OXYGEN TRANSPORT THROUGH
HEMOGLOBIN SOLUTIONS

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i

FOREWORD

The work reported herein was conducted by John B. Pierce Foundation Laboratory, New Haven, Connecticut, under Contract No. AF 33(657)-11103 with the Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio. Abbott T. Kissen, PhD, Biothermal Branch, Physiology Division of the Biomedical Laboratory, was technical contract monitor for Aerospace Medical Research Laboratories. The work was performed in support of Project 7164, "Biomedical Criteria for Aerospace Flight," Task 716409, "Human Thermal Stress." Harold T. Hammel, PhD was the principal investigator for John B. Pierce Foundation Laboratory. The research was begun in March 1963 and completed in March 1965.

This technical report has been reviewed and is approved.

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ii

ABSTRACT

Scholander discovered that the steady state flux of oxygen through a thin film of water could be enhanced many times by adding hemoglobin to the water. Several authors have ascribed this facilitated flux of oxygen to the diffusion of oxyhemoglobin down its gradient although the details of their formulations have not been rigorously supported by experimental evidence. A series of measurements of $\dot{Q}_{O_2}$ and $\dot{Q}_{N_2}$ through a film of hemoglobin solution were made for which the pO_2 and pN_2 on one side were always 16.5 and 62.5 mm Hg respectively while the pO_2 on the other side was increased from 0.5 to 14 mm Hg by adding increasing thicknesses of teflon film to this side. When the facilitated oxygen flux was small, it was found to equal the calculated flux assuming that the oxygen was carried by the diffusing oxyhemoglobin and that Henry's law and the equilibrium dissociation curve for oxyhemoglobin apply at the two surfaces of the hemoglobin film. When the facilitated oxygen flux was greatest, it was found to be only one half the calculated flux, presumably because the pO_2 on the solution side of the gas-solution interface was considerably less than the 16.5 mm Hg prevailing on the gas side.

SECTION I

INTRODUCTION

In 1956, Dr. Scholander discovered that the steady state flux of oxygen across a water film, arranged such that oxygen diffuses from a gas phase on one side to vacuum on the other, may be enhanced many times when hemoglobin is added to the water film. He discussed his findings with several investigators before publishing them in 1960 (1). Hemmingsen and Scholander (2) and more recently Hemmingsen (3, 4, 5) and Enns (6) have provided much more experimental data regarding the phenomenon. At least three theoretical papers on the mechanism (7, 8, 9) have been written based on the premise that the facilitated oxygen flux results from the diffusion of oxyhemoglobin and hemoglobin. Scholander's view of the facilitory process was also dependent upon Brownian movement of the oxyhemoglobin and hemoglobin molecules, but he supposed that the transfer of oxygen from oxyhemoglobin to hemoglobin occurred in significant amounts only by collisions between these two molecules; a process he termed "bucket brigade." Enns (6) formalized this view by suggesting that the collisions occurred between "oxygen carrying sites" for which there was presumed to be a gradient even through that portion of the film which was experimentally shown to have no oxyhemoglobin gradient. On one occasion, Fatt (10) suggested that an interfacial phenomenon was involved, but he subsequently abandoned this view in favor of diffusion of oxyhemoglobin (9).

In the opinion of the author, there is still lacking experimental data which provides rigorous support for one theory or the other. Fatt and LaForce found that the results of Hemmingsen and Scholander were in "qualitative agreement" with their diffusion theory implying that the agreement was not quantitative. In each instance, the theories based on the diffusion of oxyhemoglobin and hemoglobin predicted that the facilitated flux of oxygen, i.e., the flux that is added by the presence of hemoglobin to the oxygen flux by Fick diffusion, is directly proportional to the oxyhemoglobin gradient. But, as Hemmingsen and Scholander found in one series of measurements, if the pO_2 on the low pressure side of the membrane were no more than 8 to 10 mm Hg with 30 mm Hg on the other side, there appeared to be no facilitated oxygen flux. Since the hemoglobin would presumably be only 55 to 65% saturated at this partial pressure on the low side, the theory predicts a substantial enhancement of the oxygen flux when there appeared to be none.

1

Reviewing this situation, the author started with the premise that the facilitated oxygen transport results from an interfacial effect. Since both hemoglobin and myoglobin are composed of a highly polar component, globin, and a relatively non-polar part, heme, these molecules will orient and concentrate at the interface between the polar fluid, water, and the nonpolar fluid, air, such that the hemes are directed toward the gas and the globins toward the water. A surmise followed that should a heme at the interface lose an oxygen to the water behind the oriented molecular layer of hemoglobin, then that oxygen would more likely be replaced by one from the gas phase than by one from the water phase. Since the oriented layer would also be concentrated, there might be a high impedance to diffusion of that oxygen back to the gas phase. With such a mechanism, there seemed to exist a possibility that the water behind the interface on the high pressure side should become supersaturated with oxygen and that the concentration gradient for Fick diffusion to the other interface (held at $pO_2 = 0$) would no longer be that for water saturated at partial pressures existing in water alone but rather a supersaturated concentration gradient.

