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# SOLUBILITY OF NOBLE GASES IN PHOSPHOLIPID MEMBRANES
# A POTENTIAL PROBE FOR FUNCTION AND STRUCTURE

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**The absorptions of noble gases to suspensions of dimyristoylphosphatidylcholine in water were determined by measuring volume and pressure changes in a manometer. Absorptions are expressed in terms of thermodynamic functions of solutions, and also in terms of Bunsen coefficient and Henry's constants. The reasons for using different units and the advantages of each system of units are discussed in the text. The design of the experimental setup and the factors affecting its performance are discussed thoroughly. It is shown that the noble gases dissolve preferentially into the hydrophobic region of the membrane, and that this solution is simple. It is argued that these features make solubility measurements a powerful tool by which membranes can be probed and transport phenomena analyzed.**

## Introduction

This article deals with solubility measurements in phospholipid membranes, the information they provide and their relation to other methods of research.

Experimental methods of membrane research can be divided into two broad categories: biological and physical. While biological experiments are more relevant, they are phenomenological in character and lack the exactness that is so characteristic of the physical sciences. On the other hand, the physical methods, distinguished by their clarity, simplicity and ease of interpretation in terms of 'first principles', lose some of their power when applied to biology, because they do not correlate directly with biological phenomenology. Our knowledge of biomembranes benefitted by the combined use of physical and biological methods but, unfortunately, some information became useless by our inability to bridge the gap between the different experimental results.

Inspired by the success of theories of solution in bridging molecular parameters and macroscopic properties of liquids [1,2] and bearing in mind the essential role of solubility in biological transport [3], I decided to explore the features of noble gas solubility in membranes. My hope was that it might contribute to a better understanding of the role of lipids in membranes and how it influences transport, across membranes. By measuring noble gas solubilities in phospholipid membranes (DMPC), I try here to give answers to the following questions: (1) How do noble gases distribute inside the membrane? (2) Can membranes be thought of as bulk solvents, thus making theories of solution applicable to noble gas solubility in membranes? (3) What are the differences between noble gases and other nonelectrolyte solutes? (4) Is it possible to extend results, obtained for noble gases, to explain other solubilities? (5) How is solubility related to other methods of exploring membranes and to what extent is it complementary to these methods?

No attempt is made here to interpret membrane behaviour and transport properties from the present data, this will be done in another article.

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Abbreviation: DMPC, dimyristoylphosphatidylcholine.

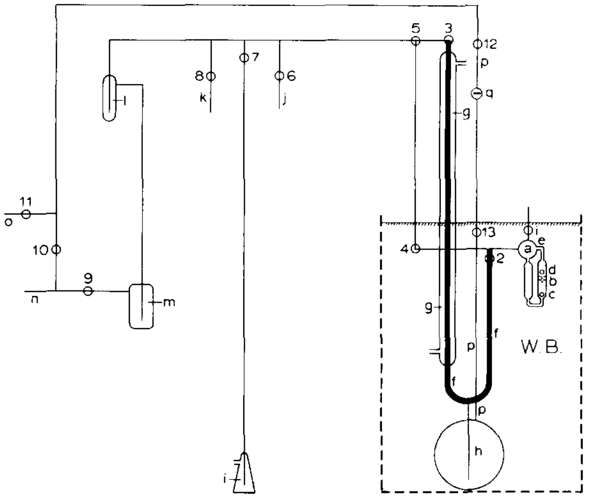

Fig. 1. A measuring system for measuring gas absorption to lecithins. a, the gas space in the mixing chamber; b, the suspension space in the mixing chamber; c,d, a valve and magnetically driven piston with a valve designed to push the suspension in one direction; e, a capillary through which the suspension is forced into the gas space from which it returns after mixing to the suspensions space b, the return is by gravity; f, precision-bore glass manometer used for filling the mixing chamber and measuring pressure and pressure volume changes; g, a water jacket used to keep the temperature of the outer arm of the manometer at a temperature close to the bath temperature; h, mercury bath which can be used to fill and empty the manometer g by forcing gas through the tube p; i, an auxiliary manometer used to keep the pressure inside the system above atmospheric pressure when gas is introduced through j. This is done to prevent contamination due to leakge of foreign gas to the system when below atmospheric pressure;j, connection for gas introduction; k, to pirani gauge; l,m, traps; n, to vacuum pump; o,p,q, a path for introducing helium, forcing the mercury in the container h up and down the manometer f. q, sintered glass moderating the flow of helium to the bath h. No contact exists between the forcing gas (helium) and the gas absorbed. W.B., water bath (120 l) in which the system is immersed. 1–13, Teflon valves in the system. Valve 1 was used to introduce the phosphatidylcholine to the mixing chamber.

