REFERENCES

1. L. Triner., Y. Vulliemoz, I. Schwartz and G. G. Nahas, *Biochem. Biophys. Res. Com.* **40**, 64 (1970).
2. G. Poch and W. R. Kukovetz, *Life Sci.* **10**, 133 (1971).
3. T. Dousa and I. Rychlik, *Life Sci.* **7**, 1039 (1968).
4. W. J. Thompson and M. M. Appleman, *Biochemistry* **10**, 311 (1971).
5. F. Ferre, M. Breuiller and L. Cedard, *FEBS Lett.* **52**, 295 (1975).
6. O. H. Lowry, U. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 264 (1951).
7. G. M. Stancel, W. J. Thompson and S. J. Strada, *Mol. Cell. Endocrin.* **3**, 283 (1975).
8. C. H. Beatty, R. M. Bocek, M. K. Young and M. J. Novy, *Prostaglandins* **11**, 713 (1976).
9. A. K. Goff and P. W. Major, *FEBS Lett.* **61**, 144 (1976).
10. P. Mitznegg, B. Hach and F. Heim, *Life Sci.* **9**, 975 (1970).
11. P. De La Fuente, R. Diaz Castellanos, A. Gonzalez, M. A. Ezcurdia, S. Dat and A. Martin De La Lastra, *J. Gyn. Obst. Obst. Biol. Repr.* **1**, 497 (1972).
12. T. Uruno, I. Takayanagi, K. Kubota and K. Takagi, *Eur. J. Pharmac.* **32**, 116 (1975).
13. J. R. Vane, *Nature New Biol.* **231**, 232 (1971).
14. S. S. Pong and L. Levine, *J. Pharmac. exp. Ther.* **153**, 237 (1976).
15. R. M. Pinto, E. Montuori, U. Lerner, H. Baleiron, M. Glauberman and H. Nemirovsky, *Am. J. Obst. Gynec.* **91**, 1084 (1965).
16. R. M. Pinto, U. Lerner and H. Pontelli, *Am. J. Obst. Gynec.* **98**, 547 (1967).

Biochemical Pharmacology, Vol. 27, pp. 1294–1296.

0006-2952/78/0415-1294$02.00/0

Partition coefficients of alkanols in lipid bilayer/water

(*Received* 22 *July* 1977; *accepted* 8 *September* 1977)

Long chain alcohols induce narcosis and anesthesia in a variety of animals. Like other anesthetics they also modulate a variety of other functions in biomembranes and in lipid bilayers: these include expansion [1], motional and phase properties [2–7], permeability [8] and functions of membrane-bound enzymes [9, 10]. Although the molecular mechanisms involved in the perturbation of any of these functions are not established, it is generally accepted that alcohol molecules localized in the membrane are responsible for such effects. Thus, a correlation is observed between the *n*-octanol/water partition coefficients of a series of *n*-alkanols and their equipotency concentrations for induction of a variety of membrane phenomena. Such correlations suggest that the incremental free energy for the incorporation of methylene residues in *n*-octanol and in membrane systems is similar [11]. Such correlations, however, do not give any information about the actual amount of alcohol partitioned into the membrane phase. In this communication, we report partition coefficients in lecithin bilayer/water for homologous alkanols.

