REVIEW ARTICLE

THE PHYSICAL CHEMISTRY OF THE LECITHINS

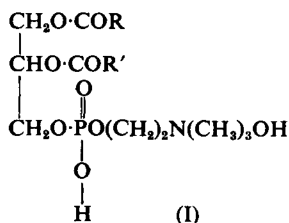
BY P. H. ELWORTHY, B.PHARM., PH.D., A.R.I.C., Wellcome Pharmaceutical Research Fellow and L. SAUNDERS, B.SC., PH.D., F.R.I.C., Reader in Pharmaceutical Chemistry

From the School of Pharmacy, University of London

THE lecithins belong to the group of natural fats or lipides known as phosphatides, which occur in nearly all living cells. It has been shown that when animals are subjected to nutritional deficiencies, the amounts of phosphatides present in the various organs remain constant[1,2], indicating that these substances are structural components.

The universal distribution of phosphatides in animal cells and the facility with which fatty films are formed by them, suggest that they constitute the central structure of the cell wall (assuming that this structure is of the form proposed by Danielli and Davson[3] and Danielli and Harvey[4]). As is described later in this review, lecithin sols in water possess the property of forming stable films at a boundary made between the sol and water. These films consisting of membranes of fatty material and separating two aqueous liquids, have some of the properties of simple cell walls. Further studies of them and of the physical chemistry of lecithin sols would seem to offer a promising approach to the elucidation of the structure of cell membranes. Such work could also lead to the development of model membranes which might be helpful in the quest for further knowledge concerning the physico-chemical mechanisms of drug action.

In this review, we summarise the present state of research into the physical chemistry of the lecithins.



CHEMICAL STRUCTURE OF THE LECITHINS

As a result of their hydrolysis experiments on lecithin prepared from egg yolk, Diakanov[5–7], and Strecher[8] in 1868 proposed the structure (I) for the compound where R and R′ are hydrocarbon chains, which in the natural lecithins, may contain one or more double bonds.

The basis of this structure is the glycerol nucleus; this is joined by ester links to two long chain fatty acid radicals and to an orthophosphate group, which in turn is linked to the strongly basic choline group by means of another ester bond. The resulting molecule has a soap-like structure with water-insoluble long chain fatty acid groups joined to the rather complex water soluble head group (the part of the molecule other than the hydrocarbon chains R and R′).

The structure (I) has the phosphate radical attached to the α-carbon atom of the glycerol; optical isomers are possible as the β- or central carbon atom of the glycerol is asymmetrical. The alternative structure in which the phosphate is attached to the β-carbon and R and R′ are identical, would not give optical isomers. The natural lecithins are always optically active. Hydrolecithins, which are prepared by reducing the fatty acid radicals of natural lecithin and have also been isolated directly from natural sources have $[\alpha]_D^{20}$ varying between $+6{\cdot}25°$ and $+7{\cdot}10°$[9,10].

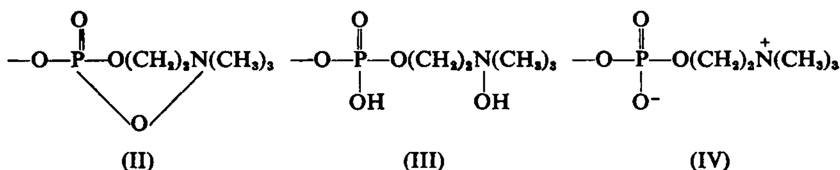
Pure (dipalmitoyl) lecithin prepared from natural sources was found to be dextrorotatory; it was shown to have the same configuration as synthetic L-α-(dipalmitoyl) lecithin. This configuration also applied to egg and brain lecithins, since the hydrolecithins obtained by reduction with hydrogen, were dextrorotatory. In addition, Long and Maguire[11] have shown that after hydrolysis of natural lecithin amounts of α-, L-α- and D-α-, glycerophosphoric acid were recovered, which were identical with those obtained by hydrolysis of synthetic L-α-lecithin, and quite different from those obtained by the hydrolysis of synthetic β- or DL-α-lecithins. It was therefore proved that natural lecithins have the L-α conformation.

Fatty Acids. A great deal of work has been done on the identification of the fatty acids obtained by hydrolysis of lecithin. Lecithin prepared from natural sources normally yields a fairly complex mixture of acids. Palmitic and stearic were the only saturated acids present[12–15] in egg lecithin, they comprised about 35 per cent. of the total fatty acid mixture. A wide range of unsaturated acids have been found[14–19]. Levene and Rolf[16] identified oleic (C_{18} with one double bond), linoleic (C_{18} with two double bonds) and arachidonic (C_{20} with four double bonds) acids in egg lecithin hydrolyzates. Subsequent work[14,15] has shown the presence of a group of highly unsaturated, long chain acids described collectively as "clupadonic acid". A typical analysis of the fatty acid mixture resulting from the hydrolysis of egg lecithin is,

Stearic acid	4·1 per cent.
Palmitic acid	31·8 per cent.
Oleic acid	42·6 per cent.
Linoleic acid	8·2 per cent.
"Clupadonic acid"	13·3 per cent.

