955. *Sorption Studies on Phosphatides. Part II.*[1] *Sorption of Water Vapour by a Synthetic Lecithin and Cephalin.*

By P. H. Elworthy.

The sorption of water vapour by L-α-dipalmitoyl-lecithin and -cephalin has been studied at 25° and 40°. The lecithin took up more water vapour than the cephalin, owing to the presence of the quaternary ammonium group in the former. The results, which were fitted by B.E.T. plots at low relative vapour pressures, show that the synthetic lecithin sorbed less than the naturally occurring material. Calculation of the differential heats and entropies of sorption gave endothermic $\Delta\bar{H}$ values, and positive $\Delta\bar{S}$ values. The thermodynamics of sorption of lysolecithin differed frome those of the other phosphatides.

By comparing the sorption of water vapour by triolein and tristearin with that of naturally occurring lecithin and lysolecithin, it was concluded[1] that the polar head groups of the phosphatide molecules were principally responsible for the sorption, as the latter pair of compounds sorbed 10—20 times more water than the former. Differences in behaviour also existed between lecithin and lysolecithin. In the solid state the phosphatides exist as bimolecular leaflets,[2] with the hydrocarbon chains parallel, and the head groups opposing, one another. The water vapour was sorbed in the space between the polar heads.

As the polar head groups of cephalin and lecithin contain $-NH_2$ and $-NMe_3\,OH$, respectively, comparison of the sorption by the two compounds will give an idea of the relative sorptive power of these groups. Also, a comparison of the synthetic lecithin, containing only saturated fatty acids, with the naturally occurring one, containing some unsaturated ones, will give information on the effect of the hydrocarbon part of the molecule on sorption. From the results obtained on the four phosphatides, the differential heats and entropies of sorption can be calculated.

EXPERIMENTAL

Materials.—Synthetic L-α-dipalmitoyl-lecithin and -cephalin (from Messrs. Lights) were chromatographed on silica[3,4] (Found, for lecithin: N, 1·9; P, 4·1. Calc. for $C_{40}H_{82}NO_9P$: N, 1·9; P, 4·1%. Found, for cephalin: N, 2·0; P, 4·5. Calc. for $C_{27}H_{74}NO_8P$: N, 2·0; P, 4·5%). Sulphuric acid used was of " AnalaR " grade.

Sorption of Water Vapour.—The apparatus used previously has been considerably modified. The phosphatides were contained in weighing bottles which fitted into wells cut in a thick brass plate. The plate rested at the bottom of a wide-mouthed glass bottle, which could be connected to a three-way tap by means of ground-glass joints. A flask of sulphuric acid was also connected to the tap. The sulphuric acid solution was outgassed at 0·01 mm., the solution being cooled in carbon dioxide–ether, and allowed to regain room temperature at intervals. The phosphatide samples were outgassed at 25° for at least 2 hr. and then connected to the vapour above the acid solution through the tap. After the whole apparatus had been placed in a thermostat-bath at 25° or 40° (±0·01°), the phosphatides were weighed every 24 hr. until equilibrium had been reached. Adsorption experiments were performed by starting with phosphatide that had been dried in a vacuum-oven and outgassed; desorption runs started with the samples which had been equilibrated over water, and finished over the most concentrated sulphuric acid solution. The acid concentration was determined by titration after equilibrium had been reached.

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[1] Part I, Elworthy, *J.*, 1961, 5385.

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

[3] Lea and Rhodes, *Biochem. J.*, 1954, **57**, xxiii; 1955, **59**, v.

[4] Lea, Rhodes, and Stoll, *Biochem. J.*, 1955, **60**, 353.