Firstly, measurements were made to test this hypothesis. The results were to show that a supersaturating mechanism was not operating to any measurable extent in transporting oxygen through hemoglobin from gas to vacuum. Secondly, measurements were made which require a revised application of the current theory based on diffusion of oxyhemoglobin.

SECTION II

METHOD

During the September and October of 1964, the author spent three weeks in Dr. Scholander's laboratory to learn from him and Dr. Hemmingsen the method for measuring the oxygen and nitrogen fluxes diffusing from air on one side of an HA millipore filter containing a hemoglobin solution to vacuum (1). The only modification of this technique was to add barriers of assorted kinds to either the high pressure or the low pressure side of the hemoglobin film.

Fresh whole human blood was separated from its plasma and twice washed with isotonic saline. Isotonic saline was then added to the red cells to return the volume to the initial whole blood volume. The red cell solution was frozen and rewarmed twice and then centrifuged to separate out the cell membrane. The resultant hemoglobin solution was always stored frozen except when used.

2

SECTION III

RESULTS

A. Pertaining to the Supersaturation Theory

The rationale for these measurements was to interfere with
or prevent the orientation and concentration of the hemoglobin at one
or other of the interfaces and observe the effects upon the oxygen
transport. Several variants of the same principle were employed, the
principle being to place a layer of water on one side of the hemoglobin
film. The problem was to support the water in a carrier which also
blocked the diffusion of hemoglobin through the water barrier to the
water-gas interface. With such a water barrier on the vacuum side
and air on the other side with a pO_2 somewhat less than for saturated
hemoglobin, say 20 mm Hg, the supersaturation theory predicts that
the enhancement, that is, the ratio of the oxygen flux with hemoglobin
to the oxygen flux with met-hemoglobin will be the same with or without
the water barrier.

The results shown in Table I clearly indicate that this is
not the case. The result with hemoglobin in an HA millipore over
0.001 inch cellophane soaked in water was, at first, not accepted as
a valid test, Table I, A. Although cellophane soaked in water is
highly hydrated and does block the diffusion of hemoglobin, the
possibility that orientation was occurring where the hemoglobin faced
the cellulose could not be ruled out. A special experimental millipore
was obtained from the Millipore Corporation which, hopefully, would block
the diffusion of hemoglobin. When placed on hemoglobin, it, too, soaked
up the hemoglobin and enhanced the oxygen flux by 2.6 times, Table I, B.
When this special VX millipore was soaked with hemoglobin which was
then thermally coagulated and placed on the vacuum side of a hemoglobin
film, there was found to be enhancement of 2.8 times even though no
trace of hemoglobin was found on the vacuum side of the coagulated
hemoglobin film, Table I, D. However, the possibility that penetration
of hemoglobin into or nearly through the coagulated layer of hemoglobin
could not be ruled out. Finally, it was possible to coagulate hemoglobin
in the standard HA millipore (used throughout as the carrier for hemoglobin)
and to prevent the penetration of hemoglobin by mass flow by placing on
the vacuum side of the coagulated hemoglobin a silicone rubber membrane[*]

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[*]   Silicone rubber membrane – received by courtesy
        from the General Electric Company, Schenectady,
        New York, Mr. N. R. Dibelius.

3

TABLE I

FLUXES OF OXYGEN AND NITROGEN THROUGH SELECTED COMBINATIONS
OF HEMOGLOBIN AND BARRIER.

All values are for $pO_2 = 16.5$ mm Hg and $pN_2 = 62.5$ on one side and approximately zero pO_2 and pN_2 on the other. Both sides saturated with water vapor at 23°C. Volumes are given for S.T.P.D. Total diffusion area = 8.0 cm^2.

Diffusing Combination (air above/vacuum below)	$\dot{Q}_{O_2}$ mm^3/min	$\dot{Q}_{N_2}$ mm^3/min	$\dot{Q}_{O_2} / \dot{Q}_{N_2}$	Enhancement Factor
A. hemoglobin in HA millipore over 0.001" cellophane	0.143	0.259	0.56	1.0
B. hemoglobin in VX special millipore	0.235	0.140	1.69	2.6
C. thermally coagulated hemoglobin in VX special millipore	0.067	0.104	0.65	---
D. hemoglobin in HA millipore over thermally coagulated hemoglobin in VX special millipore	0.181	0.100	1.80	2.8
E. thermally coagulated hemoglobin in HA millipore over silicone rubber film	0.079	0.141	0.56	---
F. hemoglobin in HA millipore over thermally coagulated hemoglobin in HA millipore over silicone rubber film	0.069	0.105	0.66	1.2
G. hemoglobin in HA millipore over protein impregnated membrane	0.095	0.136	0.71	1.3
H. hemoglobin in HA millipore over Diaplex film	0.126	0.151	0.84	1.5
I. hemoglobin in HA millipore over silicone rubber film	0.866	0.303	2.86	5.1
J. met-hemoglobin in HA millipore over silicone rubber film	0.150	0.269	0.56	---
K. 0.001" Teflon	0.322	0.467	0.69	---

4

highly permeable to oxygen and nitrogen but not to water or hemoglobin. The result of this test, Table I, F, was an enhancement of nearly one, i.e., little or no facilitated oxygen flux. This result was supported by tests using protein impregnated membrane[**], Table I, G, and Diaplex, type XM[***], Table I, H. It appears that the facilitated oxygen flux cannot be attributed to an interfacial effect.

