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Effects of Deoxycholate on the Microsome Fraction of Thymus*

E. L. Hess and D. F. Oberhauser

ABSTRACT: Ultracentrifuge patterns obtained with the microsome fraction from calf thymus extracts are clearly distinguished from similar solutions to which deoxycholate (DOC) was added. In solutions which contain DOC both the total area of the pattern and the area under the peak in the region of the meniscus is strikingly larger, whereas the peak which sediments most rapidly, usually ascribed to ribosomes, is reduced in area and in rate of sedimentation. Within the concentration ranges examined, 0.1–0.5% DOC, the above changes are dependent upon the concentration of DOC. In control experiments with the microsome fraction in the absence of DOC, there is a progressive decrease in the total area of the pattern with time of centrifugation and a decrease in the areas of individual peaks, greater than can be attributed to radial dilution effects. Area and weight relationships and ribonucleic acid (RNA) and lipid analyses are consistent with the behavior in transport experiments of two reactants in equilibrium with a product. In sedimentation experiments with the microsome fraction the boundary usually ascribed to ribosomes appears to be a reaction boundary. A destruction of isolated 74S ribosomes as a function of DOC concentration was also observed.

The microsome fraction is defined operationally as subcellular material which, after removal of the mitochondria, sediments at 105,000*g* (Loftfield, 1957; Hess and Lagg, 1963a). In accord with the suggestion of Roberts (1958) and consistent with current usage (Petermann, 1964) the term ribosome will designate ribonucleoprotein particles in the size range 20S–100S. The sedimentation properties of ribosomes observed when the microsome fraction of calf thymus extracts is centrifuged differ from those of isolated ribosomes (Hess and Lagg, 1963a). On the basis of several kinds of evidence we suggested that in the microsome fraction ribosomes, and a fraction called M rich in lipids, exist in equilibrium with an interaction product (Hess and Lagg, 1963a). The behavior of two reactants in equilibrium with a product in transport experiments has been considered by Svedberg and Pedersen (1940), Gilbert and Jenkins (1956), Schachman (1959), and Nichol *et al.* (1964). Direct evidence for an interaction between deoxycholate (DOC)[1] and the lipid-rich microsomal constituent has also been reported (Hess and Lagg, 1963b).

This report will present evidence suggesting that a component seen in ultracentrifuge patterns obtained with the microsome fraction of thymus, which would normally be considered to represent ribosomes, is in reality an interaction product. The addition of deoxycholate to the fraction displaces the equilibrium and alters the quantitative distribution of peaks seen in the pattern.

* From the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts. *Received August 23, 1965; revised November 15, 1965.* Supported by U. S. Public Health Service Grant CA-04102 from the National Cancer Institute.

[1] Abbreviations used: DOC, deoxycholate; RNA, ribonucleic acid; M, membrane; R, ribosomes.

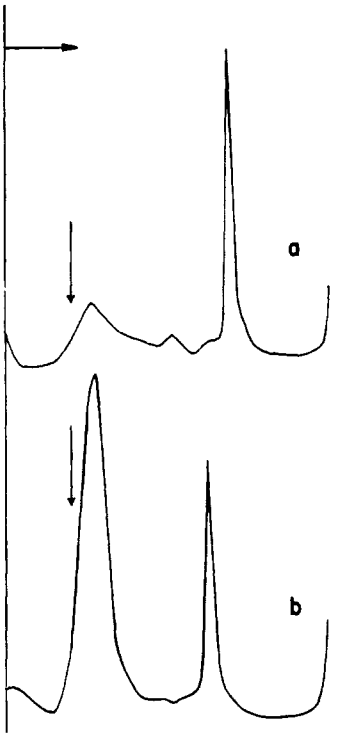

FIGURE 1: The effect of deoxycholate on the microsome fraction. Upper pattern (a) is a control solution containing microsomes in standard buffer examined in a 12-mm synthetic-boundary cell. Standard buffer was used to overlay the solution in the observation channel. Lower pattern (b) the same concentration of microsomes was dissolved in standard buffer which contained 0.5% DOC. Buffer which contained 0.5% DOC was used to overlay the solution in the observation channel. Microsome concentration approximately 1%, phase plate angle 55°. Photograph taken after 920 sec at 33,450 rpm. Vertical arrow indicates the position in both experiments of the initial boundaries.

