SOLUBILIZATION OF CHOLESTEROL IN PHOSPHOLIPID MICELLES IN WATER

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In a companion communication (Fleischer and Klouwen, 1961), we have presented data that the active component in the lipoprotein preparation of Basford and Green (1959) is phospholipid in micellular form and that the state of molecular dispersion or solubilization is an intrinsic property of the component phospholipid(s) alone. Such soluble micelles can be prepared not only from mixtures of mitochondrial phospholipids, but also from the individual phospholipids. Since micelle formation requires paraffin ions or their equivalent in polarity, neutral molecules such as cholesterol and coenzyme Q_{10} should not be solubilized by the procedures which are effective for phospholipids (for details of solubilization, cf. Fleischer and Klouwen, 1961) and indeed this has been found to be the case. However, we have found that phospholipid micelles provide an elegant vehicle for solubilizing cholesterol (and other neutral molecules such as coenzyme Q) in aqueous media. In view of the central position of cholesterol in biochemical studies and the difficulties which many investigators have experienced in dealing with aqueous suspensions of cholesterol (cf. Oncley, 1958), it should be of wide interest to describe how it is possible to prepare clear aqueous solutions of cholesterol of the order of mgs per ml.

When mitochondrial phospholipids or individual phospholipids (lecithin and cardiolipin) are treated in the presence of cholesterol with the reagents for inducing micelle formation (exposure to a butanol-water-cholate mixture

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followed by dialysis) micelles of phospholipid are formed containing one-fifth by weight of cholesterol (cf. Table I). Micelles of mixtures of phospholipids

TABLE I

Solubilization of Cholesterol in the Presence of Phospholipids

Conditions of Solubilization			Analyses of Solubilized Lipid		
Phospholipid(mg)	Cholesterol(mg)	Butanol-Cholate[o](ml)	µg P/ml	Cholesterol (mg/ml)	mg Cholesterol[oo]/mg Phospholipid
None	4.4	1.0	---	< 0.04	---
None	33.0	1.5	---	< 0.04	---
Mitochondrial Phospholipid-33 mg	17.0	1.5	242	1.20	.18
Mitochondrial Phospholipid-33 mg	6.6	1.5	303	1.65	.20
Lecithin-33 mg	33.0	1.5	178	1.07	.22
Cardiolipin-68 mg	18.0	4.5	167	0.78	.17

Cholesterol was determined by the method of Searcy, et al.(1960). Solubilized lipids were either evaporated to dryness and the cholesterol determined directly, or the lipids in water were extracted into an organic phase with 4 volumes of chloroform-methanol (2-1) and back-extracted to remove non-lipid matter (Tris and EDTA) as described by Folch et al.(1957). The extracted lipid was evaporated, taken up in chloroform and applied to a column (1 X 8 cm) of silicic acid. Cholesterol was eluted with 25 ml of chloroform and an aliquot was analyzed for cholesterol. This more complicated procedure was necessary only for the determination of cholesterol in cardiolipin, since a turbidity develops on addition of the ferrous sulfate in glacial acetic reagent to the cardiolipin. Lipid phosphorus was determined by the method of Chen et al.(1956). Mitochondrial lipids were obtained by extracting beef heart mitochondria three times (20,10,10 volumes) with chloroform-methanol (2-1) according to Folch et al.(1957) as modified by Rouser (1961). The modifications introduced by Rouser insure more complete extraction and minimize lipid peroxidation (Dr. George Rouser, personal communication). Beef cardiolipin and lecithin were obtained from the Sylvana Chemical Company, Orange, New Jersey.

[o] The composition of the butanol-cholate mixture, as well as the exact conditions for solubilization are described by Fleischer and Klouwen (1961).

[oo] Mg phospholipid are calculated from the µg P/ml assuming 37γ P/mg phospholipid.

are equivalent to micelles of individual phospholipids in regard to the extent of solubilization of cholesterol. The presence of cholesterol in the micelle does not contribute appreciably to the opalescence of the solution. Thus, the cardiolipin-cholesterol micelle solution referred to in Table I shows an O.D. of 0.005 at 700 mμ in a 1 cm cell; whereas the corresponding micelle prepared in absence of cholesterol shows an O.D. of 0.005. Solubilized phospholipid mixtures or lecithin usually show a variable degree of opalescence which is not intensified measurably by cholesterol.

The amount of cholate retained by the phospholipid micelle after extensive dialysis is not affected by the presence of cholesterol. The determination of cholate (Mosbach, 1954) is complicated by the interference of phospholipid with color production when the level of cholate is relatively low (1 part of cholate to 20 parts of phospholipid). However, subject to this limitation in the sensitivity of the method of determination of cholate, there appears to be no more cholate in the phospholipid-cholesterol micelle than in the phospholipid micelle. Since the solubilization of neutral lipids by phospholipids can be achieved by other methods not involving cholate (see sonication method below), it follows that the presence of cholate is not vital to this phenomenon.

