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Facilitated Diffusion and the Possible Role of Myoglobin as a Transport Mechanism*

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SUMMARY

This paper deals with the facilitated diffusion of oxygen in systems containing proteins, such as ferrohemoglobin, that combine reversibly with oxygen. The phenomenon is treated in terms of translational diffusion of free and bound oxygen molecules, and of the reaction rate of the oxygen molecules with the oxygen-binding protein. These considerations lead to a differential equation (see Equation 9) for the flux; analytical or numerical solutions for this equation are not yet available. However, the assumption that chemical equilibrium exists at every point in the diffusing system leads to a simple equation already derived by other workers. This in turn permits calculation of the partial pressure of oxygen as a function of distance, in facilitated diffusion across a flat membrane, for given values of the total oxygen flux. The data given by Wittenberg in the preceding paper are analyzed in these terms, with satisfactory agreement between experiment and calculation.

The possible contribution of rotary diffusion to the facilitated flux of oxygen is analyzed in detail, and it is found to be negligible compared to that of translational diffusion.

Calculations of facilitated diffusion in muscle resulting from the presence of myoglobin indicate that myoglobin may be responsible for a substantial part of the transport of oxygen in muscle, especially at low partial pressures of oxygen.

FACILITATED DIFFUSION

1. The Facts

Scholander (1) and Wittenberg (2), independently and at about the same time, discovered the phenomenon of facilitated diffusion exhibited under certain conditions by solutions of hemoglobin and myoglobin. In Wittenberg's later experiments, described in the preceding paper (3), two gas chambers, in one of which the partial pressure of oxygen was maintained as nearly as possible at zero, while in the other, it was kept at a constant value of p, were separated by a slab of hemoglobin or myoglobin solution supported on a Millipore membrane. The flux of oxygen through the slab was studied in relation to the pressure difference p between the two chambers. The results on hemoglobin are shown in Fig. 1 of Wittenberg (3). (Scholander's results are essentially the same.) In a solution of ferrihemoglobin, which does not bind oxygen, the flux of oxygen (or nitrogen) across the membrane is due purely to diffusion, and it is directly proportional to the differences of partial pressure of these gases across the membrane. The flux of nitrogen through a solution of oxyhemoglobin behaves similarly. The flux of oxygen through a ferrohemoglobin solution, however, is consistently greater than that through the ferrihemoglobin solution; the former curve is parallel to the latter over the range of pressures studied by Wittenberg (p_{O_2} from 20 to 600 mm of Hg), but it is displaced upward by a constant amount. This displacement represents the facilitated diffusion produced by the oxygen-combining form of the molecule. Of course, at sufficiently low pressures, the curve for ferrohemoglobin must on any basis turn downward to pass through the origin, but this phenomenon was not observed within the range of pressures studied by Wittenberg.

Since the original discovery, the experiments have been considerably extended by Wittenberg (4), by Hemmingsen and Scholander (5), by Hemmingsen (6), and by Mochizuki and Forster (7). It has been shown that the phenomenon can easily be inhibited by raising the oxygen pressure in the low pressure chamber to 10 to 20 mm (5, 8); also, that it is lacking, or at least very nearly lacking, in the case of carbon monoxide (3). Myoglobin produces about the same facilitation, per unit of heme, as hemoglobin (3); on the other hand, very large pigments, like some of the invertebrate hemoglobins, with molecular weights of several million, are ineffective (3). The relation between facilitated diffusion and hemoglobin concentration is shown in Fig. 5 of the preceding paper (3). The amount of facilitation rises steeply to a maximum as the concentration, expressed in terms of heme groups, increases from zero to about 9 mM; it then falls off less steeply as the concentration increases further. At 20 mM the facilitation is about half as great as at 9 mM.

Attention has already been called to the possible physiological significance of the phenomenon (1, 2), and the subject has been recently enlarged upon by Wittenberg (9). Also, several theoretical discussions of the mechanism of the effect have been given, all based on the idea that the facilitation arises from the random displacement of carrier particles (10–12). It is the purpose of this paper to consider both aspects of the phenomenon

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in somewhat greater detail, making use of quantitative data bearing on the problem. In the theoretical discussion of the mechanism involved, we do not, in the initial phase, limit ourselves to the case where there is chemical equilibrium between free oxygen and the carrier molecule, as has commonly been done in previous discussions, but leave that question open. However, we show that in the case of oxygen, although not in that of carbon monoxide, departures from equilibrium are in all probability small, and present a set of tentative calculations based on that assumption for the case of oxygen, in this respect following in the steps of earlier investigators (10–12).

2. Two Possible Interpretations

One possible explanation of the phenomenon of facilitated diffusion would be in terms of the rotary diffusion of the protein molecules, which in the net result would act to transport oxygen somewhat after the fashion of a series of water wheels. In view of the small relaxation time of the hemoglobin molecule (of the order of 10^{-6} sec (13)) and the relatively slow rate of dissociation of oxygen from the protein (the half-life of an oxygen molecule attached to the protein is of the order 0.1 sec (14)), this explanation is at least questionable (see also Section 5). Another, more plausible one, is in terms of the translational diffusion of the protein. At first glance, it would seem that this could be ruled out on the grounds that the protein molecules diffuse so much more slowly than the free oxygen molecules. How could a fly hope to increase his rate of progress by alighting on the back of a tortoise? The problem however assumes a new complexion when we take account of the fact that under the experimental conditions the amount of oxygen present in combination with hemoglobin is many times that present in free solution. A swarm of flies whose forward progress through the air was limited by the necessity of passing through a narrow orifice in a barrier might well improve their situation by riding on the backs of an army of tortoises which could pass under the barrier along the ground. This interpretation is susceptible of more exact formulation.

