Reports

Transport of Gases through Hemoglobin Solution

Abstract. A mathematical theory is developed to explain the observed enhancement of oxygen transport through solutions by hemoglobin. At high partial pressures of oxygen, ordinary diffusion through the solvent accounts for all transport of oxygen, but at low partial pressures the transport may be increased many fold by the presence of hemoglobin. This phenomenon is explained and the possible role of this phenomenon in living organisms is discussed. The theory also indicates a new method of determining dissociation curves from diffusion experiments.

Two recent articles (*1*, *2*) have described several interesting and perhaps important features of gas transport through hemoglobin solutions. Scholander (*1*) and later Hemmingsen and Scholander (*2*) reported observation of an enhanced diffusion of oxygen through solutions containing hemoglobin. Briefly these experiments are reviewed as follows.

A Millipore membrane saturated with a hemoglobin solution separated two gas chambers. In the first instance (*1*) one chamber was maintained at near vacuum while oxygen at a fixed pressure filled the other chamber. In the second case (*2*) oxygen at different pressures was maintained in the two chambers. Similar experiments were performed with nitrogen in place of oxygen. In both cases the flux of gas through the solution-filled membrane was measured.

The results of these measurements revealed that while the nitrogen flux was always simply proportional to the difference in partial pressures of nitrogen in the two chambers, as required by Fick's law of diffusion, such was not the case for oxygen. It was found that the rate of transport of oxygen was proportional to the difference of the partial pressures of oxygen in the two chambers only when the partial pressure in both chambers was appreciable. When the partial pressure of oxygen in the low-pressure chamber was very small, the rate of transport was several-fold greater for the same difference in oxygen partial pressure.

In particular, the first reported results (*1*) in which the low-pressure chamber was maintained at near vacuum showed that the O_2 transport could be represented by the equation:

$$\text{Total } O_2 \text{ flux} = N_2 \text{ flux} \times (0.56) + c$$

where 0.56 is the O_2/N_2 flux ratio in hemoglobin-free solution for the same difference in pressure, and c is a constant. The second report of results (*2*) revealed that this augmentation of transport was not constant, but was reduced by a back pressure of oxygen. Furthermore, the augmentation was shown to be proportional to the hemoglobin concentration of the solution, except at very high concentration.

Scholander (*1*) pointed out that the additional transport mediated by the hemoglobin could be due to the random Brownian motion of the hemoglobin molecules. Thus, a hemoglobin molecule could move from a region of high oxygen concentration to a region of low oxygen concentration. In doing so it would give up some of its associated oxygen which would be taken up by hemoglobin molecules in the region of low concentration. This, of course, would be accompanied by hemoglobin molecules moving in the opposite direction. In this manner a "bucket-brigade" would mediate oxygen transport. That thermal or Brownian motion plays an important role in the transport of oxygen was demonstrated by the fact that oxygen transport was greatly reduced when the solution was solidified with agar gel. Indeed, the theory which has been developed reveals that this is the mechanism of hemoglobin transport of oxygen, as well as of other gases which are associated with hemoglobin.

The theory which has been developed to describe these diffusion phenomenon is presented in detail elsewhere (*3*). It is shown that, for equal differences of partial pressure across a Millipore filter filled with hemoglobin solution, the flux of oxygen $\dot{N}_{O_2}$ and the flux of nitrogen $\dot{N}_{N_2}$ are related by:

$$\dot{N}_{O_2} = \left(\frac{D_{O_2} k_{O_2}}{D_{N_2} k_{N_2}}\right) \dot{N}_{N_2} + \frac{A\phi}{L} nXD_{Hb} \left(1 - S' [p_{O_2}(L)]\right) \qquad (1)$$

where D_{O_2} is the diffusion coefficient of O_2 in solvent; D_{N_2} is the diffusion coefficient of N_2 in solvent; k_{O_2} is Henry's law coefficient for O_2; k_{N_2} is Henry's law coefficient for N_2; A is the area of membrane; ϕ is the porosity of membrane; L is the thickness of membrane; n is moles of O_2 per mole of hemoglobin at 100 percent saturation; X is moles of hemoglobin per volume of solution; D_{Hb} is the diffusion coefficient for hemoglobin in solvent; S' is the fractional saturation of hemoglobin; and $p_{O_2}(L)$ is the partial pressure of O_2 on the low-pressure side. (Here p_{O_2} on the high-pressure side is assumed to be large enough to consider $S' = 1$ on this side.) This equation, which is derived from the theory of Brownian motion, shows that the flux of oxygen is a function of oxygen back pressure, $p_{O_2}(L)$. In particular the oxyhemoglobin dissociation curve, S' as a function of p_{O_2}, enters explicitly.

In every respect this theory agrees with the reported experimental observations. Thus the augmentation of O_2 transport is proportional to the hemoglobin concentration, X, up to concentrations great enough to modify the value of D_{Hb}. Effects of pH, temperature, CO_2, and so forth are also implied through the dependence of D_{Hb} and the dissociation curve on such factors. The reported effect of gelation of the solution is also indicated.

Thus, according to the theory of Brownian motion, one has for diffusion of hemoglobin molecules:

$$D_{Hb} \approx \frac{RT}{6\pi\eta r A_0} \qquad (2)$$

where R is the gas constant; T is the absolute temperature; η is the viscosity of solvent; r is the effective molecular radius of hemoglobin; and A_0 is Avo-

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

gadro's number. Hence D_{Hb}, and the augmentation of O_2 transport, decreases with increasing solvent viscosity.