The unsaturated fatty acid composition of egg lecithin can vary according to the diet of the hens[20].

Zwitterionic Structure. Grun and Limpacher[21] suggested that the phosphate-choline part of the lecithin molecule could have one of two



structures, the anhydro-form II or the hydrated form III. Jukes[22], however, proposed a zwitterionic structure IV and this seems most likely to be correct in view of the fact that both choline and phosphoric acid (first stage of dissociation), are strong electrolytes. This zwitterionic structure was supported by potentiometric titration evidence[22,23], and by the observations that lecithin was dielectrically active in both water[24] and ethanol[25].

Hydrolecithin. This name is given to lecithins containing only saturated fatty acid radicals[26]. They can be prepared by the hydrogenation of natural lecithins, by isolation in the pure state from natural sources[9,10], and by synthesis[34].

Lysolecithin. Lysolecithins have the same structure as the lecithins except that only one fatty acid radical is present. They occur in association with the natural lecithins, and it is only quite recently that chromatographic methods have been developed which ensure the complete separation of lysolecithin from natural lecithin.

Lysolecithin is formed by partial hydrolysis of lecithin and a highly specific catalyst for this reaction is the lecithinase enzyme; this occurs in a very active form in snake venoms[27,28]. An important development in the study of lysolecithin is the discovery by Hanahan and others[29] that the venom dissolved in water will act on an ethereal solution of lecithin. Lysolecithin is insoluble in ether and so is precipitated as it is formed. It can therefore be removed from the reaction mixture by centrifugation and treated to give a high yield of pure lysolecithin.

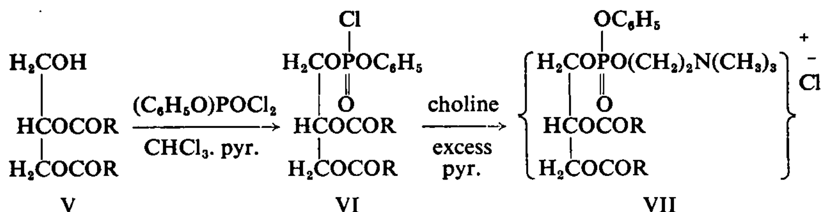
It has been found by various oxidative procedures[30,31] that the fatty acid in lysolecithin is attached to the β-carbon atom of the glycerol group. When it is prepared from natural lecithin containing unsaturated acids the lysolecithin possesses only saturated fatty acids; so it seems that in lecithin, unsaturated acids are attached to the α-carbon atom[32,33]. Hanahan and others[29] have found that the snake venom reaction occurs with pure (dipalmitoyl) and (dipalmitoleyl) lecithins giving the corresponding pure lyso-compounds.

Lysolecithin has a strong lysing action on erythrocytes, this is presumably caused by dissolving out the lecithin film which forms the membranes of the cells. The lyso-compound also probably plays an important part in the dispersion of lecithin in aqueous fluids.

Synthesis of Lecithin

The synthesis of lecithin is a difficult problem. Many of the earlier synthetic materials were in fact mixtures. A recent synthesis of hydrolecithins by Baer and Kates[34], used a D-α,β-diglyceride (V) as the starting material (prepared by the method of Sowden and Fischer[35]). This was treated with monophenyl phosphoryl dichloride in the presence of pyridine and the whole reaction mixture was treated with choline chloride in the presence of a large excess of pyridine. The resulting material was isolated as a reineckate, converted to a sulphate, catalytically hydrogenated to remove the phenyl group and then the sulphate was removed by means of barium carbonate. In this way (dimyristoyl-), (distearoyl-)

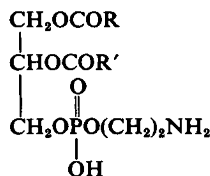
and (dipalmitoyl-) lecithins have been prepared in an optically active form. The stages in the synthesis are shown below.



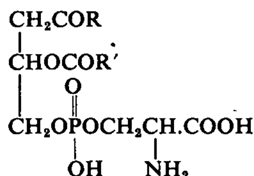
This year, Baer, Buchnea and Newcombe[36] have reported the synthesis of the unsaturated L-α-(di-oleyl) lecithin. D-Acetone glycerol was phosphorylated with phenyl phosphoryl dichloride and the product was esterified with ethylene chlorhydrin. After removal of the phenyl and acetone groups, the L-α-glyceryl-phenyl-phosphoryl-ethylene chlorhydrin was isolated as its barium salt and treated with oleyl chloride. The resulting compound was heated with trimethylamine, giving a mixture of L-α-(di-oleyl) lecithin and the corresponding lysolecithin. The pure lecithin was isolated by chromatography.

