masked under physiological conditions have been previously demonstrated; for example, certain human serums were found to contain antibodies with specificity for a hidden antigenic determinant on the immunoglobulin G molecule which could be revealed by pepsin digestion (*5*). In rabbit serums, agglutinators were found with specificity for buried antigenic determinants on the Fab fragment of enzymatically digested rabbit immunoglobulin (*6*).

Although the functional significance of the hidden C_L site remains to be established, we have demonstrated that the hidden C_L site can be exposed on the whole immunoglobulin molecule. Intact immunoglobulin G, immunoglobulin A, and immunoglobulin D myeloma proteins and immunoglobulin M Waldenström macroglobulins (all type κ proteins) were treated with 8*M* urea, *p*H 8.9; subsequent immunochemical analyses, in which an antiserum specific for the hidden C_L antigenic site was used, revealed that the hidden site was exposed on the molecules belonging to the four different classes by treatment with the dissociating agent. The urea-treated immunoglobulins gave a precipitin reaction of identity with a C_L isolated from a κ chain. Similarly, in the presence of a dissociating agent (8*M* urea), pooled human immunoglobulin G (Cohn fraction II) was unfolded to expose the hidden C_L antigenic site.

The fact that the hidden C_L site can be exposed on the complete immunoglobulin molecule without cleavage of the molecule but simply by unfolding of the molecule generates specific questions regarding the function of the hidden antigenic site on the C_L. One might speculate that this site becomes exposed when the tertiary structure of the immunoglobulin molecule is altered as a result of reaction of the molecule with antigen, and that the site participates in the stabilization of the antibody molecule. The precise location of this hidden site in the C_L and the functional role of the site remain to be determined.

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19 October 1972

Bubble Formation in Physical and Biological Systems: A Manifestation of Counterdiffusion in Composite Media

Abstract. *The counterdiffusion of gases across a composite layer can lead to supersaturation and development of bubbles within the layer. A physicochemical model has been derived to predict the extent of such supersaturation; experiments with inert liquid layers confirm predictions. These findings explain the evolution of cutaneous lesions observed in man during simulated deep-sea dives and the cutaneous lesions and intravascular bubbles experimentally induced in pigs by exchanging certain inert gases across the skin. The phenomena associated with counterdiffusion have widespread physical and biological implications.*

In an attempt to explain several puzzling physiological phenomena observed during simulated diving experiments, we have performed studies of gas counterdiffusion in composite layers. The theoretical analysis and experimental work which followed this line of inquiry have uncovered a variety of effects concerning gas exchange with potential applications in diverse fields, including hyperbaric and underwater physiology, anesthesiology, and membrane biophysics, and in studies of membrane separations and nucleation phenomena.

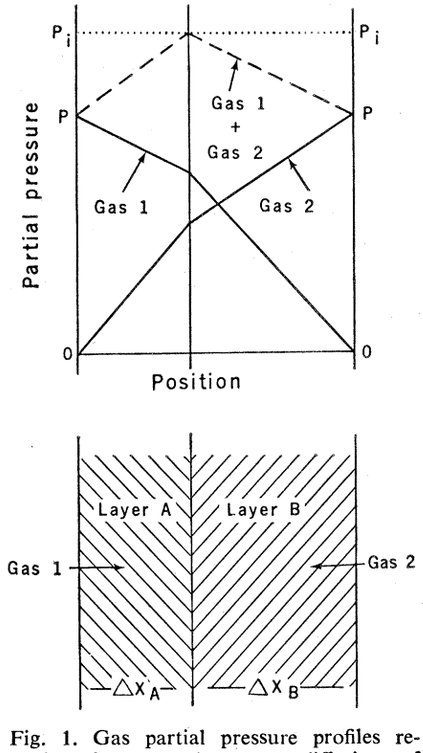

Fig. 1. Gas partial pressure profiles resulting from steady counterdiffusion of two inert gases through a two-layer composite of materials having different permeabilities for the two gases (see text).

