

required to build the ridge is unknown, but, on any reasonable assumption of rate of vulcanism, it was at least 10^7 years.

The Hawaiian region has had a prolonged and complicated history with intermittent vulcanism, formation of platforms and benches at sea level, and both local and regional subsidence. It may be anticipated that systematic surveying and dredging of drowned erosional terraces on the sides of the Hawaiian and other island groups will rapidly increase knowledge of the history of the Pacific basin and its faunas.

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Diffusion of Carbon Monoxide through Thin Layers of Hemoglobin Solution

Abstract. The facilitation of carbon monoxide flux through a thin layer of hemoglobin solution is increased in the presence of oxygen. This is consistent with the hypothesis that the facilitation results from diffusion of carbon monoxide—hemoglobin as well as carbon monoxide, but that the overall process is partially limited by the reaction rates of carbon monoxide and hemoglobin.

Scholander and Hemmingsen (1, 2) have reported that O_2 passes through thin layers of hemoglobin (Hb) or myoglobin solution at rates several times faster than N_2 . On the supposition that this phenomenon represents diffusion of HbO_2 in addition to O_2 , a supposition that appears reasonable to others as well (3), an even greater facilitation of CO

flux through hemoglobin solution would be expected, provided its partial pressure was low. Therefore we used an apparatus modeled after that of Scholander, in which a 0.015-cm-thick Millipore filter separated two gas mixtures, both at atmospheric pressure (Fig. 1), to measure the CO flux when the filter was saturated with hemoglobin solution or water. The filter holder was made of stainless steel and the filter itself was supported on a screen of similar metal. Additional circuitry was added (i) to provide convective mixing of both gas mixtures by two electrical heating coils (flow of approximately 6 ml/min), (ii) to permit replacement of the gases on either side of the filters, and (iii) to provide collection of samples by means of a needle stuck through a rubber vaccine bottle top. Both chambers were evacuated and then filled with their respective gas mixtures at atmospheric pressure. Each time a sample was removed (2 ml) an equivalent volume of mercury was added. This procedure was found to maintain the pressure in each chamber within 1 to 2 cm-H₂O of atmospheric pressure as determined with a water manometer. The gases were kept saturated with water at the vapor tension of saline. At the start of an experiment the total volume of gas in the lower circuit was 102 ml and in the upper circuit was 42 ml. The exposed area of the filter paper was 7 cm².

The filter paper was soaked with water or with hemoglobin solution, the excess was wiped off, and the paper was clamped in the holder. The two chambers were then evacuated and the gas mixtures were added. Two-milliliter gas samples were removed alternately from each chamber every 15 minutes for about 2½ hours and analyzed in a gas chromatograph. In most experiments the lower chamber contained about 1.5 percent neon and varying concentrations of CO up to 2.5 percent, in addition to O_2 and N_2 . The upper chamber contained the same O_2 concentration as the lower but no CO or neon. The hemoglobin solution was prepared from thrice-washed, freshly drawn, human red cells which were lysed by freezing and thawing, after which they were reconstituted to 120 percent of the original volume with bicarbonate buffer (pH 7.4). The average final Hb concentration was 10.8 g/100 ml. Carbon monoxide flux showed no trend after the first 30 minutes; therefore we concluded that a steady state had been reached by that time.

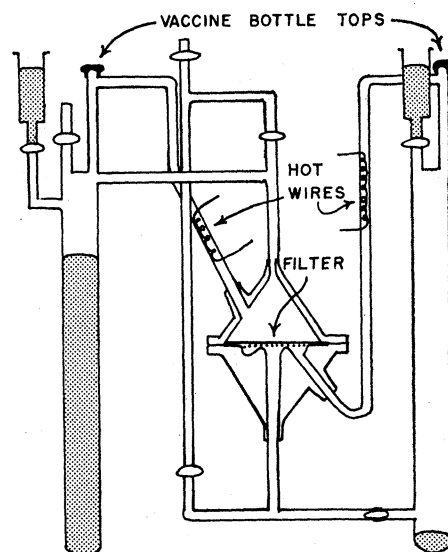


Fig. 1. Diffusion apparatus, which was immersed in a temperature-regulated bath at 37°C. Volume of the upper chamber and tubing, 42 ml; that of the lower chamber and tubing, 103 ml.