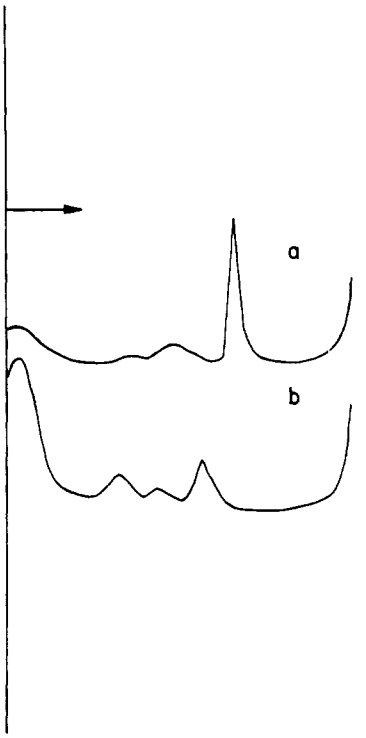

FIGURE 2: Ultracentrifugal behavior of the 79,000*g* sediment. Upper pattern 79,000*g* sediment in a cell with a wedge window. Lower pattern 79,000*g* sediment containing 0.4% DOC in the standard cell. Concentration about 0.6% in phosphate buffer. Photographed after 1800 sec at 33,450 rpm. Phase plate angle 60°.

Experimental Section

The preparation of the microsomal fraction, ribosomes, and fraction M, as well as ultracentrifugal and other analytical procedures, have been described (Hess and Lagg, 1963a). In experiments using synthetic boundary cells, standard buffer was layered over control solutions which did not contain DOC, whereas buffer containing DOC was layered over solutions which contained DOC. The Spinco Model E centrifuge was operated at 3000 rpm for a time sufficient to produce patterns for area analyses which were used as a reference. The speed of the centrifuge was increased to 33,450 rpm and photographs were taken at various time intervals. Areas from these patterns were used for comparison with the reference areas. Potassium phosphate buffer at pH 7.6 having an ionic strength 0.05 and containing 0.002 M magnesium sulfate (standard buffer) was used routinely as a solvent system.

Results

Ultracentrifuge patterns obtained with the microsome fraction are clearly distinguished from similar solutions to which DOC was added, as seen in Figure 1. In solutions which contain DOC, the peak which sediments most rapidly is reduced both in area and in rate of sedimentation, as shown in Table I. Both the total area

TABLE I: Normalized Area Relationships of the Microsome Fraction and Sedimentation Coefficients of Ribosomes as a Function of Deoxycholate Concentration.

Concn of DOC (%)	s_{ap} (S)	Total Area	Area of Lead Peak	% Area under Lead Peak
0.0	66.1	100	44	44
0.1	62.4	146	43	29
0.2	61.9	175	42	24
0.3	61.3	178	40	23
0.4	61.1	179	40	22
0.5	59.0	197	39	20

of the pattern and the area under the peak in the region of the initial boundary is strikingly larger. It is apparent that a larger quantity of material is optically recorded when DOC is present in the solution.

The areas seen in Figure 1 correspond to the concentration gradients in the boundary regions at the time of photography. Material removed from solution during centrifugation will not be optically recorded. It should be noted that since the buffer solution used to overlay the protein solution in the experiment where DOC was employed contained the same concentration of DOC as did the bulk solution, a DOC concentration gradient did not exist at any point in the cell. The larger area under the slower boundary in solutions containing DOC, Figure 1b, represents material either present in the more rapidly sedimenting boundary, or material which was removed from solution and not optically recorded in the boundaries seen in Figure 1a. Alterations in refractive indices can be excluded as a cause of area differences since it has been established (Hess and Lagg, 1963b) that the refractive increment of mixtures was the sum of the increments of protein constituents and DOC.