The initial observations were made during a series of experiments carried out at the Institute for Environmental Medicine, University of Pennsylvania (Predictive Studies III) (*1*). Subjects in a helium-oxygen environment at a constant elevated pressure developed intense itching; gross, confluent maculopapular skin lesions (*2*); and an incapacitating vestibular derangement including vertigo, nausea, and nystagmus (*3*) within an hour after beginning to breathe a nitrogen-oxygen or neon-oxygen mixture through a mask or mouthpiece. Itching and skin lesions in man under related circumstances had been reported by investigators at Duke University (*4*). The skin manifestations could be prevented by covering exposed skin areas with a relatively impermeable suit ventilated with the same gas mixture that was being breathed.

The investigators at Duke had postulated osmotic gradients and the water flux produced by these gradients (*5*) as a causative mechanism for the skin lesions. We examined the counterdiffusion of two gaseous species through a two-layer structure and concluded (*6*) that, under the proper conditions, supersaturation with the attendant possibility of bubble development would exist in some region within the two-layer system (that is, the sum

of the two gas partial pressures would exceed the ambient pressure, which is equivalent to the statement that the amount of gas dissolved would exceed that corresponding to equilibrium at the ambient pressure).

The counterdiffusion supersaturation phenomenon is most readily illustrated by assuming constant diffusivities and ideal solubility relationships for the gases. These restrictions are not necessary, but they simplify the analysis and lead to the possibility of developing quantitative conclusions and criteria through a mathematical model. Figure 1 illustrates the linear partial pressure profiles which are calculated to result during gas counterdiffusion under the conditions described. The properties of the layers and permeants have been chosen in such a way that the resistance to transport is low in the first layer that a permeant traverses and high in the second layer. The sum of the partial pressures is shown as a broken line which in this case is always above the ambient pressure and is a maximum at the interface of the two layers. More specific information can be gained by starting with a flux equation based on a combination of Fick's law for diffusive flux and Henry's law for the gas solubilities (*7*)

$$J_{1A} = -K_{1A}\frac{\Delta P_{1A}}{\Delta X_A} \qquad (1)$$

where J_{1A} is the flux of species 1 through layer A, K_{1A} is the permeability coefficient of 1 in A (the product of diffusivity and solubility), ΔP_{1A} is the partial pressure difference of 1 across layer A, and ΔX_A is the thickness of A. By writing the four equations of this type for the two permeants and the two layers and invoking restrictions such as the continuity of fluxes, one can find an expression for the sum of the partial pressures at the interface between the two layers (P_i). For pure permeants 1 and 2 at equal pressure P on opposite sides of layers A and B, the following result is obtained:

$$\frac{P_i}{P} = \frac{\Delta X_B K_{1A}}{\Delta X_B K_{1A} + \Delta X_A K_{1B}} + \frac{\Delta X_A K_{2B}}{\Delta X_A K_{2B} + \Delta X_B K_{2A}} \qquad (2)$$

The first term in Eq. 2 is the relative partial pressure of component 1 and the second term that of component 2.

Two consequences of this model are particularly interesting. The first is that the two layers do not have to exhibit opposite semipermeabilities for the two gases to produce supersaturation ($P_i/P > 1$). A necessary and sufficient condition is simply that the layers exhibit different semipermeabilities and that they be arranged in the proper sequence. Specifically, the condition is given by

$$\frac{K_{1A}}{K_{2A}} > \frac{K_{1B}}{K_{2B}} \qquad (3)$$

The second consequence is that, for a particular pair of permeants and of materials, a maximum supersaturation is obtained at a certain ratio of layer thicknesses:

$$\frac{\Delta X_A}{\Delta X_B} = \left(\frac{K_{1A}}{K_{1B}}\frac{K_{2A}}{K_{2B}}\right)^{1/2} \qquad (4)$$

The absolute thicknesses are immaterial at steady state. If at least one of the layers is liquid and if suitable nuclei are present, bubbles will form and grow continuously. With poorly adherent solid layers, blebs or gross separation could result. If mechanical restraints are imposed which prevent layer separation, an increase in pressure in the gas phase within the layers will be seen.