Results of more than 25 experiments on 14 preparations are presented in Fig. 2. The analytical error in each point is less than 0.01 mm³/min. The "unfacilitated" diffusion of CO was calculated as the diffusion rate of neon

$$(\text{mm}^3/\text{min}) \times 0.018/0.016 \times (20/28)^{1/2}$$

0.018 and 0.016 being the Bunsen solubility coefficients of CO and neon (4), respectively, in water. The transfer of CO is enhanced as much as 15-fold by the presence of the hemoglobin in solution. It seems reasonable to assume that this facilitation results from the diffusion of COHb in addition to dissolved CO, and that the [COHb] and [O₂Hb] at each surface of the film are in equilibrium with [CO] and [O₂] in the respective gas phases. Knowing these gas tensions it was possible for us to calculate the equilibrated [COHb] by means of

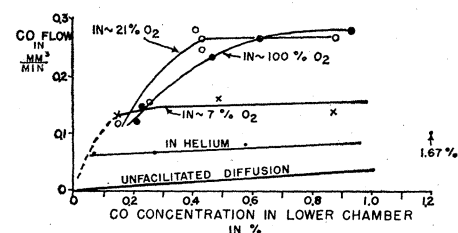


Fig. 2. Flux of CO through hemoglobin solutions as a function of CO concentration in the gas of the lower chamber, at different O_2 tensions. Gaseous CO concentration of the upper chamber was approximately zero initially. Gaseous O_2 concentrations were the same in both chambers.

the relationship of Haldane, $[\text{COHb}] = 245 [\text{O}_2\text{Hb}] [\text{CO}] / [\text{O}_2]$, and the normal hemoglobin dissociation curve (5). Although $[\text{CO}]$ in the upper chamber was zero at the start, it rose during the experiment, and its average value during the 30- to 60-minute period was used. As $[\text{O}_2]$ rose, the calculated equilibrated $[\text{COHb}]$ difference across the film fell; the mean values were 87, 68, 64, and 47 percent for the helium, 7 percent O_2 , 21 percent O_2 , and 100 percent O_2 experiments, respectively, in Fig. 2. Thus the facilitated CO transfer rises under conditions where the equilibrated $[\text{COHb}]$ difference falls.

The ratio, facilitated flux / diffusion flux of CO, obtained experimentally, is significantly less than would be predicted from the data of Scholander (1) on O_2 , assuming that facilitation is produced by the diffusion of hemoglobin + ligand, that chemical equilibrium exists at the film surface, and that the diffusion coefficients of hemoglobin and its compounds are the same. For example, when the $[\text{CO}]$ in the lower gas chamber is 0.065 percent and $[\text{O}_2]$ is zero, the difference in concentration of dissolved CO across the film is

$$0.065 \text{ percent} \times 0.018 = 0.000012 \text{ ml/ml}$$

In the absence of O_2 , 0.065 percent CO will produce a COHb saturation of 97 percent (5). Therefore the difference in $[\text{COHb}]$ across the film will be

$$0.97 \times 10.8/100 \times 1.34 = 0.14 \text{ ml/ml}$$

where 1.34 equals the total milliliters of CO bound per gram of Hb (5). Assuming that $D_{\text{CO}}/D_{\text{COHb}} = 1/50$ (6, and as calculated from the data of Scholander, 1), the facilitated flux / diffusion flux should equal

$$0.014 / (0.000012 \times 50) = 233$$

From Fig. 2 the experimentally determined ratio is

$$\begin{aligned} (0.065 \text{ mm}^3/\text{min}) / \\ (0.0028 \text{ mm}^3/\text{min}) = 23.3, \end{aligned}$$

one-tenth that predicted.