3. General Formulation in Terms of Translational Diffusion

Consider an element of volume in which the concentration of free oxygen is C moles cm^{-3}. The concentration of bound oxygen is given by $mC_p\bar{Y}$, where C_p is the concentration of protein, also in moles cm^{-3}, m is the number of oxygen binding sites per protein molecule ($m = 4$ for hemoglobin), and $\bar{Y}$ is fractional saturation.

In general the free and bound oxygen will not be in equilibrium; instead, there will be a continuing reaction everywhere.

$$O_2 + Hb \rightarrow HbO_2 \tag{1}$$

The rate of this reaction, ρ, will depend on C, C_p, and $\bar{Y}$, but it is not necessary for the moment to formulate it in detail.

The total rate of change of the *bound* oxygen, per unit volume, will be the sum of ρ and a term representing the net effect of the diffusion of the oxygenated hemoglobin molecules. Let us suppose that the experiment is so designed that the diffusion is linear, in the x direction. Then the conservation requirement gives

$$\frac{\partial(mC_p\bar{Y})}{\partial t} = D_p \frac{\partial^2(mC_p\bar{Y})}{\partial x^2} + \rho \tag{2}$$

A similar expression holds for the free oxygen

$$\frac{\partial C}{\partial t} = D_c \frac{\partial^2 C}{\partial x^2} - \rho \tag{3}$$

In these two equations, D_c represents the diffusivity of free oxygen, D_p that of hemoglobin; ρ is the rate of Reaction 1 from left to right.

In the steady state

$$\frac{\partial mC_p\bar{Y}}{\partial t} = \frac{\partial C}{\partial t} = 0 \tag{4}$$

Consequently

$$\frac{\partial}{\partial x}\left(D_p \frac{\partial(mC_p\bar{Y})}{\partial x} + D_c \frac{\partial C}{\partial x}\right) = 0 \tag{5}$$

whence, by integration

$$D_p \frac{\partial(mC_p\bar{Y})}{\partial x} + D_c \frac{\partial C}{\partial x} = A \tag{6}$$

Here A is a constant independent of x (and of course of t); it will be seen that it is minus the total flux F of oxygen, bound and free. Equations essentially equivalent to Equation 6 have been derived by earlier workers (*e.g.* References 10–12).

In order to decide whether the mechanism just analyzed is capable of meeting the facts, or should be disqualified without more ado, let us consider the data shown in Fig. 1 of Wittenberg (3). In the experiments represented there, the concentration of the hemoglobin was 19% or 0.28×10^{-5} g molecule cm^{-3}. The thickness of the membrane was 1.5×10^{-2} cm, and its area approximately 10 cm^2; the pressure was maintained at various constant values p on one side and at zero on the other. The facilitated diffusion may be taken as the difference between the observed flux and the flux when oxyhemoglobin was replaced by ferrihemoglobin. This difference was 1.3 μl per min or 10^{-10} mole cm^{-2} sec^{-1}; it remained the same over the whole range of pressures covered by the experiments as Wittenberg's figure shows. If our hypothesis is correct, this should be equal to the flux of bound oxygen. Consequently, by Equation 6,

$$D_p mC_p \left(\frac{\partial\bar{Y}}{\partial x}\right)_{\text{average}} \cong 10^{-10}$$

Taking

$$mC_p = 4 \times 0.28 \times 10^{-5} \cong 10^{-5}$$

we obtain

$$D_p \left(\frac{\partial\bar{Y}}{\partial x}\right)_{\text{average}} \cong 10^{-5}$$

Reported values of D_p at the relatively low concentrations employed in the usual diffusion measurements are close to 7×10^{-7} cm^2 per sec at 20° (15). If, somewhat arbitrarily, we take a value about one-third of this, namely 2.0×10^{-7}, as applicable to the 19% solution used in Wittenberg's measurements we obtain

$$\left(\frac{\partial\bar{Y}}{\partial x}\right)_{\text{average}} \cong 50$$

Since the thickness of the slab was 1.5×10^{-2} cm, this implies that $\Delta\bar{Y}$ across the slab was about 0.75. The expected value is 1, or somewhat less, since the pressure of oxygen at one face of the slab was in all of the cases high enough to produce saturation at equilibrium and was zero at the other face. This crude calculation is therefore presumptive evidence that our hypothesis is correct, or at least that it accounts for a substantial part of the

facilitated diffusion. It justifies developing the idea in greater detail. This involves formulating the expression for ρ.

