

## SURFACE INTERACTION OF LECITHIN AND LYSOLECITHIN

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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 activity, 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<sup>1</sup> 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<sup>2</sup> general structure of a simple cell membrane. Later Saunders<sup>3</sup> 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<sup>4</sup> 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,

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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 head-group 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 therefore 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<sup>4</sup>. Lysolecithin was prepared from a sample of the lecithin using Russell viper venom according to Saunders<sup>3</sup> modification of the method of Hanahan<sup>5</sup>. Analytical figures for the two substances are given below.

	<i>Lecithin</i>	<i>Lysolecithin</i>
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

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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

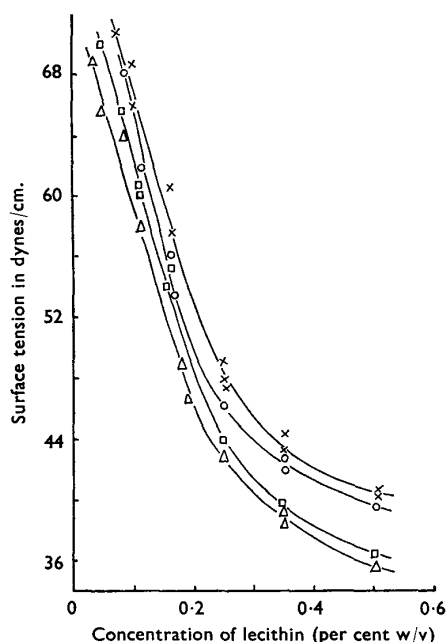


FIG. 1. Variation of surface tension of water with concentration of lecithin at  $\times 20^{\circ}$ ,  $\circ 25^{\circ}$ ,  $\square 32.5^{\circ}$ ,  $\triangle 40^{\circ}$ .

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  $20^{\circ}$  and  $40^{\circ}$  were measured by the ring method using the chainomatic balance assembly previously described<sup>6</sup>.

### 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

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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

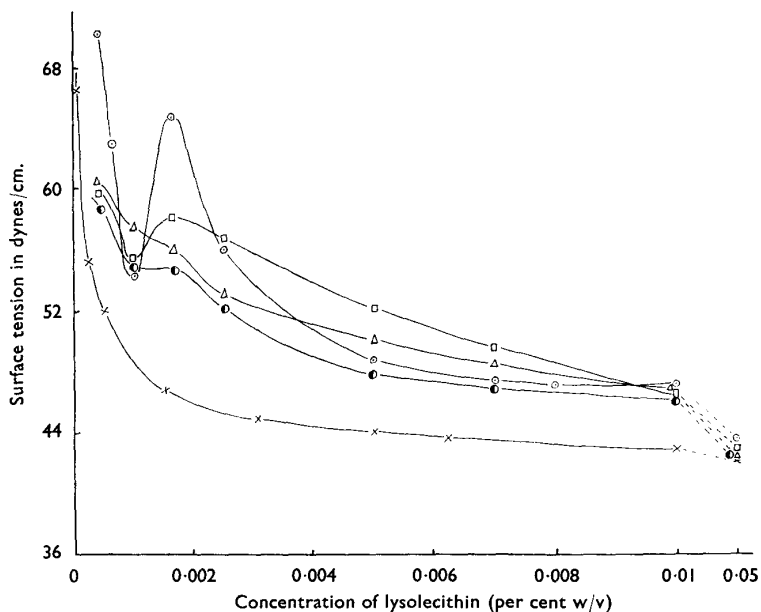


FIG. 2. Effect of  $\text{CaCl}_2$  on the surface tension of mixed lysolecithin-lecithin sols at 25°.

- × Lysolecithin
- Mixed sols of lysolecithin and 0.05 per cent w/v lecithin
- Mixed sols in  $10^{-4}$   $\text{CaCl}_2$
- △ Mixed sols in  $10^{-4}$   $\text{CaCl}_2$  after 24 hours
- Mixed sols in  $10^{-4}$   $\text{CaCl}_2$  after 96 hours

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

Earlier studies by Neuschloz<sup>11</sup>, 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

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chlorides. Price and Lewis<sup>12</sup>, 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<sup>13</sup> have since shown that the isoelectric point is in fact much higher (6.7). The experiments of Boutaric and Berthier<sup>14</sup> showed a lowering of the surface tension of water by 0.5 per cent w/v

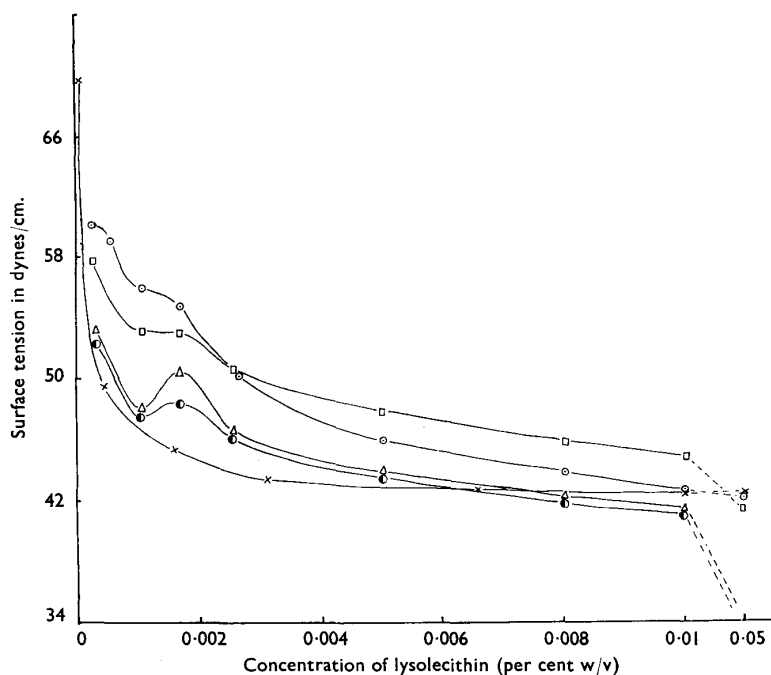


FIG. 3. Effect of  $\text{CaCl}_2$  on the surface tension of mixed lysolecithin-lecithin sols at  $40^\circ$ . For key see Fig. 2.

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  $25^\circ$  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 concentration 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

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