A systematic decrease in the total area of the pattern, and in the area under individual peaks, greater than could be attributed to radial dilution effects, was always present in control solutions. The finding indicates that material was being removed from solution throughout the period of centrifugation. In solutions which contained DOC area changes other than what could be attributed to radial dilution effects did not occur. The presence of sizable amounts of material in the untreated microsome fraction which would have been removed from solution at the time the pattern seen in Figure 1a was obtained was verified in separate experiments.

Centrifugation at 79,000*g*: Centrifugation of the microsomal fraction for 15 min at 79,000*g* should yield a pellet having a composition comparable to that of material removed from solution during centrifugation in the analytical centrifuge. Synthetic boundary experiments in the ultracentrifuge suggested that a pellet would be essentially absent in comparable studies where DOC was added to the microsome fraction. As the results in Table II indicate, essentially no sediment was obtained with the microsome fraction containing 0.5% DOC, whereas in the absence of DOC the sediment amounted to about 27% of the original fraction. Results from lipid and ribonucleic acid (RNA) analyses listed in Table II are reasonably consistent with earlier studies (Hess and Lagg, 1963a). The sediment, redissolved and examined with moving-boundary electrophoretic procedures (Hess and Lagg, 1963a; Figure 5B) and in the ultracentrifuge (Figure 2), was qualitatively indistinguishable from both the supernatant fraction (Hess and Lagg, 1963a; Figures 5 and 3B) and the whole microsome fraction (Figure 1). Quantitatively the pellet appears to contain a somewhat larger proportion of membrane material, a result in accord with data in Table II.

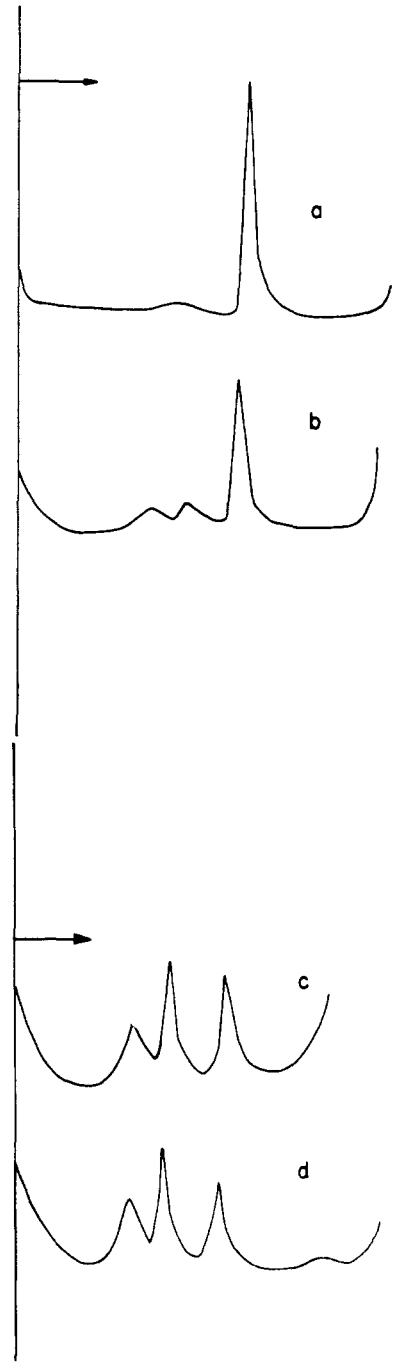

FIGURE 3: The effect of DOC on ribosomes. a, Control sample 74S ribosomes in phosphate buffer containing 0.002 M Mg^{2+} after 1750 sec at 33,450 rpm, phase plate angle 50°, s_{ap} = 64.1 S. Concentration approximately 0.8%. b, 74S ribosomes in phosphate buffer containing 0.002 M Mg^{2+} and 0.5% DOC. Concentration, time, speed, and angle same as in a above. s_{ap} (main peak) = 60.6 S. c, 74S ribosomes, concentration about 0.8%, in phosphate buffer containing 0.002 M Mg^{2+} and 1% DOC after 1620 sec at 33,450 rpm, phase plate angle 45°, s_{ap} = 61, 46, and 34 S, respectively. d, 74S ribosomes in phosphate containing 0.002 M Mg^{2+} and 1.25% DOC. Time, speed, concentration, and angle same as in c above.