Partition coefficients were determined by measuring the concentrations of alkanols in the aqueous phase in the presence (C_w) and in the absence (C_l) of liposomes. The amount of alcohol incorporated into the lipid phase (A_l) was obtained by difference. Thus $A_l = (C_l - C_w)V_w$, and the concentration of alcohol in the membrane phase $C_l = A_l/W_l$ where W_l is the weight of lipid in V_w g of water. Thus, the partition coefficient, the ratio of the concentrations of alcohol in the two phases, $P = C_l/C_w = (C_l - C_w)V_w/C_w W_l$. It may be noted that the partition coefficient is defined as: g solute/g of lipid phase divided by g solute/g of aqueous phase. It is dimensionless. As an approximation, the amount of the lipid phase was taken to be the same as the amount of lipid present in the mixture. Experimentally, 200 μl of an aqueous solution of alcohol was mixed with 100 μl of hand-shaken liposomes. The final lipid concentrations in each tube was either 0 (control), 10, 20 or 50 mg/ml. The tubes were allowed to equilibrate at room temperature (24°) for 30 min and then centrifuged for 3 min at 12000 *g*. Separate experiments showed that equilibrium was complete in this period, since no significant change in the alcohol concentration was noted even after 24 hr of incubation. About 50 μl of the supernatant fraction was withdrawn, in which the amount of alcohol was determined by gas chromatography. Five-to 10-μl samples of the aqueous phase were injected into a 6 ft (3/16 in. i.d.) column packed with Porapak Q (Supelco, Inc., Bellefonte, PA., U.S.A.) on a model 5750 Hewlett Packard gas chromatograph equipped with a flame ionization detector. The column, port and detector temperatures were adjusted and programmed to optimize linearity between the volume of the alcohol solution injected and the area of the elution profile peak. The amount of each alcohol could thus be detected with an accuracy of better than ± 5 per cent. The value of a partition coefficient was calculated from the mean of the values for three samples, with three injections from each sample. There are several possible sources of error in these measurements [12]. The range of concentrations involved, the design of the experimental setup and the magnitudes of the partition coefficients are such that, with the controls employed, the overall error in a measured partition coefficient is less than ± 10 per cent or ± 2 (whichever is larger) of the absolute partition coefficient. This method gives a considerably larger margin of error if the partition coefficients are lower than 1 or larger than 2000.

The ratio of each concentration of alcohol in lipid (obtained by difference) to the concentration in water—that is, the measured partition coefficient for each alkanol, is presented in Table 1. The following features of these data may be noted.

Partition coefficients increase monotonically with chain length. The incremental free energy for the partitioning of a methylene residue calculated from the data is − 745 and − 630 cal/mole for the dipalmitoyl and egg lecithin liposomes respectively. Similarly, it takes 2.81 kcal/mole and 1.51 kcal/mole to partition the hydroxyl group in a dipalmitoyl and egg lecithin bilayer. These values compare favorably with the values observed for the partition coefficients of alcohols in isotropic organic solvents/water systems (column 5; [13–15]) and other membrane/water systems (column 6).

The partition coefficients for dipalmitoyl lecithin liposomes are smaller than the corresponding values for egg lecithin liposomes. However, this difference decreases with increasing chain length. Although alkanols incorporated into a bilayer induce gel to liquid crystalline phase transition, the dipalmitoyl lecithin bilayer, at the concentration employed in this study, would be exclusively in the gel phase at room temperature [16]. This is also supported

Table 1. Partition coefficients of alkanols in various systems*

Alkanol	Concn (mM)	Partition coefficients DPPC/H_2O	EL/H_2O	Octanol/H_2O (Ref. 15)	Erythrocyte/water (Ref. 17)
Pentan-1-ol	100	4.8 ± 1.4	18.1 ± 2.4	25.2	3.39
Pentan-2-ol	100	1	4.5 ± 0.6	14.5	
Isopentan-1-ol	100	4.5 ± 0.7	5.3 ± 0.8	14.5	
Hexan-1-ol	24	25.2 ± 2.8	45.4 ± 3.4	101	12.9
4-Methyl = pentan-2-ol	24	1	8.95 ± 1.43		
Heptan-1-ol	10	50.4 ± 3.0	170 ± 2	340	39 ± 3
Octan-1-ol	5	201 ± 2	378 ± 13	1010	152 ± 10
Octan-2-ol	5	54 ± 6	196 ± 10	680	
Octan-3-ol	5	41 ± 2.8	162 ± 13	680	
Octan-4-ol	5	26 ± 5	131 ± 5	680	
Nonan-1-ol	5	1020 ± 100	1400 ± 300	3400	582 ± 20
Slope		0.556 ± 0.0386	0.470 ± 0.0195	0.526 ± 0.013	0.554 ± 0.01056
x-Intercept		3.7723	2.398	2.2658	4.0510
Corr. coefficient		0.993	0.997	−.999	0.999
$\delta\Delta(CH_2)$ (cal/mole)		−745 ± 52	−630 ± 26	−705 ± 17	−743 ± 14
$\delta\Delta(OH)$ (cal/mole)		2810 ± 196	1510 ± 62	1600 ± 38	3000 ± 57

* Values of the x-intercept and slope (regression of y on x) were obtained by a least square linear regression of chain length vs log (partition coefficient) plot for the *l*-isomers only. Column 2 gives the total concentration of the alcohol used in the present study. Columns 3–6 give partition coefficients against water for dipalmitoyl lecithin bilayer, egg lecithin bilayer, bulk *n*-octanol and erythrocyte membranes respectively. All the partition coefficient values are ratios: g solute/g of phase 1 to g solute/g of phase 2.

