SURFACE INTERACTION OF LECITHIN AND LYSOLECITHIN

By N. Robinson

*From the Department of Pharmaceutical Chemistry, School of Pharmacy, University of London, Brunswick Square, London, W.C.*1

Received June 3, 1960

The lowering of the surface tension of water by lysolecithin in the presence of a small constant amount of lecithin has been investigated to examine the changes in the boundary tension during the formation of a primary phosphatide membrane. In the higher concentration range of lysolecithin (above 0·05 per cent w/v corresponding to a weight fraction of 0·4) the surface activity was not affected by the presence of lecithin. Below 0·05 per cent w/v lysolecithin the surface activity was reduced but in the very dilute region of the critical micelle concentration it was restored.

The presence of calcium chloride reduced the surface activity to a greater extent above the critical micelle concentration region of lysolecithin than below. The effects of potassium chloride differed from calcium chloride for different regions of the lysolecithin concentration range. Ageing effects due to the salts affected the surface acitivity, probably by reason of the removal of lecithin from the interface. The results were complex and only a qualitative interpretation of the behaviour was attempted. The surface tension : concentration relation for aqueous lecithin sols at four different temperatures, a precursor to the main work, showed a lowering of the surface tension of water to less than 41 dyne/cm. by 0·5 per cent w/v lecithin at 25°; reducing the concentration to 0·05 per cent w/v the surface activity of lecithin steadily diminished to zero.

Since lysolecithin is an enzymatic breakdown product of lecithin, these two substances occur together in biological systems and the surface properties of one will be modified by the presence of the other.

In 1957 Elworthy and Saunders[1] suggested that when stable boundaries were formed between an aqueous phosphatide sol and water, the structure of the interfacial film was that of an extended bimolecular leaflet, with polar groups on its outside surface. This concept bore some resemblance to Danielli and Davson's[2] general structure of a simple cell membrane. Later Saunders[3] observed that lysolecithin and lecithin interacted, when present in certain proportions, to form highly viscous sols. He suggested that if sufficient lysolecithin was present in the internal fluid of a cell the lecithin present would be stable to monovalent metal ions, but precipitation of a phosphatide membrane could still occur when the internal fluid met a solution containing divalent metal ions. At the weight fraction necessary to give precipitation the system was not lytic and hence the membrane would be stable. Robinson and Saunders[4] have reported that the strong interaction of lysolecithin and monostearin to form a viscous sol may also be indicative of typical lysolecithin-lipid cohesive forces contributory to the rigidity of a cell membrane.

The strength of the membrane will be governed in some measure by the change in surface tension of the membrane boundary according to the amount of lysolecithin present within the internal fluid. The latter will,

in turn, be influenced by the concentration of lecithinase catalysing the hydrolysis of lecithin, hydrogen ion concentration, ionic strength and other environmental conditions. The surface interaction of lysolecithin and lecithin has been studied to determine the extent of modification of a boundary tension by changes in some of these conditions.

Both phosphatides possess surface activities to different extents and the lowering of the surface tension of water when both components are present will depend upon interaction in solution. Lysolecithin could exert a solubilising power on lecithin thus tending to remove lecithin from the interface. It is suggested that the physical state of the mixed phosphatide aggregate in the bulk phase will not be one of lecithin solubilised within the lipophilic region of the lysolecithin micelle in the conventional manner; this is prevented by the hydrophilic phosphoryl choline headgroup of the lecithin molecule. It is more probable that the surface of a lysolecithin micelle will be impregnated with single lecithin molecules. The physical state of this mixed phosphatide aggregate will be reported later.

Lecithin sols are sensitive to very small amounts of sodium, potassium and calcium chlorides and the presence of these salts was expected to modify the surface activity of lecithin sols. Lysolecithin sols are stable to small amounts of electrolytes and the surface tension of these sols is comparatively unaffected by their presence. In studying the surface effects of sols containing both phosphatide components, a concentration of lecithin was chosen sufficient to influence the behaviour of the lysolecithin component whilst independently lecithin exerted little or no surface activity itself. An initial study of the lowering of the surface tension of water by lecithin showed that at a concentration of 0·05 per cent w/v lecithin its surface activity was negligible. This concentration was therefor chosen for the work.