The kinetics of the oxygenation of the 4 heme groups of hemoglobin is complex (16). Phenomenologically, however, the observed constant for the "on" reaction, which, following Roughton, we denote by k', does not vary greatly with $\bar{Y}$ except when $\bar{Y}$ is very close to 1. The "off" constant, which we denote by k, can be taken as constant. If we use an average value for k', we have for ρ

$$\rho = k'mC_p(1 - \bar{Y})C - kmC_p\bar{Y} \quad (7)$$

By integrating Equation 6 we obtain, after setting $A = -F$,

$$D_pmC_p\bar{Y} = -Fx - D_cC + B \quad (8)$$

where B is a constant determined by the values of $\bar{Y}$ and C at any one value of x.[1] If we make use of Equations 3, 4, 7, and 8 we obtain

$$D_c\frac{\partial^2 C}{\partial x^2} = \rho = -\left(\frac{k'C + k}{D_p}\right)(Fx + D_cC - B) + k'mC_pC \quad (9)$$

This equation is of the form

$$\frac{\partial^2 C}{\partial x^2} = \alpha + \beta C + \gamma x + \delta xC + \epsilon C^2 \quad (9.1)$$

where the Greek letters denote constants which can be formulated in detail by comparison with Equation 9; *i.e.* it is second order and nonhomogeneous. Its solution in analytical form presents difficulties, although if it *could* be solved it would at once provide an expression for C as a function of x from which, by Equation 6, we could then obtain $\bar{Y}$ as a function of x and solve the problem completely. A numerical solution might, however, be obtained with the aid of a computer on the basis of experimental values of the various constants involved.

4. *Approximate Treatment Which Appears to Meet Facts*

In the meantime it is of interest, following the example of earlier investigators (10–12), to try the effect of solving the equations for the limiting case where there is chemical equilibrium at every point in the system. Actually, as is shown in Section 5, when account is taken of the numerical values of the various constants involved, it would seem that, in the case of oxygen at least, departures from equilibrium may be relatively small.

Provided we know $\bar{Y}$ as a function of p, then of course Equation 6, which is quite general, may be solved very simply, and it is unnecessary to resort to the differential Equation 9. Let us assume that the equilibrium is adequately described by the Hill equation with $n = 3$ and $p_{\frac{1}{2}} = 10$ mm. Then

$$\bar{Y} = \frac{(p/10)^3}{1 + (p/10)^3} \quad (10)$$

Furthermore, in Equation 6 let us replace D_c by D'_cp, where p is the partial pressure of oxygen. Actually D_c', not D_c is the quantity directly given by Wittenberg's measurements (3) of the flux of oxygen through the slab when the hemoglobin is in the oxidized form, which binds no oxygen and produces no facilitation. At 20° the measured flux was found to be 3×10^{-10} mole cm^{-2} sec^{-1} when the pressure of oxygen on the high pressure side of the slab was 400 mm and that on the low pressure side approximately zero (see Wittenberg's Fig. 1). If we take account of the area and thickness of the membrane (see legend to Fig. 1 of Wittenberg), this corresponds to a value of $D_c' = 1.1 \times 10^{-14}$ mole cm^{-1} sec^{-1} $(p)^{-1}$, where p is pressure measured in millimeters of Hg. (No allowance is made here for the fact that, according to the manufacturers, only 80% of the total area of the slab is available for diffusion). As in the preliminary calculations, we take D_p as 2×10^{-7} cm^2 sec^{-1}. On the basis of these figures, assuming the pressure of oxygen to be zero on the low pressure side of the slab, Equation 6, in integral form, may be written, with sufficient accuracy, as

$$Fx = 2 \times 10^{-12}\,\bar{Y} + 10^{-14}\,p \quad (11)$$

This is essentially the same as Equation 9 of La Force and Fatt (10).

As an easy and instructive way to implement Equations 10 and 11, construct Table I. First choose a value of p, then calculate $\bar{Y}$ by Equation 10, and next Fx by Equation 11. Then fill in the last three columns, which give the values of x corresponding to the various values Fx for each of three assumed values of F chosen to cover the range of Wittenberg's observations. (In the headings of these last three columns F is also given in Wittenberg's units, namely microliters of oxygen, measured as a gas at 0° and 1 atmosphere of pressure, which cross the whole slab per min, in order to facilitate comparison with his data.)

A graph of p against x for each of the three chosen values of F is shown in Fig. 1. The slope of these lines at any value of x gives the gradient of p, and this of course is proportional to the flux of *dissolved* oxygen at that point. When the gradient is constant the flux is constant, and in this case it is the same as the total flux, the gradient of $\bar{Y}$ (or bound oxygen) being zero. Let us erect a line corresponding to a constant value of $x = l$, as shown in the figure, which represents the high pressure side of the slab. If the curve of p against x is straight at the point where it cuts this line then the total flux of oxygen at the high pressure face of the slab must be due entirely to dissolved oxygen.