## Experimental procedure

The experimental determination of gas adsorption to liquids is well established [4]. Generally this is done by measuring the amounts of the absorbed gas and the absorbing liquids, together with the partial pressure of the gas, in a measuring system that has the following components: 1, liquid space; 2, gas space; 3, manometer; 4, mixing chamber, 5, delivery and exhaust devices for gases and liquids. The major considerations in the design of a measuring system concern accuracy, response time and the amount of material to be used in the experiment.

The setup used for determination of noble gas absorption by DMPC lammela is shown in Fig. 1. This design is required because the liquid has special properties: it is a suspension, it is very viscous and the material is rather expensive. Such properties of the medium have the effect of reducing the accuracy of the determination, of prolonging the response time and limiting the amount of material that can be used, the efforts to compensate for these disadvantages by choosing optimal components are described below.

*1. Mixing chamber.* The mixing chamber is adapted from that described by Dymond and Hildebrand [5].

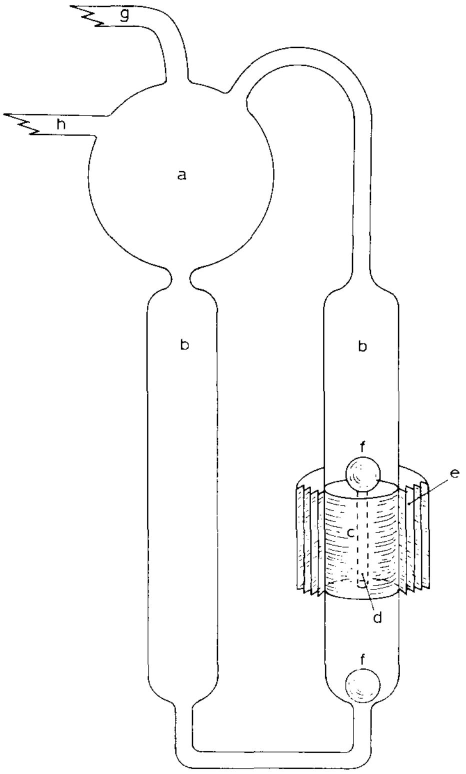

Fig. 2. The mixing chamber. a, Gas space; b, suspension space c, magnetic piston encased in glass; d, glass capillary; e, driving magnet; f, glass beads functioning as valves; g, introduction of the lecithin to the mixing chamber; h, to manometer.

Their system is fast and reasonably accurate but large amounts of liquid (250 cc) are needed. The chamber is described in Fig. 2.

The mixing chamber includes gas and liquid spaces. The liquid space is U-shaped, having a constricted connection between both arms. A glass ball, functioning as a valve, blocks the constriction in one of the arms. The same arm also contains a magnetically operated piston, on top of which lies another glass ball. The moving piston forces the liquid into the gas space, from which it flows by gravitation into the other arm. Absorption of gas into the liquid takes place in the gas space, where the liquid flows onto the wall as a thin layer. The connection between the gas and liquid phases are constricted, to minimize absorption of gas in the liquid space.

Adaptation means that the size of the Dymond-Hildebrand mixing chamber must be reduced, a goal which is very difficult to achieve because size reduction lowers accuracy and affects response time in a rather complex manner.

The modifications involve the following steps: 1, 7-fold reduction in chamber size; 2, increase in the diameter-to-length ratio of the liquid space; 3, increase in the surface-to-volume ratio in the gas space.

*2. Manometer.* A cut-off manometer which combines the functions of a gas buret and a manometer is the only measuring device in the system. The manometer can be used as an extension of the gas space.

The manometer has two arms and a mercury reservoir. Measurements are made by pushing mercury from the reservoir into the arms, and then comparing the level heights in the arms. The two arms differ in length, the shorter arm is connected to the gas space and the longer one to a vacuum pump. The arms can be disconnected from either the vacuum pump or the gas space by closing valves. The manometer together with the mixing chamber is immersed in a thermostatically controlled bath.

This arrangement is used because it is accurate, versatile and simple. The following causes that diminish accuracy, are totally eliminated or much reduced in the design: 1, diffusion of liquid vapour from the mixing chamber into the gas buret; 2, contamination of the absorbed gas by other gases; 3, a low signal-to-noise ratio in the readings; 4, cumulative errors.

