CXXV. NOTE ON THE SPONTANEOUS DECOMPOSITION OF LECITHIN.

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THE most recent work on the decomposition of lecithin is due to Fischgold and Chain [1934], who have obtained evidence that lecithin, even in the absence of air, undergoes a spontaneous change. This change was found to be due to separation of free fatty acid from the lecithin molecule.

C. W. Price and Lewis [1933, 1] noted a change in the electrophoretic properties of lecithin as it became aged even though the material was kept in the dark and in an atmosphere of dry nitrogen. In the p_H region in which the lecithin dispersion was negatively charged, it was found that there was a rapid increase in electrophoretic mobility with the age of the lecithin used for the dispersion. After a period of about 3 weeks the lecithin reached some state of equilibrium, its electrophoretic properties remaining constant (at a given p_H) for at least 5 months.

Fig. 1 shows the effect of age on the mobility of lecithin at p_H 5.02 and Fig. 2 compares the mobility- p_H curve of lecithin in its stable condition (*A*), with that of lecithin aged for 8 days only (*B*). Owing to the continuous change in the behaviour of the substance, the latter curve is approximate only. However, the points were plotted in such an order that, as nearly as possible, the curve is representative of lecithin aged for the above period.

The lecithin was prepared from egg-yolk by the method of Levene and Rolf [1927] and the measurements were carried out by the macro-electrophoretic method described by C. W. Price and Lewis [1933, 1].

From the curves it is evident that there is a very large change in the electrophoretic properties of lecithin with age, including a shift in the isoelectric point towards the acid region.

The isoelectric point of lecithin in the "aged" or equilibrium condition has been found by C. W. Price and Lewis to be at p_H 2.7. This corresponds fairly closely to the values obtained by other methods employed by workers in this field. Thus Fugii [1924] observed the isoelectric point of lecithin to be at p_H 2.7, H. I. Price and Lewis [1929] at p_H 2.6 and Remezov [1930] at p_H 2.0-2.8. It might be concluded therefore, that the material used by these authors was similar to the "aged" material used by us. Recently Chain and Kemp [1934] have reported the isoelectric point of freshly prepared lecithin as p_H 6.7; a value more nearly approximating to that calculated, namely p_H 7.5¹.

¹ This value was estimated from the values of the dissociation constants of choline and of glycerophosphoric acid (and similar phosphoric acid derivatives) and is a correction of the value (p_H 5.06) estimated in the same way by C. W. Price and Lewis but based on an erroneous value of K_b for choline. This correction is acknowledged; the error being consequent upon the assumption that the reproducibility of K_b , calculated from the p_H of an extreme range of concentrations of choline chloride, was sufficient evidence to show the absence of traces of free acid or free base. This error was likewise observed by Jukes [1934].

Fig. 1.

Fig. 2.

As previously stated, Fischgold and Chain have shown that the spontaneous change undergone by lecithin in the absence of air, is due to the separation of free fatty acid. From a knowledge of this Chain and Kemp [1934] consider, and in the writer's view quite properly, that the low value of the isoelectric p_H obtained by earlier workers is due to the presence of this material. The reason for this peculiar effect of free fatty acid in a buffered solution is, however, not considered. Clearly, the presence of free fatty acid as such, can influence the electrophoresis of the dispersion only if it is adsorbed at the surfaces of the lecithin micelle. Consequently this becomes, simply, a special case of ion adsorption, *viz.* the adsorption of fatty acid anions (or molecules subsequently ionising). This is in accordance with the view expressed by C. W. Price and Lewis [1933, 1, 2] that the charge on "aged" lecithin is a composite factor, involving an adsorption charge in addition to the ionisation charge. The source of the adsorption charge is the free fatty acid anions rather than simple OH^- ions as tentatively suggested. The reality of an adsorption charge was demonstrated by C. W. Price and Lewis [1933, 2] from determinations of the isoelectric point of the lecithin in dispersions of increasing ionic strength. It was shown that a very large shift in the isoelectric p_H from p_H 2.7 to 5.1 was consequent upon the addition of small concentrations (*e.g.* 0.03 *M*) of $BaCl_2$. Further increase in the ionic strength of the solution merely resulted in the mobility- p_H curve revolving around this point, as would be expected if, at p_H 5.1, the adsorption charge was completely nullified. The fact that the isoelectric point did not shift above p_H 5.1 to a value nearer to that calculated would seem to indicate the presence of some very small quantity (about 10^{-2} mol. %¹) of further ionising groups in the molecule formed by the inevitable slight oxidation of the lecithin. This is probably also the explanation of the discrepancy between the calculated isoelectric point and that observed by Chain and Kemp (p_H 6.7) for freshly extracted lecithin, rather than the presence of a very small quantity of free fatty acid suggested, since no shift was observed in the isoelectric point on varying the ionic strength of the dispersion. This criterion was used by Chain and Kemp to show the absence of an adsorption charge on the fresh material.

The susceptibility of lecithin for acquiring an adsorption charge can be seen from the fact that at fairly high concentrations of $BaCl_2$ (*e.g.* 0.15 *M*) it becomes charged positively at all p_H values².

It should be stressed that in the experiments of the writers only "aged" lecithin was used. It is quite probable that lecithin *in situ* resembles this equilibrium material more closely than that freshly extracted. Thus Fischgold and Chain [1934] point out that by precipitation of the lecithin with cadmium chloride (a process used in its purification) it is possible to remove the free fatty acid.

Since the physiological function of certain phosphatides is usually associated, at least in part, with their great dispersing properties, the fact that freshly extracted lecithin emulsifies only with difficulty (a phenomenon also observed by Fischgold and Chain) gives some support to the above view.

Again, the opacity of a lecithin dispersion decreases very markedly with the age of the lecithin used, until the equilibrium position is reached. This would be expected if the material becomes more hydrophilic on ageing, the refractive index of the material approaching more closely to that of water. The increase

¹ The ionisation is assumed to be from carboxyl groups formed by oxidation of unsaturated glycerides with a p_K value of 5.

² This phenomenon was observed for the fresh material by Chain and Kemp [1934] and for the "aged" material by C. W. Price and Lewis [1933, 2].

in hydrophilic nature might well be associated with the increased charge (of a negative sign) due to the presence of fatty acid adsorbed on the lecithin micelle. The process of purification would seem, therefore, to decrease, at least temporarily, the dispersing qualities of the lecithin by removing the free fatty acid and also as a consequence of this, to render it less hydrophilic.

SUMMARY.

The spontaneous change taking place in lecithin, in the absence of air, has been shown to be associated with a change in its electrophoretic properties (resulting in an increased charge of negative sign) and a decrease in the isoelectric p_H .

It is considered that lecithin aged to constant electrophoretic mobility in the absence of air, more closely resembles the state of the material *in situ* than does the freshly extracted substance, since free fatty acid present is removed in the process of purification.

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