Preparation of Lecithin from Natural Sources

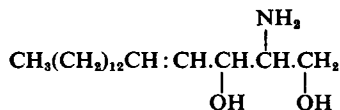
In order to prepare lecithin, the starting material is first extracted with ethanol. This breaks up the lipo-protein complexes[37] and dissolves the phosphatides together with other fats. After removal of ethanol the residue is extracted with acetone; the phosphatides are insoluble in acetone whereas the other lipides such as cholesterol, dissolve. Alternatively, the starting material can be extracted first with acetone and then with ethanol. The major components of this extract other than lecithin are:



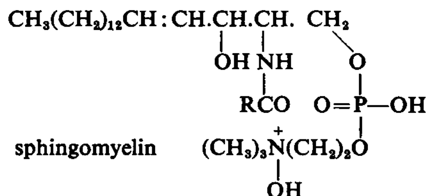
cephalin



phosphatidyl serine



sphingosine



sphingomyelin

Fractionation of this mixture to give lecithin was until recently carried out by a method devised by Bergell[38] in 1900 and developed by MacLean[39],

Levene and Rolf[13,40] and by Pangborn[41–44]. In this a complex between cadmium chloride and lecithin is formed which is only sparingly soluble in ethanol. It is precipitated by adding a saturated cadmium chloride solution to an ethanolic solution of the mixed phosphatides. The complex is further purified by extraction or by partition between immiscible solvents. Lecithin is liberated from the complex by adding methanolic ammonia to a chloroform solution[13,40], which precipitates the cadmium as hydroxide. Alternatively the chloroform solution of the complex is extracted with 30 per cent. ethanol in water[41–44], or it is broken up by using ion exchange resins[45].

Reasonably good samples of lecithin, free from amino nitrogen and with the correct N/P ratio, have been obtained. However, the phosphatides are prone to associate with one another in solution and fractionations based on precipitation are not clear cut. The ideal procedure for separating such materials is one in which the molecules are dealt with almost individually, for example, by adsorption on to the surface of a solid followed by elution. Several chromatographic procedures based on this principle have been successfully developed for fractionating phosphatide mixtures. A disadvantage of the cadmium chloride purification method is that it is almost impossible to remove all traces of cadmium from the lecithin, and traces of cadmium have a considerable effect on some of the properties of lecithin sols[46]; the chromatographic method avoids this contamination.

Hanahan[47,48] and his colleagues have used alumina for the chromatography of ethanolic solutions of phosphatide mixtures. They found that all the amino-containing substances were removed by the column and the effluent gave a solid product with a reasonable N/P ratio. Faure also used this method[49].

Lea and Rhodes[50,51] have found that the product from alumina chromatography has a low fatty acid/P ratio and they attributed this to the presence of lysolecithin. Using a mixed silicic acid and Celite column, with 20 per cent. methanol in chloroform as solvent, they obtained three chromatographic fractions from egg yolk phosphatides. The first fraction had an R_F value of 0·9 and consisted of the amino-containing compounds, mainly cephalin, the second fraction ($R_F = 0{\cdot}6$) consisted of the lecithins and had the correct N/P and fatty acid/P ratios. The third fraction ($R_F = 0{\cdot}1$) was eluted from the column with methanol and consisted of lysolecithin.

This chromatographic method of Lea and Rhodes is the only process so far developed by which lecithin can be completely separated from lysolecithin.

Direct paper chromatography of phosphatide mixtures does not seem to have been very successful[52,53]. Lea, Rhodes and Stoll[54] have, however, developed separation procedures using silica impregnated paper. An attempt has been made to separate lecithin from an egg extract by counter current partition chromatography on paper[55]; some success has been claimed for two dimensional paper chromatography using acetone-methanol and phenol[56].

Light magnesium oxide has been used as a chromatographic material; all the phosphatides were adsorbed from a solution in petroleum ether but only the lecithins were eluted by methanol[57]. Chromatographic methods are valuable for preparing lecithin as they do not involve any harsh chemical treatment. Lecithins are easily decomposed by heating or by the action of acids or alkalies.

No separation of lecithins according to the degree of unsaturation of their fatty acids has so far been achieved by chromatography. Escher[58] in 1925 reported such a separation by fractional crystallization from ether at low temperatures, but his results have not been confirmed; his separation may have been one of lysolecithin from the lecithin.

Physical Properties of Lecithin

The purified lecithin prepared from natural sources such as egg yolk, contains a variety of fatty acids, but as it is not been resolved into separate components by techniques so far developed, it can be regarded as a chemical entity.