Diffusion of solvent vapour, from the mixing chamber into the buret, occurs when gas is charged into the chamber and results in an underestimation of the amount of gas that is delivered. The error increases with the decrease of the pressure difference between buret and chamber and the increase of solvent vapour pressure. The design of the system provides two ways by which this kind of error can be avoided: 1, making the buret part of the gas space; 2, saturating the gas with solvent prior to delivery. The manometer, together with the mixing chamber, is immersed in the thermostatically controlled bath, since a good temperature control is a prerequisite for obtaining good results.

Contamination of the absorbed gas by other gases can cause serious errors. The nature and amount of the contaminating gas affect the magnitude of the error. The mercury in the arms of the manometer also functions as a barrier against contamination.

Increased reproducibility means a low noise-to-signal ratio of the readings. It is obtained either by increasing the measured signal or by decreasing the noise. Improved reading, i.e. reduced noise, is obtained when the manometer is applied as a null device. An increased signal is obtained when the manometer is used as a McLoad manometer (at low final pressures) or by connecting it to another manometer [6]. Increased accuracy in volume measurments is obtained by manipulating gas space volume. This is done by keeping the valve in the short arm open and changing the mercury level according to need.

Accuracy is further increased by making the manometer arms of 16 mm precision-bore glass tube (Corning), reading mercury levels with a cathetometer of ±0.05 mm accuracy and the use of special high vacuum Teflon needle valves (Fisher and Porter) to prevent leakage. A large diameter of the arms is chosen so that capillary effect will be minimized.

*3. Delivery and exhaust devices.* Preventing contamination is of utmost importance when gas absorptions are determined. Contamination occurs when filling and evacuation are poorly controlled. Two devices are used here to get contamination-free gas into the system. First, the manometer is used as a barrier sealing the gas from the outside. A fuller description is given above. Secondly, gas is introduced through an auxiliary system, which has an open manometer and a valve connected to the external gas source. The gas is kept in the auxiliary system above atmospheric pressure. Gas bubbles flow through the open manometer to the atmosphere. Contamination by air leakage is thus avoided.

*4. Temperature control.* Changes in vapour pressure caused by temperature fluctuations, bring about lower accuracy of partial pressure determinations. The error becomes greater with the increase of temperature and diminishes at highher absorptions. An error of ±3% is calculated for helium absorption to water at 60°C, assuming a temperature fluctuation of ±0.02°C. Errors, however, are expected to be much smaller.

The measuring system is immersed in a large water bath (120 l). The temperature of the bath is kept constant within ±0.02°C. A large water bath is used because it has a large thermal mass, which delays and diminishes the effect of temperature fluctuation The bath is heated by an uncontrolled heater to about 0.05°C below the desired temperature. Further heating is controlled by an on/off mercury toluene regulator. A smaller, faster and more sensitive thermoregulator is used because of the employment of the constant heater.

## Materials and Methods

Solubilities of five gases in DMPC lamella were measured. The gases used were the noble gases helium, neon, argon, krypton and xenon supplied by Matheson. Purity ranged between 99.9995% and 99.9999%. Purity of DMPC (Calbiochem) was tested using TLC. No impurities were found.

A chloroform solution containing a known amount of DMPC was injected to the mixing chamber. Injection was followed by evaporation. Excess water (doubly distilled) was then added, creating a suspension. The extra amount of water was removed together with the dissolved air when the suspension was boiled under vacuum. The lecithin constituted about 30% of the final suspension.

The introduction of the sample gas to the mixing chamber involved several consecutive steps: (1) The system was evacuated. The vacuum was tested with a pirani gauge and with the manometer. The manometer was used here as a null device. (2) The mixing chamber was connected to the manometer. Vapour pressure of water was then measured, and compared to literature values. After that, the connecting valve was closed again. (3) The sample gas was forced to flow through the auxiliary system and escape from the open manometer. (4) The auxiliary system was connected to the main manometer, allowing the sample gas to bubble through the mercury barrier into the short arm. The auxiliary system was then evacuated, together with the long arm of the manometer. Finally, the connecting valve was closed. (5) The mercury level in the short arm was returned to the original position. Pressure and volume measurements were made. The connecting valve was then opened and left open from then on, making the short arm part of the gas space. The gas was allowed to equilibrate for 24 h before the volume and the pressure were measured again and the absorption recorded.

The temperature dependence of the absorption was determined by changing the temperature of the bath and allowing 24 h to elapse before reading the volume and the pressure again.