B. Pertaining to the Diffusion Theory

Oxygen and nitrogen fluxes were measured in a series of tests in which pO_2 = 16.5 mm Hg and pN_2 = 62.5 mm Hg on one side for all tests while the pO_2 and pN_2 on the other side increased from nearly zero up to 14 mm Hg and 32 mm Hg respectively. The increasing pO_2 and pN_2 on the low side was achieved by interposing increasing thicknesses of teflon (in steps of 0, 1/8, 1/4, 3/8, 1/2, 3/4, 1 and 1-1/2 mil)[****] between the hemoglobin film and the vacuum. All tests were started by placing an HA millipore filter (0.45μ pore size, 150μ thickness) on the same sample of freshly made hemoglobin solution. Allowing a few minutes for soaking to capacity, the millipore filter with hemoglobin was then placed on a double film composed of a highly permeable silicone rubber film and the teflon film of desired thickness. The millipore filter with hemoglobin always lies on the silicone rubber film in such a way as to exclude all gas bubbles. When teflon film was used, the silicone rubber film was caused to adhere to the teflon film by placing bubble free water between the two and then carefully squeezing out all of the water possible while the two films were pressed between blotting paper and a smooth hard surface. The teflon film, when used, lay on a stainless steel grid in the diffusion chamber and toward the vacuum compartment.

In order to make precision measurements of the oxygen and nitrogen fluxes it was necessary to insure that no gas enter the vacuum compartment except that which diffused through the hemoglobin layer, silicone rubber and teflon. This was achieved in these tests by covering the margins of the millipore filter, silicone rubber and teflon films above and below with double thickness rubber gaskets cut from thick-walled rubber balloons.

[**]   PEM - protein impregnated membrane obtained from Gelman Instrument Company.

[***]   Diaplex, type XM - polyanion - excess membrane obtained from Amicon Corporation, Cambridge, Massachusetts.

[****]   Teflon - received by courtesy from Dilectrix Corporation, Farmingdale, Long Island.

5

The results for these measurements are shown in Figure 1. The middle line shows the decrease in the nitrogen flux with increasing thickness of the teflon barrier. The line is drawn to provide the best
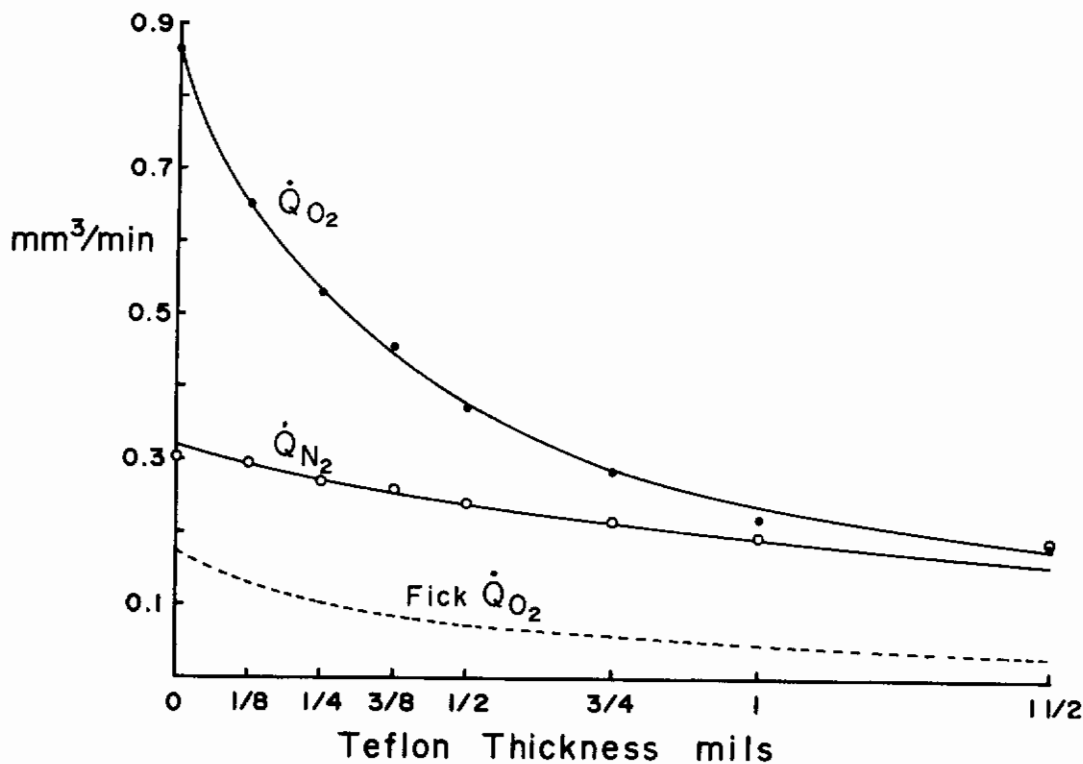

