cable to humans, continuous inhalation of air containing 0.1 ppm NO_2, or concentrations of other nitrogenous compounds giving rise to NO_2^- at equivalent levels, will lead to a concentration of NO_2^- in body fluids and tissues of about 400 ng kg^{-1} (about 30 n*M*) (*28*). Consequently, we suggest that continuous exposure to atmospheric concentrations of NO_2 or other NO_2^- precursors below 0.1 ppm are unlikely to produce an extrapulmonary health risk (for example, by production of carcinogenic nitrosamines). By comparison, a continuous exposure to more than 100 ppm NO_2 would be required to produce gastric concentrations of NO_2^- comparable to the transient micromolar values associated with the ingestion of a meal containing 2 to 3 mg of NO_2^-. The in vivo concentrations of NO_2^- that may saturate the "normal" metabolic pathways are still unknown.

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28. $(d[NO_2^-]/dt) = -k[NO_2^-]$, which is equal to the input rate. Thus, the equilibrium $[NO_2^-]$ is ~ 400 ng kg^{-1}.
29. We thank K. A. Krohn for his contributions to the ^{13}N biology program and this work. We acknowledge the assistance of M. Doughty, C. Giacelli, L. Levy, the Digital Systems Group, and the staff of Crocker Nuclear laboratory. We thank J. C. Meeks, J. P. Witter, C. A. Mathis, J. Macy, H. P. Misra, M. F. Miller, and L. S. Rosenblatt for valuable discussions. This work was supported by the State of California Air Resources Board (contract A0-031-31).
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Diffusion Coefficients of Respiratory Gases in a Perfluorocarbon Liquid

Abstract. *Although great quantities of respiratory gases dissolve in a perfluorocarbon liquid used to formulate artificial blood, their diffusion rates in this liquid do not exceed those in water.*

Some liquid perfluorocarbons can dissolve far greater quantities of gases than other liquids at comparable pressure (*1*). They do not react with the gases in solution; the high energy content of the C–F bonds [approximately 120 kcal/mole (*2*)] accounts for their lack of reactivity (*3*) and is, in part, also responsible for their lack of toxicity.

Aqueous dispersions of perfluorocarbons stabilized by nonionic detergents have been formulated as possible blood substitutes (*4*), for clinical infusion (*5*), or for the perfusion of isolated organs destined for transplantation (*6*). The pure compounds find another promising application in liquid barrier filters (*7*), highly efficient devices that permit gases to pass a continuous liquid phase by diffusion while capturing all particulate contaminants carried in them.

Discussions of the possible value of perfluorocarbon preparations as blood substitutes always refer to their enormous gas-carrying capacity. Correlations exist between the solubility of gases in fluorocarbon liquids and the boiling points, densities, molecular weights, viscosities, and surface tensions of the liquids (*8*). However, the solubility is only a measure of solute uptake and does not distinguish between the solute molecules freely available in the solvent and those irreversibly bound to it; it is not a parameter defining the gas transfer capacity of a liquid. As both the solubility and the passage of gases are important functional criteria in all the applications given above, it would appear appropriate to state not only the solubility but also the

Table 1. Diffusion coefficients of respiratory gases in perfluorotributylamine at 22°C. The last column shows the mean standard deviation of experimental points, grouped by time and diffusion distance, from theoretical values.

Gas	Diffusion coefficient (cm^2 sec^{-1})	Standard deviation (percent)
CO_2	1.3×10^{-5}	2.7 (N = 7)
N_2	1.4×10^{-5}	2.8 (N = 8)
O_2	2.0×10^{-5}	2.4 (N = 9)

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diffusion coefficient whenever the use of liquids to provide oxygen or other gases is discussed (*9*).

We have followed the diffusion of respiratory gases in perfluorotributylamine (*10*), a liquid that is the principal ingredient of a commercially available preparation of artificial blood (*11*). The diffusion of gases in the liquid phase was measured by holographic interferometry (*12*). The holographic image of the test liquid in a gas-tight cell with windows of the highest optical quality is brought to interference with a hologram of the same system at reference condition. An interferogram is produced in real time, monitored on a television screen, and recorded on film. The method is sensitive, fast, and reliable; it permits measuring transient changes of the liquid's refractive index, n, with time. One can, for instance, follow the profile of gas concentration in a solvent and measure the rate of the processes of solution and diffusion without perturbing the system.

