LXXIX. THE PHYSICO-CHEMICAL BEHAVIOUR OF LECITHIN.

II. THE CAPILLARY ACTIVITY OF LECITHIN- CHOLESTEROL DISPERSIONS.

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THE work described in the present paper is a continuation of that of Price and Lewis [1929] who measured, by means of the Sugden maximum bubble pressure method (a dynamic method), the surface tension of dispersions in water of lecithin (prepared and purified by the method of Levene and Rolf [1927]) as a function of the p_H of the system. It was found that a maximum value of the surface tension occurred at p_H 2.6 (approx.), a position which agrees well with the value 2.7 for the isoelectric point obtained by an entirely different method by Fugii [1923-24]. The surface tension fell to a very marked minimum on either side of the maximum referred to.

Employing the same technique and methods of preparation, the above results have been repeated and confirmed by the present authors. With this as a starting point the next step was to investigate what modification, if any, was produced by the presence of cholesterol in the lecithin dispersion. It was found, as might have been expected, that pure cholesterol owing to its insolubility and lack of dispersibility in water exerted no effect of itself upon the surface tension of water. This was confirmed by a series of measurements in which cholesterol was first dissolved in ether, the solution being poured into water. It was found that the capillary effect was due entirely to the ether.

Before dealing with the behaviour of lecithin-cholesterol dispersions, however, it is convenient to give the experimental evidence which is concerned with the dimensions of the capillary-active unit itself.

The capillary-active unit in lecithin dispersions.

From the fact that lecithin dispersions are visibly turbid it follows that the disperse units are, for the most part at least, of very considerable size, *i.e.* of the order of 10^{-4} – 10^{-5} cm. radius. It is a point of some theoretical interest to know whether units of this order are responsible for the capillary effects produced or whether the latter are due to the presence of much smaller units either of truly colloidal or even molecular magnitude. To determine this a series of

filtration experiments was carried out (a) with a coarse filter; (b) with an ultra-filter (collodion).

Series (a). The filter was a good commercial preserve paper. The filtrate was itself turbid but less so than the original. In this case any colloidal and molecular units would come through with ease and if these were the truly capillary-active constituents the surface tension should be unchanged. Actually the filtrate possessed somewhat higher tension than the original, *i.e.* the filtrate approximated more nearly to pure water, indicating that the large units are certainly capillary-active though this of itself does not exclude the presence of active units of small size. For the sake of comparison, filtration was carried out with sucrose solution, the concentration of which was found to be unaffected by this filter, as is shown by the constancy in the optical rotation. The experimental observations are as follows.

Sucrose solution (20 %):

$$\text{Angle of rotation} \begin{cases} \text{before filtration} = 47.53^\circ, \\ \text{of filtrate} = 47.33^\circ. \end{cases}$$

Lecithin (1 % dispersion):

Before filtration, surface tension = 32.0 dynes/cm.

Surface tension of filtrate = 51.8 dynes/cm.

Lecithin (0.5 % dispersion):

Before filtration, surface tension = 66.2 dynes/cm.

Surface tension of filtrate = 69.4 dynes/cm.

Series (b). In this case a collodion ultrafilter was employed. The filtrate now came through water-clear. As the results show, the surface tension of the filtrate was practically that of water itself. At the same time sucrose molecules, as would be expected, passed through with ease. The results are as follows.

Sucrose solution (20 %):

$$\text{Angle of rotation} \begin{cases} \text{before filtration} = 47.54^\circ, \\ \text{of filtrate} = 47.30^\circ. \end{cases}$$

Lecithin (0.5 % dispersion):

Before filtration, surface tension = 66.2 dynes/cm.

Surface tension of filtrate = 71.0 dynes/cm.

(Surface tension of pure water = 71.78 dynes/cm.)

The results of both series of measurements demonstrate that there are no capillary-active units of molecular or colloidal size in a lecithin dispersion, the capillary effect being due entirely to the large units which give rise to the turbidity.

Preparation of lecithin-cholesterol dispersions.

The desired amounts of pure lecithin and cholesterol were dissolved in a small volume of specially purified ether and the solution was evaporated to dryness. The residue was redissolved in ether and again evaporated. This was repeated two or three times, the evaporation being carried out under reduced

pressure and at low temperature to avoid decomposition of the lecithin. The final dry residue was shaken with the correct quantity of water and the resulting dispersion kept in an atmosphere of nitrogen.

Except in the case of the dispersion which contained the highest amount of cholesterol (namely, lecithin : cholesterol = 1 : 3) all the dispersions prepared were very stable, no precipitation occurring. In the one exceptional case not all the cholesterol was combined with or peptised by the lecithin, with the result that there was a certain amount of solid cholesterol present as a precipitate. (It was demonstrated that this was pure cholesterol.) It is evident that the ratio recorded as 1/3 is in fact slightly larger than this.

EXPERIMENTAL RESULTS.

All measurements were carried out at 25°. As in the work of Price and Lewis, the dynamic surface tension was found to be independent of the rate of bubble-formation, which could be varied, in the apparatus employed, between 1 bubble per 0.5 sec. and 1 bubble per 3 secs. The p_H determinations were carried out by means of the gold-quinhydrone electrode as well as by the glass electrode with valve. The composition of the dispersed material is expressed in grams of lecithin to grams of cholesterol, *e.g.* 4 : 1. Different concentrations of any given lecithin-cholesterol dispersion were employed, *e.g.* 0.5 %, that is 0.5 g. of dispersed material per 100 cc. of aqueous "solution." The surface tension of water was taken as 71.78 dynes per cm.

