XLIX. UNIMOLECULAR FILMS OF LECITHIN AND RELATED COMPOUNDS.

By ARTHUR HUGHES.[1]

From the Colloid Science Laboratory, Cambridge.

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It is a significant fact that organic molecules such as lecithin, cholesterol and protein, which are known to occur in the membrane of a living cell are all molecules capable of forming unimolecular films at an air-liquid interface. The importance in this connection of the established work on unimolecular films of organic compounds has been realised by Leathes [1923; 1925] and Adam *et al.* [1926; 1928; 1929], who have studied films of lecithin and cholesterol on the surface of water. Their results demonstrated the essential difference between the typical loosely packed expanded film of lecithin and the close-packed condensed film of cholesterol and further indicated the power of cholesterol to bring about a closer packing of lecithin in a mixed film of the two substances.

Gorter and Grendel [1925; 1926] approached the problem from a different angle and showed that the amount of lipoid material in a typical living cell is sufficient to form approximately two molecular layers in the cell membrane.

No precise information has been forthcoming as to the orientation and chemical reactivity of these substances when present in the form of unimolecular films. In view of the fresh light that has been thrown on the physical structure and chemical reactions of molecules by the method of surface potentials [Schulman and Rideal, 1931; Fosbinder and Rideal, 1933], this technique has now been applied in conjunction with force/area measurements, to study the physical and chemical properties of the following compounds: tripalmitin, triolein, lecithin, lysolecithin and cholesterol. A preliminary investigation has also been made of composite films of the above substances with protein.

Experimental.

The surface potentiometer employed in the following work was essentially similar to that previously described by Schulman and Rideal [1931]. In Fig. 1 ΔV, the change in air-liquid potential difference due to the presence of the unimolecular film, is plotted against n, the number of molecules per sq. cm. of surface. The main characteristics of the films are given in Table I and refer to dilute NaCl solution at 17–20°. The two values of n, and the corresponding area per molecule (A), are given for the points where a homogeneous film is first formed on compression and where it finally collapses. The values for the resolved vertical component of the electric moment per molecule (μ) are calculated from the equation $\Delta V = 4\pi n\mu$, and are only relative values.

The limiting areas per molecule show good agreement in those cases previously studied by Adam *et al.* [1926; 1929] by force/area measurements. They find for cholesterol 40·8 sq. Å., for lecithin 114 sq. Å. and for triolein 135 sq. Å. (15°).

Tripalmitin, a typical close-packed condensed film, gives a high moment, due to the glyceride group, of $10{\cdot}0.10^{-19}$ E.S.U. which is constant during the small compression range of the film. Triolein, an expanded film with a much greater

[1] Beit Memorial Fellow.

compression range, gives a slightly higher value of μ owing presumably to the presence of the weakly polar double bonds, but on compression the value approximates to that of tripalmitin. For cholesterol μ remains constant at $4{\cdot}2 . 10^{-19}$ E.S.U. during compression over its small range of about 3 sq. Å. The dipole moment is thus considerably smaller than for lecithin which gives an expanded film of the same type as triolein. μ for lecithin decreases on compression to a

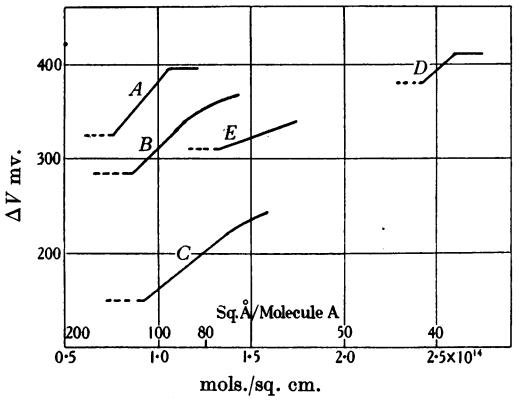

Fig. 1. Surface potentials of unimolecular layers.

A. Triolein NaCl 0·9 %, 19°
B. Lecithin NaCl 0·9 %, 18°
C. Lysolecithin NaCl 0·9 %, 18°
D. Cholesterol NaCl 0·9 %, 17°
E. Lecithin borate p_H 9, 18°

Table I.