It is a white, somewhat waxy solid and is very hygroscopic. On exposure to air, oxidation of the unsaturated acids occurs; the hydrolecithins are more stable. All the natural lecithins and lysolecithins are optically active, the former are dextrorotatory while the latter are reported to be laevorotatory[28]. No definite melting points have been reported but synthetic (dipalmitoyl) lecithin sintered at 40–42° C. and formed a meniscus at 235° C.

With water, synthetic hydrolecithins form sols which settle out quite rapidly[59] in contrast to the natural lecithins whose sols are stable over long periods. These natural lecithin sols are turbid. The lysolecithins give optically clear sols in water.

The hydrolecithins are soluble in warm ethanol and are insoluble in ether, acetone and cold ethanol[34]. The natural lecithins are insoluble in acetone but freely soluble in ethanol, ether, and chloroform. Lysolecithin is insoluble in ether and acetone but freely soluble in warm chloroform, water, and ethanol.

X-ray diffraction patterns of crystalline, synthetic hydrolecithins showed variations with the length of fatty chain in the compound[34]. From low angle X-ray diffraction studies[60], layer spacings were deduced, and on plotting these against fatty acid chain length a straight line was obtained. The slope of this line was characteristic of the arrangements of the hydrocarbon chains while extrapolation to zero chain length gave the contribution of the end group to the layer spacing (23 Å).

The natural lecithins can be prepared in crystalline form by cooling a saturated solution in a mixture of acetone and methyl ethyl ketone. The crystals show a parallel extinction under the polarising microscope[61].

When water is put on to solid lecithin, twisting cylindrical forms (myelin forms) grow out from the surface of the solid, these often develop into intricate patterns, and ultimately the solid disperses completely. Leathes[62] has studied the effects of various additives on these; he found that their appearance was changed when various acids and alcohols were

put in contact with the lecithin. Lime water prevented their growth but baryta water did not. Leathes considered that the outgrowths were due to the hydrophilic nature of the lecithin molecule. Hydrolecithins give few myelin forms, and so the unsaturated acids in natural lecithin must play some part in the process of growth. It has been suggested that the myelin forms are liquid crystals[63].

PHYSICAL CHEMISTRY OF LECITHIN SOLS

General

With water, natural lecithin forms sols which are easily precipitated by small concentrations of divalent metal salts but are stabilised by higher concentrations. Large concentrations (10 per cent.) of lecithin can be dispersed in water, the surface tension of the sol being lower than that of water. The turbidity and stability of the sol to electrolytes are both dependent on the proportion of lysolecithin in the dispersed material. It is only since Lea and Rhodes'[50] work in 1954 that natural lecithin free from lysolecithin has been made, and so it must be recognised that all earlier work has been carried out on preparations containing varying amounts of lysolecithin.

The sols, which are turbid white liquids, are made by shaking or triturating solid lecithin with water[64], or by adding an ethereal solution of lecithin to water with vigorous stirring and removing the ether by bubbling nitrogen through the liquid[65]. In the absence of inorganic salts, they are quite stable over a period of days. De Jong and his colleages[66] claim to have prepared clear sols by adding a hot ethanolic solution of lecithin to hot water and stirring, however, this procedure undoubtedly results in some decomposition of the lecithin.

The mean molecular weight of the monomer of egg lecithin can be calculated as 773[67]. Boiling point measurements with solutions in ethanol gave a value of 797, whereas in benzene a value of 3388 was found[68]. Dialysis experiments have confirmed that in polar non-aqueous solvents such as ethanol the lecithin is present as the monomer whereas in non-polar solvents, it is in a micellar form[69]. In benzene and heptane the micelles are believed to have the paraffin chains of the molecules directed outwards. In water, lecithin exists in the form of very large micelles in which the polar parts of the molecule are on the outside. X-ray diffraction studies[70,71] of concentrated sols indicate that bimolecular leaflets are present. The long spacing, representing the thickness of the bimolecular leaflet plus the distance between the leaflets, was found to be 69 Å for a 33 per cent. sol. Both potassium and calcium chlorides reduced this figure, the latter salt having the greater effect.

Surface and Interfacial Tension Studies

Neuschlosz[72,73] reported that lecithin did not lower the surface tension of water. Subsequently, more accurate work[68] has shown that the surface tensions of the sols varied as a function of pH, being a maximum at the isoelectric point; sodium and calcium chlorides shifted the maximum to lower pH values. These effects were attributed to changes of charge

on the micelles. Filtration experiments showed that the largest particles in the sol possessed the surface activity, the filtrate having a surface tension nearly as high as that of water[75]. Addition of cholesterol modified the pH/surface tension curves.

At the water/benzene interface, small amounts of lecithin dissolved in the benzene phase caused a lowering of interfacial tension; the same amount of lecithin dissolved in the aqueous phase had a much smaller effect[76].

