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THE SOLUBILITY OF ANESTHETIC GASES IN LIPID BILAYERS

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We have measured the lipid/gas partition coefficients at various temperatures of eight anesthetic agents in two sonicated lipid bilayers containing either 96% egg phosphatidylcholine/4% phosphatidic acid or 64% egg phosphatidylcholine/3% phosphatidic acid/33% cholesterol. The Bunsen lipid/gas partition coefficients in the pure phospholipid bilayer at 25°C were: methoxyflurane 820 (interpolated), halothane 150, isoflurane 140, fluroxene 52, xenon 1.4, sulfur hexafluoride 0.24, carbon tetrafluoride 0.056 and hexafluoroethane 0.34. These partition coefficients were close to those in a bulk hydrophobic solvent (olive oil) but were reduced by about 20% in the cholesterol-containing bilayer preparation. In biomembranes the partition coefficient for halothane was lower than in lipid bilayers by about half an order of magnitude. As in olive oil, the partition coefficients mostly increased with decreasing temperature. The enthalpy, entropy and free energy associated with transfer of 1 mol of these agents from the gas phase at 1 atmosphere partial pressure and 25°C into the lipid bilayers under the same conditions were calculated from the temperature variation of the partition coefficients. All of these compounds, with the exception of methoxyflurane, fit the Barclay Butler relationship between entropy and enthalpy of partitioning. The Bunsen partition coefficients were correlated with the anesthetic potencies of seven of these agents in mice and in dogs. Comparisons were made between the different bilayers and olive oil and between hypotheses of anesthesia based on concentration of anesthetic at the active site (Meyer-Overton) and based on the product of concentration and molar volume of anesthetic at the active site (Mullins). Excellent correlations between anesthetic potency and lipid bilayer partition were obtained in all cases. The most consistent fits to the predicted slopes were achieved when both molar volume and partitioning of the anesthetic into the cholesterol-containing bilayer were taken into account, but the differences between the models were small.

Introduction

The lipid solubility of compounds with biological activity is of fundamental importance to our understanding of the absorption, distribution, mode of action and elimination of substances in an organism. It is particularly important to our knowledge of the action of general anesthetics whose potency has been known to correlate with lipid solubility for many years. Traditionally lipid solubility has meant the solubility of a compound in naturally occurring oils, particularly olive oil, and the pitfalls of using substances with varying compositions have been largely ignored. More recently solubility in octanol has been preferred. However these solubilities are an unknown approximation of the solubility of a compound in the hydrophobic region of a bimolecular phospholipid membrane such as is found in many cell systems. Although the acyl chains of the phospholipids may closely resemble the acyl chains in a three-dimensional bulk solvent, the hydrophobic interior of such

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a bilayer may be considered to extend in only two dimensions and exhibits considerable anisotropy. Ideally we should measure lipid solubility in this two-dimensional region of biological membranes but in practice this is complicated by the presence of proteins and by the heterogeneous mixture of acyl chains present in any given membrane. Thus protein free model membrane systems of known lipid composition offer an attractive compromise between olive oil and a biological membrane. Non-polar solutes in the gaseous or vapor phase have been studied by Hill [1], Simon et al. [2,3], Miller et al. [4] and Mastrangelo et al. [5]. Measurement of these solubilities as a function of temperature enables estimation of the thermodynamic transfer parameters from the vapor phase to the dissolved phase which are independent of interactions between the solute and water in the aqueous phase [3,6]. This is particularly appropriate in the case of the inhalational anesthetic agents.

We have used a gas chromatographic technique to determine the solubilities of four volatile and four gaseous anesthetic agents. The solubilities, expressed as Bunsen lipid/gas partition coefficients, have been measured at two or more temperatures in a pure phospholipid bilayer and in a cholesterol containing bilayer and the thermodynamic transfer parameters have been estimated.

Methods

The appropriate phospholipids with or without cholesterol were dried down from chloroform solutions and suspended in 0.15 M KCl solution containing 0.1% sodium azide buffered at pH 7.4 with 10 mM Tris-HCl. Suspension was achieved by vortexing and then by sonication with the microprobe of a Heat Systems sonifier (Model W185) at a maximum power of 90 watts intermittently for 60 min under a nitrogen stream and with the sample in an ice bath. Centrifugation at $30\,000 \times g$ for 15 min removed multilamellar vesicles and material eroded from the tip of the microprobe to yield translucent suspensions containing 10–46 mg/ml of total lipids depending on the solubility of the agents being studied. Two different lipid compositions were used. The first (PC/PA) contained 96% egg phosphatidylcholine and 4% egg phosphatidic acid (Lipid Products, Nutfield, U.K.). The other bilayer (PC/PA/Chol.) contained the same lipids in the same proportions plus 33 mol% of cholesterol (Sigma, recrystallized from methanol).

Different apparatuses were used to equilibrate the anesthetic agents and the inert gases. The first apparatus has been described previously [4] and was used in this work to measure the lipid/gas partition coefficient of methoxyflurane, halothane, isoflurane and fluroxene in the two different lipid bilayers. Briefly lipid, buffer and water samples were equilibrated overnight at various temperatures in a humidified stream of volatile vapor in nitrogen at ambient barometric pressure. Samples were withdrawn in syringes and analyzed by gas-solid chromatography [4].

