1061. *The Adsorption of Water Vapour by Lecithin and Lysolecithin, and the Hydration of Lysolecithin Micelles.*

By P. H. Elworthy.

The adsorption of water vapour by lecithin, lysolecithin, triolein, and tristearin has been studied at 25° and 40°. The polar parts of the phosphatide molecules appear to be responsible for adsorption, and the results were fitted by B.E.T. plots at low relative vapour pressures. Some hysteresis in the adsorption isotherms was found for lysolecithin. Certain thermodynamic quantities have been calculated from the adsorption results. A value of micellar hydration of lysolecithin has been calculated from transport properties of solutions, and compared with the value found from adsorption experiments.

The adsorption of water vapour by proteins has been studied by several authors.[1,2] No reports have been made of adsorption by phosphatides and, in view of their biological importance, it was considered worthwhile to study the uptake of water vapour by these substances.

As the phosphatides form part of the animal cell membrane, the extent of their hydration may well influence the adsorption of drugs on to the membrane, and also the passage of biologically active materials through it. Choline, which is intensely hygroscopic, is present in the molecules of both lecithin and lysolecithin, indicating that the polar parts of the phosphatide molecules would be responsible for adsorption of water vapour. Adsorption studies should give an idea of the extent of hydration of phosphatide micelles.

A second line of approach to micellar hydration is to use the transport properties of the phosphatides in solution to obtain values of micellar weight, which can be compared with a value found from light scattering; as the micelles of lysolecithin are reasonably spherical, discrepancies between the two values of micellar weight are likely to be due to hydration. The extent of hydration can thus be calculated.

Experimental

Materials.—Lecithin was prepared from the mixed phosphatides of chicken's egg yolks by treatment with alumina to remove ninhydrin-reacting materials, followed by chromatography on silica to remove lysolecithin.[3] Lysolecithin was prepared from the lecithin–lysolecithin mixture obtained after the alumina treatment by Saunders's method.[4] Two samples of each material were used. Two samples of lecithin contained, respectively, N 1·77, 1·75, and P 3·92, 3·83%, and had I no. 55, 73. Two samples of lysolecithin contained, respectively, N 2·62, 2·65 and P 5·80, 5·90% and had I nos. 8.

Glycerol trioleate and tristearate were " molecularly " distilled, the former having I no. 83 (calc., 86) and the latter m. p. 70° (lit.,[5] 72°).

The sulphuric acid used was of " AnalaR " quality.

Adsorption of Water Vapour.—The phosphatide was contained in a weighing bottle over a solution of sulphuric acid under a vacuum. At the beginning of each experiment the flask containing the acid and weighing bottle was placed in carbon dioxide–ether and evacuated at 0·01 mm. for 2 hr., allowed to warm at intervals, and recooled. It was then placed in a thermostat bath at 25° or 40° (±0·01°). The sample was weighed every 24 hr. until its weight was constant. Equilibrium was nearly always reached within 1 day and, at longest, 48 hr.

[1] Altman and Benson, *J. Phys. Chem.*, 1960, **64**, 851; Mellon, Horn, and Hoover, *J. Amer. Chem. Soc.*, 1947, **69**, 827; 1948, **70**, 1144, 3040; 1949, **71**, 2761.
[2] Bull, *J. Amer. Chem. Soc.*, 1944, **66**, 1499.
[3] Elworthy and Saunders, *J.*, 1957, 330.
[4] Saunders, *J. Pharm. Pharmacol.*, 1957, **9**, 834.
[5] Heilbron and Bunbury, " Dictionary of Organic Compounds," Eyre and Spottiswoode, London, 1953, Vol. IV, p. 633.