Hirt and Berchtold[77] studied the effect of a large number of substances on the lowering of interfacial tension at the chloroform/water interface by lecithin. Adrenaline-like substances, which possessed an acidic or phenolic *ortho* dihydroxy group, had the greatest effect in reducing the lowering of interfacial tension.

Effect of Electrolytes on Lecithin Sols

Most of the work on this subject suffers from the use of impure materials. The authors have shown that small amounts of impurities, particularly divalent metals or soap-like substances, have a considerable effect on the stability of lecithin sols in the presence of electrolytes.

Early studies showed that the higher the valency of the ions in the added salt the more effective was the coagulating effect on lecithin sols[78-82]. In accordance with the usual theory of colloid precipitation, it has been found that the concentrations of salts which produced coagulation were those required to neutralise the charge on the sol particles[82]. On addition of acid, the sols flocculated at a pH value of about 2[65,81,83,84]. The rate of coagulation was increased by increase of temperature[85].

Some workers[65,86] have reported that divalent metal chlorides gave two precipitation zones with a region of peptisation between them; we have confirmed this observation with calcium chloride[46]. In an extensive study of the coagulating effects of potassium and calcium chlorides on sols of lecithin which had been prepared by various methods, Elworthy and Saunders have found that chromatographic lecithin, which was free from lysolecithin, gives sols which are much less stable to salts than did lecithin prepared by the cadmium chloride method. The sols were stabilised by the presence of small traces of soaps, for example a 0·5 per cent. sol of chromatographic lecithin was precipitated by 2×10^{-3} M potassium chloride; the addition of sodium dodecyl sulphate to a concentration of 5×10^{-5} M rendered the sol stable to 0·1 M potassium chloride. Lysolecithin exerted a similar protecting effect and it is probably the presence of this compound which stabilises lecithin dispersed in biological fluids.

Coacervation studies by de Jong and his colleagues[87-96] have been made with clarified lecithin sols, but the method of clarification almost certainly caused decomposition of the material. Horvath[97] suggested that complex coacervation was a possible form of protein-phosphatide association in living organisms, but this view has been criticised by Bull[98].

Viscosity

The addition of ethanol to lecithin sols increased their viscosity while the addition of electrolytes caused a decrease[99,100]. Old sols had a lower

viscosity than fresh ones. Divalent cations had a greater effect than monovalent ones[101]. However, when viscosity was studied by observing the time taken for a rotating sol to come to rest, it was found that calcium chloride increased the viscosity at concentrations where it reversed the charge on the sol particles[89-91]. Sodium salicylate had a similar effect.

Conductivity

It has been reported that as the concentration of a lecithin sol was increased the specific electrical conductivity passed through a minimum[103]. It was also found that further purification of lecithin decreased the specific conductivity; ageing of the sol increased conductivity[104]. We have found[61] that when a lecithin sol was passed through a column containing mixed ion exchangers, the conductivity was reduced almost to that of pure water though the lecithin content of the sol was almost unchanged. This result indicates that the conductivities previously reported were due to the presence of electrolytes as impurities in the lecithin. Lecithin itself has a very small conductivity in an aqueous sol, as is to be expected if only very large particles are present.

The mean size of the micelles in lecithin sols has yet to be determined. Some early measurements of osmotic pressure indicated a molecular weight of the order of 350,000 but the material used was of doubtful purity[99]. Ultrafiltration studies showed that the lecithin micelles were bigger than haemoglobin molecules[105]. Saunders[106] has found that the diffusion coefficient of lecithin sols clarified with small amounts of soap were of the order of 10^{-8} cm.^{2} sec.$^{-1}$, indicating a molecular weight for the micelles of several millions.

Electrophoretic Studies and Isoelectric Point

Lecithin sols showed a low electrophoretic mobility[107,108] at neutral pH values; the point of charge reversal was reported as pH 2·7 in the earlier work. Addition of barium chloride decreased the mobility and shifted the isoelectric point to a higher pH. Price[109] found that cholesterol had little effect on the mobility of lecithin, he reported the isoelectric point of lecithin as pH 2·7. Other workers reported values between 1·73 and 2·8[65,81,84].

Theoretically the isoelectric point of lecithin should be about pH 7 since both the acidic and basic groups in the molecule have the character of strong electrolytes[110]. Careful mobility studies of purified lecithin gave a value of 6·7[111], the addition of small amounts of cephalin lowered this slightly[112]. Aged lecithin sols were found to have much lower isoelectric points[113], and it has been suggested that the very low values originally obtained were due to free fatty acids present in the sol which gave the micelles an adsorption charge; the amount of free acid present would increase with the age of the sol, due to hydrolysis of the lecithin.

Dialysis

Dialysis has been used for the purification of sols since lecithin only dialyses slowly[114]. When lecithin-sodium oleate mixtures were dialysed

the lecithin did not affect the rate of dialysis of the soap; with lecithin-sodium glycocholate mixtures the rate of passage of soap through the membrane was increased by a factor of 4.

