vary considerably, with a conservative estimate for an average of 0.3 m. The attenuation coefficient of floating snow-ice was calculated from the data published by Meguro (*5*) and substituted in place of the value for sea water in the formula of Riley (*8*), to give an estimated attenuation coefficient of 12.8 for the ice and microalgae layer. The intensity of light reaching the bottom part of the ice community turned out to be about 1 percent of the incident radiation at the surface of the sea. Our calculations indicate that organic production is approximately zero at the bottom of the brown layer because of the low intensity of light. By integrating the production at the top and the bottom of the brown layer and assuming uniform distribution of the algae, we calculated that the algae under each square meter are able to fix about 0.19 g of carbon per day. It is of interest to compare this estimate with plankton productivity values which we have calculated as 0.09 g of carbon fixed per square meter per day in the Bellingshausen Sea and 0.66 g in the richer waters of the Gerlache Strait during the Antarctic summer.

It is not known how active the ice microalgae may be in the frozen condition; our experiments were conducted with slush communities transferred into sea water at about the same temperature. If the trapped ice algae have an activity *in situ* of the order calculated in our investigations, then the productivity of the algae, in a total of $2.6 \times 10^6$ km$^2$ of this kind of brown sea ice surrounding Antarctica in summer, would amount to about one half million tons of carbon fixed per day.

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11 June 1965

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# Oxygen-Hemoglobin System: A Model for Facilitated Membranous Transport

Abstract. *Enhanced transport of oxygen in a Millipore filter containing a solution of hemoglobin can be accounted for by the diffusion of free oxygen as well as of hemoglobin-bound oxygen. A model shows that, at oxygen tensions at which the hemoglobin is fully saturated in a portion of the membrane, the enhanced transport is due to a steeper gradient for free oxygen, whereas in the rest of the membrane an "(oxy)hemoglobin shuttle" operates. A similar model may be useful for explaining facilitated diffusion in other systems.*

In 1960 Scholander (*1*) showed that the presence of hemoglobin solution in a Millipore filter membrane accelerated the transport of oxygen by an amount depending on the partial pressure of oxygen at the inlet side. Later it was shown (*2*) that the extra transport of oxygen due to the presence of hemoglobin was abolished by slight amounts of oxygen at the outlet side of the membrane. Although several models were proposed, no quantitative comparisons were made between these experimental results and the oxygen transport predicted by a model. Fick's diffusion law has been applied (*3, 4*) to Scholander's system to derive a general expression for oxygen transport, but little or no effort has been made to test the equations against experimental data or to predict oxygen and oxyhemoglobin gradients within the membrane. Enns (*5*) measured the oxyhemoglobin gradient within the Millipore membrane and found that at high pressures of oxygen at the inlet nearly all the hemoglobin in the membrane existed in the oxyhemoglobin form. This finding led him to postulate that oxygen transport by hemoglobin results from oxygen exchange between binding sites of colliding hemoglobin molecules. Although Enns's calculations showed reasonable correspondence between theoretical and experimental values for oxygen transport, it was not immediately apparent by what force enhanced oxygen transport takes place in that portion of the membrane in which no gradient of oxyhemoglobin exists.

I now present a model based on the simultaneous diffusion of free oxygen and oxyhemoglobin. The results illustrate how transport can be enhanced even in that portion of the membrane that does not exhibit a gradient in oxyhemoglobin concentration.

A model for a steady-state transport of oxygen in a Millipore filter containing hemoglobin was constructed on the following assumptions: (i) one fraction of the oxygen is transported as free $O_2$ by diffusion, the rest by diffusion of hemoglobin-bound $O_2(HbO_2)$; (ii) $O_2$ and $HbO_2$ are in instantaneous equilibrium at each point (*6*); and (iii) diffusion rates are determined by the local gradients in the membrane

$$\left(\frac{d[O_2]}{dx}, \frac{d[HbO_2]}{dx}\right)$$

The basic equations for the steady state are:

$$D_1 \frac{d[O_2]}{dx} + D_2 \frac{d[HbO_2]}{dx} = K \qquad (1)$$

$$[HbO_2] = f[O_2] \qquad (2)$$

where $x$ is a variable distance through the membrane, $D_1$ is the diffusion coefficient of free $O_2$, $[O_2]$ is the concentration of free $O_2$ as a function of $x$, $D_2$ is the diffusion coefficient of $HbO_2$, $[HbO_2]$ is the concentration of $HbO_2$ as a function of $x$, and $K$ is oxygen flux through the membrane. Equation 1 expresses Fick's law; Eq. 2, the oxyhemoglobin dissociation curve. Integration of Eq. 1 gives

$$D_1 \int_0^x d[O_2] + D_2 \int_0^x d[HbO_2] = K \int_0^x dx$$

or

$$D_1 ([O_2]_x - [O_2]_0) + D_2 ([HbO_2]_x - [HbO_2]_0) = Kx \qquad (3)$$

where the subscripts 0 and $x$ refer to the location in the membrane, so that $[HbO_2]_0$, for example, is the concentration of $HbO_2$ when $x = 0$. It is interesting to note that the steady-state transport of oxygen ($K$) through a given membrane depends only on the boundary concentrations of $O_2$ and $HbO_2$.

