*MOLECULAR COLLISION-EXCHANGE TRANSPORT OF OXYGEN BY HEMOGLOBIN**

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It has been demonstrated by Scholander[1, 2] and Hemmingsen[2, 3] that the transport of oxygen across a water-filled Millipore filter membrane is increased by dissolved hemoglobin. The reasonable assumption has been made that the increment of oxygen transport in excess of that attributed to Fick's diffusion of dissolved oxygen represents transport by the hemoglobin. An attempt will be made to examine this transport and determine its mechanism.

The following pertinent facts have been established within experimental error:

(*a*) When one side of the membrane is exposed to oxygen gas and the other to wet vacuum, the additional transport due to hemoglobin increases with increase of oxygen tension until the hemoglobin saturation pressure is reached.[4] At still higher oxygen tension there is no further increase in oxygen flux attributable to the hemoglobin, i.e., the enhancement remains a constant addition to Fick's diffusion transport. An exit pressure equivalent to the saturation pressure stops the net transport.[1, 2]

(*b*) When both sides of the membrane are subjected to equal oxygen tension, the unidirectional flux as measured by adding O^{18} tracer to the gas on one side of the membrane is equal to the net flux observed when one side of the membrane is subjected to the same oxygen tension while the other side is exposed to a wet vacuum. This condition also holds at oxygen tensions at least three times higher than those required for hemoglobin saturation.[3]

(*c*) The transport attributed to hemoglobin is inversely proportional to membrane thickness.[4]

(*d*) Increase in hemoglobin concentration increases the transport, but the specific enhancement falls off with increasing viscosity at high hemoglobin concentration and if gelatin is added.[1]

(*e*) The exchange of oxygen between hemoglobin-bound oxygen and dissolved oxygen is extremely rapid[5] and complete for all hemoglobin-bound oxygen.[6]

(*f*) The oxyhemoglobin gradients along the transport path have been examined and show that the net transport enhancement takes place through oxygen-saturated hemoglobin layers. These experiments are described below.

Experiment.—An experiment was devised to determine the total fraction of oxyhemoglobin in a membrane adapting a spectrophotometric method.[4] A membrane of Millipore filter 0.15 mm thick saturated with hemoglobin solution was mounted between two transparent gas chambers. The assembly formed a cuvette with the light path successively through one gas chamber, through the membrane normal to its largest surface, and through the second gas chamber. The cuvette was placed in a spectrophotometer. The absorption of light at 5,620 Å wavelength was measured when water-saturated gases of various compositions flowed through the chambers on either side of the membrane. The hemoglobin was obtained from fresh human blood and diluted with 0.85% saline to the concentration in whole blood.

To produce oxyhemoglobin gradients, one side of the membrane was exposed to helium while the other was exposed to oxygen-nitrogen mixtures having the desired oxygen concentrations. Total gas pressures on both sides of the membrane always remained at one atmosphere.

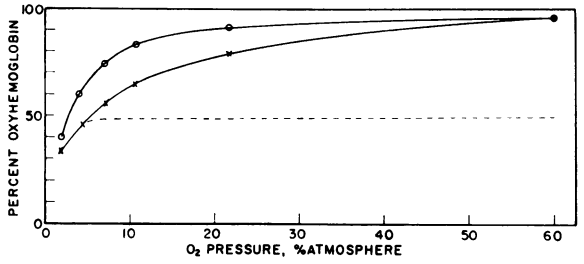

FIG. 1.—Per cent oxyhemoglobin in Millipore filter membrane plotted against oxygen tension on one side of the membrane, the other side being at zero oxygen tension. Circles—calculated values. Crosses—observed values. Dotted line represents linear oxyhemoglobin gradient.

The resultant values of total hemoglobin oxygenation calculated from the absorption measurements are shown in the center plot of Figure 1.

The hemoglobin oxygenation curve for the membrane was obtained by exposing both sides of the membrane to identical oxygen-nitrogen mixtures. If equilibration between oxyhemoglobin and dissolved oxygen is assumed in the experiments described in the previous paragraph, it is possible to construct a series of curves representing hemoglobin oxygenation along the transport path. Integration and normalization of these curves gave the values of the upper plot of Figure 1.