Turbidity

Levy[115] has studied the turbidity of lecithin sols. De Jong[92] and his colleagues have used turbidity measurements to study the coacervation of the sols.

Hydrotropism

The addition of soaps, particularly bile salts, clarified lecithin sols; double bonds in the fatty acid of a soap made it a better hydrotropic agent[116,117]. Sols have been clarified by polyethylene oxide stearates[106], sodium dodecyl sulphate[118], and sodium cholate[119]; lysolecithins can be used as clearing agent for lecithin sols.

Lecithin as an Emulsifying Agent

Lecithin has been used to emulsify various fish oils and animal fats in water[120]. Emulsions of both types can be formed, for example with sunflower oil and water the type of emulsion formed in the presence of lecithin, depended only on the relative proportions of the two liquids[121]. Studies have been made of the effect of cholesterol on the types of emulsions obtained with lecithin[122,123] and on the behaviour of lecithin as an emulsifying agent with different substances[124,125].

Bull[126] has suggested that phosphatide complexes with proteins or carbohydrates are better emulsifying agents than pure phosphatides, and this has been confirmed[127]. However, it has been reported[128] that pure soya bean lecithin is a better emulsifier in some cases than the crude material containing protein.

Oil in water emulsions prepared with lecithin resembled soap emulsions in that the oil tended to separate on standing. The effects of electrolytes on these emulsions have been studied[129].

Lecithin as a Protective Colloid

Lecithin acted as a protective colloid for dispersions of carbon and calcium carbonate in xylene[130,131], the lecithin was thought to be adsorbed at the surface of the solid particles giving them a charge which was generally negative. Red gold sols were protected from precipitation by potassium chloride[132] as were titanium oxide sols[133]. Lecithin has been used as a protective colloid in the manufacture of foodstuffs and vitamin A preparations[134].

The Effect of Lecithin on Gels

Yumikura[135] studied the rates of diffusion of some drugs in gelatin gels. He found that the presence of lecithin increased the rate of diffusion of cocaine and various surface active substances; the opposite effect was found with alypine and eucaine. Diffusion of various acids and alkalies in gels was said to be inhibited by lecithin but promoted by cholesterol[136,137].

The effects of lecithin and cholesterol on the swelling of gelatin gels at different pH values have been studied[138-141]. The results of different workers are conflicting probably due to the impurity of the lecithin samples used.

THE SURFACE CHEMISTRY OF LECITHIN

Air/Water Interface

Leathes[142,143] found that when lecithin was spread on the surface of water, an expanded film was formed. At a surface pressure of 1·4 dynes per cm. the area per fatty acid chain in the lecithin molecule was 55 Å^2 for one sample and 58 Å^2 for another. There was no sign of condensation of the film when the pressure was increased by a factor of twelve. Hydrolecithin gave a condensed film with an area per fatty acid chain of 28 Å^2 at 1·4 dynes per cm. When the pressure was increased considerably the area diminished but did not become as small as that for a palmitic acid chain in a condensed film (about 21 Å^2 in ethyl palmitate[144] and 26·3 Å^2 in α-monopalmitin[145]). This suggests that the polar part of the lecithin molecule prevents close packing of the fatty acid chains; the unsaturated acids present in natural lecithin caused the high areas per acid chain found with this material. The area occupied by a lecithin molecule in the film was found to be reduced by the addition of cholesterol to the film, due to interaction between the two lipides. It has been reported that at high compressions, the area per molecule of hydrolecithin could be reduced to 41·4 Å^2 [148].

Surface potential studies of films of this type by Hughes[146] indicated an area per fatty acid chain of 58 Å^2 for lecithin; lysolecithin gave a more distended film with an area per molecule of 108 Å^2. Values for the vertical component of the electric moment were 8·8 and 4·3 $\times$ 10^{-19} e.s.u. respectively. On altering the pH of the water on which the film was spread it was found that a rise in surface potential occurred for lecithin between pH 6 and pH 3. Lysolecithin only showed this effect between pH 2 and pH 1. Lecithin films were easily oxidised while lysolecithin films were only slowly affected. The surface potential studies confirmed the observation that the area per lecithin molecule was smaller in a mixed lecithin-cholesterol film than in a pure lecithin film. Injection of snake venom below a lecithin film on water gave a rapid decrease in surface potential due to the conversion of the lecithin to lysolecithin by the enzyme lecithinase present in the venom[147].

The sulphonamides were found to have no action on lecithin monolayers on water, and so it was concluded that the biological activity of these compounds did not depend on their influence on the permeability of the cell membrane[149]. The logic of this conclusion is rather dubious, since it seems unlikely that a lecithin film at the air/water interface bears much resemblance to a cell membrane. Plant growth hormones were found to accumulate in lecithin monolayers and increased the area per molecule in the film, but there was no relation between growth promoting activity and their effect on the monolayer[150].