The performance of the system, its accuracy and reproducibility were tested by absorbing helium to water at 25°C. By this procedure I tested the behaviour of the system as a whole, rather than the characteristics of each element. Helium and water were chosen because the combination produces low

TABLE I

RESULTS OF HELIUM ABSORPTION TO PURE WATER AT 25°C

A test for accuracy and reproducibility of the experimental system. Absorptions are expressed in terms of Bunsen coefficients. Bunsen absorption coefficient of a gas into a liquid is defined as the ratio of the volume of the absorbed gas (when measured at standard conditions: 1 atm partial pressure of the absorbed gas and 0°C) to the volume of the absorbing liquid, when absorption occurs at 1 atm partial gas pressure [4]. Average absorption (experimental) was $9.89 \cdot 10^{-3}$. S.E. of the mean was $3.19 \cdot 10^{-4}$ (±3.2%).

| Measured absorption (experimental) | Literature values | Ref. | Deviation (%) (literature values (from average |
|---|---|---|---|
| $10.08 \cdot 10^{-3}$ | | | |
| $10.72 \cdot 10^{-3}$ | $9.50 \cdot 10^{-3}$ | 7 | 4 |
| $8.57 \cdot 10^{-3}$ | | | |
| $9.91 \cdot 10^{-3}$ | $8.45 \cdot 10^{-3}$ | 8 | 17 |
| $10.16 \cdot 10^{-3}$ | $9.40 \cdot 10^{-3}$ | 21 | 5.2 |

solubility and because this solubility has been very carefully measured [7,8]. The results, presented in Table I, show ±3% reproducibility and a somewhat higher absorption than the 'best' literature values.

Repeated vapour pressure determinations gave a value of 23.76 ± 0.07 mmHg, compared to the literature value of 23.756 mmHg [9]. The uncertainty of 0.07 mmHg corresponds to an error of about 2.5% in helium absorption determination (absorption caused about 3.5 mm change in mercury level inside the manometer). The conclusion is that uncertainties in manometer readings are the major source of error at low absorptions. Accuracy can be improved by decreasing the diameter of the manometer but this was not done because higher absorbances were expected to be found in DMPC.

The response was found to be fast. Equilibrium was established within 30 min. Unfortunately, the response deteriorates badly when highly viscous fluids are used. This is the case with suspensions of DMPC in water. The outcomes were: the concentration of DMPC in suspension was limited to 30%, thereby decreasing accuracy; response was reduced appreciably, requiring several hours to attain equilibrium; absorption measurements could not be made when DMPC was below its phase transition temperature.

## Results

This section describes the results obtained when absorptions of noble gases to DMPC were measured. Solubilities of helium, neon, argon, krypton and xenon were measured at several temperatures. The outcomes are presented in three different but equivalent ways: Bunsen absorption coefficients; standard free energies of solution; and Henry's constants. Entropies and enthalpies of solution, derived for argon and krypton, are also included.

The Bunsen absorption coefficients of the noble gases in DMPC lamella are displayed in Table II. They have been calculated from experimental values of pressure and volume of the suspension, assuming ideality in the gas phase and Henry's law in solution. Defined as the ratio of the normalized volume of the absorbed gas to the volume of the absorber [4], the commonly used Bunsen coefficients measure the absorbing power of the condensed phase. This presentation has the advantage of facilitating comparison with the existing literature.

TABLE II

BUNSEN ABSORPTION COEFFICIENTS OF NOBLE GASES IN DMPC LAMELLAS

Absorption to the free water of the suspension is accounted for by assuming literature values of noble gas absorption to pure water [8].

| Helium | | Neon | | Argon | | Krypton | | Xenon | |
|---|---|---|---|---|---|---|---|---|---|
| $t$ (°C) | $\alpha$ $(\cdot 10^3)$ | $t$ (°C) | $\alpha$ $(\cdot 10^3)$ | $t$ (°C) | $\alpha$ $(\cdot 10^3)$ | $t$ (°C) | $\alpha$ $(\cdot 10^3)$ | $t$ (°C) | $\alpha$ $(\cdot 10^3)$ |
| 30.00 | 41.06 | 30.00 | 14.61 | 30.00 | 75.14 | 30.00 | 112.77 | 30.00 | 295.11 |
| 30.00 | 36.52 * | | | 32.65 | 87.44 | 39.00 | 140.64 | | |
| | | | | 34.60 | 94.33 | 41.20 | 125.24 | | |
| | | | | 37.20 | 79.82 | 43.70 | 125.56 | | |
| | | | | 38.25 | 85.53 | 47.70 | 115.89 | | |
| | | | | 40.46 | 85.66 | 51.15 | 107.45 | | |
| | | | | 41.65 | 76.97 | | | | |
| | | | | 45.15 | 83.71 | | | | |

* Experimentally measured absorption of helium to water (Table I) is used to account for gas absorption to the free water. Comparison between the two computational methods for estimating the absorption of helium to the liposomes reveals that the deviation in estimation of the absorption to free water (17%) diminishes when estimation of helium to liposomes is calculated (to about 12%). As is argued in the text, this is an estimated upper limit of the deviations resulting by using experimental and literature values to obtain the amount of gas dissolved in the free water.

