27. *The Solubilisation of Some Dibasic Fatty Acids by Lecithin in Benzene.*

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The solubilisation of twelve dibasic fatty acids by solutions of lecithin in benzene has been studied; quite large amounts of the acids were solubilised. Those acids containing an odd number of carbon atoms were taken up to a larger extent by the micelles than those containing an even number, indicating a different type of packing in the micelle for each sub-series. The number of molecules of acid solubilised per molecule of lecithin decreased as the chain length of the acid increased, but the actual volume of solubilisate increased with chain length. Using a model of the micelle, we suggest an explanation of the mode of packing of the acids from which the cross-sectional area of the lecithin molecule at the head group can be calculated; it agrees well with results from surface film experiments. The possible biological significance of this type of solubilisation is discussed.

Lecithin forms both small and large micelles in benzene.[1] The small micelles have a molecular weight of 3200, and the large ones of 55,000. Diffusion and viscosity studies [2] have shown the micelles to be laminar in structure, presumably with the polar head groups forming a double sheet in the centre of the micelle, and the hydrocarbon chains protruding outwards into the benzene. It has been suggested [1] that it might be possible to solubilise polar substances between the two sheets of polar groups in the micelle centre. Dibasic fatty acids have been chosen for study in view of their ease of assay and low solubility in benzene, and results on twelve acids have shown that solubilisation in the lecithin micelle is possible.

Experimental

Materials.—Good-quality commercial acids were used, each being recrystallised at least twice from a suitable solvent. Some physical constants of the recrystallised materials are given in Table 1; molecular weights were determined by titration.

Table 1. *Physical constants of dibasic fatty acids.*

No. of carbon atoms	2	3	4	5	6	7
Molecular weight (obs.)	89·93	104·0	118·0	132·2	146·2	160·2
Molecular weight (calc.)	90·03	104·1	118·1	132·1	146·1	160·2
Melting point (obs.)	188°	132°	185°	97°	153°	105°
Melting point (lit.[3])	189°	132°	185°	97°	153°	106°
No. of carbon atoms	8	9	10	12	13	16
Molecular weight (obs.)	173·8	188·1	202·6	230·8	244·9	286·0
Molecular weight (calc.)	174·2	188·2	202·3	230·3	244·4	286·4
Melting point (obs.)	113°	106°	132°	128°	112°	125°
Melting point (lit.[3])	114°	107°	133°	128°	113°	125°

The physical constants of the lecithin and benzene used have been reported.[1]

Solubilisation Measurements.—The solution of lecithin, together with the dry powdered acid, and a stainless-steel ball bearing, were placed in 100 ml. conical flasks, whose bottoms were slightly curved. The ground-glass stoppers were sealed in with " Parafilm " to exclude moisture. The flasks were attached to a metal frame, and were held half submerged in a thermostat bath at 25° ± 0·05°. The frame was rocked by a motor, causing the ball bearings in the flasks to move; this movement kept the solution and acid well mixed, and also ground the acid very finely. When equilibrium had been reached, rocking was stopped, and the solutions set aside for 24 hr. Samples were withdrawn into a pipette fitted with a sintered-glass filter and preheated a few degrees above 25°. The sample was run into a tared flask and weighed, the benzene was evaporated, 10 ml. of neutral alcohol were added, and the acid was titrated (phenolphthalein) with alkali of convenient concentration.

[1] Elworthy, *J.*, 1959, 813.
[2] Elworthy, *J.*, 1959, 1951.
[3] Rodd, " The Chemistry of Carbon Compounds," Elsevier, London, 1952, Vol. I, Part B, p. 954.

The time required to reach equilibrium was found by stopping the rocking of the flasks after 2, 3, 4, and 5 days, withdrawing a sample, and titrating it. Generally 2—3 days were required for complete saturation of solution with acid. Blanks on the solubility of the acid in benzene, and on the titration of lecithin alone, were performed under identical conditions.