Figure 1. Total oxygen flux, total nitrogen flux and calculated oxygen flux by Fick diffusion through an HA millipore filter containing hemoglobin and a barrier consisting of an 0.001" silicone rubber film plus increasing thicknesses of teflon. All values are for pO_2 = 16.5 mm Hg and pN_2 = 62.5 on the upper side of the hemoglobin layer and approximately zero pO_2 and pN_2 below the barrier. Both sides saturated with water vapor at 23°C; volumes at S.T.P.D.; diffusion area = 8.0 cm^2. The actual pO_2 on the solution side of the upper gas-solution interface is shown in the text to be much less than the 16.5 mm Hg prevailing on the gas side.

fit of the experimental data and fits the equation

$$^{h,t}Q_{N_2} = \frac{62.5}{^{h}R_{N_2} + {}^{t}R_{N_2}}$$

where the resistance to the diffusion of nitrogen through the hemoglobin film plus silicone film alone is

$$^{h}R_{N_2} = \frac{62.5}{0.320} = 195 \text{ mm Hg mm}^{-3} \text{ min}^{*}$$

and the resistance through teflon alone is calculated from the nitrogen flux through one mil teflon, Table I, K, to be

$$^{t}R_{N_2} = \frac{62.5}{0.467} d = 134 \text{ d mm Hg mm}^{-3} \text{ min}$$

where d = thickness in mils.

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* –   This resistance value may be used to compute the diffusion coefficient of nitrogen in hemoglobin as follows:

$$^{h}D_{N_2} = \frac{760 \times 0.015 \times 10^{-3}}{194 \times 60 \times 8 \times 0.0155 \times 0.84} \text{ cm}^2/\text{sec}$$

$$= 9.4 \times 10^{-6} \text{ cm}^2/\text{sec.}$$

The ratio of the diffusion coefficient of nitrogen in hemoglobin at 15.9 gm/100 ml to that for water is 0.45 (11) and the diffusion coefficient of nitrogen in water is 2.02×10^{-5} cm^2/sec (12). Therefore, the diffusion coefficient of nitrogen in hemoglobin ought to be 9.0×10^{-6} cm^2/sec. The above value is in good agreement with this expected value.

The partial pressure of oxygen at the interface between the silicone[**] plus teflon barrier and the hemoglobin film, pO_2 (x_j), may be calculated from the total flux of oxygen through the teflon barrier and the resistance of the barrier, i.e.,

$$pO_2 \ (x_j) \ = \ ^{h,\,t}\dot{Q}_{O_2} \ (^t R_{O_2} + \ ^s R_{O_2})$$

Using the flux of oxygen through a one mil thickness of teflon, Table I, K,

$$^t R_{O_2} \ = \ \frac{16.5}{^t\dot{Q}_{O_2}} d \ = \ 51.2\,d$$

and

$$pO_2 \ (x_j) \ = \ ^{h,\,t}\dot{Q}_{O_2} \ (51.2\,d + 0.64) \ .$$

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** –   The permeability of the one mil silicone rubber film is 69 and 149 mm^3 min^{-1} cm^{-2} mil^{-1} atm^{-1} for nitrogen and oxygen respectively (manufacturer's values). The resistances of the silicone rubber film in the experimental set-up for nitrogen and oxygen are as follows:

$$^s R_{N_2} \ = \ 1.38 \ \text{mm Hg mm}^{-3} \ \text{min}$$

$$^s R_{O_2} \ = \ 0.64 \ \text{mm Hg mm}^{-3} \ \text{min}$$

The lower curve in Figure 1 is that for the Fick diffusion
of oxygen through the hemoglobin film when backed by the silicone film
plus increasing thicknesses of teflon and is calculated from

$$\text{Fick } {}^{h,t}\dot{Q}_{O_2} = \frac{16.5 - pO2\,(x_j)}{{}^{m}R_{O_2}}$$

assuming that $pO_2 = 16.5$ at $x = 0$ and where ${}^{m}R_{O_2}$ is the resistance
of a film of methemoglobin to oxygen flow and is equal to

$$ {}^{m}R_{O_2} = \frac{16.5}{{}^{m}\dot{Q}_{O_2}} = \frac{16.5}{0.56 \; {}^{m}\dot{Q}_{N_2}} = \frac{16.5}{0.56 \, {}^{h}\dot{Q}_{N_2}} = 91.5. $$

The upper curve of Figure 1 shows the measured total flux
of oxygen through hemoglobin and increasing thicknesses of teflon. The
difference between the upper curve and the lower curve yields the facilitated
oxygen flux for each thickness of teflon. In Columns 2, 3, 5 and 6 of Table II
are recorded the ideal values of the total oxygen flux, the nitrogen flux,
the calculated oxygen flux by Fick diffusion and the facilitated oxygen flux
respectively for each thickness of teflon as obtained from the smooth fitting
curves of the experimental data. The enhancement, Column 7, is the ratio
of the total oxygen flux through hemoglobin to the calculated oxygen flux
through methemoglobin in the millipore filter, Column 2 divided by Column 5.

