AN INTERACTION OF DEOXYCHOLATE WITH A MICROSOMAL COMPONENT[1]

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The solubilization of lipophilic material by bile salts, e.g., sodium deoxycholate is well established (Wieland and Sorge, 1916; Ekwall, 1953). It has also been shown that sodium deoxycholate in aqueous systems forms coacervates (Booij and deJong, 1949) and micelles (Philippoff, 1951; de Moerloose and Ruyssen, 1959). The number of deoxycholate molecules per micelle depends upon the amount and nature of added electrolyte (Philippoff, 1951; de Moerloose and Ruyssen, 1959). Solubilization of lipids has been attributed to their inclusion within the micelle (Ekwall, 1953). Rich and Blow (1958) and Blow and Rich (1960) have studied deoxycholate systems under slightly acid conditions and observed the formation of helical complexes of macromolecular dimensions. Interaction of amino acids and peptides with the helical structure was indicated from x-ray diffraction measurements.

Sodium deoxycholate is commonly employed to separate constituents present in cellular extracts (Strittmatter and Ball, 1952; Littlefield et al., 1955). In the preparation of ribosomes, deoxycholate is presumed to solubilize the lipid rich endoplasmic reticular membrane material contained in the microsome fraction (Littlefield et al., 1955; Palade and Siekevitz, 1956; Petermann,

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1960). Direct evidence of interaction between membrane material and deoxycholate has not been reported. In the preparation of ribosomes from thymus extracts (Hess and Lagg, 1963) we have found direct evidence for an interaction between deoxycholate and a microsomal constituent rich in lipids.

Ultracentrifuge patterns obtained with the microsome fraction are clearly distinguished from similar solutions to which deoxycholate was added, as seen in Fig. 1A and B. The most rapidly sedimenting peak is reduced in area and sediments more slowly in the presence of deoxycholate, the significance of which has been considered (Hess and Lagg, 1963) and will be discussed further in a separate communication. The area under the boundary in the region of the meniscus is strikingly larger in solutions containing deoxycholate. A portion of the increase in area can be ascribed to the presence of deoxycholate in the boundary region labeled M + D in Fig. 1B. Material labeled M in Fig. 1A, which has been isolated and found to contain about 35% lipid (Hess and Lagg, 1963), sediments as seen in the upper pattern of Fig. 1C. Deoxycholate, under the conditions employed, shows the sedimentation characteristics seen in the lower pattern of Fig. 1C. The pattern seen in Fig. 1D was obtained when a mixture of deoxycholate and Fraction M was examined. It is apparent that deoxycholate is transported from the meniscus in the presence of Fraction M, whereas in the absence of Fraction M transport is negligible. We interpret the result as evidence of interaction between deoxycholate and Fraction M material.

A necessary condition of the argument requires that the refractive increment of the mixture be an approximate sum of the increments of deoxycholate and Fraction M. Using a Brice-Phoenix differential refractometer, it was established that the increment of the mixture is the exact sum of the increments of the two constituents. Further verification of the summation of refraction was provided by synthetic boundary experiments shown in Fig. 2. Synthetic boundary patterns obtained with sodium deoxycholate and Fraction M are shown in Fig. 2A and B, respectively. The pattern seen in Fig. 2C was

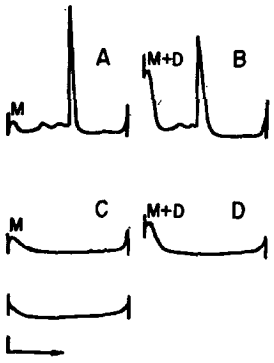

Fig. 1. Ultracentrifuge patterns after approximately 1250 sec at 33,450 rpm Buffer 0.02M phosphate at pH 7.6 containing 0.002M $MgSO_4$. Position of Fraction M indicated by letter M.

A. Microsome fraction: phase plate angle 55°
B. Same material and same concentration as A above except solution contains 0.5% sodium deoxycholate: phase plate angle 45°
C. Upper pattern in wedge cell - fraction M at 0.5% concentration. Lower pattern 0.5% solution of sodium deoxycholate: phase plate angle 45°
D. Mixture of fraction M and sodium deoxycholate both at 0.5% concentration: phase plate angle 45°

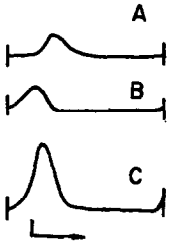

Fig. 2. Patterns obtained using a synthetic boundary cell after 510 sec at 33,450 rpm Buffer 0.02M sodium phosphate at pH 7.6 containing 0.002M $MgSO_4$. Position of the initial boundary indicated by an arrow: phase plate angle 65° in all cases.

A. Fraction M concentration 0.5%
B. Sodium deoxycholate concentration 0.5%
C. Mixture of fraction M and sodium deoxycholate each at 0.5% concentration

obtained from the mixture. The area of the pattern obtained with the mixture is, within the limits of error of enlargement and tracing, the sum of the areas of patterns seen in Fig. 2A and B.

Previously we considered evidence indicating that in the microsome fraction, ribosomes and Fraction M exist in equilibrium with an interaction product (Hess and Lagg, 1963). An interaction between deoxycholate and Fraction M which displaces the equilibrium would promote the isolation of ribosomes; a result consistent with our experience (Hess and Lagg, 1963).

The large weight fraction of lipids, 0.35, is consistent with the viewpoint that Fraction M represents membrane material (Butler, 1961; Haguenau, 1959). The observations of Tanaka (1957) who reported a small number of both rough and smooth surfaced reticular membranes in lymphocytes is likewise in accord with this assumption.

Although based upon studies of thymus lymphocytes known to contain only small amounts of membrane material, the concept of an interaction between deoxycholate and membrane material displacing an equilibrium reaction between ribosomes and membrane, may be applicable to other mammalian cells.

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