by the observation that the partition coefficient is independent of the lipid to alkanol ratio in the incubation mixture (data not presented). The absolute values for membrane/water partition coefficients for the alcohols are, however, significantly smaller than the values for isotropic solvent/water systems (column 5 in Table 1). This suggests that the more anisotropic organized gel phase (dipalmitoyl lecithin) offers greater resistance to the incorporation of lower alcohols than does a disorganized anisotropic phase as in egg lecithin liposomes and the isotropic bulk solvents. The partition coefficient of erythrocyte membrane is smaller than that of either the egg lecithin or dipalmitoyl lecithin bilayer. The protein (~ 50 per cent of the total weight) and cholesterol (~ 40 per cent of the total lipid) content of erythrocyte membrane would tend to lower measured partition coefficient values.

The constraining effect of bilayer organization is accentuated even further in the partition coefficients of branched chain alcohols. The values for the isomeric branched chain alcohols are considerably smaller than those for their straight chain analogs: compare, for example, the isomeric pentanols and octanols. Such a large systematic effect is not expected in the partition coefficients in the *n*-octanol/water system [15]. These differences have been noted in the permeability of branched chain solutes compared to their straight chain analogs [18].

The data presented in this communication are useful for computing the concentration of *n*-alkanols in the lecithin bilayer in the gel and liquid-crystalline phases. They also demonstrate that, compared to the partition coefficients in bulk solvents, the lipid bilayer/water partition coefficients are at least 3-fold smaller and are quite sensitive to the structural parameter of the solute and to the organizational parameters of the bilayer. This would imply that alcohols induce changes in biomembranes at much lower concentrations than suspected thus far, that the phase properties of the bilayer can regulate the partitioning of solutes, and that the positional isomers containing identical groups can be distinguished better than those distinguished by partitioning into an isotropic solvent.

Acknowledgement—This work was supported by the Mental Health Institute (R03MH27957).

Department of Chemistry, School of Life and Health Sciences, University of Delaware, Newark, DE 19711, U.S.A.

MAHENDRA KUMAR JAIN
LEWIS V. WRAY, JR.

REFERENCES

1. P. Seeman, W. O. Kwart, T. Sauks and W. Argent, *Biochim. biophys. Acta* **183**, 490 (1969).
2. M. K. Jain, N. M. Wu and L. V. Wray, *Nature, Lond.* **255**, 494 (1975).
3. M. W. Hill, *Biochim. biophys. Acta* **356**, 117 (1974).
4. S. H. Roth and L. Spero, *Can. J. Physiol. Pharmac.* **54**, 35 (1976).
5. S. J. Paterson, K. W. Butler, P. Huang, J. Labelle, I. C. P. Smith and H. Schneider, *Biochim. biophys. Acta* **266**, 597 (1972).
6. A. W. Eliasz, D. Chapman and D. F. Ewing, *Biochim. biophys. Acta* **448**, 220 (1976).
7. S. Matjavila, C. Lacombe and G. Carrera, *Biochem. Pharmac.* **25**, 625 (1976).
8. M. K. Jain, D. G. Toussaint and E. H. Cordes, *J. memb. Biol.* **14**, 1 (1973).
9. C. Hegyvary, *Biochim. biophys. Acta* **311**, 272 (1973).
10. K. H. Sullivan, M. K. Jain and A. L. Koch, *Biochim. biophys. Acta* **352**, 288 (1974).
11. M. W. Hill, *Biochem. Soc. Trans.* **3**, 149 (1975).
12. Y. Katz and J. M. Diamond, *J. memb. Biol.* **17**, 69 (1973).
13. K. Kinoshita, H. Ishikawa and K. Shinoda, *Bull. chem. Soc. Japan* **31**, 1081 (1958).
14. D. A. Haydon and F. H. Taylor, *Phil. Trans. R. Soc.* **252**, 225 (1960).
15. C. Hansch and W. J. Dunn, *J. pharm. Sci.* **61**, 1 (1972).
16. M. K. Jain and N. M. Wu, *J. memb. Biol.* **34**, 157 (1977).
17. P. Seeman, S. Roth and H. Schneider, *Biochim. biophys. Acta* **225**, 171 (1971).
18. J. M. Diamond and Y. Katz, *J. memb. Biol.* **17**, 121 (1974).