The measurements in these experiments were taken one hour after preparation of the sols, but systems containing salts were re-examined after 24 and 96 hours.

Experimental

Preparation of Lecithin and Lysolecithin

Lecithin was prepared from egg yolks as previously described[4]. Lysolecithin was prepared from a sample of the lecithin using Russell viper venom according to Saunders[3] modification of the method of Hanahan[5]. Analytical figures for the two substances are given below.

	Lecithin	*Lysolecithin*
Nitrogen (per cent) ..	1·75	2·72
Phosphorus (per cent) ..	3·89	5·98
N:P ratio	0·99:1	1:1·02
Iodine number	72	4·2

Preparation of lecithin sols. Lecithin sols were prepared by evaporating a sample of the stock solution of lecithin to dryness, dissolving the weighed quantity of lecithin in a minimum volume of ether, adding

distilled water and evaporating the ether with a stream of nitrogen. The sols were freed from air on a filter pump, passed down a small column of mixed strong ion exchange resins and made up to volume.

Preparation of mixed sols. The mixed sols were prepared by taking measured quantities of stock solutions of the two phosphatides of known concentrations, mixing and evaporating to dryness. The film of intimately

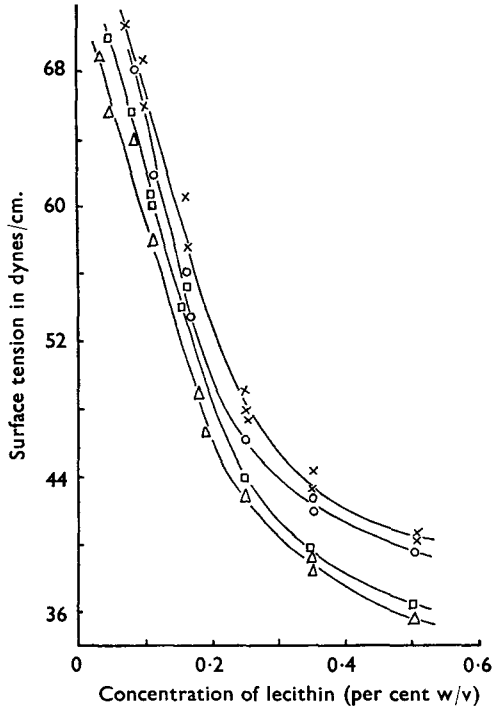

FIG. 1. Variation of surface tension of water with concentration of lecithin at ×20°, ⊙25°, □32·5°, △40°.

mixed phosphatides was dispersed in warm distilled water and shaken to give a clear sol. Traces of electrolytes and dissolved air were removed. Sols containing potassium chloride and calcium chloride were prepared as previously and made up to volume by addition of small calculated volumes of concentrated salt solutions.

Apparatus. Surface tensions between 20° and 40° were measured by the ring method using the chainomatic balance assembly previously described[6].

RESULTS AND DISCUSSION

Surface Tension Effects of Lecithin

The surface activity of lecithin at a concentration of 0·5 per cent w/v was marked, the surface tension of water being lowered to less than 41 dyne/cm. at the four temperatures investigated (Fig. 1). Smaller concentrations continued to produce a considerable lowering of the

surface tension but at 0·05 per cent w/v the effect became negligible except at 40° when the surface tension was 66·6 dyne/cm. The results did not show any abrupt change in the surface tension : concentration relationship indicating that the critical micelle concentration of lecithin in water was very low; the good balance between the hydrophilic and lipophilic groups in the lecithin molecule suggests that aggregates would be present below the concentrations range examined.