One explanation of these findings is that the reaction velocities of CO and Hb are limiting the overall process and that chemical equilibrium does not exist between hemoglobin and the gases at the film surfaces. The equations describing steady state diffusion and reaction of CO in a layer of hemoglobin solution (7) are

$$\begin{aligned} -D_{\text{CO}} \frac{\partial^2 [\text{CO}]}{\partial X^2} &= D_{\text{COHb}} \frac{\partial^2 [\text{COHb}]}{\partial X^2} \\ &= l[\text{COHb}] - l'[\text{CO}][\text{Hb}] \end{aligned}$$

D_{CO} and D_{COHb} are the diffusion coefficients (cm^2/sec) of CO and COHb respectively in hemoglobin solution; l (sec^{-1}) and l' ($\text{sec}^{-1}M^{-1}$) are the reaction velocity constants for the dissociation and association reactions respectively; $[\text{CO}]$, $[\text{COHb}]$, and $[\text{Hb}]$ are the molar concentrations of dissolved CO, carboxyhemoglobin, and reduced hemoglobin, respectively; X is distance in centimeters; l' is not a proper constant in that it increases as $[\text{Hb}]$ decreases (8).

It is impossible to obtain an analytical solution of the differential equations, and numerical methods are needed (7). It can be shown by approximate methods that the transfer of CO in these experiments is partially limited by the rate at which CO can dissociate from COHb and leave the upper surface of the film. Oxygen, by competing with the CO for the reduced hemoglobin, facilitates this process and increases the overall rate of transfer. Since there will probably be little reduced Hb in the layer, O_2 will tend to dissociate from O_2Hb at the lower surface where the CO tension is higher, and to associate with Hb at the upper surface, where the CO tension is lower. This will result in a movement of O_2 into the lower chamber in the absence of an O_2 tension gradient between the gas chambers.

Not only have Scholander and his

associates pointed out an interesting phenomenon, but their work raises the question as to whether these, or thinner, layers of solution could not be used to measure chemical reaction velocities of biological interest (9).

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Extrathyroidal Iodide Pump in Tadpoles (*Rana grylio*)

Abstract: Large amounts of iodide are accumulated in the gastrointestinal tract of tadpoles despite the presence of ligatures on the bile duct or esophagus or on both structures. These ligatures exclude the possibility that the iodine-131 tracer reaches the gastrointestinal tract by way of the bile or esophagus. The iodide in the gastrointestinal tract localizes largely in the stomach. The stomach therefore acts as an iodide pump and may be responsible for the maintenance of the iodide levels that are needed in metamorphosis.

The significance of the thyroid gland in amphibian metamorphosis has been well established (1). Swingle (2) also reported that iodide administered as either iodine or iodide was the active principle capable of inducing metamorphosis in the thyroidectomized as well as in the normal tadpole (3). The utilization of iodide by the animal is then a matter of importance since, even in the absence of the thyroid gland, the animal is said to achieve metamorphosis in the presence of adequate amounts of iodide in its environment

(2). Gudernatsch however has never confirmed the observation of a stimulatory effect of iodide or iodine on metamorphosis (4). The distribution of I^{131} in some tadpole tissues has been described, and the presence of I^{131} in the gut and pigmented tissues has been noted (5). No attempt was made to quantitate the I^{131} accumulated in these tissues (5).

In the present study *Rana grylio* tadpoles in the early hindlimb-bud stage were used. Three groups (ten tadpoles in each) were subjected, under tricaine (0.05 percent) anesthesia, to surgical procedures in which ligatures were placed on the esophagus near the cardiac sphincter, or on the bile duct, or on both the esophagus and the bile duct. In another experiment ligatures were placed around the esophagus and pylorus of ten tadpoles. Five tadpoles served as control groups for each experiment. Since the operated animals survived only 5 to 7 days after the ligatures were placed, the experiments were carried