In each case the surface tension was determined at a series of different p_H values covering a wide range of p_H . These arbitrarily chosen p_H values however differed from case to case. In order to make the results more readily comparable for dispersions of different chemical composition the actual values have been graphed and, from the resulting curves, the values of surface tension read off at a series of p_H values the same for all the dispersions for which the data are recorded. The results are recorded in the following Tables which include besides the numerical data certain comments necessary to make them self-explanatory.

Table I. 0.5 % dispersion of lecithin.

p_H	Surface tension (dynes/cm.)	
0.50	58.0	
1.00	44.2	
1.20	40.8	1st minimum
1.50	47.0	
2.00	65.8	
2.50	68.9	
2.60	69.1	Maximum
3.00	66.6	
3.50	56.2	
3.70	55.7	2nd minimum
4.00	60.0	
4.50	66.4	
5.00	66.7	
5.50	66.7	Flat portion parallel to p_H axis
6.00	66.7	
6.50	66.7	

Table II. 0.5 % dispersion.

Ratio: lecithin/cholesterol = 4/1.

p_H	Surface tension (dynes/cm.)	
0.50	67.6	
1.00	69.2	
1.25	69.6	1st maximum
1.50	69.20	
2.00	67.20	
2.50	61.0	
2.70	60.4	Minimum
3.00	62.8	
3.50	68.0	
4.00	70.2	2nd maximum
4.50	67.6	Very shallow minimum
5.00	68.0	
5.50	68.2	
6.00	68.1	Flat portion
6.50	68.0	
7.00	68.0	

Table III. 0.5 % dispersion.

Ratio: lecithin/cholesterol = 2/1.

p_H	Surface tension (dynes/cm.)	
1.00	64.2	
1.35	66.0	1st maximum
1.50	65.7	
2.00	63.0	Minimum
2.50	64.8	
3.00	67.6	
3.50	69.6	
4.00	70.8	2nd maximum
4.50	70.2	
5.00	69.8	
5.50	69.7	
6.00	69.5	Flat portion
6.50	69.6	

Table IV. 1.0 % dispersion.

Ratio: lecithin/cholesterol = 1/1.

p_H	Surface tension (dynes/cm.)	
1.00	65.4	
1.50	65.0	
2.00	63.6	
2.35	62.4	Minimum
2.50	62.5	
3.00	64.8	
3.50	66.4	
4.00	67.8	
4.25	68.1	Maximum
4.50	67.8	
5.00	67.2	
5.50	67.3	
6.00	67.6	
6.50	67.5	Flat portion
7.00	67.3	

The concentration of the dispersion is in this case 1 %, i.e. 0.5 % with respect to lecithin.
The values are thus directly comparable with those of Table I for lecithin alone.

Table V. 0.5 % dispersion.

Lecithin/cholesterol = 1/3 (approximately).

p_H	Surface tension (dynes/cm.)	
0.50	65.4	
1.00	68.4	
1.50	69.4	1st maximum
2.00	68.2	
2.50	67.0	Minimum
3.00	68.2	
3.50	70.8	
4.00	71.6	2nd maximum
4.50	71.2	
5.00	70.7	
5.50	70.7	Flat portion
6.00	70.9	
6.50	70.9	

At this concentration some free cholesterol separates out.

Inspection of the above Tables shows that addition of cholesterol, whilst leaving the general capillary behaviour of lecithin as a function of p_H unchanged as a whole, nevertheless introduces modifications in the magnitude of the surface tension values and at the same time appears to alter the location of the maxima and minima exhibited by dispersions of the pure substance. The most important modification consists in what appears to be a shift in the position of the isoelectric point (as determined by the central maximum surface tension) from p_H 2.6 to p_H 4. This very great shift into the region of higher p_H values is brought about even in the case of that dispersion in which the lecithin is still in great excess, namely, 4 : 1. In view of the fact that in blood-serum the ratio of lecithin to cholesterol is about 1 : 1 and of the order 5 : 1 in normal tissue of various kinds [Jowett, 1931] it follows that the significant reaction which would determine the electrical behaviour of the naturally occurring (impure) material would be p_H 4. Whether this is modified further by the presence of protein is a matter now under investigation.

SUMMARY.

By means of the Sugden maximum bubble pressure method (a dynamic method) the surface tensions of dispersions containing lecithin and cholesterol in different relative amounts have been determined as a function of p_H . The position of the isoelectric point (identified with that of the central surface tension maximum) is altered by the addition of cholesterol from the value p_H 2.6, characteristic of pure lecithin, to p_H 4.

It is shown that the capillary-active units are the large particles which give turbidity to the dispersion, no evidence being obtained for the existence of capillary-active units of colloidal (or molecular) dimensions.

REFERENCES.

- Fugii (1923-24). *J. Biochem.* (Tokio), **3**, 393.
 Jowett (1931). *Biochem. J.* **25**, 1991.
 Levene and Rolf (1927). *J. Biol. Chem.* **72**, 587.
 Price and Lewis (1929). *Biochem. J.* **23**, 1030.