A repetition of the experiments described above with a solution containing one fourth as much hemoglobin in a double Millipore filter produced identical results.

Discussion.—The calculated and observed integral values of Figure 1 agree at higher oxygen pressures. Figure 2*a* illustrates the concentration gradients expected at the maximum experimental oxygen pressure if the degree of hemoglobin saturation is at all times in equilibrium with the oxygen tension. Actually this is the case only when the transit time of the transport along each segment of path is long compared to the oxygen-hemoglobin reaction times. When this is not the case, equilibration lags, giving lower oxyhemoglobin concentrations. The accumulation of this effect gives the difference between the calculated and observed values on the left side of Figure 1. When the oxygen tension exceeds that necessary to saturate the hemoglobin over most of the transport path, this lowering of oxyhemoglobin concentrations becomes experimentally unobservable.

Particular attention must be given to Figure 2*a* and the measurements on which it is based. Together with facts (*a*) and (*b*) stated earlier, it indicates that the enhanced transport due to hemoglobin is independent of oxyhemoglobin gradients and remains the same when there is no gradient at all.

Prior to availability of the gradient data (*f*), it has been proposed in several recent publications[7-9] that the enhanced oxygen transport by hemoglobin is the result of oxyhemoglobin diffusion, the calculations being based on the Fick equation. This led to equations, the end results of which checked with the experimental data. At steady-state conditions, this treatment implies a linear oxyhemoglobin gradient (Fig. 2*b*) which, however, is contrary to the facts. First, the integrated oxyhemoglobin concentration would be 50 per cent as compared with the observed 96.6 per cent (point on the right edge of Fig. 1). The same calculation, applied at other oxygen pressures, gives the dotted curve of Figure 1. Second, the oxygen tension gradient would be almost zero along most of the diffusion path and could not give the large transport by diffusion of dissolved oxygen calculated.[1-3]

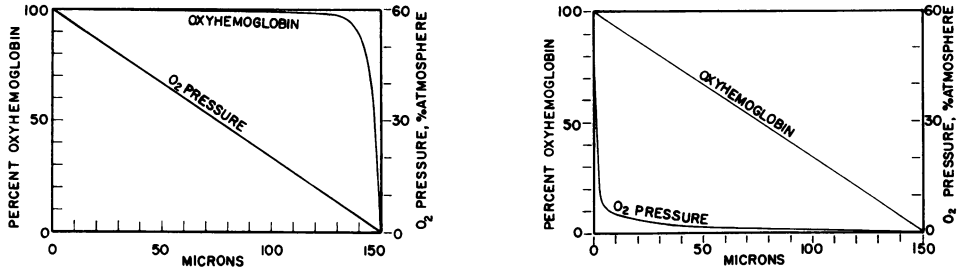

FIG. 2.—Oxyhemoglobin and oxygen pressure gradients in 0.15-mm Millipore filter membrane; (*a*) assuming equilibrium between oxyhemoglobin and linear oxygen pressure gradient, and (*b*) assuming oxygen transport by oxyhemoglobin diffusion.

The properties of the enhanced transport process may be summarized from the experimental facts. The transport depends on hemoglobin molecule kinetics (*d*). It has the characteristics of a diffusive process (*c*) but does not depend on oxyhemoglobin gradients (*a,b,f*) and hence is not due to oxyhemoglobin diffusion. The oxygen combined with hemoglobin is rapidly exchangeable at all the binding sites regardless of the degree of oxygenation (*e*).

It is postulated that the oxygen transport by hemoglobin results from exchange between binding sites of colliding hemoglobin molecules. The process itself is independent of the presence of oxygen on the sites, but the unidirectional transport is proportional to the oxygen content of the hemoglobin at the intake surface. The role of oxygen may be compared to that of an isotope tracer added to one side of an equilibrium reaction. As transport is by molecular collision, the diffusivity coefficient is probably the same as the heat diffusivity coefficient of the hemoglobin.