In Column 4 are shown the calculated values of the pO_2 at the
interface between the hemoglobin film and the silicone plus teflon barrier
of increasing thickness. Using the dissociation curve of Hemmingsen and
Scholander (2) for hemoglobin at 23°C, and assuming equilibration between
the oxyhemoglobin and the dissolved oxygen at the calculated partial pressures,
the values for the % saturation of the hemoglobin at this interface are given in
Column 8. Since the pO_2 at the air-hemoglobin interface was always presumed
to be 16.5 mm Hg and the % saturation there was always 80% (assuming equili-
bration), the difference in % saturation at the two interfaces of the hemoglobin
were those shown in Column 9. The ratio of the facilitated oxygen flux (Column
6) to the Fick oxygen flux (Column 5) is shown in Column 10.

Assuming that the calculation of the Fick oxygen flux is valid,
i.e., assuming that Henry's Law is valid at the gas-hemoglobin interface
and at the hemoglobin-barrier interface where the partial pressures of oxygen
are known, and assuming that the pO_2 at $x = 0$ is always 16.5 mm Hg then
the above results indicate that the ratio of facilitated to the Fick flux is
constant, and that the facilitated flux of oxygen is about 4 times the Fick
flux. These assumptions will be questioned in the discussion.

9

TABLE II

IDEAL VALUES OF TOTAL OXYGEN FLUX, NITROGEN FLUX, THE OXYGEN FLUX BY FICK DIFFUSION AND THE FACILITATED OXYGEN FLUX AS OBTAINED FROM FIGURE 1 FOR EACH THICKNESS OF TEFLON BARRIER.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
Teflon thickness mils	Total $\dot{Q}_{h,t}O_2$ mm^3/min	Fick $\dot{Q}_{h,t}N_2$ mm^3/min	$pO_2(x_j)$, pO_2 on low side mm Hg	Fick $\dot{Q}_{h,t}O_2$ mm^3/min	Fac $\dot{Q}_{h,t}O_2$ mm^3/min	En-hance-ment	% Sat. on low side	Δ% Sat.	$\dfrac{Fac \dot{Q}O_2}{Fick \dot{Q}O_2}$	$\dfrac{\Delta\%Sat.}{\Delta pO_2}$	$pO_2(o)*$ mm Hg	$\dfrac{Fac \dot{Q}O_2}{Fick \dot{Q}O_2}$ recalculated**
0	0.865	0.320	0.55	0.175	0.690	4.95	3.0	77.0	3.95	4.81	5.9	13.9
1/8	0.651	0.295	4.58	0.130	0.521	5.00	30.0	50.0	4.01	4.20	8.8	13.5
1/4	0.530	0.274	7.12	0.103	0.427	5.14	49.5	30.5	4.15	3.25	12.3	8.3
3/8	0.443	0.255	8.78	0.084	0.359	5.28	58.0	22.0	4.28	2.86	14.2	6.5
1/2	0.378	0.238	9.91	0.072	0.306	5.25	62.6	17.4	4.25	2.64	15.0	5.8
3/4	0.288	0.212	11.24	0.058	0.230	4.97	67.8	12.2	3.97	2.30	15.8	4.8
1	0.237	0.190	12.28	0.046	0.191	5.16	70.7	9.3	4.15	2.21	16.2	4.5
1-1/2	0.178	0.158	13.77	0.030	0.148	5.94	74.6	5.4	4.93	2.00	17.0	4.0

Avg. = 4.11

* - The necessary and only values of the pO_2 at x = 0 for which there can be agreement between experimental results and theory.

** - The ratio of facilitated flux to Fick flux for which the recalculated experimental ratio and theoretical ratio agree.

10

DISCUSSION

We may assume 1) that hemoglobin (or myoglobin) molecules are undergoing Brownian movement, both rotatory and translatory, and 2) the average time interval between successive displacements of a heme on the hemoglobin molecule by either rotation or translation is sufficient to permit each heme to achieve some fraction of full equilibration with the pO_2 of the dissolved oxygen immediately surrounding the heme. Thus when a hemoglobin molecule is in a partial pressure gradient for oxygen such that there is a difference in the % saturation of each heme before and after each displacement of the heme, there will be a net displacement of oxygen down that gradient in addition to that which diffuses dissolved in water.