Although this analysis has been presented for dissolved gaseous permeants, it is by no means limited to them. For example, in the case of two solutes which participate in a common chemical reaction, the product of chemical activities (or, in the ideal case, concentrations) may be of primary importance rather than the sum of partial pressures. Counterdiffusion in this instance might drastically alter the velocity of a chemical reaction. Specifically, consider a case where two different reactants are maintained at equal concentrations in separate compartments (for example, intracellular and extracellular) and that a reaction involving them takes place between two sequential membranes separating the compartments. If each membrane is perfectly permeable to the reactant in contact with it and impermeable to the second reactant, the concentrations within the intramembrane space would be the same as in each external compartment. If we prepare a second system having two identical membranes with some finite permeability, however, the intramembrane concentrations would be half those in the two compartments. For a reaction which is first order in each reactant, the rates in those two cases would differ by a factor of 4. This illustrates how reaction rates in a multimembrane system such as a cell might be quite different from those anticipated.

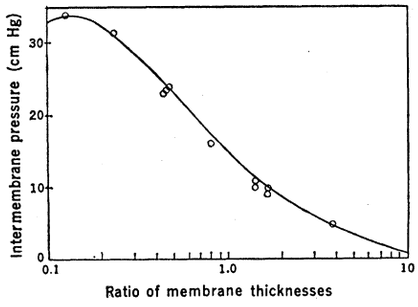

Fig. 2. Confirmation of Eq. 2 (solid curve) with an ethyl cellulose–silicone copolymer two-membrane system (data points). The ordinate represents the steady-state pressure buildup (between the membranes) over ambient pressure (outside the membranes) as a function of the thickness ratio of ethyl cellulose to silicone. The solid curve was calculated from measured permeabilities by using Eq. 2; the data points were measured in a dual-membrane cell. Note that the pressure goes through a maximum as predicted by Eq. 3.

The prediction of counterdiffusion supersaturation leading to bubble formation has been confirmed through a series of related experimental studies, both in model physical systems (*6*) and in vivo with young pigs (*8*). With the physical model we have demonstrated bubble evolution in an oil-water system seeded with crushed glass for nuclei with counterdiffusing nitrogen and helium (helium on the water side) (*6*). We also used two solid membranes as the layers [General Electric XD1 silicone copolymer (*9*), and ethyl cellulose or polyethylene] with the same counterdiffusing gases (nitrogen on the silicone side). A small gas space was provided between the two membranes with an outlet so that flow into the space or pressure buildup could be monitored. Even with a relatively crude apparatus, we measured both a continual flow of gas into the space and a pressure buildup to 25 torr (3.4×10^4 dyne/cm^2) when the outlet was closed off in the silicone-polyethylene system. A very satisfactory confirmation of Eq. 2 was obtained in the silicone–ethyl cellulose system (see Fig. 2). A substantial pressure of 336 torr was measured in one case. In the physiological studies, young pigs anesthetized with pentobarbital breathed a mixture of one inert gas such as nitrogen or nitrous oxide with oxygen at its normal 0.21-atm pressure while they were surrounded by a second inert gas, helium (*8*). In this in vivo situation the specific inert gas atmosphere surrounding the skin of the pig (source 1) contained a gas which diffused through the skin to the blood capillary (sink 1). The blood itself contained the highest tension of a second inert gas (source 2) which diffused out-

ward through the skin to the atmosphere (sink 2). These exposures led to formation of gas bubbles in the skin and in cutaneous blood vessels.

In the physiological studies (*8*), more than 50 such animals were exposed to counterdiffusion conditions for periods of at least 6 hours. In 43 cases where the pig was surrounded by helium and the normoxic (0.21 atm) breathing mixture contained nitrous oxide at atmospheric pressure, or argon or nitrogen at pressures ranging from 1 to 10 atmospheres absolute, skin lesions occurred after an average exposure time of 80 minutes. In the animals where conditions were reversed (argon or nitrous oxide surrounded the pig while the inspired gas was a helium-oxygen mixture), no lesions were observed in any animal throughout the 6-hour duration of the experiment. In the "reversal" experiments we selected those conditions of gas and pressure which had produced the most severe lesions, but reversed the direction of the gas gradients. The most pronounced effects were seen with a nitrous oxide–oxygen mixture for the breathing gas at atmospheric pressure and with an argon-oxygen mixture at 10 atm pressure. Details of the in vivo studies, including implications for gaseous anesthesia, will be described elsewhere (*8*).