TABLE III

STANDARD FREE ENERGIES OF ABSORPTION OF NOBLE GASES IN DMPC

Free energies are calculated from the definition $\Delta\mu^\circ = RT \ln p/x$. The partial pressures $p$ are in mmHg. The standard free energies of absorption are given in kcal.

| Helium | | Neon | | Argon | | Krypton | | Xenon | |
|---|---|---|---|---|---|---|---|---|---|
| $t$ (°C) | $\Delta\mu^\circ$ | $t$ (°C) | $\Delta\mu^\circ$ | $t$ (°C) | $\Delta\mu^\circ$ | $t$ (°C) | $\Delta\mu^\circ$ | $t$ (°C) | $\Delta\mu^\circ$ |
| 30.00 | 8.009 | 30.00 | 8.631 | 30.00 | 7.650 | 30.00 | 7.400 | 30.00 | 6.820 |
| | | | | 32.65 | 7.620 | 39.00 | 7.480 | | |
| | | | | 34.60 | 7.620 | 41.20 | 7.610 | | |
| | | | | 37.20 | 7.790 | 43.70 | 7.660 | | |
| | | | | 38.25 | 7.770 | 47.70 | 7.810 | | |
| | | | | 40.46 | 7.830 | 51.15 | 7.940 | | |
| | | | | 41.65 | 7.900 | | | | |
| | | | | 45.15 | 7.960 | | | | |
| | | | | 45.65 | 7.900 | | | | |

The rigour and correlating power of thermodynamics are brought in by expressing the solubilities in terms of standard free energies of solution. The free energies, defined by $\Delta\mu^\circ = RT \ln(p/x)$ in terms of the partial pressure $p$ and the mole fraction of the solute $x$, are shown in Table III. Gas ideality and the validity of Henry's law are assumed in the thermodynamic relation. This relation provides a basis for comparisons and correlations and is used extensively to deduce solution properties.

A common linear empirical relation $\Delta\mu^\circ = a + bT$, between free energies and temperatures [10], is used to improve the results and estimate the experimental errors. The constant coefficients $a$ and $b$ of the function $\Delta\mu^\circ = a + bT$ are found by least-squares and then utilized to give the calculated ratio $k_H = p/x$. $k_H$ is the much used Henty's constant, which is closely related to the partition coefficients of solutes (the partition coefficient of a solute between two solvents is equal to the ratio of two Henry's constants). Corrected $k_H$ values, describing noble gas solubility in DMPC, are presented in Table IV. The differences in solubility are more pronounced when expressed in terms of $k_H$.

TABLE IV

CALCULATED HENRY'S CONSTANTS FOR THE ABSORPTION OF NOBLE GASES IN DMPC

The numbers in the last five columns are Henry's constants, defined as $k_H = p/x$ where $p$ is the partial pressure of the gas and $x$ is the mole fraction of the absorbed gas in the liposome. Naturally $k_H$ has dimensions of pressure. The numbers in the table express the partial pressure, in mmHg, that is needed to obtain a hypothelical solution in which the mole fraction of the gas equals unity. $k_H$ values for the gases argon and krypton were calculated from the equation $\ln k_H = a + b(1/T)$. The constants $a$ and $b$ were extracted by least-squares from the measured free energies of solution.

| Temperature (°C) | $k_H$ (mmHg) | | | | |
|---|---|---|---|---|---|
| | Helium | Neon | Argon | Krypton | Xenon |
| 25.00 | | | 296 714 | 187 120 | |
| 30.00 | 594 584 | 1 669 760 | 295 148 | 190 219 | 82 601 |
| 35.00 | | | 293 642 | 193 266 | |
| 40.00 | | | 292 190 | 196 262 | |
| 45.00 | | | 290 792 | 199 208 | |
| 50.00 | | | 289 443 | 202 106 | |

That the three presentations are equivalent is seen when the following definitions are compared:

Bunsen absorption coefficients:

$$\alpha = \frac{V_g}{V_m} \cdot \frac{273.16R}{T}$$

Henry's constant: $k_H = p/x$
free energy of solution: $\Delta\mu^\circ = RT\ln(p/x) = \mu^\circ_{gl} - \mu^\circ_{gg}$
where $\alpha$ is Bunsen absorption coefficient; $k_H$ Henry's constant; $\mu^\circ_{gl}$ and $\mu^\circ_{gg}$, standard free energies of the solute in the liquid and gas phases, respectively; $V_m$, the volume of the liquid, and $V_g$, the volume of the absorbed gas under the conditions of the experiment, $p$, the partial pressure of the gas, $x$, its mole fraction in solution, $T$, the absolute temperature and $R$ the universal gas constant.