Results

The results for the twelve dibasic fatty acids are given in Table 2. Below 0·73 g. $l.^{-1}$ (0·00110 mole of lecithin per kg. of solution) small amounts of acids were solubilised by the small micelles. Very small titres were obtained in these experiments, yielding rather inaccurate results. From the mass-action law for this system,[1] the concentrations of small and large micelles at any lecithin concentration can be calculated and, from a knowledge of the amount of an acid taken up by the small micelles, the amount solubilised by the large micelles can be calculated for any solution. All results in Table 2 have been corrected for the solubility of the

TABLE 2*A*. *Amounts of oxalic acid solubilised by solutions of lecithin in benzene.*

(i) Total lecithin concentration, moles/kg. of solution.
(ii) Total acid concentration, moles/kg. of solution.
(iii) Lecithin concentration as large micelles, moles/kg. of solution.
(iv) Acid concentration in large micelles, moles/kg. of solution.
(v) Moles of acid/moles of lecithin = M_a/M_l.

(i)	0·46	0·87	1·03	14·61	17·03	22·70	29·04	34·07	43·81	45·49
(ii)	0·92	1·79	2·17	32·00	37·06	48·93	62·80	73·65	94·02	97·97
(iii)	—	—	—	13·12	16·53	21·17	27·49	32·51	42·23	43·91
(iv)	—	—	—	28·90	33·91	45·72	59·54	70·37	90·70	94·65
(v)	2·0	2·1	2·1	2·203	2·051	2·160	2·166	2·165	2·148	2·156

Small micelles, mean = 2·1 molecules of acid/molecule of lecithin.
Large micelles, mean = 2·150 molecules of acid/molecule of lecithin.

TABLE 2*B*. *Amounts of dibasic acids solubilised by solutions of lecithin in benzene.*

Acid	Mean M_a/M_l for small micelles	Mean M_a/M_l for large micelles	Acid	Mean M_a/M_l for small micelles	Mean M_a/M for large micelles
Oxalic, C_2	2·1	2·150	Azelaic, C_9	1·8	1·387
Malonic, C_3	2·3	2·013	Sebacic, C_{10}	0·9	0·799
Succinic, C_4	1·2	1·209	Decane-1,10-dicarboxylic, C_{12}	0·8	0·746
Glutaric, C_5	2·1	1·900	Brassylic, C_{13}	1·9	1·039
Adipic, C_6	1·0	1·082	Tetradecane-1,14-dicarboxylic, C_{16}	0·9	0·629
Pimelic, C_7	1·1	1·397			
Suberic, C_8	1·0	0·955			

acids in benzene. The results are finally expressed as the number of acid molecules solubilised by one lecithin molecule, M_a/M_l.

Quite large amounts of the acids of shorter chain length were solubilised, about two molecules of oxalic and malonic acid being associated with each lecithin molecule. As the chain length of the acid increased, M_a/M_l decreased, and for tetradecane-1,14-dicarboxylic acid (C_{16}) only 0·6 molecule per molecule of lecithin was solubilised.

The set of results for oxalic acid is shown in full in Table 2*A*. The data for the other eleven acids are summarised in Table 2*B*. For the small micelles each value of M_a/M_l given is the mean of three determinations, and for the large of at least six.

Discussion

It is likely that the acids, which are only slightly soluble in benzene, are solubilised between the two sheets of polar groups in the centre of the laminar micelle. Owing to the inaccuracies of measurements on small micelles, only the large ones will be considered.

Fig. 1 shows the volume of acid solubilised by two lecithin molecules as a function of chain length of solubilisate. This is the *apparent* volume solubilised between two lecithin molecules, *i.e.*, between one lecithin molecule in one sheet of polar groups, and the opposite lecithin molecule in the second sheet. The volume solubilised increases as the chain length of the acid increases, and the acids appear to fall into two separate series, those

containing an odd number of carbon atoms being taken up by the micelle to a greater extent than those containing an even number. The volumes of the acid molecules were calculated from density data.[4,5,6,7]

The packing of the acids in the micelle cannot be explained simply. It is unlikely that the acids pack so that their *c* axes (long axes) are perpendicular to the polar sheets. The cross-sectional area [4] of the acids is 18·1 Å^2 and this value is reasonably constant throughout the series; malonic and oxalic acid differ slightly. An equal number of molecules of all acids should be solubilised per molecule of lecithin if packing perpendicular to the polar sheets is assumed. Table 2 shows that this is not so.