TABLE I

Calculations based on Equation 11

p_{mm}	$\bar{Y}$	$Fx \times 10^{12}$	Values of x		
			$F = 1.5 \times 10^{-10}$ mole cm^{-2} sec^{-1} (2 Wittenberg units)*	$F = 3 \times 10^{-10}$ mole cm^{-2} sec^{-1} (4 Wittenberg units)*	$F = 6 \times 10^{-10}$ mole cm^{-2} sec^{-1} (8 Wittenberg units)*
		mole cm^{-1} sec^{-1}	$\times 10^2$ *cm*	$\times 10^2$ *cm*	$\times 10^2$ *cm*
2.5	0.0135	0.052	0.0347	0.0174	0.0087
5	0.105	0.26	0.1735	0.0868	0.0434
10	0.50	1.100	0.733	0.367	0.184
20	0.895	1.99	1.326	0.663	0.332
40	0.9865	2.373	1.584	0.792	0.396
100	1	3.00	2.00	1.00	0.500
500	1	7.00	4.67	2.335	1.167
1000	1	12.00	8.00	4.00	2.00

* One Wittenberg unit (microliters per 10 cm^2 per min) = 0.745×10^{-10} mole cm^{-2} sec^{-1}.

[1] It will be seen that Equation 8 can be alternatively written as

$$-F + D_pmC_p\frac{\Delta\bar{Y}}{\Delta x} + D_c\frac{\Delta C}{\Delta x} \quad (8')$$

where Δ indicates the difference between the values of the variable at the two faces of the slab. This form shows how F can be expressed in terms of the over-all gradients of Y and C across the slab. It was in effect anticipated in the initial exploratory calculation.

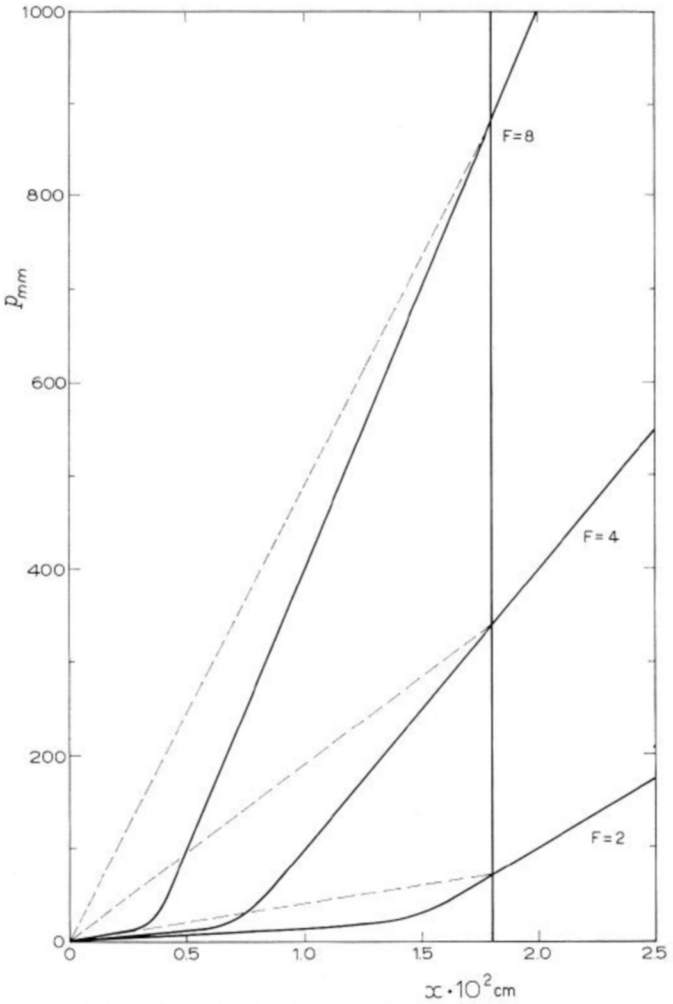

FIG. 1. Graph of data shown in Table I. Full curves give partial pressure of oxygen as a function of distance (x) within the membrane, at three values of the flux (F) of oxygen expressed in Wittenburg units. The *heavy vertical line* represents the distance corresponding to the high pressure side of the membrane; the low pressure side is at $x = 0$. *Dashed lines* show p as a function of x in the absence of facilitated diffusion.

Further to the left, where the curve of p against x is no longer straight, part of the flux is taken over by the bound oxygen. At any point, the flux of bound oxygen will of course be equal to the total flux minus the flux of dissolved oxygen, and by dividing the difference by $D_p m C_p$ we obtain the gradient of $\bar{Y}$.

If there were no protein facilitation, the flux would be everywhere due exclusively to the dissolved oxygen and the gradient of p would be constant; in other words, the curve of p against x would be a straight line through the origin and the value of p at the high pressure side of the slab, where $x = l$. Such lines are shown as *dashed lines* in the figure. The facilitation is therefore proportional to the difference between the slope of such a line and the actual value of dp/dx at $x = l$, where p is linear in x. In the case illustrated in Fig. 1, we have chosen $l = 1.8 \times 10^{-2}$ cm, somewhat arbitrarily. The actual thickness of the slab is given by Wittenberg as 1.5×10^{-2} cm, and this slightly higher value allows for the fact that the pores may not be quite straight and are reported to occupy only 80% of surface of the membrane. Over the range from $F = 2$ to $F = 8$ (in Wittenberg's units), the gradient of p is constant at $x = l$. The facilitation effect is found to be 1.45 (in Wittenberg's units) at every value of the total flux, and therefore at every value of p (the pressure at the high pressure side of the slab) over this range. The observed value is 1.3 to 1.5, and is likewise independent of p. This constancy of the facilitation, ΔF, is one of the most striking features of the experimental results. Reflection shows that it is not an accident but is indeed inherent in the phenomenon if this interpretation is correct. For it follows from Equation 11 that the total flux is

$$F = \frac{2 \times 10^{-10} \bar{Y}}{l} + \frac{10^{-14} p}{l}$$

The unfacilitated flux F_0 is of course simply $10^{-14}\ p/l$. The difference is

$$\Delta F = F - F_0 = \frac{2 \times 10^{-10} \bar{Y}}{l}$$

and is independent of p provided p is sufficiently great to make $\bar{Y} = 1$ at the high pressure face of the slab.[2]