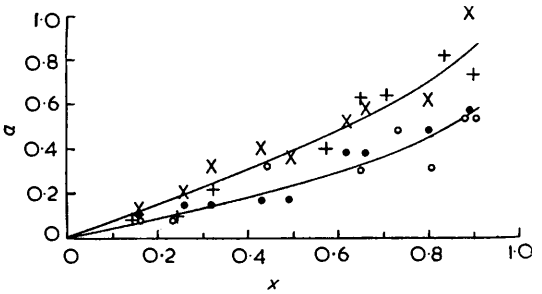

FIG. 1. Adsorption of water vapour by triolein at 40° (×) and at 25° (+) and by tristearin at 40° (•) and at 25° (○). a = g. of water adsorbed per 100 g. of phosphatide, and x = relative vapour pressure (also for other Figures).

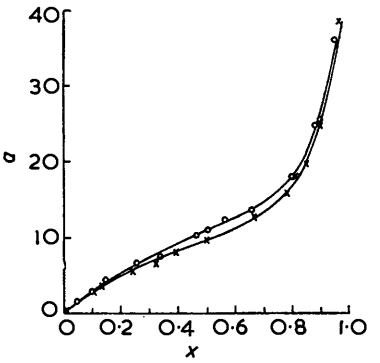

FIG. 2. Adsorption of water vapour by lecithin at 40° (○) and at 25° (×).

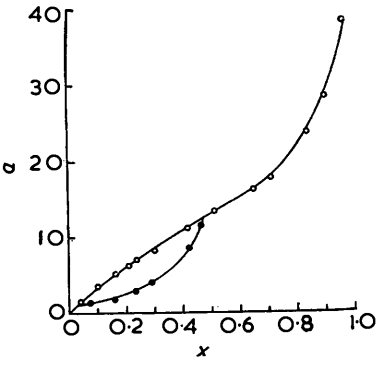

FIG. 3. Adsorption of water vapour by lysolecithin at 25°: • adsorption, ○ desorption.

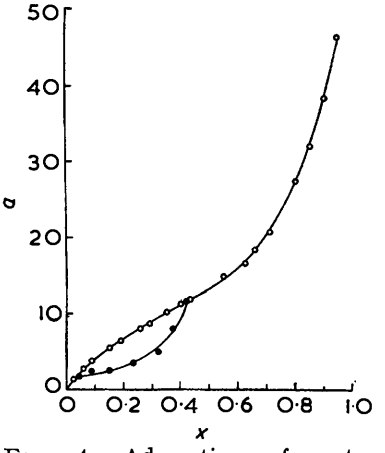

FIG. 4. Adsorption of water vapour by lysolecithin at 40°: • adsorption, ○ desorption.

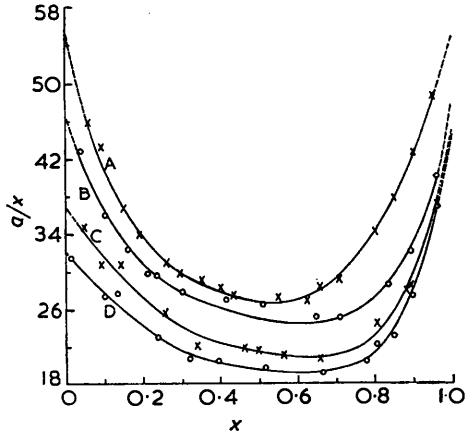

FIG. 5. Graphs of a/x against x for lecithin and lysolecithin.

Lysolecithin at (A) 40° and (B) 25°.
Lecithin at (C) 40° and (D) 25°.

The concentration of sulphuric acid solution was determined by titration. A loose flap of Polythene was placed across the neck of the weighing bottle to prevent water droplets falling in when the vacuum was released at the end of a run. Corrections for small loss on drying were obtained for the phosphatide samples by drying them at 60°/0·01 mm. The weights of the samples plus adsorbed water were reproducible to $\pm 0{\cdot}3$ mg.