If the acids lie in the micelle with their long axes parallel to the polar sheets, the area of acid apparently lying between two lecithin molecules can be calculated from volume solubilised/$\sqrt{}$(cross sectional area).

The area of acid solubilised increases with chain length, and again there is a large difference between the odd- and even-numbered acids. This difference indicates that

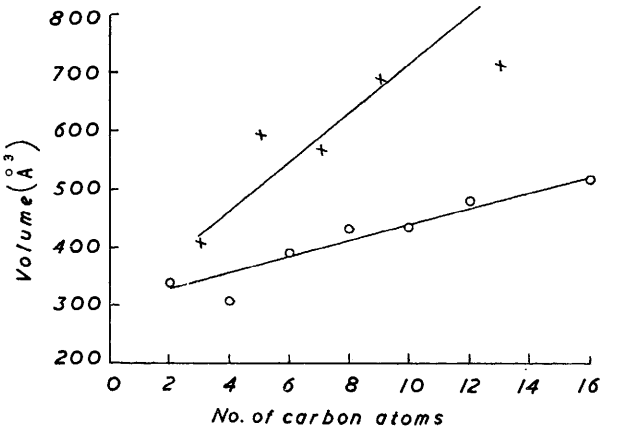


FIG. 1. *Volumes of acid solubilised by two lecithin molecules as a function of the chain-length of the acid.*
× *Odd no. of* C.
○ *Even no. of* C.

the two sub-series pack into the micelle in different ways. If the acids covered the polar sheets exactly, the areas given in Table 3 should be constant. The larger areas of longer-chain acids taken up indicate that some parts of the molecules may protrude over the edges of the polar sheet into the solvent.

TABLE 3. *Area of solubilisate associated with two lecithin molecules.*

Acid	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{12}	C_{13}	C_{16}
Area (Å^2)	90·4	104·3	74·1	140·3	96·0	131·7	101·9	160·1	102·4	110·8	167·3	120·1

If one polar head-group of a dicarboxylic acid is held in the micelle, the remainder of the acid, residing outside the micelle, is akin to a monocarboxylic acid. The latter type is freely soluble in benzene at 25°. A tentative explanation of the packing can be given based on the assumptions that the acids may protrude into the benzene, and that the acids with an odd and an even number of carbon atoms pack into the micelle in different ways.

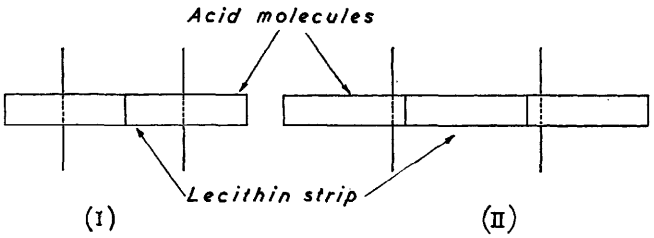
Theoretical Calculation of Number of Acid Molecules lying on a Polar Sheet.—Caspari,[4] in crystallographic studies, has shown that the gap between adjacent molecules in the line of the *c* axis is 2·09 Å, and that the oxygen and hydrogen atoms of the two molecules lie in this gap. The calculation of the number of acid molecules which can lie on a strip of lecithin molecules is based on this measurement. Thus if the length of the acid is 10 Å, a strip of lecithin molecules of length 10 Å can hold two acid molecules [Fig. 2 (I)]. If the

[4] Caspari, *J.*, 1928, 3235.
[5] Morrison and Robertson, *J.*, 1949, 980, 987, 993, 1001.
[6] Hendricks, *Z. Krist.*, 1935, **91**, 48.
[7] " Handbook of Chemistry and Physics," Chemical Rubber Publ. Co., Cleveland, 1959, p. 1027.

strip length is 12·09 Å (10 + 2·09) it can hold three acid molecules [Fig. 2 (II)], one being placed centrally, and one polar head of each of the remaining two molecules being on the strip, while the rest of the two molecules are outside the micelle. The relevant portions of graphs of lecithin strip length against the number of acid molecules taken up on the strip are given in Fig. 3.