Equivalency is demonstrated by the equations $\Delta\mu^\circ = RT\ln k_H$ and $k_H = 273.16R/\alpha V_m$, which are derived directly from the definitions. The assumptions of gas ideality and exactness of Henry's law appear in all definitions and are justified by the high dilutions and relatively low pressures found in the determinations.

The formulations are complementary to each other; Bunsen coefficients and Henry's constants are inversely proportional to each other and can, therefore, be interchanged when expansion of scale or increase in sensitivity are needed. The advantage of free energy being a thermodynamic function disappears sometimes, because it is a logarithmic function of Henry's constant, therefore reflecting solubility changes to a lesser extent.

Comparing the calculated and experimentally found free energies shows the accuracy of the system as a standard error or the mean of the deviation of the data from the expected. Errors of 0.18% and 0.4% were found for the free energies of argon and krypton, respectively, corresponding to 2.3% and 5.5% error in the absorption coefficients determinations. Two major factors account for the elevated error rates. 1, High viscosity increases the likelihood of inadequate absorption or the trapping of microscopic gas bubbles. 2, Extra measurements are needed to determine the absorption to lecithin, which is just one of the three components of the suspension.

A closer look into the distribution of solutes between the suspension's components proves useful, because it casts light and explains the difficulties and uncertainties involved in solubility determinations in liposomes. Liposomes exist only in suspensions that consist of three phases: free water; non-solvent water; and lecithin. The free water behaves as pure water, whereas the non-solvent water is bound strongly to lecithin, and is not available to solutes [11].

Absorption coefficients are obtained by measuring the total amounts of lecithin and water, the amount of non-solvent water, the total amount of the gas absorbed to the suspension and its solubility in pure water. An uncertainty in any of the measured quantities means a lower accuracy. An increased error is, therefore, expected when absorption to lecithin is determined.

Bunsen absorption coefficients apply to lecithin as a whole. A change in values of the coefficients is obtained if absorptions are assumed to take place only in one region, say the hydrophobic region of the lecithin. Ambiguity does not occur when the absorption is expressed by Henry's constants, because they relate to mole fractions rather than to the solvent volume.

Standard entropy and standard enthalpy are two important thermodynamic functions, which can be described roughly as measuring the amount of order and the energies of interaction in the solution, respectively. Their values are shown in Table V. They are related to the standard free energy by the general statement $\Delta\mu^\circ = \Delta H^\circ - T\Delta S^\circ$, and are temperature independent, as can be seen by combining together the general thermodynamic expressions $\Delta H^\circ = -T^2\partial(\Delta\mu^\circ/T)/\partial T$ and $\Delta S^\circ = -(\partial\Delta\mu^\circ/\partial T)$ with the

TABLE V

ENTROPIES AND ENTHALPIES OF ABSORPTION OF NOBLE GASES IN DMPC

The quantities are the standard partial enthalpies ($H^\circ$) and entropies ($S^\circ$) for transfer of the indicated solute from gas to DMPC. The solutions are hypothetical solutions, having unity mole fraction of the solute.

| Solute | $\Delta H^\circ_{g\to l}$ (cal/mol) | $\Delta S^\circ_{g\to l}$ (cal/mol per K) |
|---|---|---|
| Argon | 190 | −24.4 |
| Krypton | −590 | −26.1 |

empirical equation $\Delta\mu^\circ = a + bT$. The combination shows directly that the enthalpy and entropy are not influenced by temperature changes: $\Delta H^\circ = a$ and $\Delta S^\circ = -b$ are constants. The estimated errors for argon and krypton entropies and enthalpies of solution are 5 and 10%, respectively.

## Discussion

Where do noble gases partition in liposomes? What directs their distribution? Why is it simple and easy to analyze? What are the solubility laws? Which membrane properties do they reflect? How are the laws to be extended when more complicated solutes are treated? These are the main problems that are asked, discussed and analyzed in this chapter. Reference to solubility prediction and probe construction is made when the special properties of the noble gases in membranes are discussed, because solubility in membranes plays an important role in membrane permeability [12], drug activity [13] and anaesthetic potency [14].