To calculate the steady-state oxygen transport ($K$) for a 0.015-cm thick Millipore filter (*1*) which is positioned between a given pressure of oxygen on one side and vacuum on the other, Eq. 3 can be simplified by setting $[O_2]_x$ and $[HbO_2]_x$ to zero and making $x$ equal

0.015. The values of Fig. 1 are calculated with $D_1$ equaling $2.4 \times 10^{-5}$ (*5*) and $D_2$ equaling $6.8 \times 10^{-7}$ cm$^2$ sec$^{-1}$ (*7*). The shapes of the curves in Fig. 1 representing oxygen transport in the presence and absence of hemoglobin agree well with those obtained experimentally (*8*), and Table 1 shows good agreement between the calculated and measured ratios of oxygen transport in the presence and absence of hemoglobin (*9*).

Although Fatt and LaForce (*4*) showed qualitative agreement between the nitrogen and oxygen transport found by Hemmingsen and Scholander (*2*) and that predicted by diffusion equations, no attempt was made to describe the concentration gradients of oxygen and oxyhemoglobin within the membrane. The profile of the concentration gradients in the membrane can be calculated, however, from Eqs. 2 and 3. For any value of $[O_2]_x$ less than $[O_2]_0$, $[HbO_2]_x$ is determined from the oxyhemoglobin dissociation curve for wet membranes in air (*2*). Then Eq. 3 is solved for $x$, which gives the particular location in the membrane exhibiting the concentrations $[O_2]_x$ and $[HbO_2]_x$. Figure 2 shows an example of the concentrations when the $O_2$ pressure is 200 mm-Hg on one side and 0 mm-Hg on the other. Oxygen tensions and concentrations of free oxygen and $HbO_2$ are plotted against distance through the membrane. Most of the oxygen is present as oxyhemoglobin, but the diffusion coefficient for this large molecule is only about 1/35 that for free oxygen, and oxygen transport depends on both concentration gradient and diffusion coefficient. The sigmoid curve in Fig. 2 (solid line) shows the fraction of the total oxygen transport that can be accounted for by diffusion of free oxygen.

From the data of Fig. 2, we can also calculate that 75 percent of the total membrane hemoglobin should be present in the oxygenated form; this agrees reasonably with the value of 82 percent observed by Enns (*5*). Similarly, for an inlet $O_2$ pressure of 50 mm-Hg and an outlet pressure of 0 mm-Hg, the calculated percentage of oxyhemoglobin in the membrane is 57; 56 percent is observed.

It is surprising at first sight that enhancement of transport of oxygen depends on the simultaneous diffusion of free $O_2$ and oxyhemoglobin, notwithstanding the fact that at oxygen pressure of 200 mm-Hg at the inlet both theory and experiment (*5*) show the absence of an oxyhemoglobin gradient in the left half of the membrane (Fig. 2, dashed line); apparently in this portion of the membrane only the flow of free oxygen contributes to transport (Fig. 2, sigmoid curve). This flow is enhanced when hemoglobin is present in the membrane because, according to the proposed model, the concentration of free oxygen decreases from its initial value at the far left to near zero in about half the membrane thickness instead of over the full thickness, as would be the case if no hemoglobin were present. This is well illustrated by the difference in oxygen-tension gradients in the top portion of Fig. 2. In the left-hand portion of the membrane the gradient for free oxygen is increased. From about the middle of the membrane to the exit side the gradient for free oxygen becomes so small that its effectiveness in oxygen transport is negligible. In this portion of the mem-

Table 1. Ratio of oxygen transport through Millipore membrane in presence and absence of hemoglobin.

| Oxygen pressure, inlet (mm-Hg) | Ratio | |
|---|---|---|
| | Measured (*8*) | Calculated |
| 58 | 3.1 | 3.7 |
| 93 | 2.5 | 2.7 |
| 155 | 1.9 | 2.0 |
| 230 | 1.7 | 1.7 |