RESULTS AND DISCUSSION

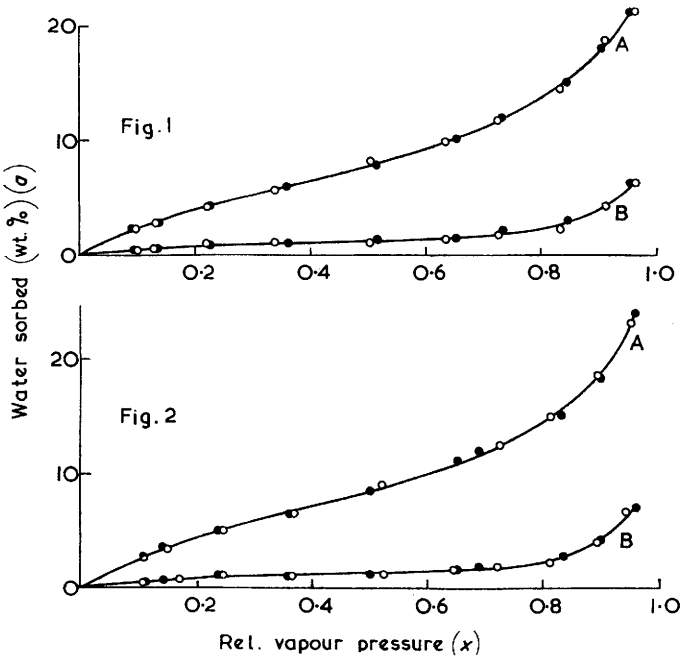
Adsorption and desorption isotherms of synthetic lecithin and cephalin are shown in Figs. 1 and 2. No hysteresis loops were observed. Good B.E.T. plots[5] were obtained for lecithin below relative v. p. (x) = 0·4 and for cephalin below $x = 0{\cdot}3$. In the annexed Table, a_1, a_2, and a_s are the amounts of water vapour sorbed in the first layer, the second layer, and at saturation, respectively; a_2 and a_s were determined from plots of a/x against x. C is the B.E.T. constant.

Constants from B.E.T. plots on synthetic lecithin and cephalin.

	a_1	a_2	a_s	C
Lecithin at 25°	4·45 (1·86)	9·18 (3·83)	25·2 (10·52)	10·71
Lecithin at 40°	4·93 (2·06)	9·94 (4·15)	26·7 (11·15)	9·67
Cephalin at 25°	1·11 (0·43)	1·31 (0·51)	9·9 (3·80)	3·96
Cephalin at 40°	1·20 (0·46)	1·31 (0·51)	9·9 (3·80)	3·55

The figures given in parentheses are moles of water per mole of phosphatide.

Results[1] on tristearin and triolein gave a_1 = 0·09 and 0·14 mole/mole, respectively, at 25°. The fully saturated cephalin sorbs 4—5 times as much water vapour as the tristearin, and this extra sorption appears to be due to the phosphatidylethanolamine grouping. The introduction of a quaternary ammonium hydroxide group into the molecule increases the sorption markedly, as a_1's for naturally occurring lecithin and lysolecithin were 2·50 and 2·37 moles/mole, respectively. In view of the importance of the quaternary ammonium group to the sorption by the lecithins, it is interesting that the hydration number derived from measurements of the activity coefficients of choline chloride by Fleming[6] was 2·65 moles/mole. The enhanced sorption in the presence of the quaternary



FIGS. 1 and 2. Adsorption (○) and desorption (●) isotherms for synthetic lecithin (A) and synthetic cephalin (B) at 25° (Fig. 1) and 40° (Fig. 2).

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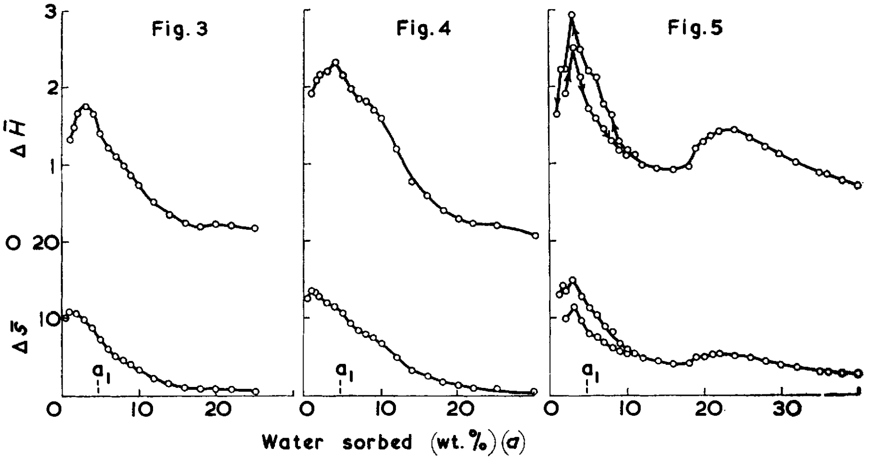
[5] Brunauer, Emmett, and Teller, *J. Amer. Chem. Soc.*, 1938, **60**, 309.

[6] Fleming, *J.*, 1961, 3100.

group may be partly due to the natural hygroscopicity of the compound, and partly due to the bulk of the group (compared with NH_2). The greater bulk may help to keep the sheets of polar groups further apart and so give more space for insertion of water molecules. With all the different types of lecithin there is a steady increase in sorption above the a_1 value, presumably resulting in an increased separation of the bimolecular leaflets, while the cephalin isotherms show an almost flat mid-portion.