The solubility of CoQ_{10} in the phospholipid micelle is much less than that of cholesterol (Table II) -- approximately 3% by weight of CoQ_{10} as compared to 20% for cholesterol. This saturation concentration of CoQ_{10} in the micelle is about three times the average concentration of CoQ_{10} in beef heart mitochondrial lipid. Mitochondrial phospholipid micelles saturated with CoQ_{10} have a yellowish tint and show the characteristic peak for the oxidized form of the quinone at 275 mμ. (Mitochondrial phospholipid solubilized in the absence of CoQ is used in the reference cell to balance the light scattering of the solubilized lipid.)

Neutral lipids can also be solubilized in phospholipid micelles prepared by sonication as follows. The phospholipid solution is combined with that of

TABLE II

Solubilization of Coenzyme Q_{10} in the Presence of Phospholipids

Conditions of Solubilization			Analyses of Solubilized Lipid		
Mitochondrial Phospholipid (mg)	CoQ_{10} (mg)	Volume Butanol-Cholate (ml)	µg P / ml	CoQ_{10} (mg/ml)	mg CoQ_{10} / mg Phospholipid
0	4.0	1.0	---	0	-----
116	2.0	4.0	252	0.084	0.012
116	4.0	4.0	223	0.175	0.029
104	3.6	4.5	185	0.154	0.031

CoQ was separated from phospholipids on a silicic acid column as described for cholesterol (cf. legend of Table I). The chloroform eluate was evaporated and taken up in ethanol. CoQ was determined by the spectrophotometric method of Crane et al.(1959). The preparation of mitochondrial phospholipids and other pertinent experimental details are also listed in the legend of Table I.

the neutral lipid in a Potter Homogenizer and the organic solvent evaporated under a stream of nitrogen. Last traces of solvent are removed in a vacuum dessicator and the residue is suspended with homogenization in Tris-EDTA (pH 8.0) 0.02 M - 0.001 M. The mixture is then exposed to 10 KC sonic irradiation and then centrifuged for one half-hour at 35,000 rpm in the Spinco preparative centrifuge. Both CoQ and cholesterol are readily solubilized in phospholipid micelles prepared in this manner. When phospholipid is absent the neutral lipids do not form soluble solutions. This method is expeditious and perhaps of more general application in that it does not depend upon the solubility of the lipid in butanol. Basford and Green (1959) have previously described the binding of various neutral and charged molecules to the Q lipoprotein such as coenzyme Q and silicomolydate and once again it can be seen that phospholipid micelles duplicate the properties that were recognized in the so-called lipoprotein.

The solubility of cholesterol in phospholipid micelles may provide an indication of how phospholipids or lipoproteins containing phospholipids can serve as carriers of normally water-insoluble substances. The observations of Byers and Friedman (1960) that sustained infusion of phospholipids induce

elevated plasma cholesterol levels may perhaps be explained in terms of this solubilization phenomenon. If, as it appears likely, lipoprotein phospholipid has a micelle structure then exchange of cholesterol between Tween-20 dispersions and plasma lipoproteins would be predictable and, indeed, Whereat and Staple (1960) have demonstrated that this exchange does take place.

The solubilization of neutral lipophilic molecules in micelles is a property well known to and documented by colloid chemists (Hartley, 1955), but by virtue of the ambiguity and uncertainty in our knowledge of the state of phospholipids in aqueous solution, the extension of this old principle to the cholesterol problem has been late in coming to the biochemical literature.

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BIBLIOGRAPHY

Basford, R. E. and Green, D. E., Biochim. Biophys. Acta, 33, 185 (1959)

Byers, S. O. and Friedman, M. J., Lipid Research, 1, 343 (1960)

Chen, P. S., Toribara, T. Y. and Warner, H., Anal. Chem. 28, 1756 (1956)

Crane, F. L., Lester, R. L., Widmer, C. and Hatefi, Y., Biochim. Biophys. Acta, 32, 73 (1959)

Fleischer, S. and Klouwen, H., Biochim. Biophys. Res. Comm. (this issue)

Folch, J., Lees, M. and Sloane-Stanley, G. H., J. Biol. Chem. 226, 497 (1957)

Hartley, G. S., in "Progress in the Chemistry of Fats and Other Lipids", Holman, R.T. Lundberg, W.O., and Malkin, T., Eds. p. 19, Pergamon Press, New York, 1955.

Holman, R. T. and Widmer, C., J. Biol. Chem. 234, 2269 (1959)

Mosbach, E. H., Kolinsky, H. J., Halpern, E. and Kendall, F. E., Arch. Biochem. Biophys. 51, 402 (1954)

Oncley, J.L., in "Chemistry of Lipids as Related to Atherosclerosis (Symposium)", I.A.Page, Ed.,p.131 - Dr. Oncley's reply to Dr. Arton, C.C.Thomas. Springfield, Ill., 1958.

Rouser, G., Bauman, A. J. and Kritchevsky, G., Am. J. Clin. Nutrition, 9, 112 (1961

Searcy, R. L., Barquist, L. M. and Jung, R. C., J. Lipid Research, 1, 349 (1960)

Whereat, A. F. and Staple, E., Arch. Biochem. Biophys. 90, 224 (1960).