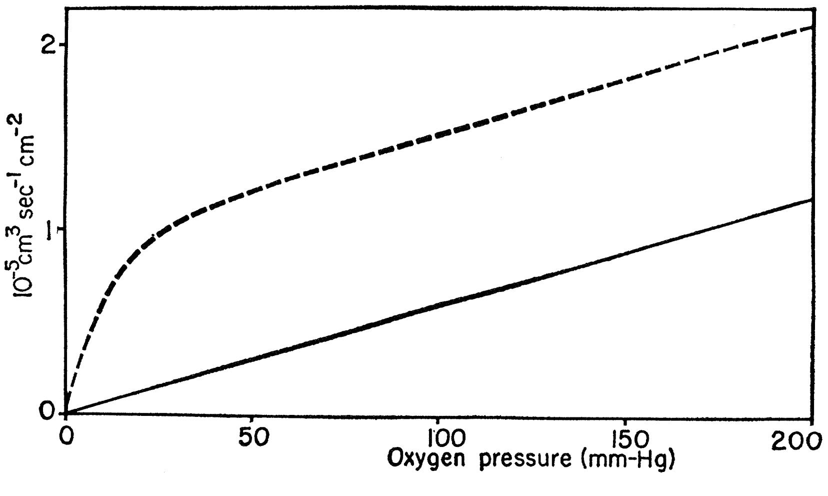

Fig. 1 (above). Calculated flux of oxygen in Millipore membrane. Lower curve, in presence of water; upper curve, in presence of 15-percent hemoglobin solution.

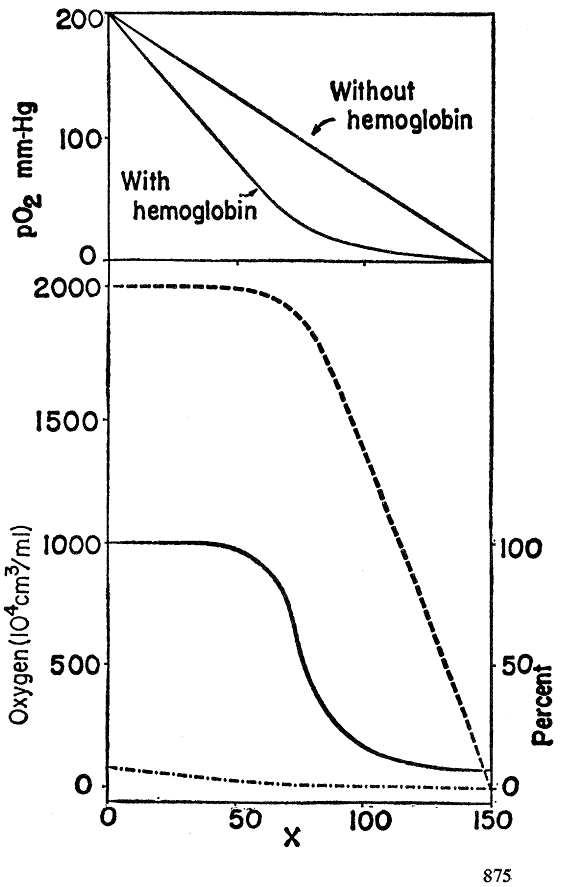

Fig. 2 (right). Free and hemoglobin-bound oxygen concentrations in Millipore membrane. Oxygen pressure ($pO_2$) at inlet ($x = 0$) is 200 mm-Hg; at outlet ($x = 150$ $\mu$), zero. (Top) Oxygen tensions in presence and absence of hemoglobin. (Bottom) Oxygen concentrations and transport in membrane containing 15-percent hemoglobin solution; – · – · – · – · –, free-oxygen concentration, ordinate at left gives volume of oxygen (at standard temperature and pressure) per milliliter of hemoglobin solution; – – – – – – –, hemoglobin-bound oxygen concentration, ordinate at left; ————————, free-oxygen transport as a percentage of total-oxygen transport, ordinate at right.

brane there is an "(oxy)hemoglobin shuttle" which, by diffusion and continuous unloading, transports the $O_2$ the rest of the way. In the middle portion of the membrane, one finds a gradual transition in the nature of the oxygen transported—from primarily free oxygen to $HbO_2$.

The proposed model easily explains the observation (*2*) that a slight back-pressure of oxygen at the exit of the membrane diminishes the enhancement by hemoglobin of the oxygen flux. Any factor interfering with the unloading of hemoglobin would, of course, diminish the gradient for oxyhemoglobin in that part of the membrane in which the hemoglobin shuttle operates, and thereby alter also the gradient of free oxygen in the first portion of the membrane.

It is possible that, in physiological systems exhibiting material transport over and above that which can be explained by simple diffusion of solute, a facilitated diffusion analogous to that described here may occur. Such diffusion requires only the presence of a carrier molecule that combines reversibly with solute and which itself is subject to diffusion gradients.