The second apparatus was specially constructed to allow the measurement of partition coefficient as a function of pressure (important because of the limited solubility of the inert gases) as well as temperature. The apparatus was built to fit inside a simple cylindrical stainless steel pressure vessel of 50 mm internal diameter. Six stainless steel tubes with an internal volume of 0.26 $\mu l \cdot mm^{-1}$ of length were passed through a 25-mm thick aluminum disc which fitted over the top of the cylinder and was held in place by a stainless steel collar. Five 18 ml glass cuvettes resting on platforms inside the cylinder each contained a small teflon coated magnetic stirring button (Bel-Art spinfin MB 10606, Markson Science Inc.). Stiff stainless steel wires were attached to the fins of the stirrers to ensure that the surface of the liquid was broken during stirring. The stainless steel tubes dipped into each cuvette and led to a high pressure selector valve (A in Fig. 1) (Valco Instruments Co.) which connected to a high pressure six port sampling valve with sampling loop (B) (Valco Instruments Co.). This valve enabled the sampling loop to be filled with a lipid suspension at pressure and then flushed out with a fixed volume of distilled water from a glass syringe after decompression. The whole apparatus including the selector and sampling valves was temperature controlled to $\pm 0.1^{\circ}C$. A large semi-circular magnet suspended from a thick plexiglass disc surrounded the pressure chamber and could be driven at 120 rev./min to provide stirring throughout the experiment.

The cuvettes contained either water, buffer solution or one of the two phospholipid suspensions. The

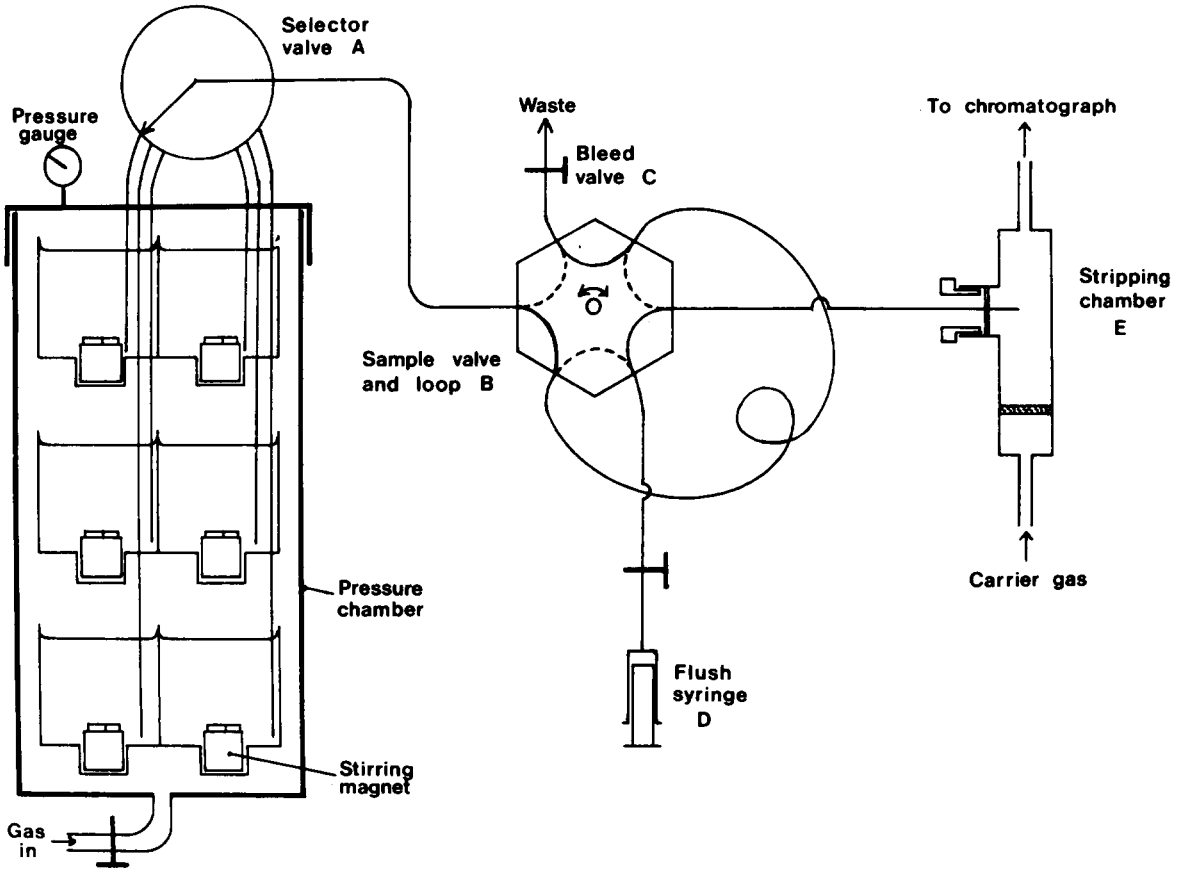

Fig. 1. Schematic representation of the apparatus for equilibrating anesthetic gases with buffer and lipid suspensions at pressure. The selector valve (A) passes the appropriate fluid to the sampling valve and loop (B). The rate of filling of the sample loop is controlled by the bleed valve (C). After decompression, the contents of (B) are flushed by a fixed volume from syringe (D) into the stripping chamber (E) of the chromatograph.

pressure chamber was flushed with the gas under study (xenon, sulfur hexafluoride, carbon tetrafluoride or hexafluoroethane) and then sealed and pressurized to the desired pressure with the same gas. Partition coefficients were measured at 2–3 times the pressures of these gases required to abolish the righting reflex in mice [7].