The general one-dimensional diffusion equation for oxygen diffusing through a film of hemoglobin in a water solution is Fick's second law of diffusion modified by subtraction of an oxygen absorbing term which states the rate of combination of oxygen with hemoglobin at x and time t and by addition of an oxygen yielding term which states the rate of dissociation of oxyhemoglobin at x and t. Thus

1) $$\frac{\partial [O_2(x,t)]}{\partial t} = -D_{O_2} \frac{\partial^2 [O_2(x,t)]}{\partial x^2} -k'[O_2(x,t)]^n [Hb(x,t)_n] + k[O_2Hb(x,t)_n]$$

where D_{O_2} is the diffusion coefficient for oxygen in the hemoglobin-water solution and k and k' are the velocity constants for dissociation and combination respectively in the reversible reaction

$$nO_2 + Hb_n \underset{k}{\overset{k'}{\rightleftarrows}} O_2Hb_n \, .$$
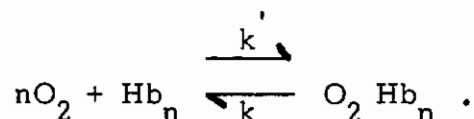
Similarly, the general one-dimensional diffusion equation for oxy-hemoglobin diffusing through a film of hemoglobin in a water solution is:

2) $$\frac{\partial [O_2Hb(x,t)]}{\partial t} = -D_{O_2Hb} \frac{\partial^2 [O_2Hb(x,t)]}{\partial x^2} -k[O_2Hb(x,t)_n] + k'[O_2(x,t)]^n [Hb(x,t)_n]$$

where D_{O_2Hb} is the diffusion coefficient of oxyhemoglobin in the hemoglobin-water solution.*

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* -   Although oxyhemoglobin does diffuse down its gradient, there is no steady state net transport of oxyhemoglobin from one side of the film to the other since as it diffuses it dissociates into hemoglobin which diffuses the opposite direction down its gradient.

In steady state the concentrations of oxygen, oxyhemoglobin and hemoglobin at every x through the film are not changing with respect to time and the above equations are equal to zero. Since these two steady state equations have a common term in the rates of combination and dissociation of oxygen, hemoglobin and oxyhemoglobin respectively, they may be combined into one equation

3)
$$-D_{O_2} \frac{\partial^2 [O_2(x)]}{\partial x^2} - D_{O_2Hb} \frac{\partial^2 [O_2Hb(x)]}{\partial x^2} = 0$$

which when integrated with respect to x becomes

4)
$$-D_{O_2} \frac{\Delta [O_2(x)]}{\Delta x} - D_{O_2Hb} \frac{\Delta [O_2Hb(x)]}{\Delta x} = \text{constant} = \dot{V}_{O_2}$$

This last equation is the equation which has been assumed by most authors (7, 8, 9) to describe the total steady state oxygen flux (combined and in solution) through layer x of the film.

The failure of the diffusion theory - as formulated and applied (7, 8, 9) - to provide a rigorous account of the data presented here may be clearly seen when analyzed in a manner suggested by Forster (13). According to the diffusion theory, the ratio of the facilitated oxygen flux to the Fick diffusion flux should be the same as the ratio of the Fick diffusion of oxyhemoglobin to the Fick diffusion of oxygen under steady state conditions.

The latter ratio may be evaluated, approximately, as follows:

$$\frac{D_{O_2Hb} \frac{\Delta[O_2Hb]}{\Delta x}}{D_{O_2} \frac{\Delta[O_2]}{\Delta x}} = 0.032 \frac{\frac{21.6 \text{ Vols.\%}}{100} \cdot \frac{\Delta \% \text{ Sat.}}{100 \Delta x}}{\Delta pO_2/\Delta x \cdot 0.0314 \times 0.84/760}$$

$$= 2.0 \frac{\Delta \% \text{Sat.}/\Delta x}{\Delta pO_2/\Delta x}$$

where 0.0314 is the Bunsen solubility coefficient for oxygen, 0.84 is the approximate fraction of the hemoglobin solution which is water, 0.032 is the approximate ratio of D_{O_2Hb}/D_{O_2} (5) and 21.6 Vols. % is the approximate value for the oxygen content of the fully saturated hemoglobin solution used in the tests of Table II. Although this ratio may be a function of x through the film, the average ratio may be obtained from the values for % saturation and pO_2 at the boundaries x = 0 and x = x_j as follows:

12

$$\text{Avg. } \frac{\text{Fac. } \dot{Q}_{O_2}}{\text{Fick } \dot{Q}_{O_2}} = 2.0 \frac{\% \text{ Sat. (o)} - \% \text{ Sat. } (x_j)}{pO_2 \text{ (o)} - pO_2 (x_j)} .$$

Assuming that pO_2 (o) is always equal to 16.5 mm Hg and that pO_2 (x_j) is the value shown in Column 4, Table II, for each thickness of barrier, and assuming that the hemoglobin at the interfaces is fully equilibrated with the oxygen according to the dissociation curve of Hemmingsen and Scholander (2), then the average $\frac{\Delta \% \text{ Sat.}}{\Delta pO_2}$ across the hemoglobin film will be the values given in Column 11 of Table II for each of the tests. Thus, according to the current application of the diffusion theory (7, 8, 9), the ratio of the facilitated to the Fick diffusion flux of oxygen will be approximately 2.0 times the values given in Column 11 for each of the tests. The range of values for the ratio are, therefore, predicted to be 10 for the maximum oxy-hemoglobin gradient down to about 4 for the minimum gradient, whereas the actual calculated ratio for all oxy-hemoglobin gradients was approximately 4. The variation in the predicted values of the facilitated to Fick ratio is due to the fact that the dissociation curve is not linear over the range from 0 to 80% saturation, that is the slope of the dissociation curve from 70 to 80% saturation is less than the average slope from 0 to 80% saturation.