That noble gases are absorbed mostly to membrane hydrophobic regions is suggested by similar solubilities in liposomes and in nonpolar solvents. A well known emprical phrase states that substances are best dissolved by others which are most like them, implying that similar solution power indicates like solvents. The rule, qualitative and ill-defined as it is, happens to be very useful when solvents have to be

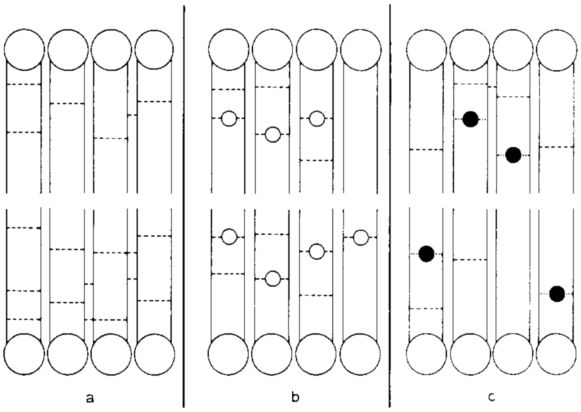

Fig. 3. Schematic representation of solution in phosphatidylcholine. ○, hydrophilic heads; ———, hydrophobic tails; - - - - -, interactions between the solvent (phosphatidylcholine) molecules; · · · · · ·, interactions between solute and solvent molecules; ○, holes created in the hydrophobic region of the phosphatidylcholine; •, solute molecules. a, The intact phosphatidylcholine; b, holes are created in the hydrophobic region; c, solute molecules were introduced into the holes. Note that for each solvent-solvent interaction abolished two solvent-solute interactions were created.

TABLE VI

SOLUBILITIES OF NOBLE GASES IN REPRESENTATIVE SOLVENTS

The numbers are the solubilities expressed as mole fraction × $10^4$. The solubilities refer to 1 atm. partial gas pressure of the solute, and, with the exception of DMPC and water, are at 25°C. Solubilities in water and in DMPC are at 30°C. The data for *n*-hexane, *n*-dodecane, cyclohexane, benzene, *n*-methylacetamide, nitromethane, methanol and dimethylsulfoxide are from Wilhelm and Battino [10]. Solubilities in water are calculated from the results obtained by Morrison and Johnstone [8]. Solubilities in DMPC are from this article.

| Solvent | Helium | Neon | Argon | Krypton | Xenon |
|---|---|---|---|---|---|
| *n*-Hexane | 2.604 | 3.699 | 25.120 | 69.600 | 258.700 |
| *n*-Dodecane | 2.223 | 3.124 | 25.660 | 75.540 | 307.200 |
| Cyclohexane | 1.217 | 1.792 | 14.800 | 46.680 | 202.900 |
| Benzene | 0.771 | 1.118 | 8.815 | 27.390 | 116.600 |
| *n*-Methylacetamide | 0.491 | | 4.434 | | |
| Nitromethane | 0.286 | 0.541 | 3.198 | 8.376 | 25.090 |
| Methanol | 0.595 | 0.814 | 4.491 | | |
| Dimethylsulfoxide | 0.284 | 0.368 | 1.540 | 4.460 | 17.000 |
| Water | 0.068 | 0.080 | 0.227 | 0.393 | 0.679 |
| DMPC | 12.780 | 4.550 | 25.750 | 39.950 | 92.000 |

chosen or substituted for one another. The representative data, presented in Table VI, shows that the nonpolar solvents *n*-hexane, *n*-dodecane, cyclohexane and benzene show a much larger dissolving power than the polar solvents nitromethane, methanaol, *n*-methylacetamide, dimethylsulfoxide and water. The similarity of solubilities in liposomes and in nonpolar solvents hints that the locus of noble gas absorption to membranes is a hydrophobic region.

Insertion of solute noble gas molecules between the hydrocarbon chains of the tails is preferable, energetically and entropically, to their introduction into the hydrophilic region. As is demonstrated in Fig. 3, abolishment of solvent-solvent interactions, creating solute-solvent interactions instead, is required for a solution to take place. A close examination of the nature of the intermolecular forces acting in the membrane reveals that solution in the hydrophobic region is preferred, because it involves replacement of a weak London interaction by a comparable one. Insertion into the hydrophilic region is inexpedient, because it involves substitution of a strong electrostatic interaction by a much weaker one. This energetic effect is compleneted by an entropic one. The larger freedom of motion, found in the tails [15] and manifested by solute and solvent mobilities, is expected to cause an enhanced partition into this region, in contrast to the ordered head groups which are likely to offer higher resistance to solute entry.