After 12 h equilibration the 300-μl sample loop was filled by slowly bleeding 0.75 ml of solution at about 0.5 ml $\cdot$ min^{-1} through a micrometer metering valve (C). The sample loop was then decompressed by switching valve (B) and its contents flushed into the stripping device with 1 ml of distilled water from a syringe (D). Other samples were collected similarly for lipid analysis. Each cuvette was sampled in turn by use of selector valve (A).

The stripping technique and gas-solid chromatographic analysis has been described by Miller et al. [4]. For use with the high pressure equilibrator the bubbling chamber above the diatomaceous earth fritted disc was enlarged to 3 ml total volume to accomodate the 1-ml sample flushed out from the high pressure apparatus. The greater volume resulted in broader peaks but integration (Spectra Physics, Model 23000-010 Santa Clara, CA) yielded identical areas. Columns of Porapak Q (Water Associates, Framingham, MA) were adjusted in length (1–3 m) and temperature (120–200°C) to provide complete separation of the agent or gas under study from water. Three consecutive 60-s stripping periods extracted more than 99% of the dissolved material.

Phospholipid content was measured by inorganic

phosphate assay [8] and total lipid content was then calculated from the known proportion of cholesterol in the membranes.

The Bunsen membrane/buffer partition coefficient (volume of pure solute gas corrected to 0°C and 1 atm pressure dissolved in unit volume of solvent) was evaluated from the ratio of gas dissolved in the suspension to that dissolved in the buffer. We also calculated the Bunsen buffer/water partition coefficient from a water standard included in the apparatus. Literature values of water/gas coefficients enabled us to calculate membrane/gas coefficients. We determined the gas solubility in water when the values were unknown, especially at the low temperatures. Gas standards were made by adding a weighed amount of liquid anesthetic into a flask of known volume to produce the desired concentration. Water standards were made up in a similar manner into flasks full of distilled water. Thus the quantity of anesthetic in both liquid and gaseous phases could be determined absolutely and the partition coefficient calculated.

Results

The Bunsen partition coefficients of the anesthetic agents in water are shown in Table I. We determined

TABLE I

BUNSEN SOLVENT/GAS PARTITION COEFFICIENTS (α) OF ANESTHETIC AGENTS IN WATER, BUFFER AND LIPID BILAYERS

Methoxyflurane is $CHCl_2CF_2OCH_3$; halothane is $CF_3CHClBr$; isoflurane is $CF_3CHClOCHF_2$ and fluroxene is $CF_3CH_2OCH{=}CH_2$.

Agent (partial pressure in atm)	Temp. (C)	Water (Ref.)	Buffer	PC/PA	PC/PA/Chol.
1. Methoxyflurane	8	11.67 [10]	10.15	1 580	752
(0.0035)	20	8.08	7.02	1 050	635
	30	5.48	4.76	744	453
	37	3.80	3.31	493	306
2. Halothane	4	4.28	3.89	613	310
(0.0087)	10	2.92	2.65	341	169
	20	1.60	1.49	244	112
	25	1.20	1.10	148	93.0
	30	0.92	0.85	114	77.3
	37	0.63	0.58	90.4	54.4
3. Isoflurane	25	1.08	0.95	140	65.3
(0.018)	37	0.54	0.47	78.2	42.0
4. Fluroxene	25	1.24	1.07	51.5	29.8
(0.044)	37	0.71	0.62	29.3	18.8
5. Butane	25	0.0273	0.0231	16.6	14.4
(1.6)					
6. Xenon	10.4	0.146 [21]	0.134	1.61	1.36
(2.2–4.8)	25	0.0965	0.0885	1.36	1.23
7. Sulfur	10.6	0.00889 [13]	0.00793	0.298	0.200
hexafluoride	25	0.00546	0.00496	0.238	0.218
(4.3–16.7)					
8. Carbon	11.5	0.00665 [13]	0.00590	0.0555	0.0329
tetrafluoride	25	0.00475	0.00416	0.0563	0.0437
(35.0)					
9. Hexafluoroethane	10.4	0.0081 [a]	0.0070	0.440	0.412
(27.7)	25	0.0050	0.0043	0.342	0.333

[a] See text.

the values for halothane, isoflurane and fluroxene. References to the literature values from which interpolations have been made are given in brackets. The values for hexafluoroethane were estimated from a plot of the energy of vaporization at the boiling point (ΔE_b) against log solubility in water for several fluorocarbon gases as described by Hildebrand et al. [9]. The mean buffer/water partition coefficient, which was the same for all agents, was 1.14 ± 0.046 ($n = 26$) and the buffer/gas partition coefficients shown in Table I have been calculated using this figure. Our value for the water/gas partition coefficient for isoflurane at 37°C was the same as that reported by Cromwell et al. [10]. Our halothane water/gas partition coefficients were slightly lower than the preferred literature values of 0.70 at 37°C reported by Steward et al. [11] and 1.30 at 25°C reported by Regan et al. [12]. Our value for fluroxene at 37°C was close to the preferred literature value of 0.75 [11–13]. These are within expected limits of error in such values and our values have been used for self-consistency. The precision was within that expected for methods based on gas-solid chromatography [14].

