_0 - P)/X\) from Fig. 5. While this method gives similar values for \(V_{O_2}\)

\[
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Similarly a value for \(DS\) was calculated by noting that the slope of the plot of equation 4 contained only \(V_{O_2}\) and \(DS\), with \(V_{O_2}\) already known. From individual plots of \((P_0 - P)/X\) versus \(X\) for each of the 13 profiles, \(V_{O_2}/2 DS\) was determined graphically. The mean was found to be 2.65 \(X 10^{-2} \pm 0.314 \times 10^{-2} \) mm Hg/\(\mu l\). Substituting \(V_{O_2} = 0.0898 \text{ ml } O_2/cm^2\cdot \text{min} , DS = 0.129 \text{ ml } O_2/cm^2\cdot \text{min} \cdot \text{atm} \) across 1 \(\mu l\), or \(DS = 1.29 \times 10^{-5} \text{ ml } O_2/cm^2\cdot \text{min} \cdot \text{atm} \) across 1 cm of tissue.

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(0.081 ml O₂/cm³ tissue-min) and $DS$ ($1.31 \times 10^{-6}$ ml O₂/cm³-atm-min across 1 cm), the estimate of $X₀$ is biased and no means of estimating the precision of $X₀$ or the slope of the $(P₀ - P)/X$ plot is available. It is easy to see that this estimate of $X₀$ is biased as the mean profile does not reach zero $P₀₂$ until all of the individual profiles have reached zero.

The values obtained for $\dot{V}O₂$ and $DS$ were then substituted back into the diffusion equation, equation 3. Using $P₀$ = the mean $P₀₂$ at the surface of the tissue slices (248 mm Hg), the equation was plotted on Fig. 4 with the laboratory data.

As would be expected, if the transfer and utilization of $O₂$ in the tissue were described by the diffusion equation, the two curves are coincident to within 1 standard error, down to about 20 mm Hg.

To further demonstrate the validity of the method it was decided to measure $P₀₂$ profiles in a substance with known properties. For this purpose, a culture of bakers' yeast was prepared in agar as described in the previous section. Microscopic inspection of a section indicated a uniform dispersion of cells. In this medium the diffusion coefficient is a constant determined by the agar concentration. Longmuir (10) and Winsler (18) have reported the $K₄$ for $O₂$ in yeast cells to be about 0.3 mm Hg; thus, the $P₀₂$ profiles in this substance when plotted as $(P₀ - P)/X$ versus $X$ should be linear down to very low $P₀₂$ levels. Figure 7 is a plot of the means of five profiles and $(P₀ - P)/X$ versus $X$ is a linear plot.

It should also be noted that the ratio $\dot{V}O₂/DS$ appears in equation 4 and although very unlikely, it would be possible for both $\dot{V}O₂$ and $DS$ to be dependent on $P₀₂$ in the same way so that the ratio remained constant. To eliminate the possibility that $DS$ is variable, cat cortex was homogenated and stirred in liquid agar to give a homogeneous respiring medium in which $DS$ was a constant fixed by the agar. Figure 8 represents the mean of five $P₀₂$ profiles in this medium, and again, the plot of $(P₀ - P)/X$ versus $X$ is a straight line. This implies that $\dot{V}O₂$ for the brain cells is constant and independent of $P₀₂$. Since we have already established that $\dot{V}O₂/DS$ is constant at $P₀₂ > 25$ mm Hg, $DS$ must also be constant for cat cerebral cortical tissue; $DS$ being constant indicates that oxygen transfer is by diffusion rather than transport at the higher $P₀₂$ values.

Knowing $\dot{V}O₂$ and $DS$ for the tissue, a value for $S$, the solubility coefficient for $O₂$ would fully characterize the physical constants of the tissue with respect to $O₂$. It was first reasoned that by introducing the electrode into the tissue some initial distance $X₁$, changing the solution flowing over the tissue from 50% $O₂$ to 2% $O₂$ and recording $P₀₂$ versus time at $X₂$, as well as several other precisely known locations ($X₂$, $X₁$, $X₄$), $S$ could be calculated from

$$DS \left[ \frac{\Delta P}{\Delta X} - \frac{\Delta P}{\Delta X} \right] \Delta t = -\dot{V}O₂ \Delta X = \Delta P S \Delta X$$

In the equation, the first term represents net $O₂$ added to an increment of tissue by flux, the second term represents $O₂$ removed from the increment by metabolism, and the third term or right side of the equation is total change in $O₂$ content of the tissue increment (all per unit area). After a
number of experiments using increments of $\Delta t$ and $\Delta X$ sufficiently small that the values of $t$ and $X$ remained relatively constant, it was decided that $S$ could not be calculated in this way. Successive calculations sometimes yielded positive and sometimes negative values for $S$, and it became evident that at unknown values of time and depth the $O_2$ flux in the tissue was reversing direction; being first positive or into the slice, later negative, and finally again positive. Individual curves in the family of data curves were so nearly parallel that graphically measuring the differences in slopes resulted in a large error.