The surface tension effects of lecithin on water under different conditions have been previously reported, but more recent preparations of

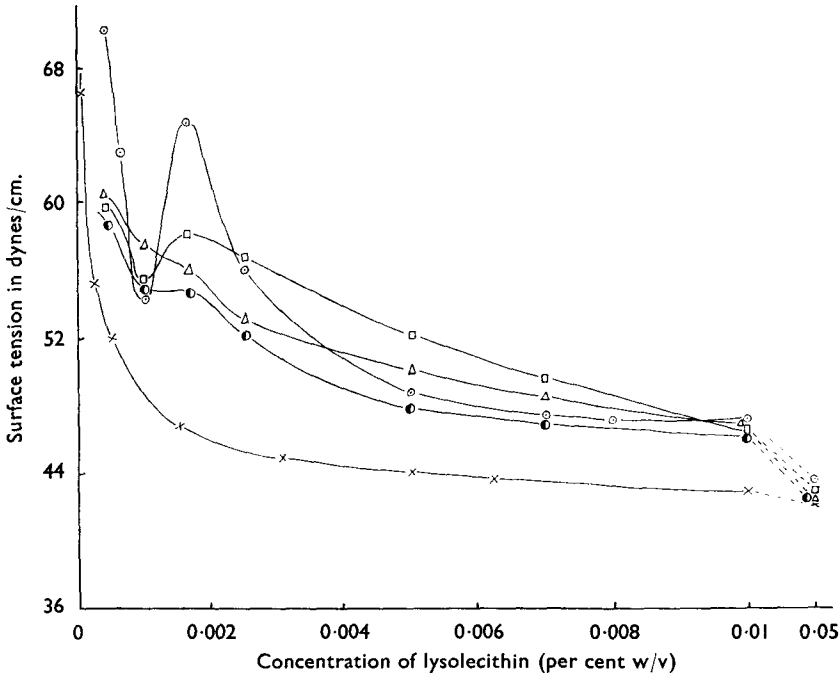

FIG. 2. Effect of $CaCl_2$ on the surface tension of mixed lysolecithin-lecithin sols at 25°.
× Lysolecithin
⊙ Mixed sols of lysolecithin and 0·05 per cent w/v lecithin
□ Mixed sols in 10^{-4} $CaCl_2$
△ Mixed sols in 10^{-4} $CaCl_2$ after 24 hours
◐ Mixed sols in 10^{-4} $CaCl_2$ after 96 hours

lecithin by chromatography indicated that small amounts of lysolecithin and other phosphatides were probably present[7–9]. An equally successful but more rapid method for the final purification of lecithin using ion exchange resins has been reported by Perrin and Saunders[10]. The high surface activity of lysolecithin could greatly affect measurements of the surface tension of aqueous lecithin sols.

Earlier studies by Neuschloz[11], using a drop number method, showed that salts brought about a change in the lowering effects of lecithin on the surface tension of water, aluminium chloride inhibiting the surface activity in smaller concentrations than calcium, sodium and potassium

chlorides. Price and Lewis[12], using the maximum bubble pressure method, obtained a maximum in the surface tension: concentration relationship at pH 2·6 which was thought to be the isoelectric point. Fischgold and Chain[13] have since shown that the isoelectric point is in fact much higher (6·7). The experiments of Boutaric and Berthier[14] showed a lowering of the surface tension of water by 0·5 per cent w/v