It will be seen from the graph that the values of p corresponding to various values of the total flux are essentially the same as those observed by Wittenberg (3) (see his Fig. 1). Indeed the approximate analysis just given, notwithstanding the oversimplification involving chemical equilibrium, agrees unexpectedly well with the facts. It explains at once why a relatively small back pressure of oxygen in the low pressure chamber, say 20 mm, which suffices essentially to saturate the hemoglobin, completely eliminates facilitation. The increase of facilitation with protein concentrations up to a sharp maximum at ~15%, followed by a rapid decline, as shown in Fig. 5 of Wittenberg (3), can be rationalized as due to a sudden increase of viscosity, possibly associated with the onset of non-Newtonian viscosity at about that concentration. It would seem therefore that linear diffusion of oxyhemoglobin must play at least a major role in the phenomenon of facilitated diffusion even if it does not explain the whole of it.

It remains however to explain why facilitation is lacking, or at least nearly lacking, in the case of carbon monoxide. Although no completely clear answer to this question can be given, two considerations come to mind. In the first place, assuming chemical equilibrium to prevail, then owing to the much higher affinity of hemoglobin for carbon monoxide than for oxygen (about 250 times higher) the *curves* shown in Fig. 1

[2] Another way of looking at the situation is to write Equation 6 in the form

$$\left(D_p m C_p \frac{d\bar{Y}}{dp} + D_c'\right) \frac{dp}{dx} = -F$$

The expression in parentheses on the left can be regarded as a generalized diffusivity which is a function of p. On the basis of Equation 10

$$\frac{d\bar{Y}}{dp} = \frac{3 \times 10^3 p^2}{(10^3 + p^3)^2}$$

and would be expected to vary from 0 to 0 as p varies from 0 to infinity, passing through a maximum equal to $2^{4/3}/30$ at $p/10 = (1/2)^{\frac{1}{3}}$, where $\bar{Y} = 1/3$. However, Equation 10 certainly breaks down for very large and very small values of p, and although, as $p \to \infty$, $d\bar{Y}/dp$ still goes to zero, at $p = 0$ it must, on any physical basis, be equal to a constant which is the same as the equilibrium constant for the first step of the reaction. (See Reference 17.)

will continue to be straight essentially down to the *abscissa*. This means that $\bar{Y}$ will be essentially 1 for all entries in Table I. In the case of oxygen, the phenomenon is destroyed by a back pressure of 10 to 20 mm, which is enough to keep the hemoglobin everywhere largely oxygenated. In the case of carbon monoxide the figure would be 250 times less, or 0.05 to 0.08 mm. It would be necessary to maintain a pressure substantially below this, say 0.01 to 0.02 mm, right up to the face of the slab to preserve the effect. Wittenberg's system for flushing out carbon monoxide from the low pressure chamber was hardly adequate for this. The other point is that the rate of dissociation of carbon monoxide from hemoglobin is nearly 1000 times less than that of oxygen. The system may well be far from equilibrium on the low pressure side and this would act to reduce facilitation. And as a matter of fact, there *is* a small observable effect even in the case of carbon monoxide (7).

The treatment just given is of course based on an approximation. Clearly, the system cannot be exactly in equilibrium for that would imply that $\rho = 0$ (exactly) and, consequently, by Equations 2 and 3, that the gradients of both p and $\bar{Y}$ were everywhere constant, something which is incompatible with the form of the equilibrium Equation 10. Nevertheless, depending on the values of the constants, it is possible for ρ to be of comparable size with $D_c(\partial^2 C/\delta x^2) = D_c'(\partial^2 p/\partial x^2)$ even when $\bar{Y}$ is fairly close to its equilibrium value. In order to get an idea of how good the approximation is, it is instructive to consider this numerically. The *curves* shown in Fig. 1 all have maximum curvature for values of p in the neighborhood of 10 mm, as would be expected. In this region graphical measurement gives

$$\frac{\partial^2 p}{\partial x^2} \cong 5 \times 10^6$$

By introducing the value of $D_c = 10^{-14}$ given above we obtain

$$D_c' \frac{\partial^2 p}{\partial x^2} < 10^{-7}$$

This is to be compared with the value of ρ corresponding to a given departure from equilibrium. To obtain this we take $k = 50$ sec^{-1} and k', assuming the concentration of oxygen to be expressed in millimeters of Hg, as 5 sec^{-1} p^{-1}. These give a value of $p_{\frac{1}{2}} = 10$ mm and lie within the range given by Gibson (16). Suppose now that $p = 10$ and $mC_p = 10^{-5}$, and that $\bar{Y}$ departs from its equilibrium value of 0.5 by 0.01. Then

$$\rho = mC_p[5(1 - \bar{Y})10 - 50\bar{Y}]$$
$$= 10^{-5}[5 \times 0.49 \times 10 - 50 \times 0.51] = 10^{-5}$$

Even in this case therefore ρ largely exceeds $D_c'(\partial^2 p/\partial x^2)$.