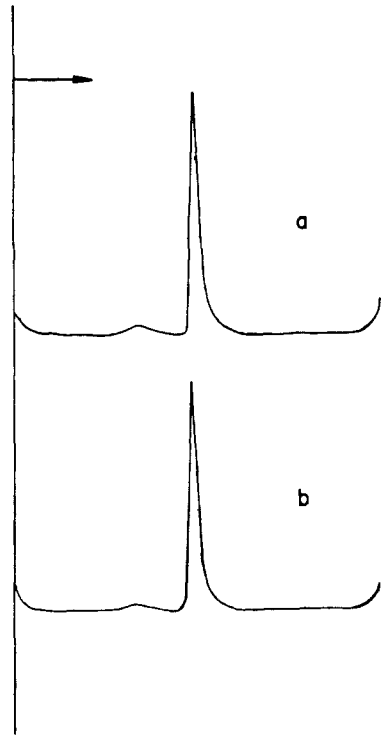

FIGURE 4: Ultracentrifugal behavior of 74S ribosomes in phosphate buffer containing 0.002 M Mg^{2+}. a, The solution contained 0.1% DOC, s_{ap} = 67.9 S. b, Control sample without DOC, s_{ap} = 68.1 S. Photographs after 1675 sec at 33,450 rpm, phase plate angle 50°, concentration approximately 0.8%.

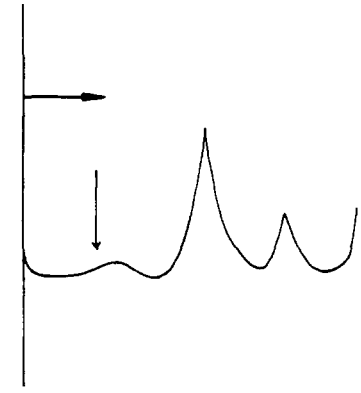

FIGURE 5: Dissociation of 74S ribosomes in absence of Mg^{2+}. Ribosomes (74S) dialyzed 24 hr against phosphate buffer in which Mg^{2+} was absent and examined in synthetic boundary cell photographed after 1380 sec at 33,450 rpm, phase plate angle 40°, s_{ap} = 71 and 34 S, respectively. Concentration approximately 0.4%. Vertical arrow indicates initial position of boundaries.

TABLE II: Weight Relationships and Analyticaal Data from Microsome Fractions Centrifuged 15 Min at 79,000*g*.

	Untreated Control Soln	Soln Contained 0.5% DOC
79,000*g* sediment (mg)	31	1.2
79,000*g* supernatant (mg)	83	111
RNA content (%)		
79,000*g* sediment	10	
79,000*g* supernatant	26	19.5
Lipid content (%)		
79,000*g* sediment	29	
79,000*g* supernatant	17	21

Discussion

An explanation is required for four observations: (1) the increase in the total area of the patterns as a function of DOC concentration; (2) a decrease in the sedimentation coefficient of the peak ascribed to ribosomes as a function of the DOC concentration; (3) a decrease in the area of the peak ascribed to ribosomes as a function of DOC concentration, and (4) a decrease in the areas of patterns as a function of time observed with solutions centrifuged in the absence of DOC.

The first two observations are easily explained as resulting from solubilization by DOC of suspended material otherwise carried down during the acceleration and early stages of centrifugation. Such material would not be optically recorded in the schlieren patterns at the time the upper pattern seen in Figure 1 was photographed. The sediment obtained when the microsome fraction was centrifuged 15 min at 79,000*g* is consistent with this interpretation.

The decrease in the sedimentation coefficients observed in the presence of DOC and shown in Table I can be ascribed to concentration dependency effects resulting from the solubilization of material by DOC. The effect of density differences resulting from the presence of DOC in phosphate buffers can be excluded as a significant factor contributing to differences in sedimentation rates. A density difference of only 0.0002 g ml^{-1} measured at 22° was found between phosphate buffer and buffer containing 0.5% DOC.