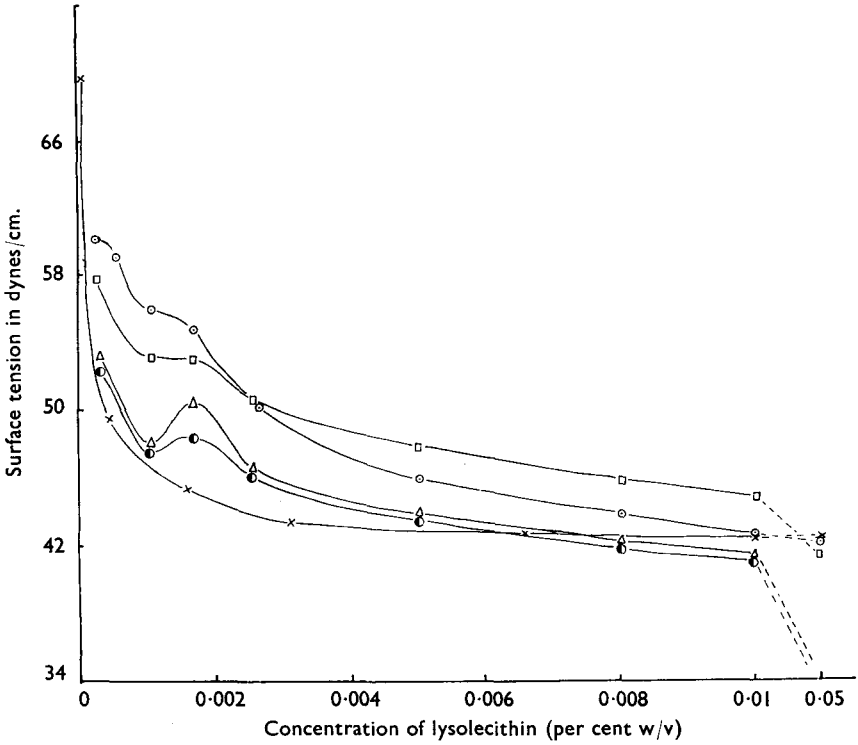

FIG. 3. Effect of $CaCl_2$ on the surface tension of mixed lysolecithin-lecithin sols at 40°. For key see Fig. 2.

lecithin to 32·6 dyne/cm. after 1 hour; the effect of salts on the surface tension of lecithin sols was, however, contrary to results obtained by previous workers.

Surface Interaction with Lecithin

Throughout the concentration range of lysolecithin at 25° its surface activity was depressed to different extents by the presence of lecithin. Above a concentration of 0·05 per cent w/v, sufficient lysolecithin was present to remove lecithin from the interface and the surface tension of the sol remained relatively unaltered. Below this concentratin the rise in surface tension suggested that the lecithin brought about a withdrawal of lysolecithin away from the air-water interface to participate in the solubilisation of the lecithin. In this concentration region the boundary

tension of a membrane could be lessened by removal of lecithin from the interface resulting in instability which may lead to some lysing action. At a concentration approaching the critical micelle concentration of lysolecithin (mol ratio of lysolecithin to lecithin approximately 1 : 20) a marked increase in surface activity took place which was attributed to small aggregates and single molecules, which possessed little solubilising