The presence of approximately one double bond per hydrocarbon chain enhances the sorption of natural lecithin over that of the fully saturated synthetic material. This effect may arise because the cross-sectional area of the molecule is increased, owing to its " kinked " structure, for this would provide more space for sorption at the head-group position. Further investigation of the effect of double bonds on sorption might throw light on the permeability of cell membranes, as a highly unsaturated lecithin might provide the basis for a pore structure in the membrane.



FIGS. 3—5. Heats (kcal./mole) and entropies (cal. mole^{-1} degree^{-1}) of sorption by synthetic lecithin (Fig. 3), natural lecithin (Fig. 4), and natural lysolecithin (Fig. 5). Arrows in Fig. 5 indicate the hysteresis loop.

Thermodynamics of Sorption.—The differential heats and entropies of sorption were determined from the isotherms, by assuming $\Delta\bar{H}$ to vary linearly over the 25—40° temperature interval: [7,8]

$$\Delta\bar{H} = \bar{H}_s - \tilde{H}_1 = \boldsymbol{R}\left(\frac{\partial \ln(p/p_0)}{\partial(1/T)}\right)_{p,N_1,N_2}$$

and

$$\Delta\bar{S} = \bar{S}_s - \tilde{S}_1 = (1/T)[\Delta\bar{H} - \boldsymbol{R}T\ln(p/p_0)]$$

where $\tilde{H}_1$ and $\tilde{S}_1$ are the molar enthalpies and entropies of water, $\bar{H}_s$ and $\bar{S}_s$ are the partial molar enthalpies and entropies of the sorbate, and N_1 and N_2 are the number of moles of sorbate and sorbent, respectively. The values of $\Delta\bar{H}$ and $\Delta\bar{S}$ are plotted against a in Figs. 3—5. As the amount of water vapour sorbed by cephalin was very small, only crude estimates of these two quantities could be made; these were, respectively, 0·8 and 6 cal. mole^{-1} degree^{-1}, and the values were approximately constant up to monolayer coverage.

The heats of sorption for the other phosphatides were also endothermic, being somewhat larger for lysolecithin than for the lecithins. The initial peaks in the $\Delta\bar{H}$ curves presumably correspond to the interaction of water with the polar head groups, and the $\Delta\bar{H}$ values are similar. At B.E.T. monolayer coverage, $\Delta\bar{H}$ was 1·6 for lysolecithin, 1·9

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[7] Barrer and Kelsey, *Trans. Faraday Soc.*, 1961, **57**, 452.

[8] Altman and Benson, *J. Phys. Chem.*, 1960, **64**, 851.

for natural lecithin, and 1·6 kcal./mole for synthetic lecithin; the $\Delta\bar{S}$ values were 8·1, 9·5, and 8·0 cal. mole^{-1} degree^{-1}, respectively. The observed positive entropies indicate that a mixing effect between the sorbed water molecules and the polar head groups predominates over any effect due to a specific arrangement of water molecules near these groups, as the second effect would be expected to produce a less random arrangement of molecules than in liquid water. A process analogous to dissolution appears the most likely explanation.

For the lecithins, both $\Delta\bar{H}$ and $\Delta\bar{S}$ decrease after the initial peak in the curves. This corresponds to the region of the isotherms where more and more water is being incorporated in the space between the polar sheets. Natural lecithin is, at best, dispersible in water, rather than soluble, while the synthetic lecithin is only poorly dispersible. If a true solution were formed, a further increase in $\Delta\bar{H}$ and $\Delta\bar{S}$ would be expected. The lecithins do not show any large changes in the thermodynamic properties at large *a* values, but with lysolecithin there are increases. This secondary peak presumably corresponds to a further mixing effect, with breakdown of the swollen structure and formation of a solution. As lysolecithin forms micelles of molecular weight ~100,000 in aqueous solution, this secondary effect will be due to the mixing of these large colloidal particles with water; if monomers had been formed in this process, very much larger changes in the thermodynamic properties might have been expected.

Lysolecithin shows a hysteresis loop on adsorption between $a = 1$ and $a = 12$ g. of water per 100 g. of phosphatide. There is some doubt [7,9] about the application of the thermodynamic equations to the hysteresis region of isotherms, but a qualitatively correct picture is believed to result. The values of $\Delta\bar{H}$ and $\Delta\bar{S}$ obtained were very similar to those from the desorption isotherm, being a little smaller.

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[9] Barrer and Kelsey, *Trans. Faraday Soc.*, 1961, **57**, 625.