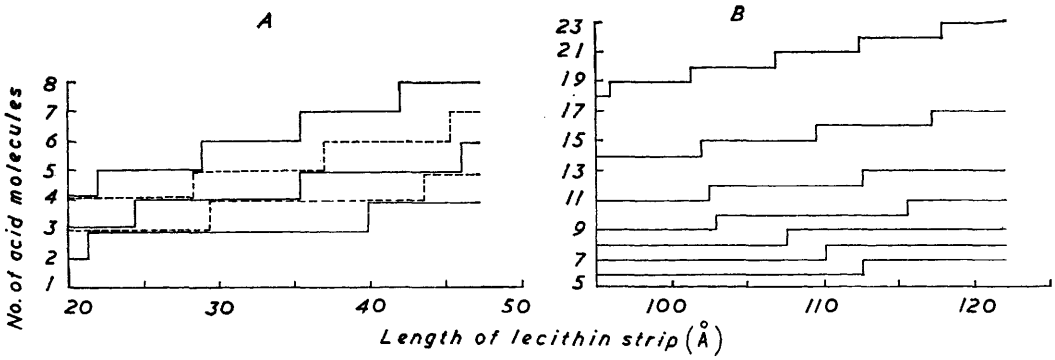
Application of Theory to Solubilisation, and Calculation of Dimensions of Polar Sheet.— Considering first those acids containing an odd number of carbon atoms, we calculate from results in Table 2 the ratio (number of C_n acid molecules)/(number of C_{13} acid molecules solubilised by one lecithin molecule), where n is the number of carbon atoms in the acid. Fig. 3 is used to find the length of a lecithin strip upon which the acids can pack so that the ratio of C_n/C_{13} is in accord with experiment.

FIG. 2. (I) *Packing of two acid molecules (length* 10 Å*) on a strip of lecithin* 10 Å *wide.* (II) *Packing of three acid molecules (length* 10 Å*) on a strip of lecithin* 12·09 Å *wide.*



The best agreement between experiment and theory was found by considering the packing of acids on a strip of lecithin molecules whose length lay between 29·6 and 35·4 Å (Fig. 3). This type of packing, with part of the acids protruding outside the micelle, will explain why the same amount of acids of different chain lengths can be solubilised (C_7 and C_9). The total number of acid molecules in the micelle is calculated from $(M_a/M_l) \times$ (number of monomers in the micelle); the number of monomers is 70, from diffusion and

FIG. 3. *Theoretical number of acid molecules of different chain lengths packing on a lecithin strip*: *A, odd-numbered acids from* C_3 *reading downwards; B, even-numbered acids from* C_2 *reading downwards.*



viscosity results. For simplicity a simple rectangular shape is assumed for the polar sheet. Each row of acid molecules consists of six molecules of malonic, five of glutaric, four of pimelic acid, etc. The rows of acid molecules lie parallel to one side of the rectangular model of the polar sheet (Fig. 4). In this case the side has a length between 29·6 and 35·4 Å (mean 32·5 Å). The total number of acid molecules in the micelle divided by the number in one row gives the number of rows in the micelle. The width of each row is the same as the width of the acid molecule, $\sqrt{(18{\cdot}1)} = 4{\cdot}24$ Å. The number of rows of acid molecules of different chain lengths lying on the polar sheet should be constant, and is

reasonably so (Table 4), with a mean value of 24·6. The number of rows multiplied by 4·24 gives the second dimension of the rectangular polar sheet, which is 103 Å.

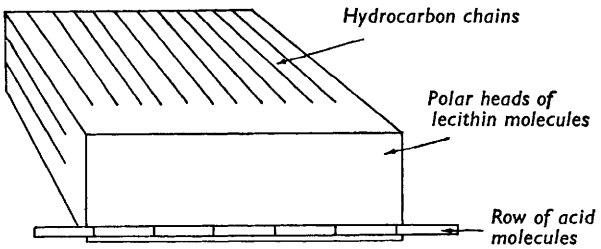
The difference between the numbers of odd- and even-acid molecules solubilised can be explained by supposing that the odd-numbered acids pack across the shortest dimension of the rectangular polar sheet, while the even-numbered acids pack across the longest dimension. This is shown diagramatically in Fig. 4. Results from the odd-numbered acids predict, on this basis, that the long dimension of the rectangle should be 104 Å. The calculated and theoretical number of acid molecules packing on a strip of this length should agree.