In view of this difficulty, $S$ was estimated by measuring the rate of change of $P_{O_2}$ at points deep in the tissue where the initial $P_{O_2}$ was between 10 and 25 mm Hg. As can be seen from Fig. 4, the gradient for $O_2$ is small at low $P_{O_2}$ values and the first term (net flux) in the equation for calculating $S$ may be neglected without great error at low $P_{O_2}$. Even stronger support for dropping the flux term comes from the fact that the decay of $P_{O_2}$ with time is linear down to about 5 mm Hg. This is consistent with $O_2$ utilization at a constant rate with negligible transfer by flux. Dropping the flux term, the equation becomes $-V_{O_2} \Delta t \Delta X \approx \Delta P \Delta X$ and $S \approx (-V_{O_2} \Delta t) / \Delta P$ where $\Delta P$ is the mean $P_{O_2}$ change of the tissue increment over the time interval, $\Delta t$. Eighteen determinations were made in tissue from two animals and $S$ was estimated as 0.0144 ± 0.004 ml $O_2$/cm$^3$ tissue per atmosphere.

**DISCUSSION**

From equation 4 it is evident that a plot of $(P_0 - P)/X$ versus $X$ will reflect changes in $V_{O_2}$ or $DS$ with $X$, as a curvature of the plot. Thus, if $V_{O_2}$ decreased with depth and $P_{O_2}$ the curve would be convex with the slope decreasing as $X$ increased. Similarly, if $DS$ increased with decreasing $P_{O_2}$, one might expect if there were a transport system for $O_2$, the curve would be concave with the slope increasing as $X$ increased.

As can be seen from the graphs of the data, there is considerable variation. However, there are a number of nonsystematic sources of variation which are not intrinsic to the tissue slices or variation between animals. The small glass tip of the oxygen electrode could not be visualized against the white brain tissue with our dissecting microscope. Thus, the point at which the electrode was believed to have entered the tissue surface was probably in error by several microns. Temperature of the tissue chamber also drifted with time. It was clearly evident that the critical depth decreased with small increases in temperature. While those profiles in which the temperature was known to have varied as much as 0.1 C from 37 C were not used for analysis, temperature must be considered as having contributed to the variation of the data. A factor which undoubtedly contributed to variation in $S$ was tissue movement due to switching from one perfusing solution to another. On some occasions the tissue slice could be observed (through the microscope) to move vertically as one perfusing solution was turned off and another turned on. Penetrations in which movement was obvious were discarded before analysis. While these are all important sources of variation, one would not expect them to introduce any systematic error or change in calculated means.

Several factors which probably did introduce systematic error are the value used for the diffusion coefficient for the perfusing solution and the failure to consider flux in the calculation of $S$. However, when the values calculated for $DS$ and $S$ are used to calculate $D$, one obtains a value of $1.54 \times 10^{-5}$ cm$^2$/sec. When this figure is compared to $D = 1.7 \times 10^{-8}$ cm$^2$/sec as reported by Thews (16) for human gray matter, it is seen that they differ by only 10%. As the value calculated for $DS$ was in the range of values determined by other means for tissues (8) this comparison lends confidence to the estimate of $S$.

The value determined for $V_{O_2}$ is several times the value reported for whole human brain by the method of Kety and Schmidt (14), but this is to be expected as the metabolic rate of gray matter is known to be greater than in the white fiber tracts.

Examination of Fig. 6 reveals that the maximum slope of the plot of $(P_0 - P)/X$ versus $X$ for tissue below 25 mm Hg is greater than the slope of the plot in Fig. 5 for tissue at higher $P_{O_2}$. From equation 4 it is evident that these slopes should be the same. The low $P_{O_2}$ profile (Fig. 6) was a separate experiment done several weeks after the experiments which determined the data for Figs. 4 and 5. While the time difference would not be expected to affect the results, the experiment was done using five tissue slices from one animal of a new group of younger cats. The difference in the slope of Fig. 6 was attributed to the fact that only one animal was used and it was from a group of younger animals. As the primary purpose of Fig. 6 was to describe the relationship at low $P_{O_2}$ it is used only to indicate the probable dependence of $V_{O_2}$ on $P_{O_2}$ at low $P_{O_2}$. The slope of Fig. 5 which was determined from 13 tissue slices in three animals is considered as a more meaningful measure of the mean $V_{O_2}$/2$DS$ in cat cerebral cortex. The greater slope at low $P_{O_2}$ would be consistent with facilitated transport as discussed by Wyman (19). Facilitated transport was, however, dismissed after considering its effect on the plot of $(P_0 - P)/X$. According to Wyman, the one-dimensional diffusion equation becomes:

$$\frac{\partial C}{\partial t} = DS \frac{\partial^2 C}{\partial x^2} + D_p \frac{\partial (mC_p y)}{\partial x} - V_{O_2}$$

where $D_p$ = diffusivity of the oxygen carrier, $C_p$ = concentration of the carrier in moles per unit volume, $m$ = the number of binding sites per molecule of carrier, and $y$ = the fractional saturation of carrier. The solution to this equation for an infinite plane of tissue supplied with oxygen from the surface and sufficiently thick that oxygen does not diffuse to the tissue center is:

$$P - \frac{V_{O_2}}{2DS} X^2 \left( \sqrt{\frac{2V_{O_2}}{DS} \left( P_b + \frac{mD_p C_p y}{DS} \right)} + P_b \right)$$

$$+ \frac{mD_p C_p (y_s - y)}{DS} + P_b$$
The transformation of this equation is:

$$P = P_0 - \frac{Vo_2}{4DS} \left[ r_C^2 \ln \frac{r_C^2}{r_o^2} - r_C^2 + r_o^2 \right]$$

(9)

If a carrier is present in the tissue one might expect it to be saturated ($y = y_0$) as hemoglobin and myoglobin are at unphysiologically high PO$_2$ near the surface of the tissue slices (248 mm Hg). If this is true the last term of equation 8 becomes zero and the slope of $(P_0 - P)/X$ versus $X$ would be the same, $Vo_2/2DS$, with or without facilitated diffusion. However, if facilitated diffusion is present the y intercept would be greater as indicated by equation 9 versus equation 4. Having previously determined $Vo_2/DS$ from the slope of $(P_0 - P)/X$ in Fig. 5 the y intercept was calculated from 2 $Vo_2/DS$ $P_0$, equation 4. The calculated value was 4.86 and the value determined from the plot of the data in Fig. 5 was the same as far as could be determined graphically. This is not consistent with an elevated y intercept due to facilitated diffusion.

Figures 6 and 8 would both be expected to reflect dependence of $Vo_2$ on PO$_2$ in cortical cells. However, Fig. 8 (cells in agar) does not indicate a dependence $Vo_2$ while Fig. 6 (tissue slice) clearly does. This is consistent with the buildup of metabolic wastes in lower layers of the tissue. As the cell density in the tissue slice was much greater than in the agar medium, a greater production of CO$_2$ and other products of metabolism would be expected in the tissue. pH was measured in the brain agar homogenate after the experiment by slicing off the upper layer and pressing a pH electrode firmly on the surface. pH was 7.0 in the agar tissue media, but no method was available for making similar measurements in the thin cortical slices and it is conceivable that pH in the lower tissue levels may have been markedly depressed.

It is satisfying that the parameters determined from the in vitro studies seem reasonable in terms of in vivo PO$_2$ and capillary distribution. The equation

$$P = P_0 - \frac{Vo_2}{4DS} \left[ r_C^2 \ln \frac{r_C^2}{r_o^2} - r_C^2 + r_o^2 \right]$$

as given by Hill (6) describes the PO$_2$ profile in a capillary domain (cylindrical model) where $P_0$ is the PO$_2$ in the capillary, $r_0$ is the capillary radius, $r_C$ is the radius at which $P = 0$ (the maximum tissue radius which can be oxygenated by a capillary as opposed to $r_d$, the tissue radius normally oxygenated by a capillary), and $Vo_2$ and $DS$ are as previously defined. If we consider the cylindrical model with metabolism independent of PO$_2$ in cerebral cortex (knowing full well that the model is not strictly appropriate) and insert previously calculated values for the parameters in the above equation, $r_C$ may be calculated as follows:

Let $y_0 = 50$ mm Hg (estimated mean capillary PO$_2$)

$$\frac{Vo_2}{4DS} = 1.32 \times 10^8 \text{ mm Hg/cm}^2 \text{ from previous calculations}$$

$$r_C^2 = 9 \times 10^{-8} \text{ cm}^2 \text{ (square of estimated capillary radius)}$$

at $r = r_o$, $P = 0$ by definition

$$0 = y_0 - \frac{Vo_2}{4DS} \left[ r_C^2 \ln \frac{r_C^2}{r_o^2} - r_C^2 + r_o^2 \right]$$

or

$$37.8 \times 10^{-4} \text{ cm}^2 - \left( r_C^2 \ln \frac{r_C^2}{9 \times 10^{-8}} - r_C^2 + 9 \times 10^{-8} \right) = 25 \text{ mm Hg}$$

Solving by trial and error, $r_C \approx 31.8 \mu$. In the absence of additional information it is very appealing to suggest that tissue PO$_2$ at the edge of a capillary domain is probably near zero and thus the capillary domain is approximately equal to $r_C$. Making this approximation ($r_C \approx r_d$) the capillary domain would be estimated as 31.8 $\mu$. These (16) suggests 30 $\mu$.

From in vivo studies, however, we have the additional information necessary to calculate the average actual capillary domain in a cylindrical model. The average in vivo cortical PO$_2$ was found to be 25.3 mm Hg (unpublished observations). Consider again the solution to the cylindrical diffusion equation for a metabolizing medium, equation 9. The mean PO$_2$ in a capillary domain can be calculated by integrating this equation from zero to $r_d$ and dividing the result by $r_d$: $r_d$ was determined as follows:

$$P = P_0 + \frac{\int_{r_d}^P dP}{r_d}$$

and if we consider the capillary PO$_2$ independent of radial position,

$$P = P_0 + \frac{\int_{r_d}^P dP}{r_d}$$

Substituting for $P_0$, $r_0$, $Vo_2/4DS$, $r_0$, and using $P = 25$ mm Hg:

$$0.27 \times 10^{-4} = 25 \mu \cdot 26.84(r_d - 4.09a) + 1.32 \times 10^8 \left( \frac{r_d^3}{3} - 9 \times 10^{-4}r_d \right)$$

Solving for $r_d$ by trial and error, $r_d = 18.6 \mu$. Silver's (13) in vivo studies indicated PO$_2$ peaks at approximately 40 $\mu$ intervals. He indicated that histological studies showed that these peaks occur as the electrode passed a capillary. Thus $r_d$ in rat brain was about 20 $\mu$.

Teleologically we would expect $r_d$ to be considerably less than $r_d$ in brain tissue. If, as seems to be the case, the tissue has a critical PO$_2$ of about 2 mm Hg, it would seem unlikely that some cells would be respiring at a much lower rate than others. Such would be the case if $r_d$ was nearly equal to $r_d$.

The minimum domain PO$_2$ can be calculated from the
cylindrical diffusion equation at \( r = r_d \). Substituting numerical values for the parameters and using \( r = r_d = 18.6 \mu \)

\[
P_{\text{min}} = 50 \text{ mm Hg} - 1.32 \times 10^{16} 10.16 \times 10^{-4} \ln \left( \frac{348 \times 10^8}{9 \times 10^4} - 348 \times 10^{-8} + 9 \times 10^{-8} \right) + 0.09 \times 10^{-8} = 50 - 44.9
\]

\[P_{\text{min}} = 5.1 \text{ mm Hg}\]

If the half-maximum respiration rate occurs at 2 mm Hg and the minimum tissue \( P_{O_2} \) is greater than 5 mm Hg, assuming a constant metabolic rate would not likely introduce a large error into the calculation of \( r_d \). However, the only significant evidence for the adequacy of a cylindrical model is that \( r_d \) calculated from this model agrees very well with the capillary domain as measured by Silver (13).

We believe the techniques described in this paper provide a method for determining whether a transport system for \( O_2 \) exists in a tissue and for characterizing the relationship between \( V_{O_2} \) and \( P_{O_2} \) if no transport mechanism other than diffusion is present. In addition, the critical thickness for tissue slices is easily determined in a given \( P_{O_2} \) and the physical constants determining the mass transfer of \( O_2 \) in a tissue can be evaluated. To the best of our knowledge, this is the first technique which provides a method for determining the regional \( V_{O_2} \) and the diffusion coefficient in soft tissues such as brain. The disadvantages of manometric and Krogh diffusion studies in soft tissue are obvious while this method utilizes only data from the uncut surface of fresh tissue slices at 37°C.

We express our appreciation to Dr. I. S. Longmuir whose visit to our laboratory inspired this study.

This study was supported in part by Public Health Service grants HE 11006 and FR 05631, and by Public Health Service Fellowship 5F1GM-34, 062-03 from the National Institute of General Medical Sciences.

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Received for publication 10 September 1969.

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