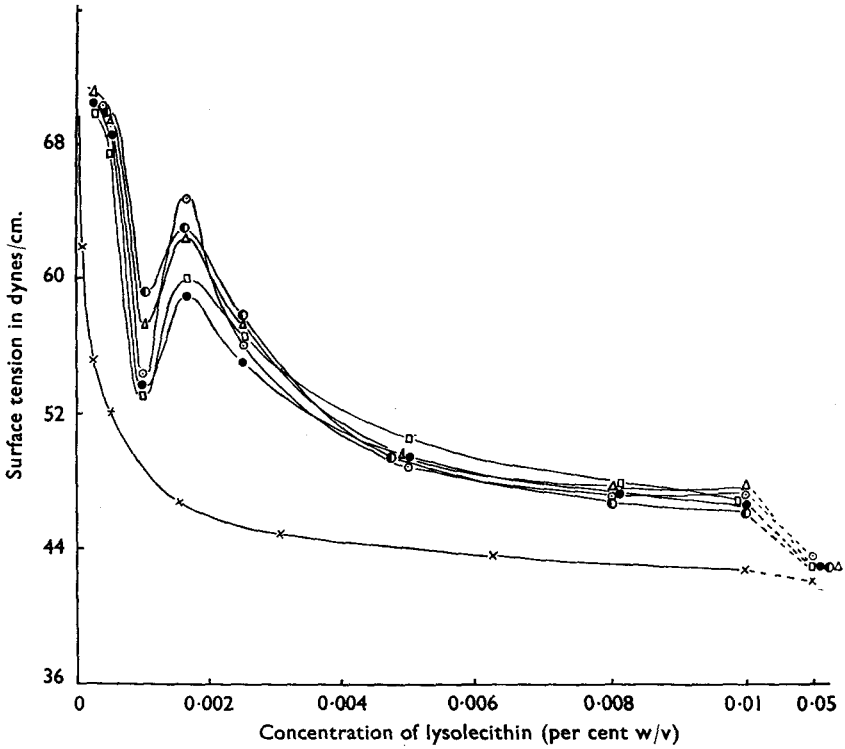

FIG. 4. Effect of KCl on the surface tension of mixed lysolecithin-lecithin sols at 25°.

× Lysolecithin
⊙ Mixed sols of lysolecithin and 0·25 per cent w/v lecithin
□ Mixed sols in 10^{-2}M KCl
● Mixed sols in 10^{-1}M KCl
△ Mixed sols in 10^{-1}M KCl after 24 hours
◐ Mixed sols in 10^{-1}M KCl after 96 hours

power, present at the interface. The surface tension at high dilution was slightly less than that of a pure lysolecithin sol.

At 40° a similar lowering of the surface tension of water took place except below the region of 0·001 per cent w/v. The plateau in this region contrasted strongly with the behaviour at 25°, the higher temperature favouring greater solubilisation with a small reduction of phosphatide in the surface layer. Although this reduction in surface activity appeared to continue into the most dilute region examined at 40°, there was no marked change compared with that observed at 25°.

Effect of Calcium Chloride

The lecithin and lysolecithin molecules have two sites of charges at the phosphoryl and the cholyl groups. Adsorbed molecules such as soaps must also be considered since a primary ageing effect will be hydrolysis at the ester linkages.

Above 0·002 per cent w/v lysolecithin the lowering effect was slightly less than in the absence of calcium chloride (Fig. 2). The calcium chloride would normally be expected to lower the critical micelle concentration of lysolecithin and it was probable that above 0·002 per cent w/v lysolecithin the salt assisted aggregation of molecules and consequently increased solubilisation of the lecithin. From the small change in surface behaviour

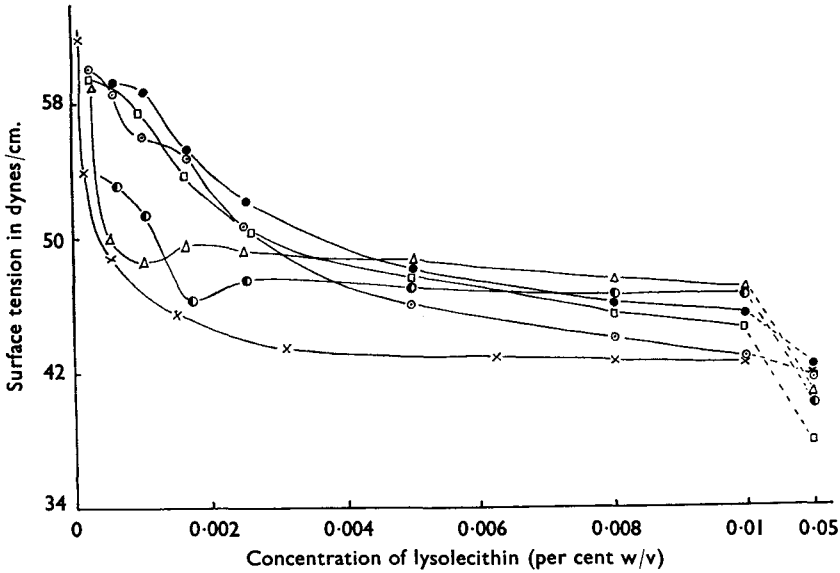

FIG. 5. Effect of KCl on the surface tension of mixed lysolecithin-lecithin sols at 40°. For key see Fig. 4.

it appeared that sufficient lysolecithin was present to prevent substantial removal of the lecithin component from the surface layer by calcium chloride.