A similar calculation for the case of carbon monoxide indicates that departures from equilibrium should be very much greater for that ligand.

5. *Negligible Role of Rotational Diffusion*

It was pointed out near the start that it seemed unlikely that rotational diffusion could play a significant part in the phenomenon of facilitated diffusion, and this has been borne out by the success of the calculations just presented in meeting facts. The following argument may be of interest in showing in detail why it should be so.

Clearly there can be no direct long range transport of oxygen by a set of molecules with centers of gravity fixed in space and with random displacements confined to rotations.

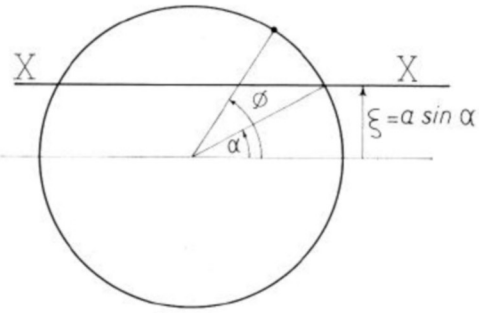

FIG. 2. Explanation of notation used in calculations of two dimensional rotatory diffusion.

Assume each molecule to bear a single site located at a distance a from its center of rotation. The transport of oxygen across a plane X perpendicular to the x axis due to rotations can only result from molecules with centers lying within a distance a above or below the plane. For simplicity, we reduce the problem to a two dimensional one; the principle involved remains the same.

Consider the *average* contribution to the total downward flux due to a molecule for which the distance below the plane is

$$\xi = a \sin \alpha$$

Let ϕ be the angle between the radius vector through a site and a line parallel to X through the center of rotation of the molecule (see Fig. 2). All of the orientations being equally probable, the fraction of the time spent by the site in any small angular element $d\phi$ will be $d\phi/2\pi$. The molecules move so fast in relation to the velocity of the reaction of the site that the site may be assumed to remain always at the constant saturation $\bar{Y}$, which is the equilibrium value corresponding to the pressure p of oxygen in the surrounding medium at the level of the center of the molecule. Since there is no accumulation of oxygen by the molecule, the contribution f to the downward flux of oxygen across X due to the molecule will be the difference between the net amounts of oxygen taken up by the site during the times spent above and below the plane. Therefore if $\dot{p}$ denotes the gradient of p, which may be taken as constant over the small distance involved, f is given by

$$f = \int_{\alpha}^{\pi-\alpha} [k'(1 - \bar{Y})(p + \dot{p}a \sin \phi) - k\bar{Y}] \frac{d\phi}{2\pi} - \int_{-\alpha}^{\pi+\alpha} [k'(1 - \bar{Y})(p - \dot{p}a \sin \phi) - k\bar{Y}] \frac{d\phi}{2\pi} \quad (12)$$

When account is taken of the equilibrium condition this yields

$$f = \frac{4k'a\dot{p}(1 - \bar{Y}) \cos \alpha}{2\pi} \qquad (0 < \alpha < \pi/2) \quad (13)$$

In order to obtain the total downward flux per unit area produced by the rotary diffusion of *all* of the molecules whose centers are located below the plane we have to evaluate the integral

$$\int_0^a f C_p d\xi \quad (14)$$

where C_p is the concentration of the protein molecules. If we introduce the relation $\xi = a \sin \alpha$, this may be more conveniently written as

TABLE II

Calculations of facilitated diffusion of oxygen into cylindrical muscle cell

p_{mm}	$\bar{Y}$	$1.5 \times 10^{-13}\ \bar{Y}$	$10^{-14}\ p$	$\frac{qr^2}{4} + A$	r cm*		$1.5 \times 10^{-13}\ \bar{Y}$
					$q = 4 \times 10^{-8}$ $Q = 16.2$	$q = 40 \times 10^{-8}$ $Q = 162$	$\frac{qr^2}{4} + A$
		$\times 10^{13}$	$\times 10^{13}$	$\times 10^{13}$	$\times 10^{4}$	$\times 10^{4}$	$\times 10^{4}$
0.3	0.0909	0.136	0.03	0.166	12.90	4.08	0.819
0.9	0.241	0.361	0.09	0.451	21.25	6.72	0.801
1.5	0.333	0.499	0.15	0.649	25.48	7.99	0.769
2.1	0.412	0.618	0.21	0.828	28.8	9.11	0.753
3	0.500	0.750	0.30	1.05	32.4	10.25	0.714
6	0.667	1.000	0.60	1.60	40.0	12.65	0.625
9	0.75	1.125	0.90	2.03	45.0	14.2	0.556
15	0.833	1.250	1.50	2.75	52.4	16.6	0.455
20	0.870	1.304	2.00	3.304	57.5	18.3	0.395
30	0.909	1.364	3.00	4.364	66.1	20.9	0.313
45	0.937	1.405	4.50	5.905	76.8	24.3	0.238
60	0.953	1.430	6.00	7.430	86.2	27.3	0.1925
150	0.983	1.474	15.00	16.474	129	40.8	0.0895