If full equilibration at either boundary was not achieved in these tests or if the pO_2 at x = 0 was not always 16.5 mm Hg, one might suppose that departure of the experimental from the predicted ratio would be greatest when the enhanced flux was greatest. The results appear to bear out this supposition. The observed ratio of facilitated to Fick flux best fits the predicted ratio when the enhanced flux is least and is less than half the predicted ratio when the enhanced flux is greatest.

The following argument suggests that the predicted ratio can be made to agree with the experimental results only if the pO_2 at x = 0 is assumed to be something less than 16.5 mm Hg when the facilitated flux is greatest.

The argument is based on the following beliefs:

1) The calculated values of pO_2 at the boundary between the hemoglobin and the barrier, $x = x_j$ are correct (Column 4, Table II).

13

2)   Under steady state conditions (which pertain in the measurements reported here) full equilibration between the oxygen and hemoglobin in accordance with the equilibrium dissociation curve occurs at all levels throughout the film. This must be true because the velocity constants for combination and dissociation, k' and k respectively, determine not only the rates of combination and dissociation (which are equal in steady state) but also the ratio of these same constants (k'/k, the dissociation constant) determines the equilibrium relationship between % saturation and pO_2, i.e., the equilibrium dissociation curve. This is true even though the so called velocity constants and dissociation constant are not constant but depend upon the degree of saturation.

If these statements are true, then there is only one way to make the predicted ratio of facilitated flux to Fick flux agree with the calculated experimental flux, and that is to assume that the pO_2 in the water at the boundary $x = 0$ is less than 16.5 mm Hg. The pO_2 at $x = 0$ for each test in Table II may be determined empirically by choosing that value which satisfies the following equation

$$\frac{\text{Total } \dot{Q}_{O_2} - \dfrac{pO_2 \, (o) - pO_2 \, (x_j)}{R}}{\dfrac{pO_2 \, (o) - pO_2 \, (x_j)}{R}} = 2.0 \frac{\% \text{ Sat. } (o) - \% \text{ Sat. } (x_j)}{pO_2 \, (o) - pO_2 \, (x_j)}$$

where Total $\dot{Q}_{O_2}$ and $pO_2 \, (x_j)$ are the experimental values shown in Table II, % Sat. (x_j) is obtained from the equilibrium dissociation curve (2) for $pO_2 \, (x_j)$ and R is the resistance to Fick diffusion of oxygen through hemoglobin or methemoglobin and is 91.5 in these tests. The term on the left is recognized to be the total oxygen flux minus the Fick diffusion flux divided by the Fick diffusion flux, i.e. the ratio Fac $\dot{Q}_{O_2}$/Fick $\dot{Q}_{O_2}$, and the term on the right is the theoretical value for the same ratio. $pO_2 \, (o)$ is chosen to satisfy this relation and the equilibrium dissociation curve relating $pO_2 \, (o)$ and % Sat. (o). In Column 12, Table II, are shown the values of $pO_2 \, (o)$ which satisfy these conditions and provide agreement between the recalculated experimental ratio of Fac $\dot{Q}_{O_2}$/Fick $\dot{Q}_{O_2}$ and the predicted ratio based on the theory that the facilitated flux of oxygen results from the diffusion of oxyhemoglobin. The last column of Table II shows the Fac $\dot{Q}_{O_2}$/Fick $\dot{Q}_{O_2}$ ratio for which there is agreement between the theory and experiment.

14

The empirical fact appears to be that oxygen molecules are unable to pass the interface between the gas and the water phase at a sufficient rate to maintain the pO_2 on the water side of the interface at the same level as on the gas side when hemoglobin is facilitating the movement of oxygen through the film. Any mechanism which serves to increase the flux through the solution and away from the interface can only act to increase the gradient across the interface. Perhaps the orientation and concentration of hemoglobin molecules at this interface reduce the transfer coefficient across the boundary and thereby accentuate the effect. This interpretation of these results may also account for results reported by Mochizuki and Forster (13) for the diffusion of CO through hemoglobin. They found the experimental Fac $\dot{Q}_{CO}$/Fick Q_{CO} to be one tenth the predicted ratio when assuming that the pCO (o) was the same on both sides of the interface between gas and hemoglobin solution.