In a one-dimensional steady-state system the "oxygen site" transport by hemoglobin may be expressed by the simplified diffusivity equation:

$$\frac{dQc}{dt} = -\Delta \cdot \frac{dc}{dx} \cdot A, \tag{1}$$

where dQc/dt = rate of "oxygen site" transport,
Δ = diffusivity coefficient,
c = "site" concentration = oxygen capacity of hemoglobin, and
A = membrane area.

There is no dc/dx except in the sense of unidirectional transport. For unidirectional transport:

$$-\frac{dc}{dx} = \frac{C_{\text{in}}}{l} \cdot a, \tag{2}$$

where C_{in} = site concentration at input face,
l = membrane thickness, and
a = hemoglobin activity coefficient. The oxygen transport is given by

$$\frac{dQ_{O_2}}{dt} = \Delta \frac{C_{\text{in}}}{l} \cdot a \cdot F \cdot A, \tag{3}$$

where F = saturation fraction of hemoglobin. For heat conductivity:

$$\frac{dH}{dt} = -\Delta \cdot s \cdot d \cdot A \cdot \frac{dT}{dx}, \tag{4}$$

where s = specific heat, and d = density. If k = heat conductivity,

$$\Delta = \frac{k}{s \cdot d}. \tag{5}$$

From (3) and (5)

$$\frac{dQ_{O_2}}{dt} = \frac{k}{s \cdot d} \cdot \frac{A}{l} \cdot a \cdot C_{in} \cdot F. \tag{6}$$

An estimate of the value of k/sd may be made from the value for water. This is 0.00143 cal. cm/°C/sec at 20°C. Calculating the thermal diffusivity from the values of s and d gives:

$$\Delta_{H_2O} = 0.00143 \text{ cm}^2 \text{ sec}^{-1}.$$

To adapt this value to hemoglobin it is assumed that the diffusivity varies as the frequency of molecular collisions. This in turn is the mean molecular velocity divided by the distance between molecules. The mean molecular velocity varies inversely as the square root of the molecular weight. The distance between molecules varies inversely as the cube root of molecular concentration.

Let C_{Hb} = hemoglobin concentration in gm/cc,
M_{Hb} = 68000 = gram molecular weight of hemoglobin,
M_{H_2O} = 18 = gram molecular weight of oxygen, and
Δ_{Hb} = thermal diffusivity of hemoglobin.

Then

$$\frac{\Delta_{Hb}}{\Delta_{H_2O}} = \left[\frac{M_{H_2O}}{M_{Hb}}\right]^{1/2} \div \left[\frac{1}{M_{H_2O}} \div \frac{C_{Hb}}{M_{Hb}}\right]^{1/3}$$

$$= \left[\frac{M_{H_2O}}{M_{Hb}}\right]^{5/6} \cdot (C_{Hb})^{1/3} \tag{7}$$

$$\Delta_{Hb} = \Delta_{H_2O}\left[\frac{M_{H_2O}}{M_{Hb}}\right]^{5/6} \cdot (C_{Hb})^{1/3}$$

$$= 1.493 \cdot 10^{-6}(C_{Hb})^{1/3} \text{cm}^2 \text{ sec}^{-1}. \tag{8}$$

From (6)

$$\frac{dQ_{O_2}}{dt} = 1.493 \cdot 10^{-6} \cdot \frac{A}{l}(C_{Hb})^{1/3} \cdot C_{in} \cdot a \cdot F \tag{9}$$

Example: (Data from one experiment[4])

P_{O_2} = 50 mm on one side of membrane (zero on the other side),
C_{in} = 0.2 cc O_2/cc Hb solution,
F = 1 because P_{O_2} is above hemoglobin saturation pressure,

$\alpha_{O_2} = 0.028$ cc/cc/atmosphere, and
$C_{Hb} = 0.15$ gm/gm.