The Bunsen coefficients for each of the agents in the two artificial lipid bilayer systems are also shown in Table I. The solubility of butane at 1.6 atm in this series of experiments agrees with that found at 1.0 atm in an earlier series [4]. Simon et al. [3] found 119 ± 8 for the partitioning of halothane into multilamellar egg phosphatidylcholine liposomes and 64 ± 5 into 2 : 1 phosphatidylcholine/cholesterol liposomes at 25°C. These values are slightly lower than our values in unilamellar liposomes but not as low as predicted by Gruen and Haydon [15] who proposed that partitioning into multilamellar liposomes should be 17–65% of that into a planar bilayer. Mastrangelo et al. [5] obtained a partition coefficient of 82 for halothane between 2 : 1 phosphatidylcholine/cholesterol multilamellar liposomes and water at 20°C.

At 25°C the four volatile agents had Bunsen partition coefficients in PC/PA ranging from 820 (interpolated) for methoxyflurane to 52 for fluroxene. The four gases not previously studied had much lower partition coefficients ranging from 1.4 for xenon to 0.056 for carbon tetrafluoride. The partition coefficients of all agents except carbon tetrafluoride were decreased as the temperature increased. Partition coefficients also were decreased by addition of 33 mol% of cholesterol to the bilayer and this effect was greater for the more lipid soluble agents. Partition coefficients for xenon and sulfur hexafluoride were independent of the partial pressure of gas used over the ranges quoted in Table I.

Discussion

Comparison with other solvents

It is of interest to compare the solubility of simple solutes in lipid bilayers to that in olive oil both because the latter solvent provides a good model of the anesthetic site [7,16] and because oil solubility provides a good index of relative cellular permeability to such solutes [17]. It is also interesting to note that historically olive oil played these roles before the nature of cell membranes was well defined.

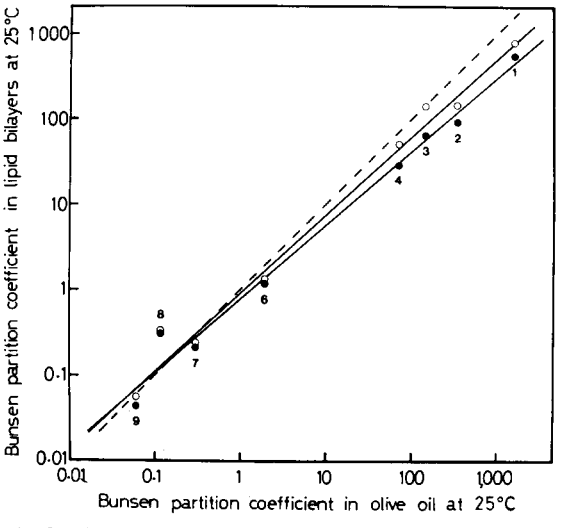

Fig. 2. The relationship between Bunsen parition coefficients in 96% phosphatidylcholine/4% phosphatidic acid bilayers (○, upper solid line) and 64% phosphatidylcholine/3% phosphatidic acid/33% cholesterol bilayers (●, lower solid line) and in olive oil at 25°C. The dashed line represents the situation where the partition coefficient in olive oil equals that in the lipid bilayer. The values of α in olive oil for methoxyflurane, halothane, xenon and sulfur hexofluoride were obtained from Miller et al. [32], fluroxene from Regan and Eger [12], hexafluoroethane from Miller et al. [7]; isoflurane was extrapolated from Cromwell et al. [10] and carbon tetrafluoride was estimated from Eger et al. [30]. The numbers identify the agents as coded in Tables I and II.

We have used Bunsen partition coefficients for this comparison for reasons which have been discussed fully in an earlier publication [4]. One of these reasons is simply that molecular units are inappropriate in solvents of ill defined composition. The second is that for a given gas in a series of solvents volume fraction, rather than mol fraction, provides the best basis for comparison when the solvent and solute have very different molar volumes [9].

The comparison of Bunsen coefficients for our two bilayers with those for olive oil at 25°C is shown in Fig. 2. Sources of data are given in the legend. The dashed line is that expected if solubility in either bilayer were equal to that in the oil. Although olive oil provides a very good first approximation to the bilayer, only one point lies above this line indicating that olive oil is generally a somewhat better solvent (i.e. it will dissolve more solute) than the bilayers. The same is true for simple apolar solvents compared to bilayers [2,4,6]. The best fits to the actual data are shown by the solid lines in Fig. 2. The slope of the upper solid line is slightly less than unity and the intercept is almost equal to zero. The relationship is:

$$\log \alpha_{(PC/PA)} = 0.908(\pm 0.046) \log \alpha_{(Olive\ oil)} - 0.015(\pm 0.085) \quad (1)$$

In PC/PA/Chol. the equivalent relationship is:

$$\log \alpha_{(PC/PA/Chol.)} = 0.859(\pm 0.047) \log \alpha_{(olive\ oil)} - 0.083(\pm 0.086) \quad (2)$$

The slope in Eqn. 1 is not significantly different from one but that in Eqn. 2 is significantly less than unity ($P < 0.05$). This indicates a tendency towards a greater decrease in partition coefficient for the more soluble agents. Since the slopes of these equations tend to be less than one, olive oil gives its best estimate for bilayer/gas partition coefficient when α is close to one because the intercepts are not significantly different from zero. The origins of this small effect may arise from the increasing molar volume, the increasing polarity and increasing dispersion forces of the more soluble anesthetics. The differences between the interaction of these properties with the bilayer and the oil must underlie the disproportionate, but weak, decrease in partition coefficient for the more soluble agents.