Enns (6) observed that the measured average % saturation through a film of hemoglobin, for which the pO_2 on one side was 0 and ranged from 15 to 450 mm Hg on the other, was always less (except at the highest value where the difference was not measurable) than was calculated on the assumption that the equilibration between oxyhemoglobin and dissolved oxygen was in accordance with the measured equilibrium dissociation curve. Perhaps this observation may also be explained by suggesting that the pO_2 on the high side of the hemoglobin film was actually less than in the gas phase outside the film and not the same as was assumed in his calculation. If this view is correct then there is clear understanding of the mechanism by which an enhanced flux of oxygen is transported through that portion of the hemoglobin film which is fully saturated. Oxygen would be transported with an enhanced flux by Fick diffusion down a steepened pO_2 gradient over the saturated part for which there would be no oxyhemoglobin gradient. Then the same oxygen flux would be primarily transported by hemoglobin facilitation through the unsaturated portion of the film. The transport mechanism would gradually shift from exclusively Fick diffusion to primarily (approximately 14 to 1) hemoglobin facilitated transport as the same oxygen flux moved across the film.

If any conclusions may be drawn from these results, they would be that:

1) The facilitated flux of oxygen across a hemoglobin membrane does appear to be a consequence of the diffusion of oxyhemoglobin (7, 8, 9), that is

$$\text{Total } \dot{Q}_{O_2} = -D_{O_2} \frac{\Delta[O_2(x)]}{\Delta x} - 0.032\, D_{O_2} \frac{\Delta[O_2 Hb(x)]}{\Delta x}$$

15

where D_{O_2} is the diffusion coefficient for O_2
in the hemoglobin solution, $0.032 = \dfrac{D_{O_2 Hb}}{D_{O_2}}$, $[O_2(x)]$
is the molar concentration of oxygen and $[O_2Hb]$ is
the number of gram atoms of iron per liter.

2) The steady state ratio of the facilitated flux of
oxygen and the flux by Fick diffusion of oxygen at
any level x through the hemoglobin solution will
depend upon the kinetics of the reactions between oxygen,
hemoglobin and oxyhemoglobin inasmuch as the dissocia-
tion constant k'/k determines, in part, the shape of the
dissociation curve (Hill's equation) and the ratio
Fac $\dot{Q}_{O_2}$ / Fick $\dot{Q}_{O_2}$ is proportional to the average slope

of the equilibrium dissociation curve between two pO_2's,

at the entrance and at the exit from the film. Thus

$$\text{Fac } \dot{Q}_{O_2} / \text{Fick } \dot{Q}_{O_2} = \frac{0.032V}{s} \frac{\% \text{ Sat. (o)} - \% \text{ Sat. } (x_j)}{pO_2 \text{ (o)} - pO_2 \text{ } (x_j)}$$

where V = oxygen carrying capacity of the hemoglobin
solution in volumes percent and s is the Bunsen solubility
coefficient for oxygen per mm Hg. It may be noted here
that it is the ratio of the combination and dissociation
velocity constants which determines the magnitude of
the facilitated flux relative to the Fick diffusion flux.
The absolute values of k' and k determine only the
time required to achieve steady state transport.

3) There is a pO_2 gradient across the gas-solution inter-
face on the entrance side of the hemoglobin solution,
the magnitude of which is determined by the magnitude
of the facilitated flux of oxygen through the film. Like-
wise, if any part of the pathway is oxygen saturated
hemoglobin, then the gradient across this part is
steepened by the facilitated flux of oxygen through
the unsaturated part of the pathway.

A final generalization may be recognized from these results.
Any ligand molecule which reversibly combines with another molecule to
form a third molecule will be facilitated in its transport down its concentration
gradient if

1)   All three molecules are found in such a
physical state such that each molecule
is free to diffuse down its respective
concentration gradient; e.g., in a gaseous
state or in solution; and

2)   The velocity constants for combination
and dissociation and more importantly the
dissociation constant are such as to provide
less than full saturation over some portion
of the diffusion pathway for the ligand molecule.

       The generalization is true whether the velocity constants
(but not their ratio) are increased by enzymatic activity and thereby reducing
the time to achieve steady state fluxes, as is the case for the enhanced
diffusion of carbon dioxide through a water solution of bicarbonate and
carbonic anhydrase (14), or whether the velocity constants are already large
enough without enzyme assistance, as for example, in the enhanced diffusion
of oxygen and carbon monoxide through a hemoglobin solution (1, 13).

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18

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13. ABSTRACT   Scholander discovered that the steady state flux of oxygen through a thin film of water could be enhanced many times by adding hemoglobin to the water. Several authors have ascribed this facilitated flux of oxygen to the diffusion of oxyhemoglobin down its gradient although the details of their formulations have not been rigorously supported by experimental evidence. A series of measurements of $\dot{Q}_{O_2}$ and $\dot{Q}_{N_2}$ through a film of hemoglobin solution were made for which the pO_2 and pN_2 on one side were always 16.5 and 62.5 mm Hg respectively while the pO_2 on the other side was increased from 0.5 to 14 mm Hg by adding increasing thicknesses of teflon film to this side. When the facilitated oxygen flux was small, it was found to equal the calculated flux assuming that the oxygen was carried by the diffusing oxyhemoglobin and that Henry's law and the equilibrium dissociation curve for oxyhemoglobin apply at the two surfaces of the hemoglobin film. When the facilitated oxygen flux was greatest, it was found to be only one half the calculated flux, presumably because the pO_2 on the solution side of the gas-solution interface was considerably less than the 16.5 mmHg prevailing on the gas side.

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