The tissue layers involved in the development of lesions were found to include the adipose subcutaneous tissue, the capillary plexus, and the keratinized outermost layers of the skin, each of which is traversed by the permeating gases (*8*). Constituents of such structures include lipids, lipoprotein membranes, and aqueous solutions, and we have demonstrated bubble formation in a lipid-aqueous system. Finally, the in vivo experiments with pigs were found to obey the condition demanded by Eq. 3, namely, lesions form when the gases counterdiffuse in one direction but do not form when the gases are reversed.

The consequences for physiology and anesthesiology of bubble formation in tissues and vessels are apparent. Less obvious, perhaps, is how membrane transport phenomena might be affected by factors such as the increased reaction rates suggested above. These brief examples should alert specialists in various disciplines to a few of the unusual properties of countertransport in composites. We suggest three potential applications. The study of nucleation phenomena should profit from the opportunity now presented to achieve known steady-state supersaturation levels in specific regions of a system. The conversion of free energy of mixing to work has already been achieved in our crude two-membrane device and remains to be further exploited. By operating the two-membrane device "backward" (that is, supplying a gas mixture to the intramembrane space and removing two relatively purified product streams from either side of the membranes), a separation device with interesting properties can be constructed.

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27 November 1972

Amphotericin B Methyl Ester Hydrochloride and Amphotericin B: Comparative Acute Toxicity

Abstract. *In single-dose parenteral studies with mice and dogs, the methyl ester hydrochloride of amphotericin B proved to be significantly less toxic than the parent compound, especially to the kidney.*

Intravenously administered amphotericin B (IAB) is the most effective agent currently available for the treatment of many systemic fungal infections in man. This drug is used in the treatment of a number of mycoses that, prior to its availability, were invariably fatal (*1, 2*). The use of IAB, however, is restricted to a significant degree by a variety of toxic side effects (*2, 3*), the most serious of these being nephrotoxicity. Over 80 percent of the patients treated with IAB develop decreased renal function, and it is often the degree of kidney malfunction, and not the patient's therapeutic response, that determines the duration of treatment (*2*).

Amphotericin B methyl ester hydrochloride (AME), a water-soluble derivative of amphotericin B, was recently developed by scientists at the Rutgers Institute of Microbiology. This compound not only retains the antifungal activity of the parent compound, but also, based on limited single-dose studies in mice, appears to be less toxic than IAB (*4*). The objective of this report is to summarize results of more extensive acute toxicologic comparisons of AME and IAB in mice and dogs. The studies were carried out with AME supplied by Rutgers (*5*) and with IAB supplied as marketed Fungizone Intravenous (*6*). Doses were given as milligrams of AME or IAB per kilogram of body weight.

Table 1. Acute intravenous (IV) and intraperitoneal (IP) lethalities of amphotericin B methyl ester hydrochloride (AME) and amphotericin B (IAB) in mice.

Drug	Route	LD_{50} (mg/kg)	LD_2 (mg/kg)	Slope function
AME	IV	106	75	1.20
IAB	IV	4	2	1.45
AME	IP	1320	620	1.45
IAB	IP	88	40	1.42

The results of acute lethality tests in male Charles River CD-1 mice are presented in Table 1, which shows that AME is 1/25 and 1/15 as toxic as IAB by the intravenous and intraperitoneal routes of administration, respectively.

Studies in dogs were conducted with a total of 12 unanesthetized and six anesthetized (pentobarbital sodium) purebred beagles. They received single intravenous doses of either AME or IAB injected during a period of 1 minute. In unanesthetized animals, AME was administered to two dogs each, at dosages of 6, 12, or 24 mg/kg, and to