Compound	Nature of film	Compression range n	Compression range A sq. Å.	ΔV mv.	μ E.S.U.
Tripalmitin	Solid	$1{\cdot}67 \times 10^{14}$	60·0	636	$10{\cdot}0 \times 10^{-19}$
	Condensed	1·78	56·0	662	9·9
Triolein	Liquid	0·76	132	324	11·3
	Expanded	1·05	95	395	10·0
Cholesterol	Liquid	2·42	41·3	380	4·2
	Condensed	2·60	38·5	410	4·2
Lecithin	Liquid	0·86	116	284	8·8
	Expanded	1·37	73	364	7·1
Lysolecithin	Liquid	0·93	108	150	4·3
	Expanded	1·55	65·5	240	4·1

much greater extent than for lysolecithin probably owing to the absence of the unsaturated hydrocarbon chain from the latter. In comparing lecithin and lysolecithin the most significant feature is the area of surface occupied per hydro carbon chain, which is 58 sq. Å. for lecithin and 108 sq. Å. for lysolecithin, *i.e.* lysolecithin forms a relatively much more distended film than lecithin. Further, μ for lysolecithin is about half that for lecithin, owing possibly to the removal of one ester linkage.

The molecular weights of lecithin and lysolecithin.

The molecular weights of these two compounds assumed in calculating the area per molecule were the formula weights 805 and 540, corresponding to oleyl-stearyl-lecithin and stearyl-lysolecithin respectively. Price and Lewis [1929]

give values for the molecular weight of lecithin by the boiling-point method as 797 in ethyl alcohol and 3388 in benzene. A cold solution of freshly prepared lecithin in alcohol or benzene, or of lysolecithin in chloroform, shows a strong Tyndall cone, which decreases in intensity on boiling. At ordinary temperatures it would appear that both substances are highly polymerised. The rapid spreading of these large particles on an aqueous surface, the ease of compression and extension and the fluid nature of the films suggest that such polymerisation is not due to stable chemical linkages but to a physical process of dipole association between the lecithin or lysolecithin molecules, this union being broken down by the strong attraction of the dipoles to the aqueous surface.

Chemical properties of the films.

(i) *Effect of* p_H *of the underlying solution.* Films of tripalmitin, triolein and cholesterol are unaltered over the p_H range 2–11. Tripalmitin is hydrolysed slowly and triolein about four times more quickly on N NaOH. Fig. 2 shows the effect of p_H on the surface potentials of lecithin and lysolecithin films, using dilute HCl and $M/25$ phthalate, phosphate and borate buffers. The curves for myristic acid and triolein are included for reference. Triolein is a non-ionising

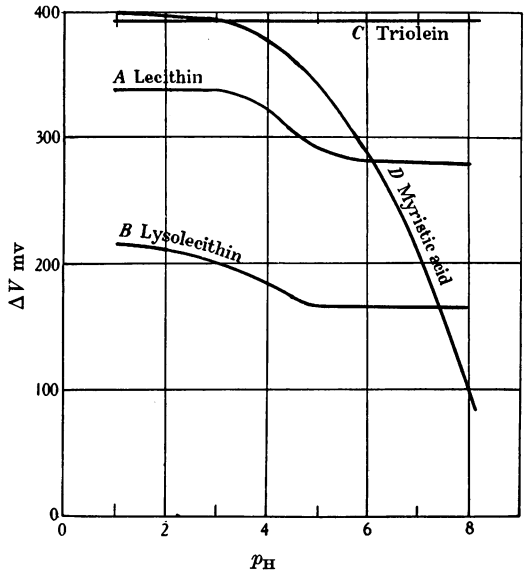

Fig. 2. Effect of p_H on surface potential (ΔV).