The results of the adsorption experiments are shown in Figs. 1—4. There is considerable scatter of results for tristearin and triolein, owing to the small amounts of water vapour taken up (at a relative vapour pressure of 0·91, tristearin adsorbed only 5·6 mg. of water per g.). For these substances adsorption appeared to be independent of temperature. No differences in adsorptive power were observed between the samples of lecithin or of lysolecithin used. For lecithin, triolein, and tristearin, the amount adsorbed was independent of the way in which equilibrium was reached, *i.e.*, from under- or over-saturation. Lysolecithin gave hysteresis loops (Figs. 3 and 4) between relative vapour pressures (x) of 0·05 and 0·5. The adsorption isotherm for the phosphatides is S-shaped; this type has also been observed for proteins.[1,2] More water vapour was adsorbed at higher than at lower temperatures.

Fig. 5 gives a plot of a/x against x, where a is g. of water per 100 g. of phosphatide. This plot allows extrapolation to $x = 0$ and $x = 1$, and was used for the calculation of some thermodynamic quantities and for determination of water-vapour uptake at saturation.

The results on phosphatides gave good B.E.T.[6] plots below a relative vapour pressure of 0·5. In the Table, a_1, a_2, and a_S are the amounts of water vapour adsorbed in first layer, second

Constants from B.E.T. plots on lecithin and lysolecithin.

	a_1	a_2	a_s	C
Lecithin at 25°	5·63 (2·50)	12 (5·1)	44 (19·5)	7·71
Lecithin at 40°	6·12 (2·71)	14 (6·0)	48 (21)	9·63
Lysolecithin at 25° (Desorb)	8·05 (2·37)	17 (5·0)	48 (14)	6·16
Lysolecithin at 40° (Desorb)	8·20 (2·42)	15 (4·5)	55 (16)	7·30

layer, and at saturation, respectively, in g. of water per 100 g. of phosphatide. Figures in parentheses give the a value in terms of moles of water per mole of phosphatide.* C is the B.E.T. constant. Although a_1 is larger for lysolecithin than for lecithin on a weight basis, this is reversed when the amount adsorbed is calculated as mole/mole. Water adsorbed in excess of a_1 corresponds to the moderately linear portion of the adsorption isotherms (Figs. 2—4) between $x = 0{\cdot}25$ and $x = 0{\cdot}55$. The points where the isotherms start their upward swing can be determined from Fig. 5, and these values are given as a_2 in the Table. They are roughly twice the a_1 figures. Above a_2, the amount of water vapour adsorbed increases very sharply, and a_S was determined from Fig. 5.

Discussion

Any interpretation of the adsorption of water vapour requires some knowledge of the molecular structure of solid lecithin and lysolecithin. *X*-Ray diffraction studies on lecithin [7] showed that the molecules were arranged in bimolecular leaflets, the polar groups forming the outer surfaces of the leaflets, and the hydrocarbon chains being parallel to one another on the inside. This type of structure also appears to be present in lecithin micelles in aqueous solution. No *X*-ray diffraction studies have been made on lysolecithin, but it seems reasonable to assume that a roughly similar structure to that of lecithin exists in the solid.

Compared with lecithin and lysolecithin, triolein and tristearin adsorb only very small amounts of water vapour (Fig. 1), indicating that the phosphorylcholine part of the phosphatide molecule is principally responsible for adsorption. Triolein, containing double bonds, adsorbs slightly more than tristearin, but the effect is small. The adsorption of water vapour can therefore be expected to take place around the polar head groups of the phosphatide molecules. From the value of a_1 in Table 1, there are 2·5—2·7 molecules of water adsorbed on each head group of lecithin and 2·4 molecules for lysolecithin, the exact

* The molecular weights of phosphatides were calculated from nitrogen and phosphorus contents.

[6] Brunauer, Emmett, and Teller, *J. Amer. Chem. Soc.*, 1938, **60**, 309.