Comparisons of olive oil with bilayers is complicated by the heterogeneity in the bilayer particularly in the interfacial region [2,6]. The acyl chains form approximately only 68% by weight of the phosphatidylcholine molecule but probably provide the major region for solvation of small non-polar molecules. If the volume of lipid available for solvation is multiplied by 0.68, an effective partition coefficient into the hydrophobic core can be calculated which is 1.5 times the measured coefficient for PC/PA and, allowing for cholesterol, 1.3 times that for PC/PA/Chol. This would add +0.17 and +0.13 log units to the intercepts in Eqns. 1 and 2 thus more than compensating for the decrease in the uncorrected partition coefficient on going from olive oil to a bilayer. However this treatment assumes no partitioning whatsoever into the interfacial region of the bilayer, which is clearly an oversimplification. These values thus represent an upper limit for solubility in the acyl region. The actual solubility must lie between the normal and the corrected values. One way to estimate the amount of solute in the polar interface is to use the value of the partition coefficient in a gel phase bilayer, since spectroscopic data suggest that under these conditions much of the solute is located in the interface [18–20]. Using the data of Simon et al. [3] for partitioning of halothane into dipalmitoylphosphatidylcholine at 25°C, we estimate 25% of the halothane to be in the interfacial region. The effective Bunsen coefficient in the acyl region of PC/PA at 25°C corrected solely for the volume of this region (as above) is reduced from 222 to 167 when the halothane estimated to be in the interfacial region is subtracted. These two values probably define a range of values within which the actual partition coefficient of halothane in the hydrophobic acyl region lies. The partition coefficient between the interfacial region and the hydrophobic core must vary with the polarity of the solute, but for the solutes examined here we can conclude that the above corrections bring the partition coefficient in the acyl region closer to that of olive oil whose Bunsen coefficient is 224.

For more hydrophilic solutes Katz and Diamond [6] found that isoamylalcohol was a better predictor of solvent power than olive oil and suggested that their solutes preferred the membrane surface to the

hydrophobic core. Comparison of their hydrophilic solutes with our more hydrophobic solutes yields a cross sectional view of the solvent properties of the bilayer that are qualitatively reasonable. Note that the ability of a bulk solvent to model a bilayer will depend on the polarity of the solute.

Although olive oil produces a good estimate of solubility in bilayers, it may be less successful at predicting solubility in the lipid regions of biomembranes since they appear to be consistently poorer solvents than bilayers for small solutes such as butane [4] or barbiturates [21]. We confirmed these findings by measuring the Bunsen membrane gas partition coefficients for halothane at 25°C in human erythrocyte ghosts and rat liver mitochondria and found them to be 35 and 50, respectively. These values are much lower than those in lipid bilayers (Table I), olive oil or other simple solvents. The cholesterol content was approx. 33 mol% in the erythrocyte ghosts and 8 mol% in the mitochondrial membranes. The partition coefficients for halothane are thus in accord with the decrease in partitioning of pentobarbital into phosphatidylcholine membranes as the cholesterol content was increased [21]. The approximate protein/lipid ratios were 0.6 and 0.8 w/w for the erythrocytes and mitochondrial membranes, respectively. This finding has obvious implications for the estimation of absolute values of cellular permeability but is unlikely to affect relative values.

Recently, Conrad and Singer [22] have claimed that small amphipathic compounds have partition coefficients in lipid bilayers which exceed those in biomembranes by several orders of magnitude. Their claim is based on results obtained with a novel experimental technique. Our results in this work for halothane and in earlier work for butane [4] and the two barbiturates [21,23,24] using conventional techniques yielded values consistently lower in biomembranes than in bilayers but only about half an order of magnitude. Other literature data for chloropromazine and decanol [25] suggest that the methodology used by Conrad and Singer yields seriously erroneous results with biomembranes.

Thermodynamics of solution in bilayers

One advantage of obtaining bilayer/gas partition coefficients is that the gaseous standard state is very nearly equal for all solutes. This obviates problems of interpretation that arise when partition between water and lipid phases are considered where differential entropy effects due to the hydrophobic effect on water by the solutes are large and notoriously unpredictable. Our analysis thus reflects enthalpic and entropic changes associated with solvation rather than the sum of dehydration and subsequent solvation.

We have calculated the thermodynamic parameters associated with the transfer of 1 mol of gas at a partial pressure of 1 atm and at 25°C into the lipid bilayers under the same conditions by plotting the natural logarithm of the Bunsen partition coefficient against the reciprocal of the absolute temperature (Fig. 3). The fan of slopes with carbon tetrafluoride giving a slope of zero is similar to that shown by Hildebrand et al. [9] for the temperature dependence of the solubility of gases in cyclohexane where both argon and carbon tetrafluoride have slopes equal to zero. The slope of this plot is $-\Delta H/R$ and the intercept is $\Delta S/R$ where R is the gas constant. ΔF can be calculated either from $\Delta F = \Delta H - T\Delta S$ or from $\Delta F = -RT\ln\alpha_T$ where α_T is the partition coefficient at absolute temperature T. The free energy of transfer (ΔF) of a gas into a solvent is a balance between the