An interesting possibility arising from the theory described here is in the determination of the oxygen-hemoglobin dissociation curve from diffusion experiments. Thus, from Eq. 1

$$S'(p_{O_2}) = \frac{(\dot{N}_{O_2})\ \max - \dot{N}_{O_2}(p_{O_2})}{(\dot{N}_{O_2})\ \max - (\dot{N}_{O_2})\ \min}$$

where the difference in p_{O_2} is always constant, but p_{O_2} on the high-pressure side is always great enough for $S' = 1$ to be assumed, and p_{O_2} in the equation is on the low-pressure side. Such experiments could be performed with a fixed CO_2 partial pressure on both sides of the membrane. This shows that measurements of $\dot{N}_{O_2}$, and p_{O_2} on the low-pressure side, can be used to determine the dissociation curve.

While the theory described has accounted for all features of enhanced transport of gases O_2, CO_2, or CO which associate with hemoglobin, it in itself does not indicate the role of this mechanism within living organisms. Since the dissociation curves for O_2 and CO_2 are similar in form, it is evident that the mechanism is the same for both of these gases within the living organism. In particular transport of gases within the red blood cell should be described by this theory.

A result of the theory described here is that the effective diffusion coefficient for O_2 in a hemoglobin solution is:

$$D_{O_2} = D_{O_2} + nXD_{Hb}\frac{dS'}{dC_{O_2}}$$

where C_{O_2} is O_2 concentration in the solvent. It is evident that in regions of low concentration of the gas in question the transport of the gas is much greater for a given gradient of concentration than in a region of high concentration. This follows since dS'/dC_{O_2} decreases rapidly with increasing C_{O_2}. Thus an important role of this mechanism in living organisms could be to maintain O_2 and CO_2 transport even with lowered gradients of gas concentration. Thus oxygenation of hemoglobin in red blood cells should be more rapid at low oxygen partial pressures in blood plasma. It could also mediate an equitable distribution of gases to tissues, that is, as the concentration of O_2 falls in a given region of tissue the transport of O_2 to that region is spontaneously increased by this mechanism.

In conclusion it should be noted that the theory described here must be modified to include the reaction rate of gas with hemoglobin if the gas concentration in the solution changes rapidly with time.

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References

1. P. F. Scholander, *Science* **131**, 585 (1960).
2. E. Hemmingsen and P. F. Scholander, *Science* **132**, 1379 (1960).
3. R. E. Collins, "Transport of gases through hemoglobin solutions," *Bull. Math. Biophys.*, in press.

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Pollination of Saguaro Cactus by Doves, Nectar-Feeding Bats, and Honey Bees

Abstract. In a large cage, free-flying western white-winged doves, nectar-feeding *Leptonycteris* bats, and honey bees were each effective as cross-pollinators of self-sterile saguaro flowers. Seed production and seed viability were not significantly different in fruit from flowers pollinated by these agents. Pollination is not a limiting factor in saguaro repopulation.

Since the turn of the century the saguaro, or giant cactus [*Carnegiea gigantea* (Engelm.) Britt. & Rose], has failed to repopulate itself in certain areas within the range of its habitat in Arizona (*1*). This report concerns the effect of pollination and of certain pollinating agents on the production of viable seed needed for plant establishment.

The saguaro flower begins to open shortly after nightfall and is usually in full bloom by midnight, when both pollen and nectar are available. The flower usually closes by late afternoon of the same day. Previous work (*2*) showed that the flower may be pollinated both at night and in the daytime and that it is self-sterile and is not wind-pollinated. These findings led to the question: What agents are responsible for pollen transfer?

Preliminary observations indicated that severed saguaro branches (arms), 2 to 5 feet long, would bud, flower, and set fruit satisfactorily. In our experiment 45 arms, removed mainly from windfall plants just before flowering, were placed upright in a 12-mesh plastic-screen cage, 12 by 24 by 9 feet high, located in a cactus forest (*3*).

The prevalence of the day-flying western white-winged doves [*Zenaida asiatica mearnsi* (Ridgway)] during the period of saguaro flowering, and the presence of pollen on their heads, led us to include them as test agents. Six birds of unknown sex were released into the cage (*3*). They soon accepted confinement and were not injured the few times they collided with the plastic screen. These doves fed at will on the nectar in the flowers and on available rolled oats and water.

Saguaro pollen has been found in the stomach and fecal material of nectar-feeding bats (*Leptonycteris nivalis* Saussure) (*4*). This finding, and our observations that pollination may occur at night, suggested that we try these bats. Nine females, three of which were gravid and four with living young, were collected in nearby Colossal Cave (*3*). They were kept in a 12 by 24 by 24 in. screen-bottomed box. Screen was also provided in the ceiling of the container for them to cling to. Because of their sensitivity to excessive temperatures, the bats were kept in an air-conditioned building during the daytime. At nightfall the box with bats was suspended in the cage and gently opened. However, the bats did not break their cluster for an hour or more; they always returned to the box voluntarily before dawn.

Flowering stalks of the century plant (*Agave schottii* Engelm.) were placed in the cage as an attractant for the bats in the early evening before the saguaro

Table 1. Saguaro fruit set, seed production, and seed viability as related to mode of pollination, Tucson, Arizona, 1960.

Mode of pollination	Flowers exposed			Seeds*	
	Dates (inclusive)	No.	Set (%)	Per fruit† (No.)	Germination‡ (%)
Doves	9–18 May	338	44.7§	2634	83.5
Bats	24–26 May	86	61.6§	1887	80.2
Honey bees	27 Apr.-8 May	193	51.8§	1751	90.4
Natural, field-pollinated	8–16 May	132	53.8	2721	83.4
Hand cross-pollinated	26 Apr.–23 May	79	70.9§	2439	91.9
Self-pollinated	26 Apr.–20 June	124	0	—	—

* Data not significantly different at 5-percent level. † Based on counts of seed in ten fruits. ‡ Based on three 100-seed replicates from each of ten fruits. § Includes all fruits harvested plus injured buds that dropped after 7 days.