[7] Baer, Palmer, and Schmitt, *J. Cell. Comp. Physiol.*, 1941, **17**, 355.

values depending on temperature. Using 10·5 Å^2 as the area of the water molecule [8] gives head group areas of 26—28 and 25 Å^2, respectively. As might be expected of allied molecules containing the same polar group which is responsible for the adsorption, these values are similar. Molecular models give a head group area of 60—65 Å^2 and surface film [9] studies 80—100 Å^2. Generally, the surface areas of proteins measured from the adsorption of water vapour are too small,[2] and the same appears to be true for phosphatides.

The completion of the second layer roughly doubles the amount of water adsorbed, and there are now about five water molecules associated with each phosphatide head group. The adsorption is a complex process and there are several interpretations of the way it could occur. Opposing head groups, one on each side of the gap between the bimolecular leaflets, may share the water molecules between them, giving approximately ten molecules between two head groups at the completion of the second layer. Alternatively each individual phosphatide molecule might conduct its adsorption independently of its neighbour. There might be adsorption into cavities on the phosphorylcholine group in this process. Calculations based on the transport properties of lysolecithin in solution suggest that cavities might play a part for this compound (see below).

Continued uptake of water vapour expands the gap between the bimolecular leaflets and gives a colloidal solution at saturation in which the leaflets are detached from one another. It is interesting that, mole/mole, lecithin adsorbs more water vapour than lysolecithin at saturation, although the latter is freely soluble in water while the former is only dispersible. This effect may be allied to the differences of micellar structure in solution. Several differences were found between the two phosphatides in the adsorption studies: in the amounts of water adsorbed, in the B.E.T. constant C, and in the presence of a hysteresis loop for lysolecithin. The exact spatial arrangements of the phosphorylcholine group, while being generally similar, may not be exactly the same in the two compounds, causing the ease of access of water vapour to this group to differ. The desorption isotherms are alike in general shape, indicating that the removal of water from the gap between the leaflets is a similar process for each substance.

An idea of the affinity of solid adsorbent for water vapour can be obtained by calculating the free energy required to transfer one mole of vapour from the vapour state to the solid surface.[2] Such calculations were made with the aid of the plots of a/x against x (Fig. 5) and show that the affinity of lecithin for water vapour is higher than that of lysolecithin on a mole/mole basis; this again indicates some difference between the interactions of water vapour with the two compounds.

Transport Properties of Lysolecithin.—A further insight into the hydration of lysolecithin may be gained by considering properties of its solutions. Saunders and Thomas [10] reported the diffusion coefficient at 25° as $6{\cdot}544 \times 10^{-7}$ cm.^{2} sec.$^{-1}$. Robinson and Saunders [11] gave the density as 1·021 g. ml.$^{-1}$. If the micelle is assumed to be spherical and unhydrated, these results give a micellar weight of 136,000 from the Stokes–Einstein equation. Light-scattering studies [12] showed that the micelles were reasonably spherical (observed dissymmetries close to unity, and small depolarisations) and that the micellar weight was 97,000. The discrepancy between the two values of the micelle size is likely to be due to the hydration of the micelle, causing the observed diffusion coefficient to be smaller than expected.

By taking the light-scattering molecular weight as that of an unhydrated sphere, the diffusion coefficient (D_0) can be calculated as $7{\cdot}317 \times 10^{-7}$ cm.^{2} sec.$^{-1}$. The ratio of the

[8] Brunauer, " Physical Adsorption of Gases and Vapours," Oxford Univ. Press, 1945, p. 287.
[9] Hughes, *Biochem. J.*, 1935, **29**, 430; Alexander and Teorell, *Trans. Faraday Soc.*, 1939, **35**, 727; Cheesman, *Arkiv Kemi, Min., Geol.*, 1946, **22**, *B*, No. 1.
[10] Saunders and Thomas, *J.*, 1958, 483.
[11] Robinson and Saunders, *J. Pharm. Pharmacol.*, 1959, **11**, 304.
[12] Robinson, Thesis, London, 1959, p. 41.

frictional coefficient (f) of the hydrated micelle to that of the unhydrated micelle (f_0) is related to the ratio of the diffusion coefficients by

$$f/f_0 = D_0/D, \quad (1)$$

giving $f/f_0 = 1{\cdot}118$.