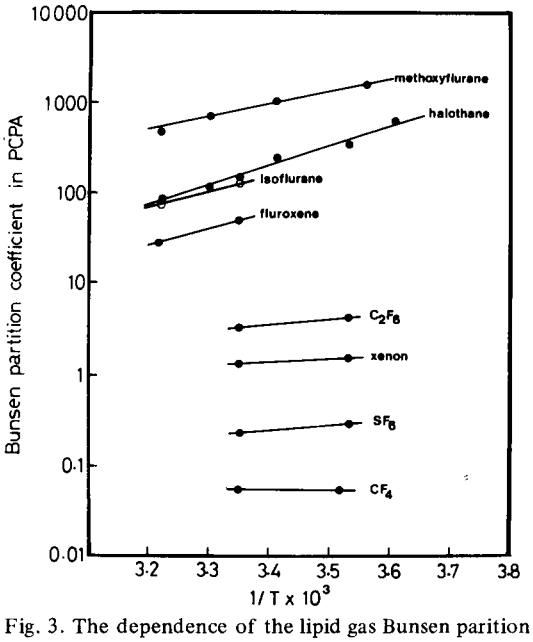

Fig. 3. The dependence of the lipid gas Bunsen parition coefficients of anesthetic agents in 96% phosphatidylcholine/4% phosphatidic acid (PCPA) bilayers on temperature.

TABLE II

THERMODYNAMIC PARAMETERS FOR TRANSFER OF GAS INTO BILAYERS AND OLIVE OIL AT 25°C

Standard errors are included where appropriate.

Anesthetic agent (Ref.)	PC/PA			PC/PA/Chol.			Olive oil [12,30,34,38]		
	ΔH (kcal/mol)	$-T\Delta S$ (kcal/mol)	ΔF (kcal/mol)	ΔH (kcal/mol)	$-T\Delta S$ (kcal/mol)	ΔF (kcal/mol)	ΔH (kcal/mol)	$-T\Delta S$ (kcal/mol)	ΔF (kcal/mol)
1. Methoxyflurane	−7.5 ± 0.7	+3.5 ± 0.7	−4.0	−6.5 ± 1.1	+2.8 ± 1.2	−3.7	−8.8 ± 0.2	+4.4 ± 0.2	−4.4
2. Halothane	−9.8 ± 0.7	+6.8 ± 0.7	−3.0	−8.4 ± 0.7	+5.7 ± 0.7	−2.7	−8.1 ± 0.9	+4.6 ± 0.9	−3.5
3. Isoflurane	−8.9	+6.0	−2.9	−6.8	+4.3	−2.5	–	–	–
4. Fluroxene	−8.6	+6.3	−2.3	−7.1	+5.1	−2.0	−7.8 ± 0.9	+5.2 ± 0.8	−2.6
5. Butane [4]	−7.6 ± 0.5	+5.9 ± 0.5	−1.7	−9.6	+7.9	−1.7	–	–	–
6. Xenon	−1.9	+1.8	−0.1	−1.2	+1.1	−0.1	−0.8 ± 0.4	+0.4 ± 0.4	−0.4
7. Sulfur hexafluoride	−2.6	+3.5	+0.9	+1.0	−0.1	+0.9	−2.5	+3.2	+0.7
8. Carbon tetrafluoride	+0.2	+1.5	+1.7	+3.6	−1.7	+1.9	–	–	–
9. Hexafluoroethane	−2.9	+3.5	+0.6	−2.4	+3.1	+0.7	–	–	–

TABLE III

PARAMETERS OF LINEAR REGRESSION FOR BARCLAY-BUTLER PLOTS OF ΔS VS. ΔH FOR PARTITION OF GASEOUS SOLUTES INTO OLIVE OIL, PC/PA AND PC/PA/CHOL. AT 25°C

Values for dimyristoylphosphatidylcholine, chlorobenzene and benzene are taken from Katz and Diamond [6].

Solvent	Slope b (K^{-1}) $\times 10^{-3}$	Intercept a (cal · K^{-1} · mol^{-1})	Correlation coefficient (r)
Olive oil	1.6 ± 0.44	−3.2 ± 2.9	0.900
PC/PA	1.7 ± 0.27	−5.3 ± 1.8	0.917
PC/PA/Chol.	2.1 ± 0.25 [a]	−1.8 ± 1.5	0.954
Dimyristoylphosphatidylcholine	2.26	−2.4	–
Chlorobenzene	1.42	−9.2	–
Benzene	1.33	−8.1	–
Water	1.82 ± 0.39	−11.2	0.893

[a] Significantly different from PC/PA ($P < 0.01$) and olive oil ($P < 0.01$).

enthalpic (ΔH) and entropic (ΔS) terms which pull in opposite directions. Table II shows the enthalpy (ΔH), the entropy expressed in energy units ($-T\Delta S$) and the free energy of transfer of the gas into PC/PA and PC/PA/Chol. bilayers and into a bulk lipid using partition coefficient data for olive oil at different temperatures from the literature. The data for methoxyflurane were fitted by least squares quadratic regression and the slope at 25°C was determined by differentiation. Standard errors for ΔH and ΔS are from the least-squares fits of the lines in Fig. 3 for methoxyflurane and halothane. For the other agents, data were obtained at two temperatures only and therefore the entropies and enthalpies must be regarded with some caution.