The hydrophobic region is a bulk solvent when solubility of noble gases is dealt with, since the noble gases are small compared to the dimensions of this region, which is made of long stretched hydrocarbon chains. The statement is wrong when large solute molecules are considered, because then it is necessary also to take into account surface effects at the boundary with the bordering polar regions. The solution is affected only by a limited number of factors, a freedom from complexity seldon existing for other solutes. Inspection of the general statistical mechanical expression, describing the standard free energy of a solute in solution in terms of all possible molecular configarations and interactions [16], reveals that this extremely complex function is very much simplified when solutions of noble gases in hydrophobic solvents are considered. Thus, terms representing solute rotational and vibrational contributions to the free energy disappear because noble gases are spherically symmetrical monoatomic molecules and potential energy contributions which are generally very complicated

TABLE VII

CHARACTERISTICS OF NOBLE GAS SOLUBILITY IN MEMBRANES AS COMPARED TO THE BEHAVIOUR OF OTHER SOLUTES

| Solute:<br>Feature | Noble gases | Small paraffin molecules | Large paraffin molecules | Rigid aromatic molecules | Polar molecules |
|---|---|---|---|---|---|
| Typical solute | Krypton | *n*-Hexane | *n*-Hexadecane | Naphtalene | Ethanol |
| Region where solution occurs | Hydrophobic | Hydrophobic | Hydrophobic region and surfaces | Hydrophobic | Hydrophilic + hydrophobic |
| Intermolecular forces | London | London | London | London | London + H bond + ion dipole |
| Molecular size of solute (Å) | 3.59 | 5.92 | 16 (approx.) | 4.71 · 2.75 | 5.1 |
| Disturbance of order in membrane | Small | Small | Large | Large | Small |
| Terms of partition function [a] | Potential energy | Rotational + potential energy | Rotational + potential energy | Potential energy | Rotational + potential energy |

[a] A general statistical mechanical expression [16] states that the standard free energy of a solute in solution depends on the kinetic energy as well as on rotational and vibrational contributions of the solute molecules; it depends also on potential energies due to interactions with neighbour molecules. The contributions can be separated in most cases.

become simple as a result of the weak short-ranged London intercations [17] between solute and solvent. The outcome of the metioned simplifications is that an analytical expression for noble gas solubility in hydrophobic solvents can be formulated to a good approximation [2], describing the macroscopic thermodynamic functions in terms of microscopic (i.e. molecular) parameters.

Some insight about the properties of the hydrophobic region and the membrane features that can be probed by noble gas solubility measurements is gained by comparing with simple solutions of noble gases in nonpolar hydrocarbon solvents. By analogy, one might anticipate that measurements of free energies of solution of the noble gases, together with knowledge of the energies of vaporization of the pure solute, will allow extraction of the cohesive energy of the hydrophobic region, i.e. that net molecular attractions between the hydrocarbon chains of the hydrophibic region. This theoretically important factor is unknown experimentally, because phospholipid membranes are inhomogeneous, having distinct but inseparable polar and nonpolar regions. Solubility is thus related to other methods of exploring membranes and is complementary to them. Comparison with the main physical techniques [22] shows that the molecular basis of solubility is less developed and further removed from the 'first principles' that characterize physics but this disadvantage is compensated by the ability to correlate remote results, providing the much needed bridge between physical exactness and biological relevance.

It may be concluded that noble gases are unique among all solutes in being simple and easy to analyze. Outcomes of solubility measurements, cited and compared in Table VII, demonstrate that this uniqueness originates from the noble gases being confined to a single membrane region, having a small size and being affected by a limited number of factors. It is seen that the existence of an extra factor, a partial freeze of the rotational degrees of freedom of *n*-hexane by the built-in order of the membrane [18], complicates analysis because the increase of unknown factors is not accompanied by new relations. Even more complicated are solubilities of larger paraffins, e.g. *n*-hexadecane [19], where surface effects must be considered in addition to other parameters. The solubility of polar molecules in liposomes offers another example [20]. The existing data is of limited use, because the partitioning of solute molecules between the membrane regions is beyond reach and because interpretation of solubilities in polar solvents is extremely difficult.

The simplicity is useful to the understanding of transport phenomena. The role of solubility in membrane transport is well established [3] and inspection of Table VII makes clear that noble gas solubility data provides essential information about some of the factors involved when more complex molecules are considered. For example, it is seen that the extra complexity of small paraffin solubility, their loss of internal degrees of freedom, can be established once the solubility pattern for the noble gases is estimated and the average potential energy of a molecule in the hydrophilic region found. It can also be seen how knowledge of noble gas solubility may help in deducing polar solute distribution in membranes by providing the much needed information about the solvent properties of the hydrophobic region compared to bulk solvents. Other examples of the usefulness of noble gas solubility data may be easily found.

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