Oncley [13] gives a relation between the ratio of the frictional coefficients and the extent of hydration (w)

$$f/f_0 = (1 + w/\bar{v}\rho)^{\frac{1}{3}} \quad (2)$$

where $\bar{v}$ is the specific volume of solute, and ρ is the density of solvent. From equation 3, $w =$ 0·39 g. of water per g. of lysolecithin. The accuracy of this estimate will not be high, as the diffusion coefficient will have an error of approximately $\pm 1\%$, while Saunders and Robinson [14] assessed the error of their light-scattering molecular weight as $\pm 7\%$. However, the agreement between this value for the hydration, and that obtained from the water-vapour experiments, 0·48 g. of water per g. of lysolecithin, is reasonable.

A further value of micellar hydration can be obtained from results of viscosity experiments on lysolecithin.[15] The intercept of a graph of η_{sp}/ϕ against ϕ gave $(\eta_{sp}/\phi)_{\phi=0} = 3{\cdot}9$, where η_{sp} = specific viscosity and ϕ = volume fraction of solute. For unhydrated spherical particles the intercept should be 2·5. From the larger value found experimentally for hydration, w takes [13] the value of 0·58 g. of water per g. of lysolecithin, again in reasonable agreement with the other values.

In attempts to decide whether the hydrating water forms a unimolecular layer around the micelle, the most accurate experimental result, the diffusion coefficient, is used to calculate the micellar radius of 37·5 Å. In a simple model of the micelle, the centres of the water molecules will be on a plane of distance (radius micelle—radius water molecule, considered as a sphere) from the centre of the micelle. A unimolecular film of water in this position around the micelle gives 0·28 g. of water per g. of lysolecithin for the hydration, which is much less than the estimates from the other methods. Assuming a bimolecular layer we have 0·50 g. of water per g. of lysolecithin. There is a serious drawback to the second assumption, in that the radius of the micelle remaining for occupation by lysolecithin molecules is only 30 Å^2, which is much smaller than the length of the lysolecithin molecule, as measured from molecular models.

Probably the hydration of the micelle consists of two distinct parts: first, a unimolecular layer of water as a sheath around the micelle; secondly, water which possibly hydrates the charged head group. An estimate of the second quantity can be obtained by subtracting the quantity present in the unimolecular layer from the mean estimate of the total hydration, *i.e.*, $(0{\cdot}48 + 0{\cdot}39 + 0{\cdot}58)/3 - 0{\cdot}28 = 0{\cdot}20$ g. of water per g. of lysolecithin. Examination of a model of the micelle shows that the polar head group is not flat, but may contain considerable cavities capable of holding water in position close to the nitrogen and phosphorus atoms. It is interesting that 0·17 g. of water per g. of lysolecithin was the amount required to complete the second layer in the water-vapour adsorption process. It may be that the first and the second layer of adsorption represent the hydration of the polar groups, and the remaining uptake occurs in separation of the bimolecular leaflets.

Calculations of the hydration of lecithin micelles from properties of its solutions are not possible, as their diffusion coefficients have not been determined and the interpretation will be complicated by the asymmetry of the micelles.[16] An approach is being made to this problem by using synthetic lecithin.

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SCHOOL OF PHARMACY, ROYAL COLLEGE OF SCIENCE AND TECHNOLOGY,
GLASGOW, C.1. [*Received, June 5th*, 1961.]

[13] Oncley, *Ann. N.Y. Acad. Sci.*, 1940—1941, **41**, 121.
[14] Robinson and Saunders, *J. Pharm. Pharmacol.*, 1959, **11**, 115T.
[15] Robinson, Thesis, London, 1959, p. 46.
[16] Robinson, *Trans. Faraday Soc.*, 1960, **56**, 1260.