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# Tension Gradients Accompanying Accelerated Oxygen Transport in a Membrane

Abstract. *Facilitated transport of oxygen through hemoglobin has been studied by use of a membrane sandwich of hemoglobin, Teflon, and water. Transport is enhanced only when there is a hemoglobin-bound-$O_2$ gradient in the pigment. When passing through a fully oxygenated layer, the accelerated transport is carried by an increased diffusion gradient of oxygen pressure through the solvent. The results substantiate experimentally the diffusion gradients proposed by Zilversmit.*

The absolute rate of enhancement of oxygen transport through a membrane containing hemoglobin reaches a maximum when the input side is saturated with oxygen and the output side is fully reduced; it makes no difference if the oxygen pressure is considerably beyond saturation on the input side. At an oxygen tension of 450 mm-Hg on the input side in one experiment some 95 percent of the membrane was fully oxygenated; it seemed clear, therefore, that the additional oxygen must be able to pass through a layer of fully saturated oxyhemoglobin (*1, 2*). If oxygen pressure on the output side was allowed to increase, it appeared that enhancement vanished when full saturation was reached (*3*).

The last point has been studied with a more direct approach: optical oxygen saturation has been compared with gasometric enhancement; the same hemoglobin solution was used. For these measurements specially designed membranes each incorporated two Millipore

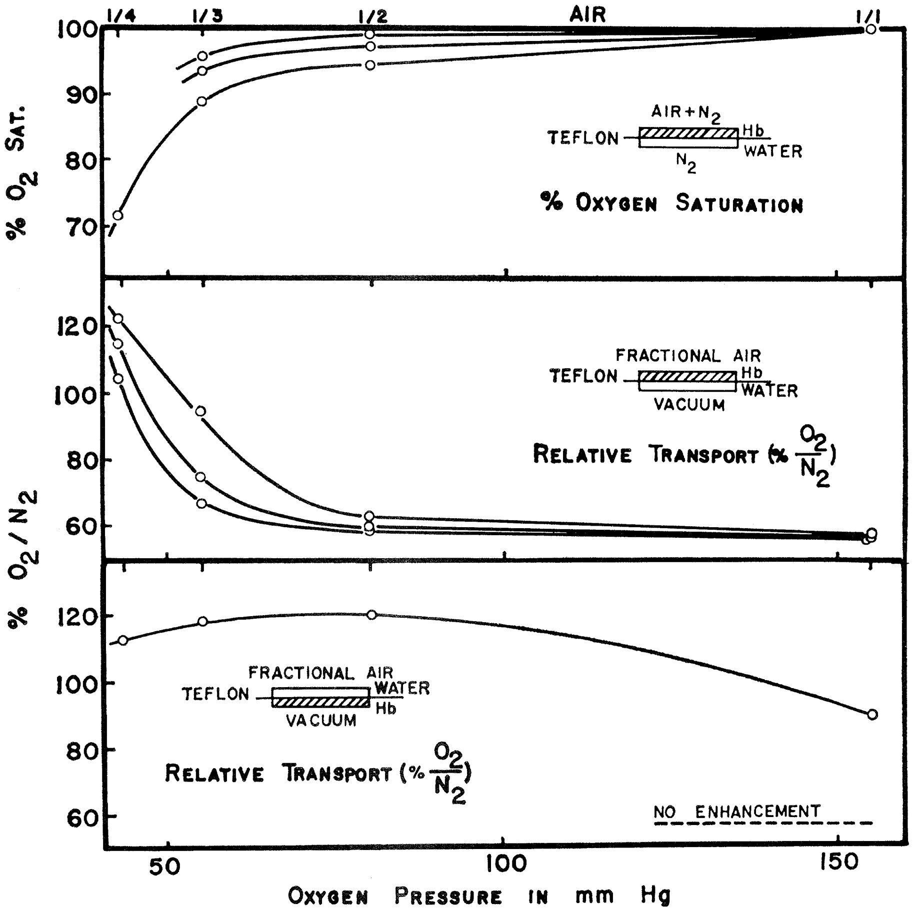

Fig. 1. Hemoglobin oxygenation and relative enhancement of oxygen transport in sandwich membranes. Each curve represents a separate experiment. The same hemoglobin solution was used in all experiments; it was prepared from human blood (*1*) having an oxygen capacity near 21 percent by volume. (Top) Oxygenation determined spectrophotometrically at a wavelength of 562 mμ; total gas pressure on each side of membrane sandwich was 1 atm. (Center) Relative enhancement determined gasometrically (*1*); value of no enhancement (methemoglobin or water), $O_2:N_2$ = 56 percent. Air at various pressures on input side. (Bottom) Relative enhancement when the hemoglobin membrane faced vacuum.