For the hemoglobin-enhanced transport, equation (9) gives:

$$\frac{dQ_{O_2}}{dt} = 1.493 \cdot 10^{-6} \cdot \frac{A}{l} \cdot (0.15)^{1/3} \cdot (0.2) \cdot a$$

$$= 1.587 \cdot 10^{-7} \cdot \frac{A}{l} \cdot a \text{ cm}^3 \text{ sec}^{-1}.$$

For O_2 diffusion in water, let transport rate be dQ_{O_2}'/dt and C_{in}' be the dissolved oxygen concentration at the input surface. Henry's Law gives

$$C_{in}' = \frac{50}{760} \cdot 0.028 = 0.001843 \text{ cm}^2/\text{cm}^2$$

using $\Delta = 2.4 \cdot 10^{-5}$ cm^2 sec^{-1},

$$\frac{dQ'_{O_2}}{dt} = \Delta \frac{A}{l} C_{in}'$$

$$= 2.4 \cdot 10^{-5} \cdot \frac{A}{l} 0.001843$$

$$= 0.442 \cdot 10^{-7} \cdot \frac{A}{l} \text{ cm}^2 \text{ sec}^{-1}.$$

The ratio of the enhanced O_2 transport to the diffusion transport is

$$\frac{dQ_{O_2}}{dt} \div \frac{dQ_{O_2}'}{dt} = \frac{1.587 \cdot 10^{-7} \cdot \frac{A}{l} \cdot a}{0.442 \cdot 10^{-7} \cdot \frac{A}{l}} = 3.58a.$$

The observed ratio is 3.0.

If the calculated and observed transport data are equated, the activity coefficient $a = 0.837$.

The activity coefficient "a" is fractional, and combines completeness of oxygen exchange on collision, and possible reduction of hemoglobin mobility by viscosity. Empirically this factor is near 0.8. Hence, the oxygen randomization between binding sites of colliding hemoglobin molecules is at least 80 per cent complete.

Conclusion.—(1) The transport takes place in the absence of oxyhemoglobin gradient. (2) The transport can be described as due to exchange of oxygen molecules by collision of hemoglobin molecules.

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*SINGLE-ACTIVE-X HYPOTHESIS: CYTOLOGICAL EVIDENCE FOR RANDOM INACTIVATION OF X-CHROMOSOMES IN A FEMALE MULE COMPLEMENT**

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According to the dosage compensation hypothesis, postulated by Lyon,[1, 2] one of the two X-chromosomes in a normal mammalian female complement becomes genetically inactivated at an early stage of embryonic development, and this inactive X, which is usually condensed or heteropycnotic at prophase, could be either paternal or maternal in origin in different cells of the same individual. This hypothesis is supported by evidence from a biochemical study of glucose-6-phosphate dehydrogenase activity in the human female[3] and has been confirmed by a recent study of Davidson, Nitowsky, and Childs.[4] In an attempt to test this hypothesis cytologically, Ohno and Cattanach[5] studied the prophase skin cells of a stock of mice whose wild-type alleles for the coat color genes of linkage group I had been translocated to the X. The male mice with X^tX^nY and females with X^tX^n chromosomes had a variegated coat color with light and dark patches, and a distinct heteropycnotic element was observed in their prophase skin cells. These investigators interpreted the heteropycnotic elements as inactive X-chromosomes and claimed that they could distinguish the heteropycnotic chromosome from the albino patches as the translocated-X (X^t) and its counterpart in the wild patches as normal-X (X^n) by comparing their total lengths. They thus concluded that random inactivation of X-chromosomes does occur in mammalian females as postulated by Lyon.[1, 2] But considering the extent of condensation and other morphologic changes that chromosomes undergo during early to late prophase, one could not be absolutely sure of distinguishing a heteropycnotic X-chromosome from a heteropycnotic X^t in prophase figures, particularly since the only criterion of identification is based merely on the comparative lengths of these two chromosomes, when X^n was derived from one prophase figure and X^t from another.

Autoradiographic studies on chromosome duplication[6–9] have revealed that in cultured cells from normal mammalian females, or in diploid polysomic-X cells, only one X completes its duplication along with the autosomes, while replication continues in the remaining X-chromosome or chromosomes after it is complete in the rest of the complement. Although the exact morphologic identification of the late-replicating chromosome in the human complement is difficult, autoradio-