Saponins caused a rise in surface pressure when injected under mixed lecithin-cholesterol films and this effect was more pronounced in the presence of calcium chloride. This result fitted in with the observation that the haemolysis of red blood cells by saponins was more rapid when calcium chloride was added[151].

Injection of sodium penicillin beneath compressed lecithin monolayers gave a rise in surface pressure and a change in surface potential; the penicillin remained in the surface layer after repeated expansion and compression of the film[152].

Oil/Air Interface

Lecithin formed a condensed film at the glyceride/air interface in which the polar parts of the molecule were thought to be protruding upwards with the fatty acid chains in the liquid phase. An estimate of the thickness of the film at this interface gave a value of 0·55 microns, very much bigger than the maximum dimension of the lecithin molecule[153].

Oil/Water Interface

At the benzene/water[154] interface both lecithin and lysolecithin films were found to be of the vapour expanded type; at high pressures, the areas approximated to those found at the air/water interface which suggested that there was the same type of packing at the two surfaces. The results were interpreted by the equation of Langmuir's theory of expanded films

$$(F - F_0)\ (A - A_0) = \text{const.}$$

where F is the force on the film, F_0 is the negative spreading force due to the hydrocarbon chains, A is the area per molecule in the film and A_0 is a constant to allow for the area occupied by the head groups. Up to $F = 15$ dynes per cm. the results fitted this equation. As was expected, the value of F_0 which was quite considerable at the air/water interface, was very small at the benzene/water surface. A_0 had almost the same value at both interfaces.

Further work on lecithin films at the benzene/water interface showed that injection of salicylic acid above the lecithin film caused a large increase in interfacial tension. Other substances such as benzoic acid, phenol and *ortho*-cresol caused only small increases in tension. The effect of salicylic acid was more pronounced when the acid was undissociated, and is thought to be due to the formation of a complex with the lecithin at the interface[155].

At the xylene/water interface a changing contact angle was found when the ring or plate method was used to study lecithin films; this difficulty was overcome by coating the plate with a deposit of carbon from a benzene flame. The compressibility of a lecithin film at this interface was greater than that at the air/water surface[156].

The Lecithin Sol/Water Interface

Many attempts have been made to correlate the action of substances on films at various interfaces with their biological activity. Most of these

attempts have failed because the interface studied bore only a very remote resemblance to the boundary which separates a living cell from a liquid environment. The simple cell membrane is a film built on a fatty structure which contains lecithin and cholesterol and which separates two *aqueous* fluids. Air/water and oil/water interfaces having a non-aqueous fluid on one side of the boundary, cannot be expected to give a very close representation of a cell membrane, since many substances cannot pass across the boundary from one fluid phase to the other.

Langmuir and Waugh[157] tried to overcome this difficulty by transferring pre-formed protein films to make boundaries between two aqueous phases, believing that such boundaries would have some of the properties of cell surfaces. Their efforts were not successful as the protein films broke up on transfer; addition of lecithin tended to strengthen them.

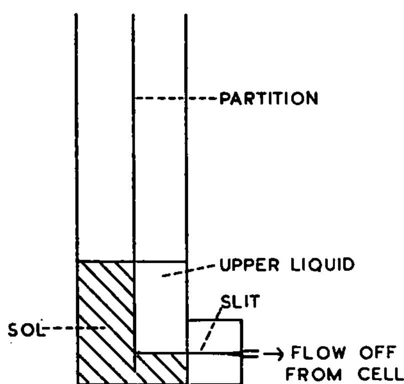


FIG. 1. Original cell for boundary formation.

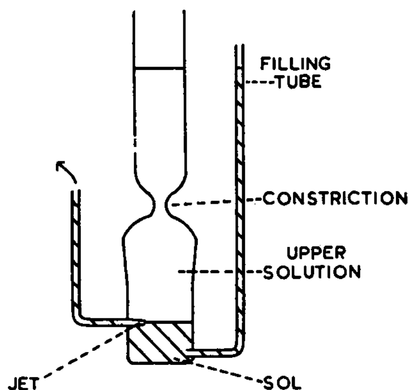


FIG. 2. Infra-red cell for surface force measurements.

Saunders[158] studied this problem of forming stable films between two aqueous liquids by examining the sharp boundaries formed when two such liquids were caused to flow through a common orifice. The apparatus used is shown in Figure 1. The cell was filled with the denser liquid on both sides of the central partition to the depth of the slit, the denser liquid was then added to the left hand side of the cell at the same rate as the lighter liquid was added to the right-hand side and both liquids were drawn off together through the slit. With care, a sharp boundary between the liquids could be made and maintained while flow through the slit continued. After flow was stopped, diffusion normally occurred and the progress of this diffusion could be followed by an optical technique (the Gouy interference method). With a lecithin sol as the lower liquid and water as the upper one, it was found that a boundary which was stable for a period of several days was formed. The presence of a coherent film at this boundary was demonstrated by the fact that when a platinum ring was drawn up through the interface, a definite pull of the order of 0·2 dynes per cm. was required to cause the ring to break through the boundary.