* Calculated on the assumption that $A = 0$.

$$\int_0^{\pi/2} fC_p a \cos \alpha d\alpha \tag{14.1}$$

The value of $\bar{Y}$ at any distance ξ from X is given by

$$\bar{Y} = \bar{Y}_0 - \dot{\bar{Y}}\xi = \bar{Y}_0 - \dot{\bar{Y}} a \sin \alpha \tag{15}$$

where $\bar{Y}_0$ is the value of $\bar{Y}$ on the plane X, *i.e.* at $\alpha = 0$, and $\dot{\bar{Y}}$ is the gradient of $\bar{Y}$. By making use of this equation, we obtain for the Integral 14.1 the expression

$$k'C_p a^2(1 - \bar{Y}_0)\dot{p} + \frac{4}{3\pi} k'C_p a^3 \dot{p}\dot{\bar{Y}}$$

A similar expression applies to the *upward* flux through X produced by the molecules with centers lying *above* X, the only difference being that the signs of $\dot{p}$ and $\dot{Y}$ are both reversed. The total net *downward* flux, F_r, through X due to the rotational diffusion of all of the protein molecules, both those below and those above the plane, is thus given by

$$F_r = 2k'C_p a^2(1 - \bar{Y}_0)\dot{p} \tag{16}$$

This may be compared with the downward flux due to the dissolved oxygen, namely

$$F_s = D_c'\dot{p} \tag{17}$$

By making use of the numerical values of the various constants given above[3] and taking a, rather arbitrarily, as 5×10^{-7} cm (50 A) we obtain

$$\frac{F_r}{F_s} \cong 5 \times 10^{-4}(1 - \bar{Y}_0) \tag{18}$$

Thus the effect of the rotary diffusion would seem to be wholly negligible.

6. *Role of Myoglobin in Muscle Respiration*

In view of the considerations just presented it seems worthwhile to reassess the possible role of myoglobin in the respiration of the cells in which it occurs; chiefly certain types of muscle cell. Up to now myoglobin has been supposed to act mainly as a reservoir of oxygen, but the question arises whether its more important function may not be as a mechanism of transport.

[3] These are: $D_c' = 1.1 \times 10^{-14}$ mole cm^{-1} sec^{-1} p^{-1}; $C_p = 0.28 \times 10^{-5}$ mole cm^{-3}; $k' = 5$ sec^{-1} p^{-1}.

TABLE III

Maximum metabolism which cell of radius 25 μ can support in relation to oxygen pressure in surrounding fluid

p_{mm}	Q with myoglobin	Q without myoglobin
20	86.4	51.7
9	52.8	23.4
6	41.5	15.6
2.1	21.5	5.46
1.5	16.8	3.89

In man, myoglobin occurs to the extent of ~1.4%, dry weight, in heart muscle and ~2.5%, dry weight, in skeletal muscle (18). The latter figure corresponds to 2.6×10^{-7} mole of myoglobin per cm^3 of live tissue. On the other hand, the resting metabolism of skeletal muscle, in terms of the usual Q units (microliters of oxygen gas, measured at 1 atmosphere and 0°, per mg of tissue, dry weight, per hour) is given as about 20. This means a consumption of oxygen of 4.96×10^{-8} mole per sec per cm^3 of live tissue.[4] Since 1 mole of myoglobin combines with 1 mole of oxygen, it follows from these figures that the reserve of combined oxygen carried by the myoglobin could not support even the resting metabolism of the muscle for more than $2.8 \times 10^{-7}/4.96 \times 10^{-8} = 5.5$ sec. From this result (even allowing for a 2- or 3-fold error), the function of myoglobin as a significant storage reservoir for oxygen would seem to be at the least dubious.

In order to consider the possible role of myoglobin as a transport mechanism, we shall treat the muscle as an indefinitely long cylinder of radius r_0 containing a solution of myoglobin at a concentration of 2.8×10^{-7} mole cm^{-3} and having a metabolism (oxygen consumption) of q mole cm^{-3} sec^{-1}. If we introduce cylindrical coordinates then, since there must be complete radial symmetry, the diffusion equations, corresponding to Equations 2 and 3, become, for the steady state,

$$\frac{D_c}{r}\frac{d}{dr}\left(r\frac{dC}{dr}\right) = q + \rho \tag{19.1}$$

and

$$\frac{C_p}{r} D_p \frac{d}{dr}\left(r\frac{d\bar{Y}}{\delta r}\right) = -\rho \tag{19.2}$$

These equations presuppose that it is only the dissolved oxygen which is directly drawn upon for metabolic purposes. As before, ρ gives the rate of transfer of oxygen from the free to the bound form; D_c and C_p have likewise the same significance as before. The quantity m drops out since myoglobin contains only a single oxygen binding site.