A. Lecithin at 100 sq. Å., 20°.
B. Lysolecithin at 100 sq. Å., 20°.
C. Triolein at 95 sq. Å., 20°.
D. Myristic acid at 20 sq. Å., 20°.

molecule and shows no change of surface potential with p_H. Myristic acid, with a single acid ionising group, shows a large fall of surface potential on solutions more alkaline than p_H 3 due to soap formation. With lecithin a fall is again noticed at a p_H higher than 3 due presumably to the ionisation of the phosphoric acid group, which appears to be of the same order of strength as the carboxyl group in myristic acid. The fall is not maintained beyond p_H 6 owing to the amphoteric nature of the molecule. In the case of lysolecithin ionisation begins

at a lower p_H, between 1 and 2, than for lecithin indicating that lysolecithin acts as a stronger acid. An effect peculiar to lecithin films is the change in character on solutions more alkaline than p_H 8, the range of compression becoming much smaller, and implying a much closer packing of the polar head groups (*cf.* Fig. 1).

It is impossible as yet to relate these surface potentials to results of cataphoretic measurements since the latter are not concerned with the total dipole system of the molecule.

(ii) *Oxidation by potassium permanganate.* Fig. 3 shows the change in surface potential with time for films of triolein, lecithin and lysolecithin on 0·1 % $KMnO_4$ in $N/100$ H_2SO_4 at room temperature. Lecithin is attacked rapidly, lysolecithin only very slowly, confirming the absence of an unsaturated hydro-

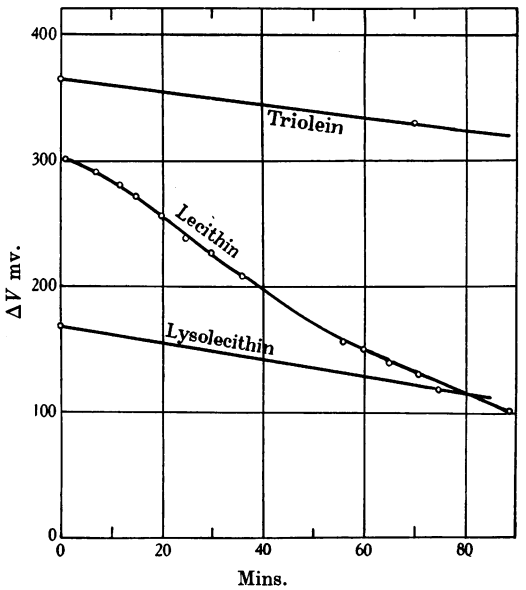

Fig. 3. Rate of oxidation of unimolecular films by 0·1 % $KMnO_4$ in $N/100$ H_2SO_4, 18°.

carbon chain from lysolecithin. Triolein is also attacked only very slowly even at the maximum extension of the film to 132 sq. Å. per molecule. The explanation lies in the fact that the area of surface per hydrocarbon chain, 44 sq. Å., is too small to permit the presence of the unsaturated double bonds in the aqueous surface and thus oxidation is inhibited. A similar effect has been observed with oleic acid itself [Hughes and Rideal, 1933].

Mixed films of lecithin and cholesterol.

A series of mixed films of lecithin and cholesterol was examined by the method of surface potentials to ascertain whether any chemical union takes place between the molecules. This would be reflected in a corresponding alteration of the electric moments and hence of the surface potentials. The actual results indicate merely a gradual contracting effect of the cholesterol on the lecithin, except that in the region of equimolecular proportions the film is compressible to values of ΔV higher than the maximum value for lecithin or cholesterol alone, implying a retention of the molecular orientation to higher compressions in these mixtures

(Figs. 4 and 5). Under 20 dynes compression the lecithin in an 80 % cholesterol mixture has an area almost as small as that of cholesterol itself, ca. 40 sq. Å., so that under these conditions the lecithin molecule must be orientated with both hydrocarbon chains nearly vertical, since the minimum area of each chain when vertical is 18·5 sq. Å.

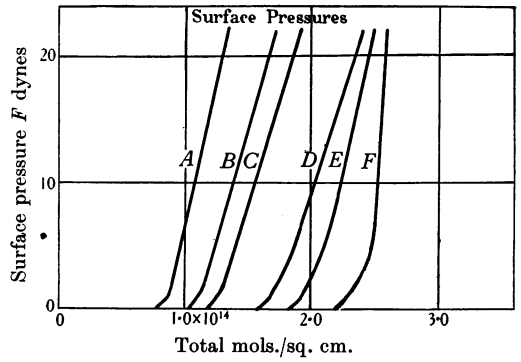

Fig. 4.