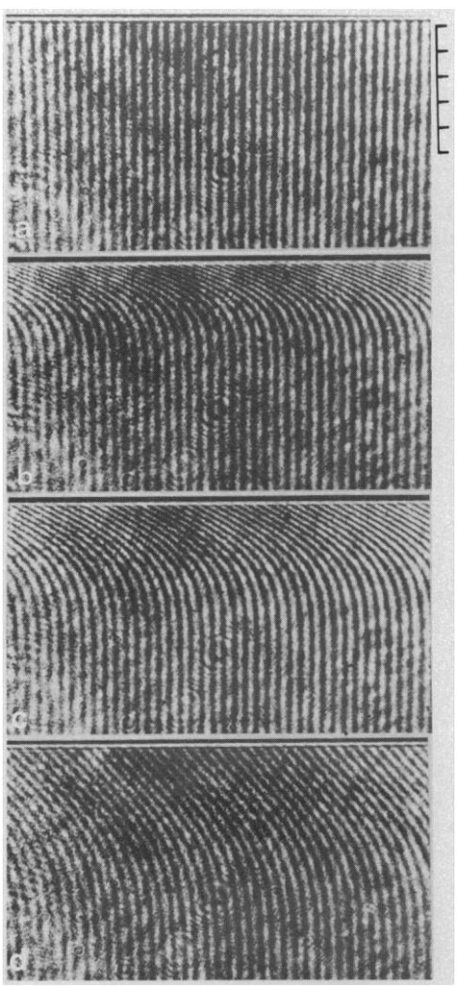

Fig. 1. Interferograms demonstrating the gradual diffusion of oxygen into perfluorotributylamine at times (a) 0, (b) 5, (c) 10, and (d) 30 minutes. The O_2 pressure applied was 11.8 pounds per square inch (8.4×10^4 N m^{-2}); temperature was 22°C. The scale (0 to 5 mm) gives the distance from the gas-liquid interface.

Figure 1 is a composite of the holographic interference patterns photographed in a single experiment at times from zero up to 30 minutes after pure oxygen was applied to the surface of the perfluorocarbon. At 5 minutes the liquid's surface is saturated with gas and any heat that might have been generated by the pressure jump is dissipated (*13*). As predicted by the Lorentz-Lorenz expression for solutions (*14*), the continued gas ingress produces proportional decrements in the liquid's refractive index, linear with concentration (*12, 15*). This change in the optical characteristics with time is visible as a fringe shift, or the progressive displacement of the fringes from an initially orthogonal intercept with the gas-liquid interface. The shift is equivalent to a change in the optical path length through the cell, directly proportional to the refractive index decrement, and linear with concentration (*15, 16*). The point where the fringes deviate from the vertical shows directly and immediately the calibrated depth to which a gas has diffused into the liquid until that time. The photographs attest to the remarkable stability of the holographic interferometer system as used. At depths beyond the point to which diffusion has progressed, the fringes are perfectly vertical; there is no indication of convection flow from any of the possible causes.

Since the fringe shifts express the refractive index decrement with concentration, one could calculate the absolute quantities of dissolved materials interferometrically and obtain a solubility coefficient (*17*). This would require determining Δn in a differential refractometer fitted, for gas solubilities, with a jacketed high-pressure measuring cell. Without the provision to measure at different pressures, a regular gas-tight differential refractometer could be used and the gas solubility determined in the usual way, assuming that Henry's law applies. The difference in refractive index between the gas and liquid phases can also be obtained from the ratio of the number of fringes counted above and below the interface (*12*).

Pure oxygen, carbon dioxide, and nitrogen were applied to the surface of the degassed liquid at pressures below 13.2 pounds per square inch (that is, $< 1 \times 10^5$ N m^{-2}). The absolute pressure values are irrelevant as long as the liquid's topmost layer is saturated with gas: the concentration difference within the liquid itself, in a direction perpendicular to the gas-liquid interface, constitutes the driving force for diffusion, not the concentration difference across the interface. Fick's second law for these boundary conditions (semi-infinite diffusion) leads to an equation that gives the concentration profile with time and depth as an error function of D, the diffusion coefficient (*18*). The theoretical concentration profiles for D between 0.3×10^{-5} and 3.0×10^{-5} cm^2 sec^{-1} were calculated and are available as tables (*19*). Evaluation of an experiment involves only a comparison of the curved fringes with the tabulated data to a best fit. Since a diffusion coefficient can be obtained from the percent change in saturation at the gas-liquid interface with depth and time, neither the absolute quantity of gas dissolved nor the absolute value of the refractive index is required.

A typical result is shown as Fig. 2. The oxygen concentration profile in the perfluorocarbon liquid was determined by measuring the horizontal shifts of five fringes each in three separate experiments, 10 minutes after admitting oxygen to the interferometer cell. Experimental and theoretical concentration profiles were compared as described and a diffusion coefficient selected on the basis of least square differences between grouped experimental data and the corresponding points on the theoretical diffusion curves. The results of these and other experiments were consistent, although the data taken farther from the interface were more scattered. This is a consequence of an increased compilation

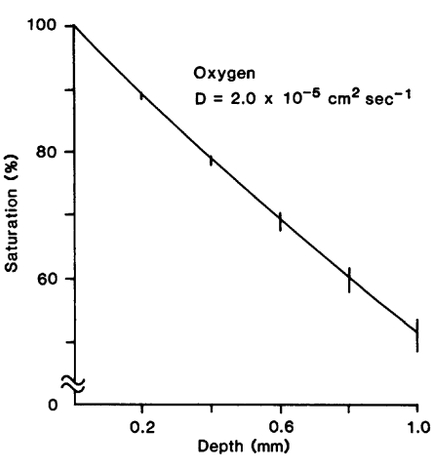

Fig. 2. Percentage of oxygen saturation of perfluorotributylamine at depths to 1 mm from the gas-liquid interface 10 minutes after application of O_2. Data are from three experiments at pressures of 8.1, 11.8, and 13.2 pounds per square inch, respectively; temperature was 22°C. Solid line represents theoretical values for the diffusion coefficient $D = 2.0 \times 10^{-5}$ cm^2 sec^{-1}. Vertical bars give the standard error of the mean.

error as the deviation of the fringes from the vertical becomes smaller.