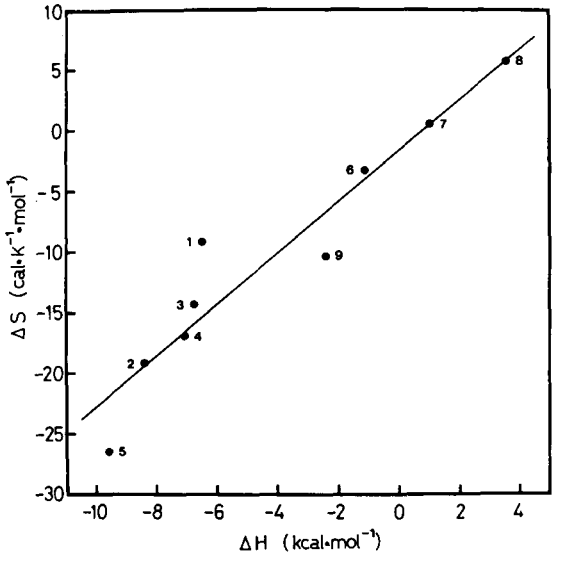

Fig. 4. Barclay-Butler plot of the entropy vs. the enthalpy of partition between 64% phosphatidylcholine/3% phosphatidic acid/3% cholesterol bilayers and the gaseous phase at 1 atm partial pressure and 25°C. The numbers identify the agents as coded in Tables I and II.

An empirical linear relationship between the entropy and enthalpy of transfer of different solutes from the vapor phase into the same solvents was discovered by Barclay and Butler [26]. For dilute solutions obeying Henry's law, the slope and intercept of this plot has been interpreted in terms of the structure of the solvent [6]. This plot is shown in Fig. 4 for PC/PA/Chol. and the results of linear regression analysis for both bilayers and olive oil are shown in Table III for the eight solutes that we have studied plus values for butane taken from Miller et al. [4]. This plot reveals that ΔH and ΔS for methoxyflurane are different from the other gases and this suggests that some additional factor influences the dissolution of this solute. Slopes (b) obtained by Katz and Diamond [6] for partition of nonelectrolytes into dimyristoylphosphatidylcholine above 25°C, chlorobenzene and benzene have also been included in Table III. Their intercepts are based on a standard state of 1 mmHg partial pressure of gas and these have been converted to our standard state (1 atm partial pressure) to yield the intercepts (a) shown in Table III. The figures for water were derived from literature values for the temperature dependence of gas/water partition coefficients for the solutes we used. In olive oil the large errors reflect

the lack of data, but if data for other agents (chloroform, cyclopropane, diethyl ether, nitrogen, neon, argon and krypton) are added the slope becomes $(1.5 \pm 0.24) \cdot 10^{-3}$ and the intercept becomes -3.8 ± 1.2. These figures suggest that olive oil lies in between the organic solvents and the bilayers.

The molecular interpretation of the Barclay-Butler plot is not well understood but amongst other factors, the polarity of the solvent, the ratio of size of solute to solvent and the nature of the solute itself are important. In general the slope of these plots discriminates poorly between solvents. The intercepts however are more negative for the organic solvents and water than for olive oil or any of the amphipathic lipid bilayer preparations but the reasons for this are unclear.

Correlations with anesthetic potency

The correlation of anesthetic potency with olive oil solubility has stood the test of time and proved remarkably precise despite numerous attempts using more sophisticated indices to gain a better correlation with more accurate predictive power [7,16]. We here compare correlations of anesthetic potency with our partition coefficients in PC/PA and PC/PA/Chol. to that with olive oil. We did not expect to improve on the correlation given by olive oil because this correlation is so good. In fact, because it is experimentally more difficult to measure partitioning into lipid bilayers in an aqueous suspension than it is into a bulk solvent, we would not have been surprised to see a slightly worse correlation. Clearly, the bilayer provides a more rational model of a putative site of anesthetic action than does olive oil. It should also obviate ambiguities of interpretation. For example, octanol may be a good model of a lipid bilayer for non-polar sources [6] but when alcohol anesthetics are considered, hydrogen bonding may change the partition coefficients in a way which is not possible either in a non-polar solvent such as hexadecane or in the interior of a bilayer [27].

We have used ED_{50} values for loss of righting reflex in mice for seven of the compounds studied [7,28] excluding butane for which the ED_{50} values were unavailable and hexafluoroethane for which the ED_{50} value in dogs was unknown. Lipid/gas Bunsen partition coefficients at 37°C for xenon, sulfur hexafluoride and carbon tetrafluoride were obtained by linear extrapolation of plots such as those shown in Fig. 3. Log ED_{50} values were plotted against log lipid/gas Bunsen partition coefficients for olive oil, PC/PA and PC/PA/Chol. bilayers as shown in Fig. 5a. These Meyer-Overton plots should have a slope of -1 and linear regression gave slopes and correlation coefficients shown in Table IV. The alternative model, first proposed by Mullins [29], that the