Addition of the Equations 19.1 and 19.2 gives

[4] The following figures are convenient to have at hand in considerations of this kind. One gram dry weight corresponds to about 5 cm^3 of live tissue. One per cent of myoglobin, dry weight, (mol. wt. 18,000) therefore corresponds to $10^{-2}/(5 \times 18{,}000) = 1.11 \times 10^{-7}$ mole cm^{-3} of live tissue. Also, an oxygen consumption of 1 Q unit corresponds to $10^{-6}/(5 \times 10^{-3} \times 22.4 \times 60^2) = 2.48 \times 10^{-9}$ mole per cm^3 of live tissue per sec.

$$\frac{1}{r}\frac{d}{dr}\left(D_c\frac{rdC}{dr}+C_p D_p\frac{rd\bar{Y}}{dr}\right)=q \tag{20}$$

which corresponds to Equation 5. Integration of this yields

$$D_c\frac{dC}{dr}+C_p D_p\frac{d\bar{Y}}{dr}=\frac{qr}{2}+\text{const} \tag{21}$$

which corresponds to Equation 6. Since the radial flux must vanish at $r = 0$, the constant may be set equal to zero.

If, as in the case of linear diffusion, we integrate once more we obtain

$$D_c C+C_p D_p\bar{Y}=\frac{qr^2}{4}+A \tag{22}$$

where A is a constant which is determined by the values of C and $\bar{Y}$ at $r = 0$.

As before we assume, as an approximation, that $\bar{Y}$ has its equilibrium value at every point in the system. For myoglobin this is given by

$$\bar{Y}=\frac{(p/p_{1/2})}{1+(p/p_{1/2})} \tag{23}$$

Also, we replace C by p in Equation 22, which then becomes

$$D_c' p+C_p D_p\bar{Y}=\frac{qr^2}{4}+A \tag{24}$$

In order to implement Equation 24, we take D_c' as 10^{-14}, as in the linear case. The value of D_p at 20° for myoglobin in water is close to 10×10^{-7} cm² (15); in the cell, at 37°, we take it, somewhat arbitrarily, as half this to allow for the viscosity of the cell fluid. If we assume $C = 1.8\times10^{-7}$, we obtain as a conservative value, $D_pC_p = 1.5\times10^{-13}$. Equation 24 then becomes

$$10^{-14}p+1.5\times10^{-13}\bar{Y}=\frac{qr^2}{4}+A \tag{25}$$

Both sides now have the dimensions of mole cm^{-1} sec^{-1}.

In order to implement Equation 23, we take $p_{\frac{1}{2}}$ as 3 mm, which is slightly above the experimental value at 37° (19).

On the basis of Equations 23 and 25, we now fill in the first five columns of Table II. The third and fourth columns give the contributions to $qr^2/4$ due to the bound and free oxygen, respectively. It will be seen that when the pressure of oxygen is 15 mm, these two contributions are about equal. At lower pressures, the contribution due to the bound oxygen is the greater, and it becomes rapidly greater as the pressure drops. The oxygen pressures which have been measured in tissues are in the range of 5 to 20 mm, but they do not often rise much above 10. If we assume that at the axis of the cylinder (cell) the pressure is zero ($p = \bar{Y} = 0$), we obtain a limiting value for the rate of metabolism which can be supported for a given value of p at the surface of a cell having an assumed radius of 2.5×10^{-3} cm. The results are given in Table III; they bring out clearly the potential significance of myoglobin for the respiration of the cell.

CONCLUSIONS

The calculations presented in Sections 2 to 5 would seem to show that the facilitated diffusion of oxygen observed in solutions of hemoglobin and myoglobin is due primarily to the translational diffusion of the protein molecules. Nevertheless, it would be reassuring to have numerical solutions of the full differential Equation 9 as a final check on the extent to which the assumption regarding the prevalence of chemical equilibrium is justified.

The treatment given in Section 6 suggests that myoglobin, if present in sufficient quantity, is capable of taking over a substantial part of the burden of oxygen transport in the cells whenever the pressure of oxygen drops to ~10 mm or less. In fact, it would seem that it is bound to do so and that its role at the microscopic level of transport within the cells is the counterpart of that of hemoglobin at the macroscopic level of transport in the whole organism, the only difference being that in one case the driving mechanism is a pump (the heart), in the other a molecular process (translational diffusion). The old observation of Millikan (20) that in rhythmically contracting muscle myoglobin undergoes a cyclic change of saturation is in no way inconsistent with this view, although it should be recognized that if during a certain part of the cycle the myoglobin were completely deoxygenated throughout the muscle its role as a mechanism of transport would be temporarily suspended, the muscle passing through a phase of oxygen debt.

The calculations of Section 6 are of course applicable to any ligand which combines reversibly and with sufficient speed with any carrier molecule. Their practical significance in any given case depends on the extent to which the carrier molecule raises the total concentration of the ligand above the level which it would have in the absence of the carrier and on the affinity of the carrier for the ligand in relation to the prevailing concentration of the ligand. It will be noticed that the equations given in Section 6, which are based on a cylindrical cell, are applicable equally well to a spherical cell if the factor 4 in Equation 22 and any of its progeny is replaced by 6.

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