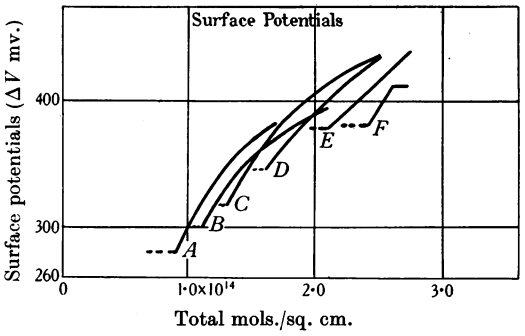

Fig. 5.

Figs. 4 and 5. 15–17°, 0·9 % NaCl. *A*. Lecithin. *B*. 20 % cholesterol. *C*. 40 % cholesterol. *D*. 60 % cholesterol. *E*. 80 % cholesterol. *F*. Cholesterol (% expressed in g. molecules).

Mixed films of protein and fatty molecules.

The possibility of obtaining mixed films of protein and lipoid molecules, and the nature of such films if obtainable, are problems of some importance in connection with current theories of the composition of cell membranes. The work of Mudd and Mudd [1926] on the surface composition of normal and sensitised mammalian blood cells indicates an increased polarity of the cell surface after sensitisation, but there is no information whether the molecules of cholesterol, lecithin and protein can form a composite membrane with properties differing from those of its constituents. With this aim some preliminary experiments have been carried out using a typical protein, egg albumin.

The procedure adopted was first to spread a unimolecular film of the fatty substance on the surface of an aqueous solution and then to inject beneath the film a dilute solution of the protein. It was found that subsequent changes in

the physical nature and in the surface potential of the film were affected by the following factors: (i) the p_H of the solution, (ii) the nature of the film-forming molecules, (iii) the surface concentration of the film molecules.

The effect of p_H. On the acid side of the isoelectric point of egg albumin injection of the protein (concentration in trough 0·001 %) causes a rapid rise in the surface potential of a triolein film to a value above that of triolein or of egg albumin. At the same time the liquid film of triolein sets to a rigid gelatinous film similar to that of the protein alone. On the alkaline side of the isoelectric point the rate of gelation of the film becomes progressively slower and is accompanied by a small fall in surface potential. This change, which is complete in 4 minutes at p_H 4·0, takes about 1 hour at p_H 7·0; it cannot be simply a replacement of triolein by protein since the potential of the complex film is always much higher than that of the egg albumin film alone.

Effect of the nature of the film-forming molecule. Films of tripalmitin, hexadecyl alcohol, cholesterol, and lecithin do not appear to be changed by injection of protein at any p_H. Oleic acid behaves in a similar manner to triolein. Lysolecithin is peculiar in that, although gelation of the film takes place very slowly, over a period of hours, an immediate and rapid rise of potential occurs, in 20 minutes at p_H 5. A further study of these complex films is in progress.

Summary.

1. The method of surface potentials has been applied to a study of the physical and chemical properties of unimolecular films of lecithin, lysolecithin, cholesterol, tripalmitin and triolein. The relative electric moments per molecule thus deduced are 8·8, 4·3, 4·2, 10·0, $11{\cdot}3\times10^{-19}$ E.S.U. respectively.

2. Lysolecithin forms a liquid expanded film with an upper limiting area of 108 sq. Å., the area per hydrocarbon chain being about twice that for lecithin.

3. Tripalmitin, triolein and cholesterol show no change in properties over a p_H range 2–12. For lecithin a fall in surface potential is noted between p_H 3 and 6, and for lysolecithin between p_H 2 and 5, due to ionisation.

4. On dilute $KMnO_4$ solutions lecithin is oxidised more rapidly than triolein or lysolecithin.

5. Mixed films of protein and fatty molecules have been examined. Homogeneous mixed films are obtainable from egg albumin and triolein.

The lecithin used in these investigations was prepared by Dr Chain by Levene's method through the cadmium chloride compound. It was nearly colourless and gave 3·96 % phosphorus, 1·79 % nitrogen. My sincere thanks are due to Dr Chain for this preparation and to Dr E. J. King for a specimen of pure lysolecithin.

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