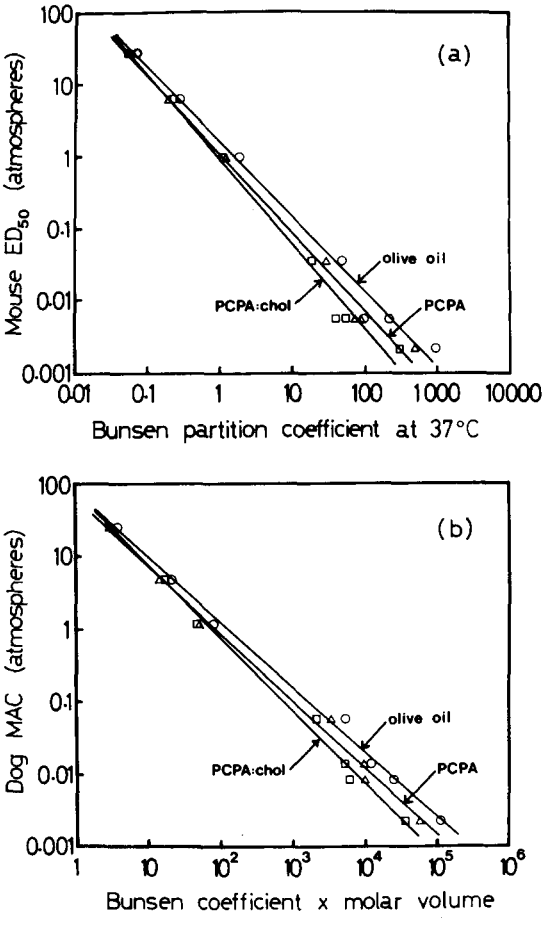

Fig. 5. (a) Correlation of log ED_{50} for loss of righting reflex in mice with log lipid/gas Bunsen parition coefficient in olive oil (○), PC/PA (PCPA, △) and PC/PA/Chol. (PCPA/Chol., □) bilayers at 37°C. Olive oil partition coefficients were obtained from the literature [10–12,30,34–37]. (b) Correlation of log minimum alveolar concentration (MAC) for loss of response to painful stimulus in dogs with lipid/gas Bunsen partition coefficient × molar volume in olive oil (○), PC/PA (PCPA, △) and PC/PA/Chol. (PCPA/Chol., □) bilayers at 37°C. Molar volumes at the boiling points were taken from Hildebrand et al. [9] or calculated from molecular weight divided by density.

TABLE IV

SLOPES AND CORRELATION COEFFICIENTS FOR MEYER-OVERTON CORRELATIONS OF log ANESTHETIC DOSE WITH log LIPID/GAS BUNSEN PARTITION COEFFICIENTS AT 37°C AND MULLINS CORRELATIONS WITH log PARTITION COEFFICIENT × MOLAR VOLUME AT 37°C

Species and anesthetic endpoint	Lipid	Type of correlation	Slope mean ± S.E.	Correlation coefficient (r)
Mouse ED_{50} for loss of righting reflex	Olive oil	Meyer-Overton	−1.04 ± 0.05	0.9930
		Mullins	−0.95 ± 0.05	0.9939
	PC/PA	Meyer-Overton	−1.09 ± 0.05	0.9954
		Mullins	−0.99 ± 0.05	0.9948
	PC/PA/Chol.	Meyer-Overton	−1.18 ± 0.06 [a]	0.9926
		Mullins	−1.06 ± 0.06	0.9929
Dog MAC for loss of response to painful stimulus	Olive oil	Meyer-Overton	−0.98 ± 0.03	0.9974
		Mullins	−0.90 ± 0.03 [a]	0.9975
	PC/PA	Meyer-Overton	−1.02 ± 0.03	0.9972
		Mullins	−0.93 ± 0.04	0.9961
	PC/PA/Chol.	Meyer-Overton	−1.11 ± 0.04 [a]	0.9972
		Mullins	−1.00 ± 0.04	0.9966

[a] Slope significantly different from −1 ($P < 0.05$).

product of lipid partition coefficient and molar volume correlates with potency is also examined in Table IV. All models yielded excellent correlation coefficients, showing the power of the lipid bilayer model.

Which of our bilayers is the better model of the anesthetic site and which of the two anesthetic models (Meyer-Overton or Mullins) is best? To decide these questions the ability of the models to predict a slope of one is the critical parameter. Since the usefulness of correlations such as these depends upon the accuracy and self-consistency of the anesthesia data, a second series of correlations was performed for the same seven agents using the minimum alveolar concentration of anesthetic required to eliminate movement in response to a painful stimulus (tail clamp) in 50% of dogs tested [10,30,31]. Both sets of anesthetic data in Table IV yield similar conclusions. The slopes of Meyer-Overton plots are consistently about 10% higher than the slopes of the Mullins plots for both mouse and dog data. The mouse data generally produce slightly higher slopes than do the dog data. Only the Mullins correlation for olive oil and the Meyer-Overton correlation for PC/PA/Chol. using both mouse and dog data yield slopes significantly different from −1. In olive oil, the slopes of the Meyer-Overton correlations for both mouse and dog data are within one standard deviation of unity. In PC/PA there is no consistent distinction between the two hypotheses, but in PC/PA/Chol. the Mullins model correlations yield slopes within one standard deviation of unity for both the mouse and dog data and the Meyer-Overton model consistently fails ($P = 0.003$ for the combined data). Thus the Mullins correlation in PC/PA/Chol. is the most consistently favored of these bilayer models. Clearly the statistical significance of this conclusion warrants caution, but it is consistent both with the findings from pressure reversal of anesthesia studies, which require the inclusion of volume [32] and with the known cholesterol content of synaptic membranes [33].

Although the detailed arguments above are not conclusive, the correlation between the anesthetic potency of these gaseous and volatile agents and their lipid bilayer solubilities is consistent with a lipid bilayer being a possible site of general anesthetic action, although they cannot unequivocally distin-

guish between the Meyer-Overton and Mullins correlations. It remains possible that binding to some site on a protein might follow a similar pattern, but this has yet to be demonstrated.

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