Elworthy and Saunders[61] have studied these surface forces at the lecithin sol/water interface in detail and have examined the effects of various substances on them. A different cell was used for most of this work and this is shown in Figure 2. This cell was filled to the constriction with the lecithin sol and then water or an aqueous solution was carefully put into the upper part of the cell, in this way a sharp boundary was produced in the constriction. Sol was allowed to flow out through the side arm causing the boundary to move down to the level of the jet, inside the cell. The boundary was finally sharpened at this position by continued flow through the jet; during these operations a platinum ring suspended from the arm of a torsion micro-balance was immersed in the lower liquid. After the boundary had been sharpened the force required to pull this ring through the sol/water interface was measured (the surface force). No appreciable force was required to raise the ring through the liquid until it reached the boundary, then an increasing force had to be applied until the ring suddenly broke through.

Using egg lecithin purified through the cadmium chloride complex we found that there was no surface force if all small ions were removed from the sol by passing it through mixed ion exchange resins, the force originally obtained with lecithin alone was almost certainly due to traces of cadmium chloride remaining in the lecithin. Addition of 0·01 M potassium chloride gave a small force of 0·01 dynes per cm. In the presence of small concentrations (0·0001 M) of divalent metal chlorides, surface forces of 0·1–0·25 dynes per cm. were obtained. The order of activity in producing these forces for the metal chlorides was Cd>Mg>Ca>Ba.

Sols of lecithin prepared by the chromatographic method of Lea and Rhodes[50,51] were unstable to electrolytes and they were rapidly flocculated by 0·01 M potassium chloride. They also gave appreciable surface forces in the presence of this salt, differing in both these respects from the sols made with cadmium complex prepared lecithin. The sols of chromatographic lecithin could be stabilised by adding small traces of soaps and these additions caused the disappearance of the surface force. This suggested that there was a relation between the occurrence of a surface force at the sol/water boundary and the stability of the sol. Further work has confirmed this view. By studying sol stability and surface force in the ternary system of lecithin-soap-salt, the authors have found that for a given soap concentration in the lecithin sol, the lowest concentration of a salt which causes an appreciable surface force when placed in the upper solution in the cell, is very much the same as the concentration of salt which causes precipitation of the sol. The surface force therefore appears to be due to a film of insoluble lecithin-salt complex formed at the boundary between the sol and the upper liquid[46].

It should be noted that this surface force differs from a surface or interfacial tension; there are no unbalanced molecular attractive forces at the sol/water interface, as there are at the benzene/water interface. Surface force is more akin to the work of extension of a membrane than to the work of extension of a liquid/liquid interface.

It is not difficult to imagine that cell membranes are formed in some such

way as this. The intracellular fluid is rich in phosphatides but contains very little calcium, and the lecithin in it is presumably stabilised to the monovalent metal salts present by soap-like substances, probably lysolecithins. When it meets a fluid of higher calcium content, an insoluble lecithin-calcium complex film is formed around the cell and this is fixed and rendered more insoluble by adsorption of proteins and insoluble lipides such as cholesterol. We hope that studies of the lecithin-lysolecithin-divalent metal salt systems will give a more definite picture of cell membrane formation and hence a better understanding of the structure and properties of these membranes.

Correlation of Some Properties of the Sol/Water Interface with Those of Some Biological Membranes

Both these two boundaries form surfaces of separation between two aqueous fluids. The magnitudes of the surface forces at the boundaries are very similar; the surface forces of the lecithin sols can be varied between 0·01 and 0·25 dynes per cm. while those of some simple cells are, *Arbacia punctulata* egg 0·03–0·2 dynes per cm., *Busycon caniculatum* egg 0·5, *Triturus viridescens* egg 0·1, erythrocytes 0·25, *Amoeba dubia* 1·0[159–162].

Ionic effects can be compared. Immersion of various Amoebae in potassium chloride solutions caused dispersion of the plasmalemma and this effect could be prevented by adding calcium chloride[102,163]. We have found that at certain calcium chloride concentrations, addition of potassium chloride reduces the surface force but at higher calcium chloride concentrations this effect does not occur. Another similarity is in the toughening effect of calcium salts on the membranes of some Amoebae species and Fundulus eggs[74], and on the lecithin films.

In the present state of our knowledge it is inadvisable to carry this comparison too far but it seems reasonable to forecast that the lecithin sol/water interface will serve as a useful model for the study of mechanisms of drug action.

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