The instability of the sols over a period of time was believed to be brought about by the electrolytes affecting the charge on the colloidal particles resulting in a tendency to coagulation.

On standing for 24 hours the calcium chloride appeared to interact with the system in this concentration region of lysolecithin (0·002 per cent) resulting in an increase in surface activity of the sols. After another 72 hours this behaviour spread throughout the concentration range of lysolecithin examined. At 40° (Fig. 3) the effect after 24 hours was more pronounced but a further 72 hours showed little change. The effect of calcium chloride with time on the general behaviour of the system was to be expected from the sensitivity of lecithin to this electrolyte. Calcium

chloride was active in much smaller concentrations than potassium chloride in producing instability of the sols which was thought to be due to the divalent calcium ions linking the phosphoryl groups of two single molecules or molecules within the aggregates.

Effect of Potassium Chloride

The effects of potassium chloride differed from those of calcium chloride due largely to the degree of sensitivity of lecithin to these two electrolytes. Above a weight fraction of lysolecithin of 0·1 the mixed sols were fairly stable to potassium chloride.

At 25° (Fig. 4) and concentrations greater than 0·0025 per cent w/v lysolecithin, addition of potassium chloride produced only a slight change in surface tension, whereas between 0·001 and 0·002 per cent w/v lysolecithin the surface tension lowering was considerably increased. This deviation was unexpected and thought to arise from ionic forces suppressing the aggregation process of lysolecithin molecules. Below this concentration region the lowering effect was unchanged. After standing, concentrations above 0·002 per cent w/v lysolecithin showed little change but below this value the surface tensions steadily increased.

At 40° (Fig. 5) the effect of potassium chloride was to depress the surface activity of the mixed phosphatide system containing more than 0·003 per cent w/v lysolecithin but below this concentration there was little change. After standing, however, the system showed a considerable lowering in the surface tension indicating that removal of lecithin from the surface layer took place resulting in small lysolecithin particles producing increased surface activity.

The surface activity of the system is thus very sensitive to both calcium chloride and potassium chloride particularly in the region of the critical micelle concentration of lysolecithin where changes are likely to be emphasised. Here the presence of electrolytes shows a tendency to increase the surface activity of the mixed phosphatide system, especially at 40°, compatible with changes in metabolism within the environment of a cell.

Acknowledgement. I thank Dr. Saunders for his interest in this work.

References

1. Elworthy and Saunders, *J. chem. Soc.*, 1957, 330.
2. Danielli and Davson, *J. Cell. Comp. Physiol.*, 1935, **5**, 495.
3. Saunders, *J. Pharm. Pharmacol.*, 1957, **9**, 830.
4. Robinson and Saunders, *ibid.*, 1959, **11**, 346.
5. Hanahan, Rodbell and Turner, *J. biol. Chem.*, 1954, **206**, 431.
6. Robinson and Saunders, *J. Pharm. Pharmacol.*, 1958, **10**, 384.
7. Lea, Rhodes and Stoll, *Biochem. J.*, 1955, **60**, 353.
8. Lea Proc. 2nd International Conference on Biochemical Problems of Lipids, 1955.
9. Rhodes, *Chem. and Ind.*, 1956, **75**, 1010.
10. Perrin and Saunders, *J. Pharm. Pharmacol.*, 1960, **12**, 253.
11. Neuschloz, *Kolloid Z.*, 1920, **27**, 292.
12. Price and Lewis, *Biochem. J.*, 1929, **23**, 1031.
13. Fischgold and Chain, *ibid.*, 1934, **28**, 2044.
14. Boutaric and Berthier, *C.R. Acad. Sci. Paris*, 1940, **211**, 100.