TABLE 4. *Fit of theory to experiment for odd-numbered acids.*

No. of C atoms in acid (n)	3	5	7	9	13
No. of molecules of C_n/no. of molecules of C_{13} (expt.)	1·94	1·83	1·35	1·34	1·00
No. of molecules of C_n/no. of molecules of C_{13} (calc.)	2·00	1·67	1·33	1·33	1·00
Molecules of acid in micelle	141	133	98	97	73
Molecules of acid in one row	6	5	4	4	3
No. of rows of acid molecules in micelle	23·5	26·6	24·5	24·3	24·2

The results for two strips of lengths 101 and 104 Å are given in Table 5, as the position at 103 Å is complicated, as shown in Fig. 3, by the strip's being of such a length that several acids are changing from one packing number to another. The agreement between observed and calculated ratios of C_n/C_{16} is reasonable, being a little better at 101 than

FIG. 4. *Diagram of one half of a lecithin micelle, showing one way in which a row of acid molecules may be packed.*



104 Å. As a check on the dimension of the rectangular polar sheet at right angles to the 101 Å length, the mean number of rows of acid molecules is 7·1, giving a length of 7·1 × 4·24 = 30 Å, in agreement with 32·5 found by considering the odd-numbered acids.

This explanation of the solubilisation, although tentative, shows why a larger volume

TABLE 5. *Comparison of theory and experiment for even-numbered acids.*

No. of C atoms in acid (n)	2	4	6	8	10	12	16
Ratio, no. of molecules C_n/C_{16} (expt.)	3·42	1·92	1·72	1·52	1·27	1·19	1·00
Ratio, no. of molecules C_n/C_{16} (calc. at 104 Å)	3·33	2·50	2·00	1·67	1·33	1·17	1·00
Ratio, no. of molecules C_n/C_{16} (calc. at 101 Å)	3·33	2·33	1·83	1·50	1·33	1·17	1·00
Molecules of acid in micelle	151	85	76	67	56	52	44
Molecules of acid in one row, at 104 Å	20	15	12	10	8	7	6
Molecules of acid in one row, at 101 Å	20	14	11	9	8	7	6
Rows of acid molecules in micelle, at 104° Å	7·5	5·6	6·3	6·7	7·0	7·5	7·3
Rows of acid molecules in micelle, at 101 Å	7·5	6·0	6·9	7·4	7·0	7·5	7·3

of the acids of longer chain length can be taken up by the micelles. The rectangular model of the polar sheet, with sides 32·5 and 101—104 Å, has an area of 3330 Å.^{2} As 35 monomers of lecithin are present in one polar sheet (half the micelle) the cross-sectional area of the lecithin molecule at the position of the head-group is 95 Å^2. This value agrees well with figures from surface-film studies. At the air–water interface [8,9] the mean of several

[8] Leathes, *J. Physiol.*, 1923, **58**, vi; *Lancet*, 1925, **208**, 853.
[9] Hughes, *Biochem. J.*, 1935, **29**, 430.

determinations was 109 Å,[2] at the benzene–water interface [10] 116 Å^2, and at the xylene–water [11] interface 80 Å^2. No assumption about the cross-sectional area of the molecule was made in treating the solubilisation data.

Possible Biological Significance of Solubilisation.—Finean,[12] on the basis of *X*-ray studies on nerve myelin, suggested a structure for the myelin containing four phosphatide molecules arranged in two pairs. In each pair the molecules are arranged so that their polar heads form a double sheet. It is possible that polar materials could lie in the double sheets, as they can in the lecithin micelles. This space may serve as a storage place for materials to be transferred to or from the interior of the nerve.

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[10] Alexander and Teorell, *Trans. Faraday Soc.*, 1939, **35**, 727.
[11] Cheesman, *Arkiv Kemi, Min., Geol.*, 1946, *B*, **22**, No. 1.
[12] Finean, *Experientia*, 1953, **9**, 17.