The results of 24 diffusion experiments with three gases at nine pressures are given in Table 1. The diffusion coefficients rank the gases in the inverse order of their molecular dimensions (*20*), the same order as in water solutions (*13*), as expected from Stokes' law (*21*). Graham's inverse relation between the root of a solute's molecular weight and its diffusion coefficient (*22*) was not observed.

Neither the absolute nor the relative solubilities, in perfluorotributylamine, of the three gases examined [$CO_2 > O_2 > N_2$ (*3*)] were related to the diffusivities found. This emphasizes the point that the two parameters must be considered as distinct physical entities, a fact which is also evident from their opposite temperature coefficients in certain instances (*23*).

The diffusion coefficients of respiratory gases in a perfluorocarbon liquid will make it possible to estimate the time needed to saturate pure fluorocarbon liquids or their dispersions under a given partial gas pressure, and also the time or gradient, or both, required to release these gases again. As a consequence, the rate of flow necessary for maintaining a desired gas pressure in a flow system and the optimum quantity of liquid in circulation can be calculated.

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Localization of a Circadian Pacemaker in the Eye of a Mollusc, *Bulla*

Abstract. *The eye of the marine mollusc* Bulla *contains a circadian pacemaker which, along with critical entrainment pathways, is located among a small group of neurons at the base of the retina. Long-term intracellular recording from cells of the organized photoreceptor layer, which constitutes most of the retinal volume, indicates that these cells are not involved in generating the rhythm since rhythmic changes in membrane potential were not observed. In addition, surgical removal of the entire photoreceptor layer does not alter the period of the circadian rhythm and does not prevent phase shifts by light pulses.*

The eye of the marine gastropod *Aplysia*, which expresses a circadian rhythm in the frequency of spontaneous optic nerve impulses (*1*), has been important in studying the cellular basis of circadian rhythms. The retina exhibits robust free-running circadian rhythms in vitro which can be phase shifted by the application of light pulses (*2*). The exact location of the pacemaking system is not known (*3*), but it is clear that a small fragment of retinal tissue containing photoreceptors and higher order cells will support a circadian rhythm (*4*).

In *Bulla*, the cloudy bubble snail, the eye expresses a circadian rhythm similar to that of *Aplysia* (*5*), but the retina of *Bulla* has fewer and larger cells than that

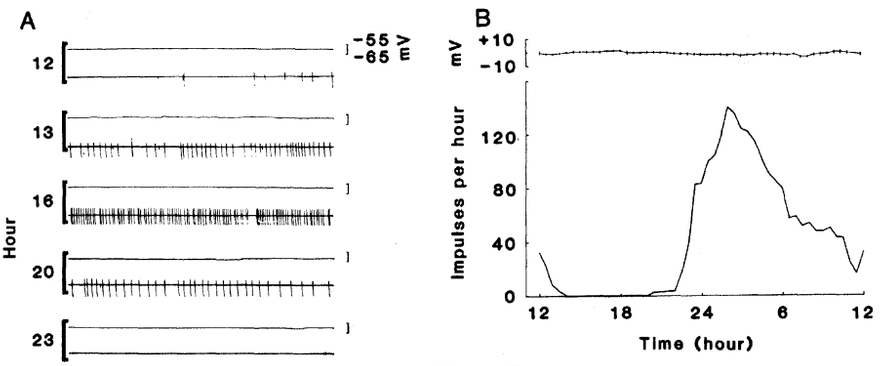

Fig. 1. Simultaneous recordings of photoreceptor membrane potential and optic nerve impulse activity. (A) Each line is a 30-minute sample taken from a continuous polygraph record at the hour specified. The upper trace for each pair is the actual measured resting membrane potential of a photoreceptor; the lower trace is an extracellular recording from the optic nerve. For long-term recording the lens was removed to make the receptors accessible, and the eye was suspended in petroleum jelly to minimize vibrations. Intracellular recordings were obtained with 20- to 40-megohm glass microelectrodes. The bathing medium was artificial seawater (Instant Ocean, 30 m*M* Hepes) maintained at 16°C. All recordings were made in continuous darkness, although eyes received 5 to 10 minutes of high-intensity light during impalement; the previous dawn was at 1400 hours in a light-dark cycle of 12 hours of light and 12 of darkness. (B) Average membrane potential and optic nerve impulse frequency from four preparations. The upper trace is a moving average (bin width = 0.25 hour, window = 0.5 hour) showing relative resting membrane potential, vertical bars represent standard deviations. The lower trace is the mean optic nerve impulse frequency from the four preparations. All records were made in darkness; previous dawn was at 2400 hours.

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