The decrease in area as a function of DOC concentration of the peak ascribed to ribosomes seen in Figure 1b and listed in Table I, however, is not compatible with this simple explanation. The ribosome peak, if anything, should be larger in the presence of DOC, since as shown in Figure 2, ribosomes are present in the 79,000*g* sediment, material not optically recorded in Figure 1a.

Destruction of 74S ribosomes by DOC would account for the decrease in area of the peak ascribed to ribosomes. The sedimentation characteristics of 74S ribosomes are substantially altered, as shown in Figure 3, when centrifuged in the presence of DOC. The extent of alteration, which depends upon the concentration of

DOC, barely detectable at a concentration of 0.1% as shown in Figure 4, is striking at 0.5% and higher concentrations of DOC. It is also of interest that the effect of DOC on the 74S ribosome can be clearly distinguished from dissociation resulting from removal of magnesium ions as seen in Figure 5.

The increase in total area of patterns and the decrease in the area of the peak ascribed to ribosomes is already manifested, as seen in Table I, at DOC concentrations of 0.1%, a condition where the 74S ribosome is essentially unaffected, as shown in Figure 4. An explanation for the decrease in the area of the ribosome peak in Figure 1 and Table I, therefore, is not attributable solely to destruction of ribosomes by DOC. The destruction of ribosomes by DOC, which has been noted previously by Petermann (1964), becomes important only at DOC concentrations 0.3% or greater.

Binding of cell sap proteins to ribosomes offers a partial explanation, which cannot be excluded by our studies, for the effect of DOC on the sedimentation coefficient and areas seen in Table I. For this explanation to apply, however, it is necessary to assume both that, in the microsome fraction, cell sap proteins are bound to ribosomes, and that the bound material dissociates in the presence of DOC.

The progressive decrease, in the absence of DOC, in the total area of the pattern with time of centrifugation and the decrease in the areas of individual peaks, greater than can be ascribed to radial dilution, is not explained by the removal of suspended material, nor by binding of cell sap proteins to ribosomes. The properties of the material in the 79,000g sediment are not consistent with the concept of insoluble suspended material in the microsome fraction. As mentioned above, the pellet obtained at 79,000g, redissolved in phosphate buffer, is remarkably similar in ultracentrifugal and electrophoretic behavior to the original microsome fraction.

The area changes observed both in the presence and absence of DOC are compatible with results and with an interpretation discussed previously (Hess and Lagg, 1963a). According to the previous interpretation, the microsome fraction of thymus consists of two molecular species M (membrane) and R (ribosomes) reversibly interacting to form a series of products M_iR_j. Where i and j are of sufficient magnitude, the products sediment rapidly and are removed from the reaction. Additional large aggregates continue to form during centrifugation and leave the boundary regions, thereby reducing the areas of the schlieren pattern. A portion of the interaction products consist of a complex MR, permitting qualitative description in terms of the Gilbert and Jenkins (1959) treatment of a bimolecular interaction. In accord with this concept, when the microsome fraction is examined in the ultracentrifuge (Figure 1a), the boundary usually ascribed to ribosomes is in reality a reaction boundary. The area under the boundary, and its shape and sedimentation coefficient, will depend upon the concentration of reactants and the rate constants (Gilbert and Jenkins, 1959).

In the presence of DOC the equilibria are displaced by the interaction between M and DOC (Hess and Lagg, 1963b). As a consequence, the reaction products dissociate, increasing the amount of optically recorded material, as observed in Figure 1b and shown in Table I. The decrease in the area under the lead peak, the boundary usually ascribed to ribosomes, results from the elimination of the reaction boundary. The reaction boundary in Figure 1a which consisted in part of an MR complex is replaced by a ribosome boundary seen in Figure 1b.

The area under the ribosome boundary seen in Figure 1b represents the quantity of ribosomes previously present in the reaction boundary plus the quantity of ribosomes carried down in the heavy aggregate and not optically recorded in Figure 1a. The area fraction under the lead peak listed in Table I reflects the weight fraction of ribosomes in the microsome fraction. The weight fraction, subject to errors resulting from differences in the refractive increments of M and R as well as by any destruction of ribosomes by DOC, can be estimated as about 0.25 for microsomes from calf thymus.

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